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EVALUADORES

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EDITORIAL

Colombia con sus 114.174.800 millones de hectáreas, posee una posición geográfica privilegiada, bañada por el costado occidental por el océano pacífico y por el norte con el mar caribe, además con el recorrido que realiza por su territorio la cordillera andina, le brinda al país una gran diversidad de climas aptos para la producción agrícola de diversos cultivos debido a los diferentes pisos térmicos, sumado a la riqueza hídrica y a las precipitaciones normalmente bien distribuidas en el tiempo y con la cantidad de agua requerida por algunos productos.

De acuerdo al último censo agropecuario realizado por el Ministerio de Agricultura y Desarrollo rural para el año 2016, donde se monitorearon 111.500.000 millones de hectáreas correspondientes al 97,7% del territorio nacional, de las cuales 63,2 millones de hectáreas corresponden a bosques, equivalentes al 56,7% del área estudiada, además se pudo evidenciar que 43 millones de hectáreas correspondientes al 38,6% del área estudiada corresponden a uso agropecuario de los cuales se tienen 7,1 millones de hectáreas cultivadas, equivalente al 6,2% de la superficie total nacional, Antioquia es el Departamento de mayor área relativa en cultivos correspondiente a 719.037 hectáreas, para uso no agropecuario se tienen 2,5 millones de hectáreas equivalentes al 2,2% y para otros usos se encontró que se tienen 2,8 millones de hectáreas correspondientes al 2,5%.

Se ha manifestado por estudios recientes la gran posibilidad y necesidad que tiene el país de aumentar la frontera agrícola en hasta un 50% de su uso actual, es decir crecer en más de 3 millones de hectáreas, aspecto que posicionaría la nación como un gran actor en la producción agrícola para cubrir las demandas nacionales, del continente y el mundo, máxime donde se espera que para el año 2050 se tenga unos 10.000 millones de habitantes, los cuales demandarán alimentos abundantes y de alta calidad.

En el país se sigue cultivando café como principal producto agrícola, además de caña de azúcar, plátano, banano, cacao, cereales, leguminosas, oleaginosas, tubérculos, raíces, frutas y hortalizas, entre otros vegetales, todos ellos con gran potencial agroindustrial y susceptibles de ser transformados con el fin de darles valor agregado, utilizar más eficientemente las producciones estacionales, posibilitar oportunidades de empleo digno en las áreas rurales, generar y propiciar investigación e innovación para el desarrollo tecnológico y social del sector agrario, además de generar divisas para los sectores productores.

Para la Facultad de Ciencias Agrarias de la Universidad Nacional de Colombia sede Medellín, en sus 103 años de fundación a través de la oferta de sus programas de pregrado y posgrado, y de todos estos años formando profesionales integrales de las más altas calidades académicas, es motivo de gran satisfacción haber contribuido de manera decidida al desarrollo agrario de la nación, y se constituye en un reto que se asume con firmeza, el aportar con todo su capital científico y técnico para que se logre un adecuado desarrollo rural con las demandas que exige el siglo XXI, con el fin de garantizar la producción, transformación y mercadeo de los productos provenientes del sector agrario, bajo un marco sostenible, cuidadoso del medio ambiente, respetuoso y valorador de los sectores sociales y de los recursos hídricos, con el firme propósito de garantizar la seguridad y soberanía alimentaria de sus habitantes.

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Effects of light intensity on the morphology and CAM photosynthesis of *Vanilla planifolia* Andrews

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Efectos de la intensidad lumínica sobre la morfología y la fotosíntesis CAM de *Vanilla planifolia* Andrews

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ABSTRACT

Key words:
Chlorophyll fluorescence
Gas exchange
Orchids
Photosynthetic pigments
Titratable acidity
Tropical crops

Vanilla planifolia is a neotropical orchid, whose fruits produce the natural vanilla, a fundamental ingredient for the food and cosmetic industry. Because of its importance in the world market, it is cultivated in many tropical countries and recently its cultivation has started in Colombia. This species requires shade for its development; however, the optimal of light conditions are unknown. This work evaluates the effect of different light intensities on CAM photosynthesis, physiology, morphology, and growth of this species. For this, vanilla seedlings were subjected to four treatments of relative illumination (RI) (T1=8%, T2=17%, T3=31% and T4=67%). Most CO_2 assimilation occurred along night in all treatments, which confirms that vanilla is a strong CAM species. Plants grown under high lighting (67% RI) had almost half of the photosynthesis in treatments of intermediate lighting (17 and 31%), which is consistent with the lower nocturnal acid accumulation in that treatment. Likewise, the photochemical efficiency of photosystem II (Fv / Fm) showed that in plants of the 67% RI occurred high radiation stress. On the other hand, vanilla plants reached greater length, leaf area, and total biomass when grown under intermediate radiation (17 and 31% RI). These results suggest that high radiation alters the functioning of vanilla plants, inhibiting photosynthesis and growth, and that highly shaded environments not significantly affected the CAM photosynthesis of vanilla; however, in the long-term this species showed higher photosynthesis and growth under intermediate levels of radiation.

RESUMEN

Palabras claves:
Fluorescencia de la clorofila
Intercambio de gases
Orquídeas
Pigmentos fotosintéticos
Acidez titulable
Cultivos tropicales

Vanilla planifolia es una orquídea neotropical, cuyos frutos producen la vainilla natural, un ingrediente fundamental para la industria alimenticia y cosmética. Debido a su importancia en el mercado internacional, se cultiva en varios países tropicales y recientemente ha comenzado su cultivo en Colombia. Esta especie requiere sombra para su desarrollo; sin embargo, se desconocen sus rangos óptimos de condiciones de iluminación. En este trabajo evaluamos el efecto de diferentes intensidades lumínicas sobre la fotosíntesis CAM, la fisiología, la morfología y el crecimiento de esta especie. Para esto, plantas jóvenes de vainilla se sometieron a cuatro tratamientos de iluminación relativa (IR) (T1=8%, T2=17%, T3=31% y T4=67%). La mayor proporción de asimilación de CO_2 ocurre durante la noche en todos los tratamientos, lo cual confirmó que la vainilla es una especie CAM fuerte. Las plantas que crecieron bajo alta iluminación (67% IR), tuvieron casi la mitad de la fotosíntesis de las plantas en los tratamientos de iluminación intermedia (17 and 31%), lo cual coincide con la baja acumulación nocturna de ácidos en este tratamiento. Así mismo, la eficiencia fotoquímica del fotosistema II (Fv / Fm) mostró que en las plantas del 67% IR ocurrió estrés por alta radiación. De otro lado, las plantas de vainilla alcanzaron mayor longitud, área foliar, y biomasa total cuando crecieron bajo radiación intermedia (17 y 31% IR). Estos resultados sugieren que la alta radiación altera el funcionamiento de las plantas de vainilla, inhibiendo la fotosíntesis y el crecimiento, y que los ambientes altamente sombreados no afectan significativamente la fotosíntesis CAM de la vainilla; sin embargo, en el largo plazo, esta especie muestra mayor fotosíntesis y crecimiento bajo condiciones intermedias de radiación.

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Vanilla (*Vanilla planifolia* Andrews) is a hemiepiphyte orchid that faces seasonal water stress throughout its life cycle under natural conditions, which explain its CAM photosynthetic pathway (Goh and Kluge, 1989); likewise, this species faces different light environments: initially it grows in the shady interior of neotropical forests, and then, leaning on the tree, reaches the canopy where flowers and fruits at more light (Fouché and Jouve, 1999). Since vanilla plants are exposed to spatial and temporal (daily and seasonal) variations in the amount of radiation received (Fouché and Jouve, 1999), it is expected that this species had high acclimation capacity to different light environments, such as other epiphytes and hemiepiphytes of tropical forests (Zotz and Winter, 1994; Zotz and Andrade, 2001; Haslam *et al*., 2003).

Because of its importance in the world market of spices, vanilla is cultivated in many tropical countries (Bory *et al*., 2008). The success of this crop depends, among other factors, of the proper management of light because it is the environmental factor with the greatest influence on plant photosynthesis and therefore on growth, survival, and ultimately, the ability of acclimation to different habitats (Puthur, 2005). Hence, the importance of establishing the acclimation capacity of vanilla plants to different light environments and determining the most favorable for their growth.

The role of radiation on photosynthesis of CAM plants is related to the processes that occur during the four phases of this metabolism (Osmond, 1978), since the radiation can modulate their magnitude and duration (Cushman, 2001). The intensity of photosynthetically active radiation (PAR) during the day (Phase III), determines the rate of mobilization of organic acids from the vacuole (Barrow and Cockburn, 1982). On the other hand, the amount of radiation during the day also influences the extent of CO_2 absorption at night (Phase I), affecting the abundance of carbohydrates generated through the Calvin cycle and glucogenesis, which are required for the provision of PEP at night (Nobel and Hartsock, 1983). Thus, the radiation during the day affects the extent of acid accumulation during the following night, and therefore, the amplitude of daily fluctuations of acids in CAM plants depends on radiation intensity (Lüttge, 2004).

Sometimes low lighting can be limiting for CAM photosynthesis, such as in epiphytes of cloud forests during the rainy season (Pierce *et al*., 2002). Excess of radiation and over-energization of the CAM photosynthetic apparatus can also be limiting; systems of energy dissipation exhibited by C3 plants, such as photorespiration and the presence of carotenes, have also been reported in CAM plants (Lüttge, 2004).

In regions where vanilla is cultivated, located mainly in the subtropical zone, different values of lighting have recommended for the proper development of plants: 30 - 80% of relative illumination (RI) in Mexico (Hernandez-Hernandez, 2011), 50 - 60% in Costa Rica (Varela, 2011), 60% in Reunion, Comoros and Madagascar Islands (Kahane *et al*., 2008), 30 - 50% in India (Anilkumar., 1994; Sarma *et al*, 2011; Zaubin *et al*, 2011), 50 - 60% in Australia (Exley, 2011), and 30 - 50% in Indonesia and Islands in the south Pacific (Bianchessi, 2004). The lower values of these ranges are generally considered appropriate for vegetative growth and the higher ones to stimulate flowering (Fouché and Jouve, 1999; Puthur, 2005). However, these recommendations are rarely based on published research or results that support higher growth or development of plants in these environments.

According to former reports, our hypothesis is that RI close to 30% is the most favorable for photosynthesis and growth of vanilla plants in the initial phase of establishment. To test it, the objective of this study was to evaluate the behavior of *V. planifolia* seedlings under different lighting levels in terms of: i) vegetative growth and morphological characteristics; ii) characteristics of CAM photosynthesis; iii) the response to extreme light environments, expressed as acclimation to low light conditions as well as the susceptibility and defense mechanisms to photo inhibition by high lighting.

MATERIALS AND METHODS

Experimental site

The trial was conducted in an area of alluvial terraces of flat topography in the inter-andean valley of the Cauca river (6º33'32'' N and 77°51''04'W), altitude of 540 m, on lands of the Agricultural Center Cotové (Universidad Nacional de Colombia). Data from the weather station located on the same site are: annual mean temperature of 27 °C, average annual precipitation of 1058 mm (bimodal distribution, with dry seasons from December to March and from June to August) and mean relative humidity of 75% (data of the Cotové Station, IDEAM).

The site corresponds to the life zone tropical dry forest (*sensu* Holdridge, 2000).

Vanilla plants

Vanilla cuttings used in this research were about 80 cm long, taken from the apical portion of the branches of healthy and adult plants of *V. planifolia* from a crop established in San Pedro de Urabá (Antioquia, Colombia). Cuttings were hardened in the shade for two weeks, and then planted in plastic containers (6 L capacity); a wooden stand was previously installed in each container to serve as a tutor for subsequent plant growth.

Lighting treatments

Shade houses of four different RI conditions were built: 8%, 17%, 31%, and 67%. Maximum values of photosynthetic active radiation (PAR) in the shade houses occurred between 13 and 14 h, and were 142, 369, 577, and 1285 µmol m^{-2} s^{-1} for the treatments of 8, 17, 31, and 67% RI, respectively. These values were obtained with quantum sensors (LI-190, LI-COR, Lincoln, Nebraska, USA) connected to a data logger (LI-1000, LI-COR, Lincoln, Nebraska, USA) installed for three days in one shade house by lighting treatment, with measurements every hour in cycles of 24 h, at the end of the first dry season of the year. The total daily radiation at full sun light averaged 2.4, 12.8, 28.7, and 43.8 mol m^{-2} d^{-1} in the treatments of 8%, 17%, 31%, and 67% RI, respectively. Shade houses were large enough (width: 4.5 m, length 6 m, and height 2.4 m) to ensure aeration and spacing between plants and prevent the occurrence of diseases. Vanilla plants were grown in the shade houses for six months before starting the measurement of variables to ensure acclimation to the corresponding light environment; then the monitoring of biometric parameters was done for twelve months. Plants received permanent irrigation to avoid differences by drought. Average temperatures in the shade houses varied between 25.3 °C in the treatment of 8% RI and 26.2 in the treatment of 67%.

Variables evaluated

CO_2 exchange. Data of CO_2 exchange were taken in fully expanded and healthy young leaves, located in the fourth internode from the terminal bud with a portable gas exchange system (LI 6400 XT, LI-COR Biosciences, Lincoln, NE, USA). The CO_2 concentration was kept constant at a value of 400 µmol mol^{-1} and the block temperature at 26 °C. Automatic records were taken every two hours during cycles of 24 hours to three plants per treatment. For each RI treatment, one curve was estimated as the average of the three curves performed. The net absorption/release of CO_2 was quantified for the day, night and the whole day, by integrating the areas under the 24 h curves of CO_2 exchange (Griffiths, 1989).

Night accumulation of H^+. To determine the nocturnal acid accumulation, the change of titratable acidity of leaf tissues between dusk and dawn of the next day was evaluated. To this, samples of 4.5 cm^2 area were taken with a metal punch in young fully expanded leaves of five plants per treatment. Immediately after collected, samples were frozen in liquid nitrogen, brought to the laboratory and stored in an opaque container at -80 °C until processing, which consisted in immersing of samples in 70 mL of ethanol (20%) and boiling for 20 min. Titratable acidity of the solution was measured as the volume of NaOH (5 mM NaOH) necessary to reach a pH of 7.0 (Silvera *et al.*, 2005) using a digital burette (Titrette, Brand, Wertleim, Germany) and a pH meter (Hanna 211, Woonsocket, RI, USA). With the values of titration, the H^+ μ equivalents were calculated (expressed in terms of leaf area). The change of titratable acidity of leaf tissue (ΔH^+) was calculated as the difference between the μ equivalents of H^+ at dawn and the μ equivalents of H^+ at dusk the day before.

Biometric parameters of plants. After 18 months of growth of vanilla plants in each lighting treatment, leaf area was measured with a portable meter (LI-3000C, LI-COR Biosciences, Lincoln, NE, USA) and the total stem length with metric tape of all plants. Besides, three plants per repetition were harvested in each treatment to assess biomass of leaves, stems, and roots; these samples were oven dried (70 °C to constant weight) and weighed. Then, total biomass and specific leaf area (leaf area per gram of biomass) were calculated.

Chlorophyll fluorescence. The maximum quantum efficiency of photosystem II (PSII), called quantum yield potential, was measured with a portable fluorometer (OS30p, Optisciences, Hudson, NH, USA). This variable expresses the relationship between maximal

fluorescence and variable fluorescence (Fv / Fm); Fv is the difference between maximal fluorescence (Fm) and the basal fluorescence (Fo). Measurements were performed in fully expanded and healthy young leaves, located at position three to seven from the terminal bud in three plants per repetition in each RI treatment; before sampling, leaves were dark-adapted for 30 min. Data were taken every two hours during 24 h.

Pigment analysis. Leaf discs of 1.5 cm^2 were taken in three plants per repetition per RI treatment. After collected, discs were frozen in liquid nitrogen, transported to the lab, and stored at -80 °C until processing. Pigment extraction was performed with 80% acetone (Lichtenthaler and Wellburn, 1983; Poorter *et al*., 2011). Samples were centrifuged (5 °C for 5 min) and the supernatant was taken for reading in a Visible - UV spectrophotometer at 470, 647 and 663 nm (Evolution 600 UV-Vis, Thermo Scientific, Waltham, MA, USA). The following equations developed by Lichtenthaler (1987) were used to obtain the concentration of chlorophylls (*Chlo*) and total carotenes (*Tot car*) (mg/g):

$$Chlo\ a = (12.25 * A_{663nm}) + (2.79 * A_{647nm}) \quad (1)$$

$$Chlo\ b = (21.5 * A_{647nm}) + (5.1 * A_{663nm}) \quad (2)$$

$$Total\ Chlo = (chlo\ a + chlo\ b) \quad (3)$$

$$Total\ car = \frac{(100 * A_{470nm}) - (1.82 * chloa) - (85.02 * chlob)}{198} \quad (4)$$

Experimental design and statistical analysis. Data were analyzed for a completely randomized design, with four RI treatments (67%, 31%, 17% and 8%) and four repetitions per treatment. Each repetition consisted of a shade house where six vanilla plants were installed (24 plants per treatment) for a total of 120 plants for the whole trial. For the statistical analysis, initially the assumptions of normality and homoscedasticity of each variable were evaluated with the tests of Shapiro-Wilk, Kolmogorov-Smirnov, and frequency histograms. Then, an analysis of variance (ANOVA) for each variable was performed. Finally, *post hoc* tests to determine significant differences among means were used. In order to evaluate the effect RI treatments on chlorophyll fluorescence throughout the day, a split plot model over time was used; variances of fluorescence were homogenized by ln (x) transformation (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

CO_2 exchange. Most CO_2 assimilation occurred at night in all treatments. At dawn, around 6:00 h, with the sunlight of the early hours of the morning, CO_2 assimilation began to decline drastically until 10:00 h. From this time, with high solar radiation, CO_2 assimilation was negative; after 16:00 h the uptake began to increase, but usually did not reach positive values until 18:00 h when it was already dark, so carbon assimilation did not occur at the end of the afternoon (Figure 1). The average values of maximum instantaneous assimilation (± SE) were 1.17 ± 0.10, 1.71 ± 0.19, 1.65 ± 0.42 and 0.98 ± 0.09 µmol m^{-2} s^{-1} for plants grown under 8, 17, 31 and 67%, RI respectively, and occurred late in the evening between 0 and 6 h. At night, during the Phase I of CAM metabolism (Osmond, 1978), plants absorbed the largest proportion of CO_2 per unit of leaf area, equivalent on average to 81.2% of the total absorption for the 24 h period (Table 1), which confirms that vanilla is a strong CAM plant (Silvera *et al*., 2005).

Nocturnal acid accumulation. No significant differences occurred among treatments in acidity values at the end of the afternoon (18:00 h), but they did early in the morning (6:00 h) (P=0.0005). Values of nocturnal acid accumulation (ΔH^+) were significantly higher in the treatments of 8, 17 and 31% RI as compared with the treatment of 67% (P=0.0007) (Figure 2).

Biometric variables

Mean liana length was significantly higher (P=0.0017) in the intermediate RI treatments (17 and 31%) compared to low and high ones (8 and 67%): 860.1 cm vs. 589.8 cm, respectively (Figure 3), which represents an increase of 45.8% in plants grown under intermediate light; however, internodes became longer with the decrease in RI (P=0.0002); values were 9.7 and 7.9 cm in plants that grew at RI of 8 and 67%, respectively. Mean values of leaf area were also significantly higher (P=0.0003) in plants under intermediate RI treatments (17 and 31%) compared to low and high ones (8 and 67%): 3737.4 and 1510 cm^2, respectively (Figure 3), which represents an increase of 147.4% in plants grown under conditions of intermediate light.

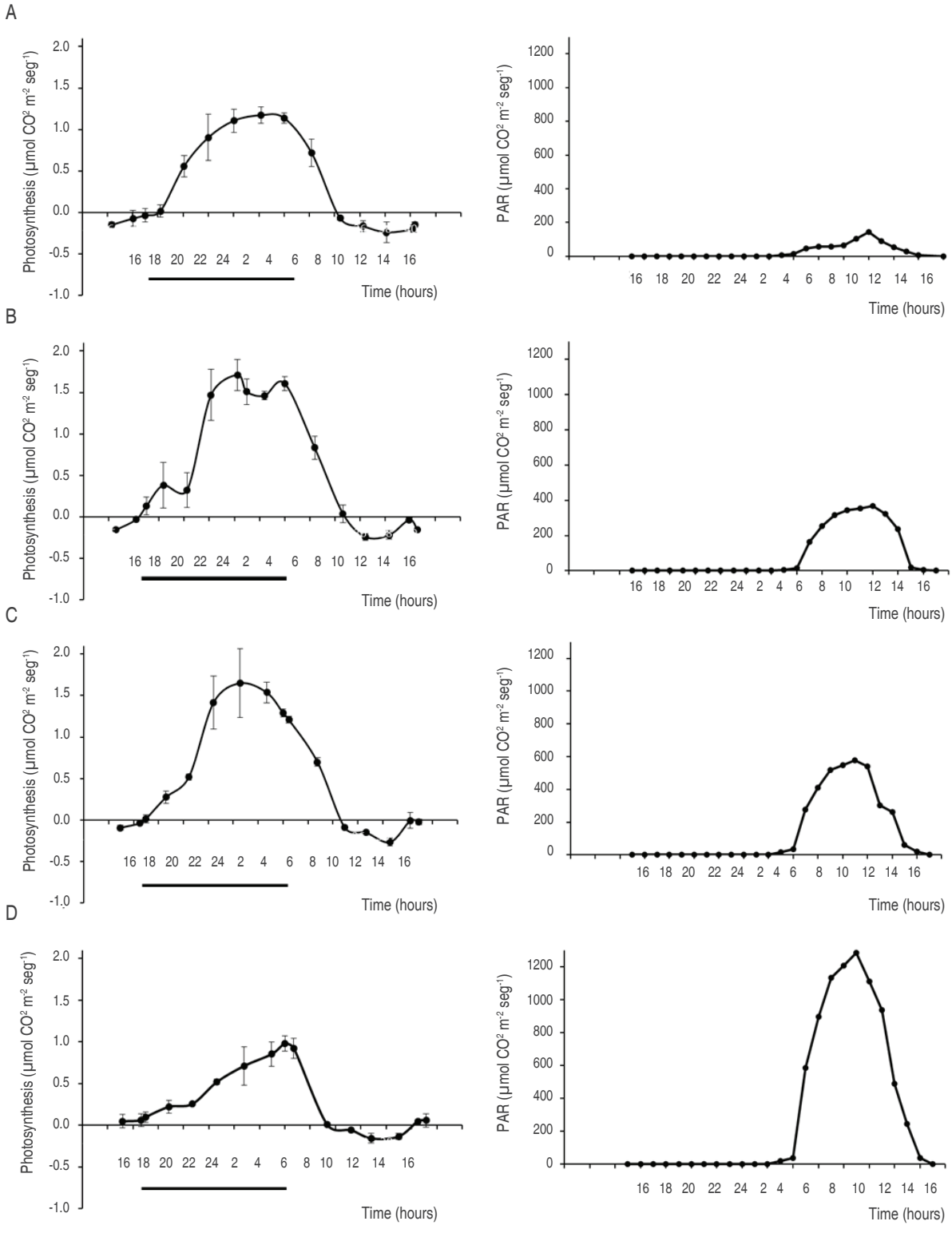

Figure 1. Daily course of photosynthesis of vanilla plants growing in different light environments (n=3, SE in bars): A. 8% RI, B. 17% RI, C. 31% RI, and **D.** 67% RI (left). Radiation values during photosynthesis data recording (right). Black horizontal lines in graphs on the left represent night hours.

Table 1. Daily photosynthesis in vanilla plants growing in different light environments.

Relative illumination (%)	Total photosynthesis (mmol m²)*			Proportion of nigth photosynthesis (%)
	Night	Day	24 h	
8	31.6 ± 4.1 ab	5.2 ± 0.8 b	36.8 ± 4.9 ab	85.9 ± 0.4 a
17	42.8 ± 6.3 a	10.8 ± 1.1 a	53.6 ± 7.2 a	79.6 ± 1.5 a
31	41.1 ± 6.9 a	8.9 ± 0.7 a	50.1 ± 6.2 a	81.3 ± 3.3 a
67	18.2 ± 1.8 b	5.3 ± 0.9 b	23.5 ± 2.4 b	77.5 ± 2.6 a

* Mean values ± SE (n=3). Different letters between light treatments in each column indicate significant differences (*P*=0.05, Duncan test).

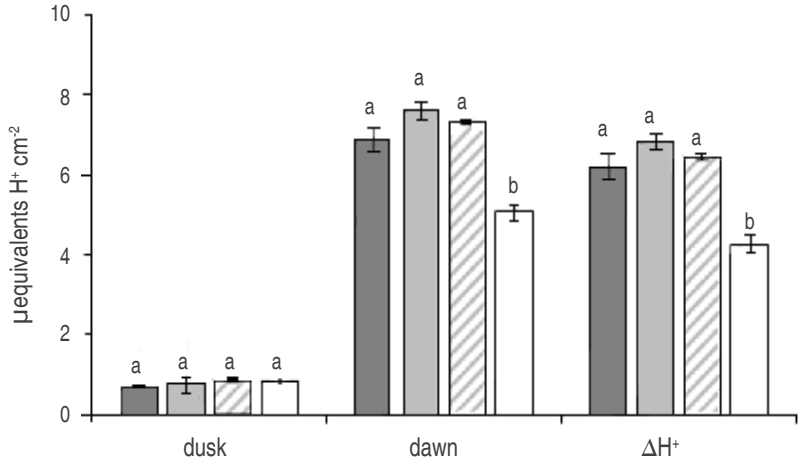

Figure 2. Titratable acidity at dusk (18:00 h), at dawn (6:00 h), and nocturnal acid accumulation (ΔH^+) in vanilla plants growing in different light environments: 8% RI, 17% RI, 31% RI and 67% RI (n=5 per light treatment).

Specific leaf area was significantly greater in the most shaded treatment (8% RI) (*P*=0.0018), with a mean value of 104.4 $cm^2\ g^{-1}$, which represents an increase of 51, 8% with respect to the most illuminated treatment (67% RI= 68.7 $cm^2\ g^{-1}$). Similarly, root biomass was significantly higher in intermediate light treatments (17% and 31% RI) compared with treatments of high and low lighting (*P*=0.0002), with mean values of 21.55 g vs. 5.17 g, respectively. Finally, also the total biomass was significantly higher (*P*=0.0013) in the intermediate RI treatments (17 and 31%) compared with low and high illumination (8 and 67% respectively): on average 149, 2 vs. 53.9 g (176.8% increase).

Chlorophyll fluorescence. Significant differences in values of chlorophyll fluorescence among illumination treatments (*P*=0.0000), as well as among hours along day (*P*=0.0000) and for the interaction treatment x hour (*P*=0.0032). The values were significantly higher in the RI of 8%, followed by 17%, 31% and 67% (Figure 4).

Leaf content of photosynthetic pigments. The concentrations of chlorophyll a, chlorophyll b, and total chlorophyll were significantly higher in plants established in the most shaded treatment (8% RI) (*P*=0.0000 in all cases), followed by the intermediate (17 and 31% RI), and high illumination treatment (67% RI), which had the lowest values (Table 2). The concentration of carotenoids showed an inverse trend: it was significantly higher in plants developed in high illumination (*P*=0.0001), and decreased in more shaded treatments. The ratio chlorophyll a/b had no significant differences between lighting treatments (*P*=0.6266), while the ratio total chlorophyll / carotenoids was significantly higher in plants established in low light (*P*=0.0001).

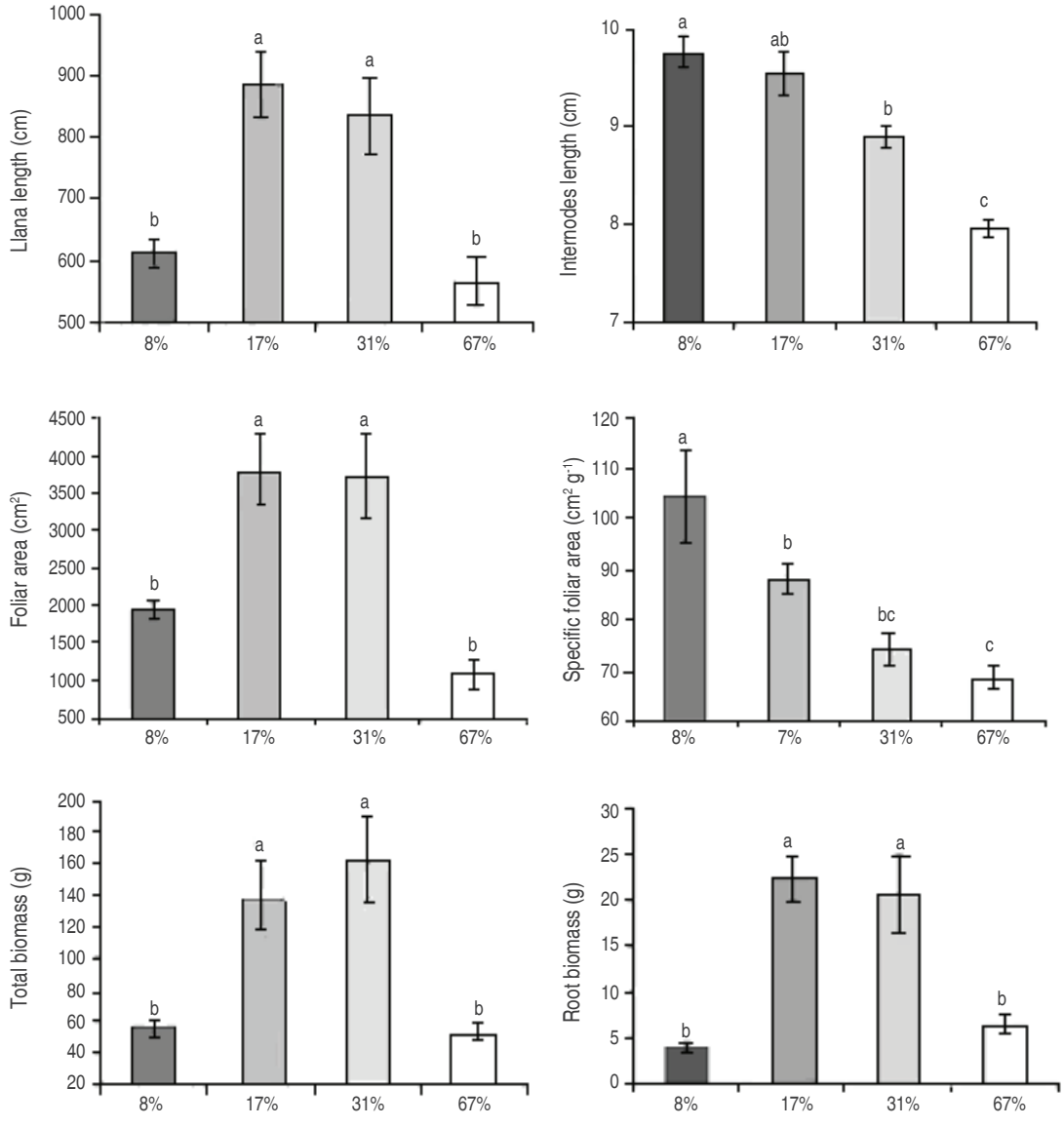

Figure 3. Biometric variables evaluated in vanilla plants growing in different light environments: 8% RI, 17% RI, 31% RI and 67% RI. Different letters on the bars indicate significant differences (Duncan, 95%, n = 5).

The results clearly show that the light environment of growing affects the CAM photosynthesis and vegetative growth of vanilla, with optimum illumination range at intermediate to low levels (8.6 - 12.8 mol m^{-2} d^{-1} of PAR, which the study area correspond to RI between 17 and 31%). Most values reported as suitable for vanilla growth in other regions where this species is cultivated, range between 30 and 60% RI (Anilkumar, 1994; Bianchessi, 2004; Kahane *et al.*, 2008; Exley, 2011; Hernández-Hernández, 2011; Sarma *et al.*, 2011; Varela, 2011; Zaubin *et al.*, 2011); however, comparisons based on RI probably are biased without knowing the PAR values at these sites. Results suggest that vanilla has better acclimation capacity to low light environments (though with suboptimal growth) than to high lighting conditions.

The highest absorption values occurred in the intermediate to low light treatments. These results agree with those of nocturnal acid accumulation (Δ H^+), which were higher in the treatments of 8, 17 and 31% RI; i.e.,

plants grown in environments RI intermediate to low (which absorbed more CO_2) transformed that CO_2 into organic acids along night (Dodd *et al*., 2002) and thus showed greater accumulation of acids in the vacuoles. During the day, the highest CO_2 uptake occurred in the treatments of 17 and 31% RI, which specifically occurred early in the morning (Phase II of CAM metabolism), because during the rest of the day (Phases III and IV) only respiration occurred.

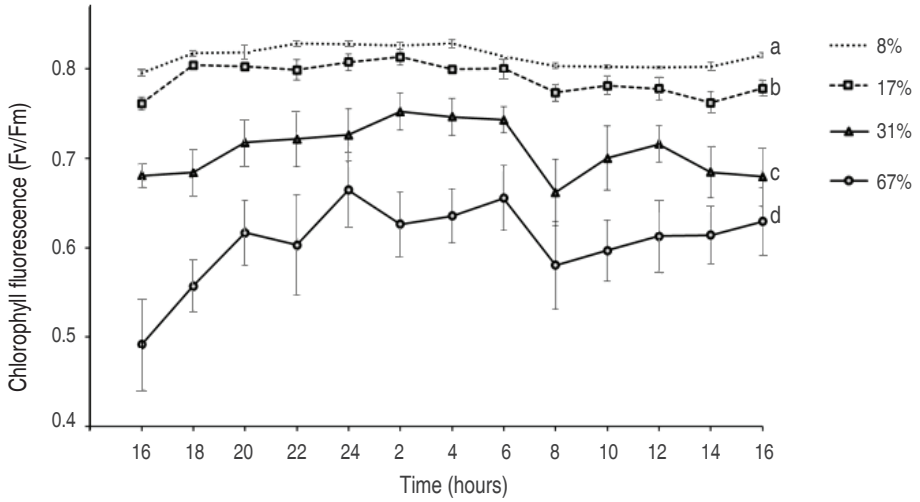

Figure 4. Daily course of chlorophyll fluorescence of vanilla plants growing in different light environments: 8% RI, 17% RI, 31% RI and 67% RI. Different letters besides each line indicate significant differences (Duncan, 95%, n=3 per treatment. SE in bars).

As a result of this balance of carbon absorption during the day and night, the largest net amount of CO_2 absorbed along the 24 h period occurred under the intermediate -low light treatments, with much lower values in the high lighting treatment. Consequently, the highest values of growth were obtained in plants developed in the intermediate treatments RI (17 and 31%), with daily PAR of 8.6 and 12.8 mol m^{-2} d^{-1}, expressed as liana length, leaf area, root biomass, and total biomass (Figure 3). In these treatments, the maximum values of instantaneous radiation were 369 and 577 µmol m^2 s^{-1}, respectively, and occurred between the 13:00 and 14:00 h. Accordingly, the environments which favor the photosynthesis and growth of vanilla are apparently those with PAR levels at noon between these two values, while environments of greater radiation (for example, the ones in the 67% or RI: 1201, 4 µmol m^2 s^{-1} at noon) seem to limit the photosynthesis and development of this species. These results are consistent with values reported in India as favorable for the vegetative development of vanilla, of 300 -600 µmol m^2 s^{-1} (Puthur, 2005), although it would be necessary to know the total daily PAR for this region, in order to evaluate whether the data are comparable.

Plants growing under extreme lighting conditions (high and low), had the lowest values of growth, although showed some mechanisms of acclimation. On the one hand, plants that grew in 8% RI showed several adaptations to be more efficient under low light conditions, such as longer internodes and higher specific leaf area, since in low-radiation, an elongated morphology and larger leaf blades increase light uptake (Walters, 2005). However, the total leaf area per plant decreased probably as a consequence of the lower resources available under that condition; perhaps for that reason, plants under this light condition showed the lower growth rate over a long time period (Shipley, 2002), despite no significant differences of total daily photosynthesis per unit leaf area were found with intermediate RI treatments (Table 1).

At the other extreme, the low CO_2 assimilation and growth in plants developed under high light (67% RI) could be explained by the signs of chronic photo inhibition detected, which damages the reaction centers of PSII and causes photo destruction of photosynthetic pigments (Powles, 1984). The PSII efficiency (Fv / Fm) in this study declined from 0.81 ± 0.001 in the treatment of 8%, indicative of absence of photo inhibition (Powles, 1984; Werner *et al*.,

2001), to 0.61 ± 0.001 in the 67% RI treatment, indicative of presence of photo inhibition.

Plants from shadow environments are especially susceptible to chronic photo inhibition when are exposed to high light intensities. Under such conditions, plants show low potential quantum yield of photosynthesis as a result of fewer reaction centers in the PSII, resulting in a lower Fv / Fm throughout the day (Murchie *et al*., 2009). A further indication of irreversible damage is that leaves of plants grown in high light (67%) had white spots by chlorosis, probably as a result of a higher chlorophyll degradation, because leaves could not effectively channel the high energy received in photochemical reactions, which leads to chlorophyll whitening (Anderson, 1986).

The concentration of chlorophylls (a, b and total) was higher in leaf tissues of plants growing in lower lighting (8% RI). Under these conditions, the synthesis of greater amount of chlorophyll is a strategy of acclimation to increase the efficiency of photon capture and counteract the lower radiation reaching the leaf (Anderson, 1986). Perhaps this high chlorophyll content was effective to avoid very low values of total daily photosynthesis per unit leaf area, despite the limitations imposed by low radiation; this response is a further evidence in favor of the shade-tolerant character of vanilla.

It has been reported that plants adapted to low radiation have low values of the chlorophyll a / chlorophyll b ratio (approximately 2) and that plants adapted to high radiation have high values (about 2.8 and more) (Anderson, 1986). In this study no significant differences in this ratio were found among vanilla plants growing under different RI, whose average value was 2.2 (Table 2). Low values of this ratio are correlated with high degree of stacking of thylakoid membranes in the chloroplast, which increases the area of the cross section of the grana and produces increased risk of photo inhibition (Anderson and Aro, 1994). This inability of vanilla plants growing under high light intensity to increase chlorophyll a / chlorophyll b ratio, suggests that there were not significant reorganization of thylakoid membranes, which would allow lower stacking and thus avoid photo destruction of photosynthetic apparatus in plants exposed to high radiation (67% RI), which is a further evidence of the shade- tolerant character of this species.

Table 2. Leaf content of photosynthetic pigments (µg cm^{-2}) in vanilla plants growing in different light environments.

RI	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Carotenoids	Chlorophyll a/b	Chlorophyll / Carotenoids
8%	23.6 ± 1.2 a	11.4 ± 0.7 a	34.9 ± 1.9 a	3.9 ± 0.5 b	2.1 ± 0.1 a	9.6 ± 1.1 a
17%	14.2 ± 1.2 b	7.2 ± 0.4 b	21.4 ± 1.1 b	4.6 ± 0.2 b	2.0 ± 0.2 a	4.7 ± 0.4 b
31%	17.0 ± 1.6 b	7.2 ± 1.1 b	24. 2 ± 1.1 b	5.8 ± 0.1 a	2.5 ± 0.5 a	4.2 ± 0.2 b
67%	8.5 ± 0.7 c	3.9 ± 0.4 c	12.4 ± 1.1 c	6.3 ± 0.1 a	2.2 ± 0.1 a	1.9 ± 0.2 c

Mean values ± SE per treatment are shown in the table body (n=5). Different letters between light treatments in each column indicate significant differences (*P*=0.05, Duncan test).

Carotenoids protect chlorophyll pigments against high radiation under conditions of light stress (Yamamoto and Bassi, 1996) and therefore, the ratio chlorophyll / carotenes is an indicator of the protection against the high radiation, since carotenes contribute to dissipate the excess of radiation (Cazzonelli *et al*., 2011). Low values, indicative of greater photo oxidative damage, were found in plants exposed to 67% RI (Table 2), whereas in plants developed under 17 and 31% RI, were intermediate, and higher values in plants under 8% RI. Vanilla plants showed ability to capture and use light by increasing the concentration of pigments when grown at low radiation; the decrease of chlorophyll / carotenes ratio under high radiation suggests their inability to minimize the damage caused by high radiation, which is a further evidence of their inability to acclimate to high radiation environments. Since the proportion of carotenoids vs. chlorophyll decreased with increasing radiation intensity (Table 2), vanilla plants grown in high light did not have enough mechanisms to protect against the photo-destructive

damage from high radiation. Such failure was evidenced in the low chlorophyll content, low CO_2 assimilation, less chlorophyll fluorescence, and low productivity of plants exposed to radiation above 800 µmol m^{-2} seg^{-1}. These results confirm that shade plants, do not have well developed mechanisms to counteract the effects of photo inhibition.

Therefore, shade plants such as vanilla are very sensitive to high radiation; this causes inactivation of reaction centers and inhibition of electron transport, since the antennas of their photosynthetic apparatus are unable of channeling the light energy into the photochemical reaction centers. The excess of energy can induce the production of reactive oxygen species and free radicals (Powles, 1984) which break the DNA, destroy the function of proteins, and are responsible for peroxidation of lipids, thus causing damage to the plant metabolism and decreasing the rate of photosynthesis and growth.

CONCLUSIONS

Most CO_2 assimilation occurred along night in all treatments, which confirms that vanilla is a strong CAM plant. Results suggest that high radiation alters the functioning of vanilla plants, inhibiting photosynthesis and growth, and that highly shaded environments not significantly affected the CAM photosynthesis of vanilla; however, in the long-term this species showed higher photosynthesis and biomass growth under intermediate levels of radiation (17-31% RI).

ACKNOWLEDGEMENTS

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Weed population dynamics in rice crops resulting from post-emergent herbicide applications

UNIVERSIDAD NACIONAL DE COLOMBIA

Dinámica poblacional de malezas en cultivos de arroz por aplicaciones herbicidas post-emergentes

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ABSTRACT

Key words:
Weed control
Echinochloa colona
Community structure
Importance Value Index (IVI)

Studies on weed population dynamics are based on observing and recording changes in weed communities in response to effects of disturbances in crop management. The present study aimed to evaluate weed population dynamics in rice crops in Tolima, Colombia, resulting from post-emergent herbicide applications. Sampling was carried out in 0.1% of the cultivated area, marking out a 1 ha area in each commercial lot. Samples were taken before and after post-emergent applications. Evaluated variables were frequency, density, and cover. The data were analyzed using the Importance Value Index (IVI). Results demonstrated that *Echinochloa colona* was the most important weed of all of the evaluated zones, before and after post-emergent herbicide applications. Other notable species included *Digitaria ciliaris*, *Cyperus iria* and *Ischaemum rugosum*. Relative frequency variable was the most influential on the importance index of the species. Furthermore, herbicide applications generated changes in the community structure in the evaluated zones and in each evaluation.

RESUMEN

Palabras claves:
Control de malezas
Echinochloa colona
Estructura de la comunidad
Índice de Valor de Importancia (IVI)

Los estudios de dinámica de poblaciones se basan en el conocimiento y registro de cambios en las comunidades de malezas en respuesta a efectos de disturbio propios del manejo del cultivo. En el trabajo se evaluó la dinámica de poblaciones de malezas del cultivo de arroz en el departamento del Tolima, Colombia, por efecto de aplicaciones con herbicidas post-emergentes. Se muestreó el 0,1% del área sembrada, demarcando un área de 1 ha en cada lote comercial. Los muestreos se realizaron antes y después de las aplicaciones post-emergentes. Las variables evaluadas fueron frecuencia, densidad y cobertura y los datos se analizaron mediante el índice de valor de importancia (IVI). Los resultados muestran que *Echinochloa colona* fue la maleza más importante en todas las zonas evaluadas, antes y después de las aplicaciones herbicidas post-emergentes. Igualmente sobresalieron especies como, *Digitaria ciliaris*, *Cyperus iria* e *Ischaemum rugosum*. La frecuencia relativa fue la variable estimada más influyente en la determinación de la importancia de las especies. Las aplicaciones de herbicidas generaron cambios en la estructura de la comunidad en las zonas evaluadas y en cada evaluación.

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Weeds are the principal limiting biological factor in global rice production, with losses that vary from country to country, depending on the cultivation system, predominant weed communities and weed control methods employed by the farmers (Labrada, 2003). Worldwide, it is estimated that weeds cause 9% of rice crop losses (Rodenburg and Johnson, 2009), with decreases in rice paddies of 94% to 96% in the Philippines (Chauhan and Johnson, 2011); in Colombia, losses of 30% to 73% have been reported (Cobb and Reade, 2010). Appropiate control methods in rice crops are essential to minimize the negative effect of weeds (Fuentes, 2010).

Use of herbicides has become the most used weed control method worldwide, on a large number of species. However, there are many concerns related to excessive use of herbicides. Although it does solve the problem of manual labor in many countries, incorrect use causes problems such as resistance in weeds, changes in weed populations, less availability of new broad-spectrum herbicides and environmental problems (Labrada, 2003; Singh, 2012).

Weed communities are affected by farming practices through variations in the flow of material, energy, and data. These changes modify the diversity and composition of species in weed communities, as well as abundance (biomass and density of individuals) (Holst *et al*., 2007; Poggio, 2012).

Population structure refers to organization of individuals within a population based on specific phenotypic and genotypic characteristics; therefore, studies on populations look at the variation that exists within them (Booth *et al*., 2003). These changes in populations or population dynamics refer to the changes in composition of a weed community, considering relative dominance of each species in the agroecosystem (Jakelaitis *et al*., 2003).

Mathematical models are widely used to study weed population dynamics in crops; these models can be developed for determined descriptions of populations, allowing for the creation of management strategies for the future (Holst *et al*., 2007). Calculating the Importance Value Index (IVI) leads to the description of population changes in communities. This index expresses the relationship between weed populations and community components that consider species frequency and dominance and number of individuals (Carvalho *et al*., 2008). Community studies and phytosociological studies of weeds compare populations over a time period, considering the consequences of management and relating them to results found in the field (Pitelli, 2000; Carvalho *et al*., 2008; Moreira *et al*., 2013). Numerous studies have calculated sociological parameters in order to establish the effects of management on the communities. Changes in the importance value index (IVI) of determined species have been reported by post-emergent herbicide applications (Jakelaitis *et al*., 2003), by establishment of associated plants (Moreira *et al*., 2013), soil management systems (Soares *et al*., 2012), climatic conditions (Andreasen and Streibig, 2010), crop rotation practices (Erasmo *et al*., 2004) and soil management and use (Concenço e*t al*., 2011).

In response to these control practices, not all species present in an agricultural system are equally important because they do not interfere with the crop at the same level. Differences in frequency, density and growth habit lead to the detection of principal species that generate larger negative effects on the crop, along with secondary species. Therefore, implementation of weed management strategies in agroecosystems requires knowledge of the community structure (Pitelli, 2000) and, before designing a management program, priorities must be established for growth suppression of determined weeds that, in general terms, are more abundant and more competitive without ignoring secondary species (Erasmo *et al*., 2004).

This study aimed to determine the population dynamics of weeds in a rice crop resulting from the effect of post-emergent herbicide controls in Centro, Meseta and Norte zones of department of Tolima, Colombia, using a plant sociology approach.

MATERIALS AND METHODS

This study was carried out on commercial rice crops in the department of Tolima, Colombia. Field sampling was conducted in 96 hectares throughout the department, which is 0.1% of total area cultivated, according to the methodology proposed by Spiegel (1988). Hectares sampling by subregions, were distributed proportionally to the total area, 53% in Centro subregion (51 ha), 21% in Meseta (20 ha) and 26% in Norte (25 ha). One hectare

was marked in each lot, within this area weeds and crop plants were evaluated through a sampling unit of 0.04 m^2, which was throwed randomly five times.

The three times of evaluation were: first, 7 to 22 days after the sowing (d.a.s.) (before the first post-emergent application); second, 22 to 35 d.a.s. (after the first post-emergent application) an the last, 37 to 52 d.a.s. (after the second post-emergent application), according to the methodology reported by Plaza and Hernandez (2014). The herbicide applications evaluated were made by farmers according with particular recommendations for each field. As evaluated variables included frequency, density and percentage weed cover through DOMIN scale. Identification of weed species was made according with Fuentes *et al*. (2006a and 2006b) and Montealegre (2011).

Data analysis that allowed knowing population dynamics was through the calculation of the following phytosociological parameters: absolute density (Da), relative density (Dr), absolute frequency (Fa), relative frequency (Fr), cover (Ca), relative cover (Cr) and Importance Value Index (IVI), calculated by the sum of the relative values of each of the variables (Curtis and McIntosh, 1950; Mueller-Dombois and Ellenberg, 1974).

RESULTS AND DISCUSSION

Weed communities of the rice crops in the department of Tolima included 42 species from 20 families and 31 genera. Centro zone contained 27 species (14 families and 21 genera), Meseta zone had 31 species (12 families and 23 genera) and Norte zone included 38 weed species (18 families and 29 genera) (Ramírez *et al*., 2015).

Phytosociological analysis in the entire department presented ten species that represented 50% of the maximum IVI (Figure 1). Predominant species before first post-emergent application were *Digitaria ciliaris* (DIGSP) and *Echinochloa colona* (ECHCO), for which the variables with the most contribution to IVI were relative density and relative frequency, respectively (Figure 1a). After first post-emergent application, index for *D. ciliaris* decreased drastically (IVI=0), indicating that control was effective for this species, while importance of *E. colona* remained the same. Species *Murdannia*

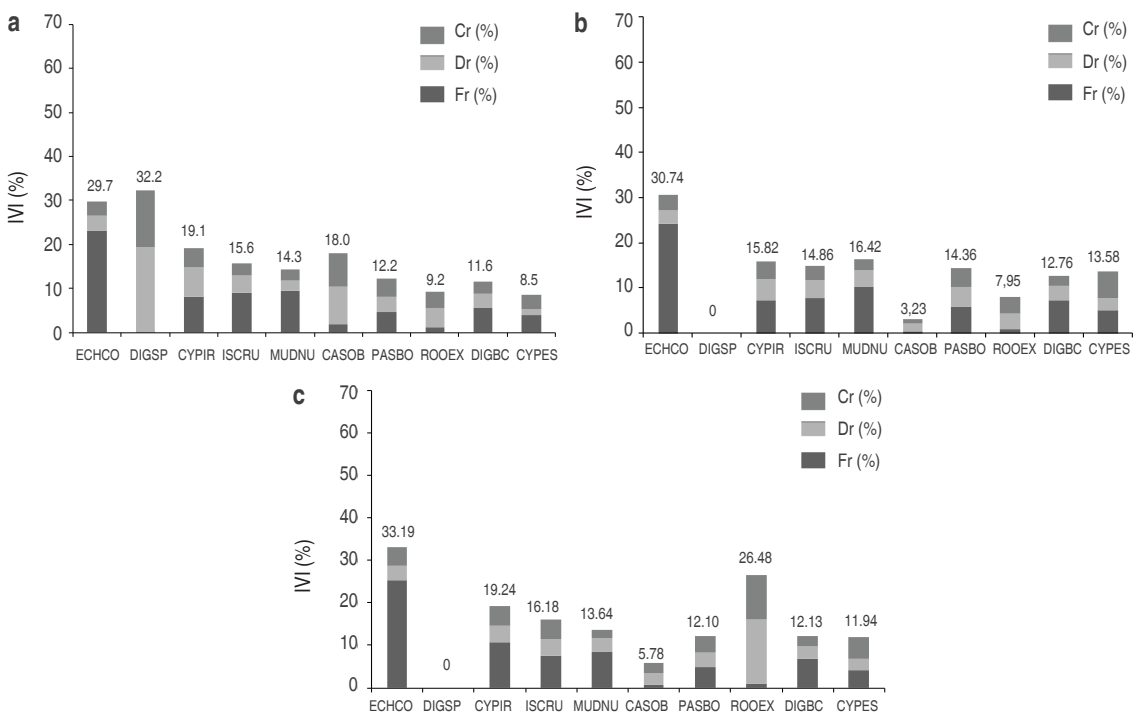

Figure 1. IVI of the principal weeds associated with rice crops in the department of Tolima: (a) before the first post-emergent application, (b) after the first post-emergent application, and (c) after the second post-emergent application. Relative frequency (Fr), relative density (Dr) and relative cover (Cr). ECHCO; DIGSP; CYPIR; ISCRU; MUDNU; CASOB: *Senna obtusifolia*; PASBO; ROTCO; DIGBC; CYPES.

nudiflora (MUDNU), *Paspalum boscianum* (PASBO) and *Cyperus esculentus* (CYPES) registered increases in IVI after first application (Figure 1b). After second post-emergent application, in last evaluation, importance level of *Rottboellia cochinchinensis* (ROTCO) increased mainly due to its relative density. On the other hand, *E. colona* remained as the principal species, demonstrating that it was the most important weed in study area (Figure 1c). Our results are in agreement of those of Puentes (2003), who reported *E. colona* in 87% of evaluated lots, being the most frequent grass within weeds of rice crops in Tolima.

Most notable component in determination of importance for more relevant species was relative frequency, before and after post-emergent applications (Figure 1). *E. colona* had the highest relative frequency in all evaluations with values of 23% before the first application, 24.2% after the first application, and 25.3% after the second post-emergent herbicide application. The importance of this species comes from its high competiveness, decreasing rice grain production by 86%, with reductions of 76% due to competition aboveground (aerial part) and 44% below the surface (radical) (Chauhan and Johnson, 2009a; Chauhan and Johnson, 2010). Results of this study indicated that frequency of this species within cultivation system determined its importance as a noxious plant, given its adaptation to environment and competition for resources with crops. Likewise, Norsworthy *et al*. (2001) stated that, in environments subjected to disturbances, weeds adapted to ecological conditions would exhibit higher frequencies.

Centro zone had six species that represented 50% of the maximum IVI (Figure 2). *E. colona* and *Ischaemum rugosum* (ISCRU) were the predominant species before first post-emergent application (Figure 2a). After this application, there was an increase in importance of *E. colona* and a decrease in importance of *I. rugosum* (2b). After second post-emergent application, *R. cochinchinensis* and *E. colona* were the principal species (Figure 2c); both registered increases in importance in regards to previous evaluation. According to Jakelaitis *et al*. (2003), increases in importance level of some weed populations

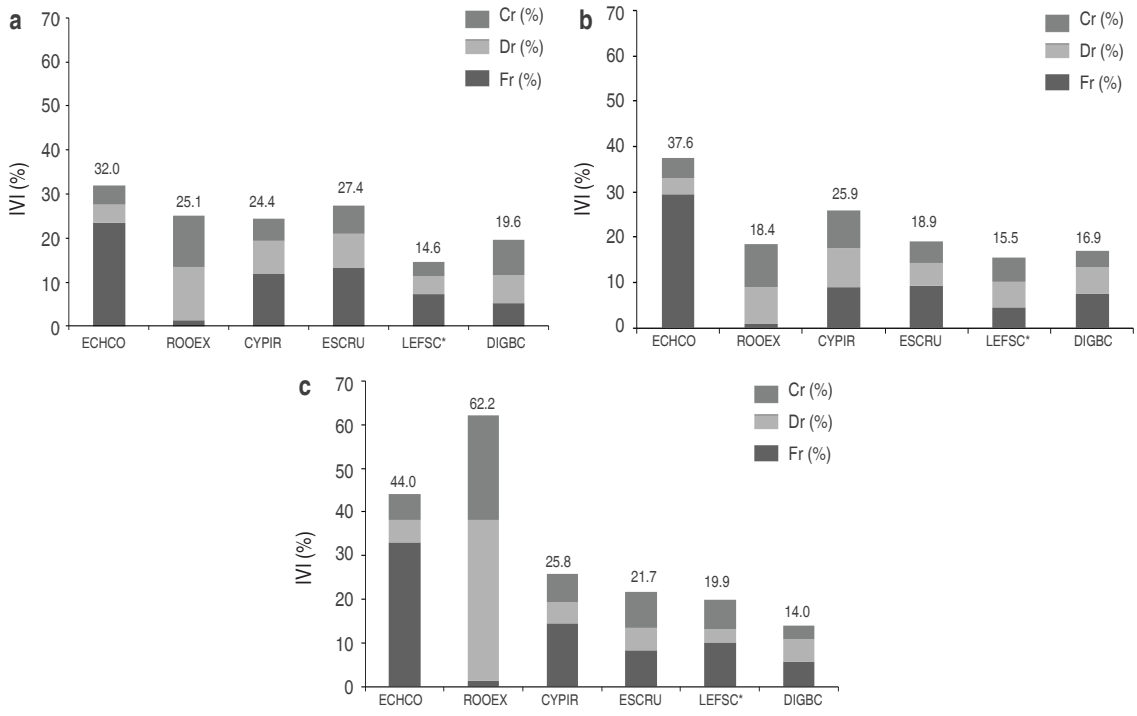

Figure 2. IVI of the principal weeds associated with rice crops in the Centro zone: (a) before the first post-emergent application, (b) after the first post-emergent application, and (c) after the second post-emergent application. Relative frequency (Fr), relative density (Dr) and relative cover (Cr). ECHCO; ROTCO; CYPIR; ISCRU; LEFSC*: *Leptochloa scabra;* DIGBC.

cause decreases in importance of others when affected by control treatments, which decreases the diversity of species; situation presented in this study.

Relative frequency component had the most influence on determination of importance of the relevant species, before and after the herbicide treatments in this region (Figure 2). *E. colona* was notable as the most frequent weed in the region with relative frequency values of 23.5%, 29.4% and 33.1%, for the three evaluation time points. On the other hand, in *R. cochinchinensis,* relative density was the component that contributed the most to its importance, mainly after the second post-emergent herbicide application, indicating little control of it with this control practice.

Phytosociological analysis of the weed community in the Meseta zone demonstrated that ten species represented 50% of the maximum IVI (Figure 3). Before first post-emergent application, *E. colona* was the most important species with highest IVI value (Figure 3a). After first post-emergent application, the only species with variation in IVI was *Heteranthera limosa* (HETLI), which had increases in its importance (Figure 3b); however, after second post-emergent application, importance of this species decreased to zero due to herbicide effectiveness and susceptibility of species (Figure 3c). *E. colona* continued to be the most important species in the region (Figure 3c); *Cyperus iria* (CYPIR) registered an increase in IVI, making it the second most important species at the start of reproductive phase of crops (Figure 3c).

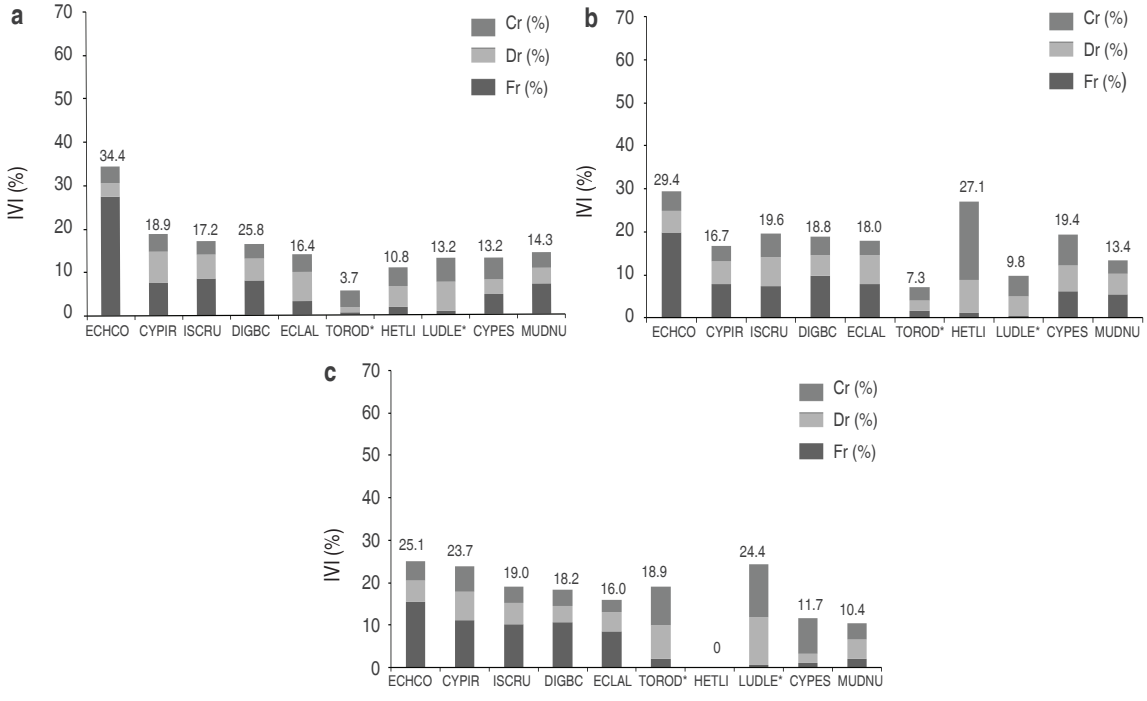

Figure 3. IVI of the principal weeds associated with rice crops in the Meseta zone: (a) before the first post-emergent application, (b) after the first post-emergent application, and (c) after the second post-emergent application. Relative frequency (Fr), relative density (Dr) and relative cover (Cr). ECHCO; CYPIR; ISCRU; DIGBC; ECLAL: *Eclipta alba*; TOROD*: *Torulinium odoratum*; HETLI; LUDLE*: *Ludwigia leptocarpa*; CYPES; MUDNU.

Relative frequency had the most influence on the determination of importance in principal species, such as *E. colona, C. iria, I. rugosum, Digitaria bicornis* (DIGBC) and *M. nudiflora,* before and after post-emergent applications (Figure 3). *E. colona* recorded the highest frequency at the three evaluations with 27.4%, 19.8% and 15.5%, respectively. Lower frequencies after herbicide applications have also been reported by Jakelaitis *et al*. (2003) and are possibly due to mortality generated by treatments (Mascarenhas *et al*., 2012).

In Norte zone, eight species represented 50% of maximum IVI value (Figure 4). *C. iria*, *E. colona* and *D. ciliaris* were the more important species in this region before first

post-emergent application, as opposed to other regions; *C. iria* was the most important species with highest IVI (Figure 4a). After first herbicide treatment, IVI of *C. iria* and *D. ciliaris* decreased, while index of *E. colona* remained the same, making it the most important species at that time. Furthermore, increases were recorded for importance of *P. boscianum, M. nudiflora* and *C. esculentus* (Figure 4b). After second post-emergent application, *E. colona* continued to be the most important weed (Figure 4c). Like in other zones, relative frequency was the component that most contributed to importance of principal weeds, before and after post-emergent applications (Figure 4). *E. colona* had the highest frequency in all evaluations with values of 19.5%, 20.5% and 22.8%, respectively. In addition, participation of relative density was notable in importance of *D. ciliaris* and *C. iria* before first application (Figure 4a) and in importance of *C. iria* and *I. rugosum* after second post-emergent application (Figure 4c).

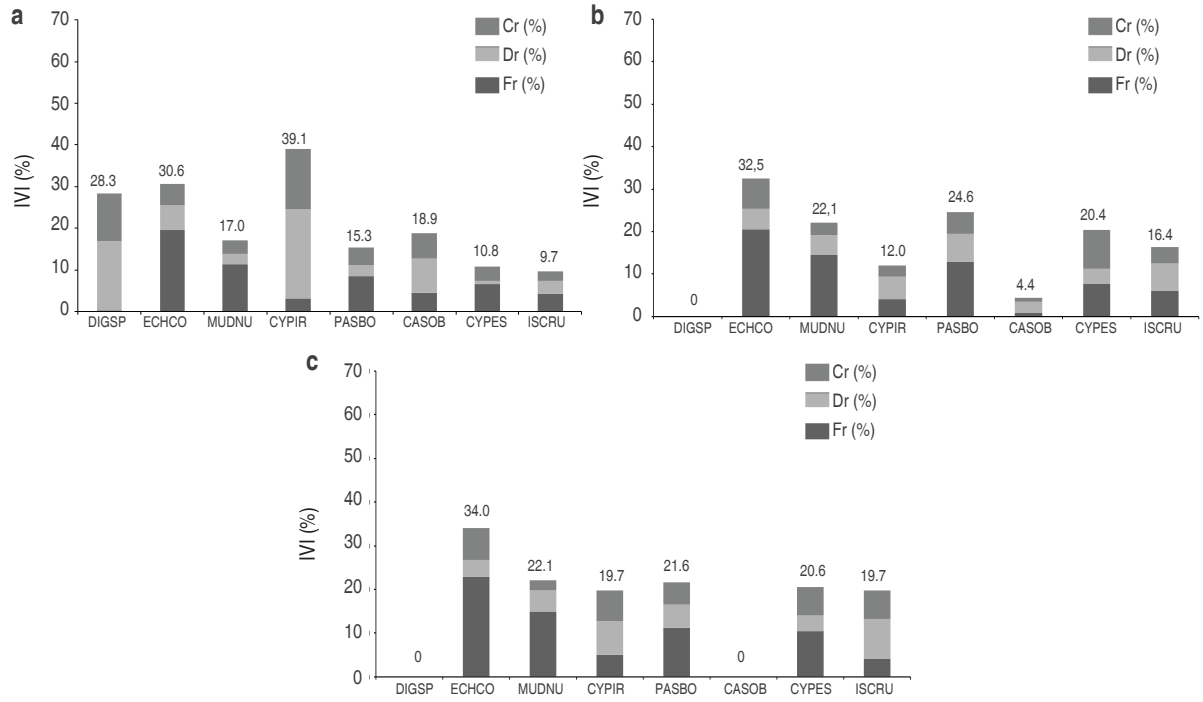

Figure 4. IVI of the principal weeds associated with rice crops in the Norte zone: (a) before the first post-emergent application, (b) after the first post-emergent application, and (c) after the second post-emergent application. Relative frequency (Fr), relative density (Dr) and relative cover (Cr). DIGSP*;* ECHCO; MUDNU; CYPIR; PASBO*;* CASOB*;* CYPES*;* ISCRU

In all zones, contribution from relative cover to importance of the species was limited. This was possibly due to low biomass accumulated by species at sampling times (early stages of development) and even when plants were able to emerge because the effect of herbicide applications impeded accumulation of biomass. Concenço *et al*. (2012) suggested that this effect on the cover could also be the result of competitiveness of the crop, blocking light from weed plantlets.

As stated above, results of this study showed that *E. colona* was the most frequent weed species and, commonly, the most important species in evaluated rice crops, before and after post-emergent applications. Its establishment after herbicide treatments, pre-sowing and pre-emergence, could have been due to complete adaptation to conditions of this environment because germination of its seeds is favored by moist environments (Chauhan and Johnson, 2009a). Germination of seeds occurs over 80% (Chauhan and Johnson, 2010) due to seed viability generally oscillates from 84% to 100% (Mendoza, 2007; Vega-Jarquín *et al*., 2010). Rao *et al*. (2007) suggested that adaptation level of species from *Echinochloa* genus to direct sowing conditions of rice

crops is due to its versatility in germination of the seeds and in establishment of plantlets in response to changes in hydric regime. This situation high production of *E. colona* seeds (7,800 seeds per plant) (Chauhan and Johnson, 2010) and the end of effect of the post-emergent herbicide applications, possibly facilitated development of new individuals. Similarly, is known the susceptibility of genera *Echinochloa* to acquire resistance to different herbicides, this supported in 83 reports, of which 30% are *E. colona* (Heap, 2016).

Species from *Digitaria* and *Cyperus* genera, *Paspalum boscianum* and *Ischaemum rugosum,* were also notable as important species throughout evaluations in rice crops of Tolima, results that agree with those of Bakar and Nabi (2003), Rao *et al*. (2007) and Chauhan and Johnson (2009a and 2009b), in studies related to weed species in rice crops. Recording of new individuals and level of importance of the *Poaceae* species after use of specific herbicides for their control (Cobb and Reade, 2010; Clavijo, 2010) resulted from their level of adaptation to and infestation of lots.

Use of phytosociological parameters for study of population dynamics is common in weed control studies. Jakelaitis *et al.* (2003) evaluated population dynamics of weeds in maize and bean crops before and after herbicide applications and they found higher densities and frequencies of dicotyledonous species in both crops before application of herbicides; however, after selective herbicides application, *Cyperus rotundus* was the species with highest importance, dominance, and density in both crops. Vaz de Melo *et al*. (2007) reported similar results for weed populations in maize, where a change in floristic composition was evidenced in response to chemical and mechanical treatments.

Composition of weed populations in an agroecosystem is a reflection of characteristics of soil, climate, and agronomic practices, including herbicide application (Booth *et al*., 2003). Selective herbicides influence population dynamics of species in agroecosystems; these effects contribute to increases in density, dominance and relative importance of weeds. This is due to the fact that application of selective herbicides results in efficient control of some species, but deficient control of others, selecting for those for which there is not effective control (Jakelaitis *et al*., 2003). Andreasen and Streibig (2010) suggested that herbicides play an important role in determination of composition, diversity, and abundance of weeds.

Under the conditions of this study, herbicides more frequently used in first post-emergent application were inhibitors of the joining of microtubules (pendimethalin), inhibitors of photosystem II (propanil) and inhibitors of cellular division (butachlor). Herbicides more frequently used in the second post-emergent application were bispyribac sodium (ALS inhibitor), pendimethalin and propanil (Ramírez and Plaza, 2015). Use of these agrochemicals, with a principal control spectrum that includes *Poaceae* weeds, possibly contributed to population changes that wereobserved in all zones, affecting to a large extent establishment and development of some susceptible species. It was observed that weeds from the *Cyperus* genus achieved establishment towards the end of control period, probably in response to lack of activity of these active ingredients on them. In this sense, changes in importance index and in components could be explained by specificity of control that herbicides had for some of populations.

Rao *et al*. (2007) stated that hydric condition of a rice crop is the main selecting factor for weed species. In this sense, Plaza and Hernández (2014) and Puentes (2003) reported differences in terms of most important species of crops in zones with divergent hydric regimes. However, Rao *et al*. (2007) suggested that lack of crop rotation in rice fields, the introduction of practices such as direct sowing and, above all, repeated use herbicides are also causal factors of changes in weed populations in rice agroecosystems.

CONCLUSIONS

The methodology used in this study allowed to determine weed population dynamics, affected by chemical controls. For this study, the most relevant species was *E. colona*, both at department land zone level, and the variable that most influenced this result was relative frequency. The IVI and components values for some weeds after herbicide applications suggest adaptability and high number of weed seeds in the soil bank. The constant values of relative cover after post-emergent applications suggest sequential weed emergence and an acceptable control

of sprayed individuals. Weed population dynamics in response to post-emergent applications have a common pattern between zones; it is related with the most important weeds and their importance after treatments.

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Characterization of traditional production systems of sugarcane for panela and some prospects for improving their sustainability

Caracterización de sistemas de producción artesanal de caña panelera y algunas perspectivas para mejorar su sostenibilidad

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ABSTRACT

Sugarcane used for the production of "panela" (unrefined cane sugar) is a crop of great importance for Colombia's rural economy. Additionally, it serves a fundamental role in the food security and sovereignty of the Colombian population and daily consumption. However, the small production system presents problems of sustainability, as a direct consequence of its technological arrears and loss of interest in this crop. In this study, a characterization of 30 small productive units located in three municipalities in Antioquia was performed with the objective of identifying the problems associated with this production system and stablish the causes associated with loss of area dedicated to this crop in the study area. The results demonstrate that in the region of study, this production system and its associated agro-industry have problems associated with low technological level, poor infrastructure, deficient agro-industry processing and low levels of associativity and marketing. This situation has generated a low economic solvency for the farmers, leading many to abandon this activity and migrate towards other economic sectors. The findings of this study indicate the need to reengineer this production system, for which they could make technological adaptations that improve productivity and product quality and generate added value. On the other hand, must the rural countryside attractive to avoid the loss of labor and make young people become interested in this economic activity. As strategies to improve productivity, we suggest the effective use of information technologies, improve rural living conditions, increase associativity and value added, involve the consumer in the production chain and design development policies for the entire chain of value.

Key words:
Panela
Economic sustainability
Rural emigration

RESUMEN

La caña de azúcar para la producción de panela es un cultivo de suma importancia para la economía del sector rural, además de su papel fundamental en la seguridad y soberanía alimentaria de la población colombiana. A pesar de esto, los pequeños sistemas productivos en algunas regiones del país presentan problemas de sostenibilidad, consecuencia directa de los rezagos tecnológicos y la perdida de interés por este cultivo. Con el fin de identificar la problemática de este cultivo, se realizó la caracterización de 30 pequeñas unidades productivas ubicadas en tres municipios de Antioquia. Los resultados encontrados demuestran que este sistema productivo y su agroindustria en la zona de estudio presentan grandes problemas asociados a bajos niveles tecnológicos, deficiente infraestructura para el procesamiento agroindustrial y bajos niveles de asociatividad y comercialización. Esta situación ha generado una baja solvencia económica de los productores, generando el abandono de esta actividad y la migración hacia otros rubros económicos. Basado en lo encontrado en este trabajo se hace necesaria la reingeniería de este sistema productivo mediante la utilización de adaptaciones tecnologías que mejoren la productividad y calidad del producto además de generar un mayor valor agregado. Por otra parte hay que buscar estrategias para volver más atractivo el sector rural y atraerlo hacia los jóvenes evitando la perdida de mano de obra y garantizando el relevo generacional. Como estrategias para mejorar la productividad se sugiere el uso efectivo de las tecnologías de información, mejorar las condiciones de vida rural, aumentar la asociatividad y valor agregado, involucrar al consumidor en la cadena productiva y el diseño de políticas de desarrollo para toda la cadena de valor.

Palabras claves:
Panela
Sutentabilidad economica
Emigracion rural

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The cultivation of sugarcane used for the production of "panela" (unrefined sugarcane) in Colombia is concentrated in the departments of Cundinamarca, Antioquia, Boyacá, Santander, Nariño and Caldas, representing 70% of the national total. These regions are also responsible for 72% of panela production in the country (FAO, 2004; Castellanos *et al*., 2010). In the country it is considered that about than 83% of the land area planted with this crop, the production system is made up of small plots that have undergone artisanal transformation processes, located in high mountain zones, with natural soil fertility limits, low mechanization, a family-based labor force and very low levels of technology or investment capital, In addition to presenting quality problems by low levels of incorporation of good practice from manufacture (GPM) (Castellanos *et al*., 2010). This situation forces families involved in this industry to complement their income with other agricultural or ranching activities (FAO, 2004; Rodríguez, 2005; M.A.D.R, 2005; Velásquez *et al*., 2006; Osorio, 2007). Meanwhile it is reported that small producers of panela in the country present low technological levels, regardless of the region to which it belongs (Castellanos *et al*., 2010).

This production system is of great socioeconomic importance for the rural sector, Rodríguez (2005) and Castellanos *et al*. (2010) propose that it serves multiple functions, not only from an economic point of view, but also from social, environmental, cultural, food security and nutritional perspectives. Additionally, it has a revitalizing effect in these territories, as it contributes to other non-agricultural sectors of the local economy, such as manufacturing workshops, infrastructure and machinery maintenance, production of packaging and financial activities linked to transport and distribution.

The characterization of production systems are very important as it sheds basic information for the design of state policies, basic input of various investigations, in addition to achieving identify technological constraints and to implement strategies that lead to the sustainability of agro-ecosystems (Martinez, 2013; Cariño *et al*., 2012; Moreno *et al.,* 2012) .

Donmatías, Barbosa and Girardota are municipalities located in the Altiplano Norte (Northern High Plateau Region), close to the Aburrá Valley of the Antioquia department. The economy of these towns is based on various economic activities. In the rural sector, livestock farming plays an important role, marked by the production of hogs, milking cows and beef cattle, and poultry. In regard to the agricultural sector, production is focused on tree tomato and garden tomato crops, and to a lesser extent, potatoes, beans, garden vegetables, sugarcane, coffee, plantains and yucca, among others. These crops are produced under a peasant economy system (Anuario Estadistico de Antioquia, 2014).

Among these agricultural activities, sugarcane production has been an important economic sector in these three municipalities. However, in recent years it has lost a great deal of importance as a result of multiple problems, leading to this land being taken over by extensive livestock farming, indicating a decrease in the area dedicated to this crop in the study region (Anuario Estadistico de Antioquia, 2010.

Considering the importance of this crop in the country's socioeconomic sphere, this study had the objective of characterizing the production system for the aforementioned region, in order to determine the reasons why growers have abandoned this economic activity and thus to be able to propose concrete strategies that would improve the economic situation of this farmers.

MATERIALS AND METHODS

Collection of samples and characterization of production system

The sampling and collection of information was performed in 30 production systems (10 in each municipality), which were located at random throughout the area dedicated to the cultivation of sugarcane for the production of panela. The number of samples was obtained by a simple random sampling, using the formula of maximum variance (Cochran, 1976). In each production unit, a survey was carried out with questions related to socioeconomic factors, planting, maintenance, harvesting, transport, agro-industrial transformation and the marketing system. The methodology for the characterization of production systems was participatory Diagnostic Checks, where producers responded to a survey of descriptive type, with structured and unstructured questions, plus extra contributions that these generated during the interview and visit lots cultivated. The participatory diagnosis was

made with producers and processors of sugarcane and within these included his family. In the design of the survey was seeking to cover all activities associated with the production and processing, but it was not very technique in order to achieve a better approach to the producer.

For the group of related socioeconomic factors, the following parameters were considered: area dedicated to cultivation, associated economic activities, land ownership, labor force used and age, economic system and significance of the crop. For the section dedicated to planting, maintenance, harvesting and transport, a characterization was performed of the following factors: the planting system, origin of the seed, cultural practices performed during the crop's life cycle, field yield in biomass and transformation process, varieties planted, time until harvest and transport to a processing center. Additionally, in each production unit, stem and leaf samples were taken from 15 plants at random, with the goal of identifying the existing phytosanitary problems and the varieties planted. For the agro-industrial transformation and marketing system factors, the parameters associated with the infrastructure of the sugar mills were determined and the various links in the marketing chain were identified.

Problems perceived by the growers and economic and financial information regarding cultivation

Based on the information obtained in the field and from the growers, the most significant limiting factors were identified and an analysis of the principal problems associated with the production system for panela sugarcane in the region of study was performed. Additionally, an economic analysis was performed using the absorption costing method (Álvarez and Sánchez, 2010). This analysis was performed on two production systems located in the municipality of Donmatías, Antioquia that had similar parameters in regards to the production stage, such as area (5 ha) and agronomic practices performed. However, in one of the systems agro-industrial processing was performed using a traditional sugar mill, while the other used a more modernized one. Using this information, the cost/benefit relationship was determined and the capacity of the infrastructure used for each system was identified. Later, the financial indicators of gross profit margin (GPM), operating profit margin (OPM) and return on assets (RA) were determined.

Processing of the information

The information compiled in the field and in the laboratory was systematized. For each qualitative variable evaluated the absolute frequency (AF) was determined and with the sample size (SZ) relative frequency was determined (RF) (RF=AF/SZ). Posterior relative frequency was converted in percentage (%), to multiply this value by 100 (%= RF*100). A schematic representation was made. The test statistic used was chi square, with a significance level of 95%. Statistical tests were run on the R program.

RESULTS AND DISCUSSION

Characterization of the production system related socioeconomic factors

One hundred percent (P<0.05) of the small farms evaluated corresponded to areas planted with less than 5 hectares of sugarcane (Figure 1A), which were supplemented by other agricultural and livestock activities such as the cultivation of coffee, plantain and subsistence crops; cattle ranching; and the farming of pigs and minor species (Figures 1B and 3A). In regard to land ownership, the majority of the land was owned by the farmers, with a small percentage rented or communally owned (P<0.05) (Figure 1C). This production system in many cases utilizes a family-based labor force (P<0.05) (Figure 1D), and the most common economic model is of family agro-industry, followed by sharecropping and, to a lesser extent, communal businesses (P<0.05) (Figure 1E). Although the latter system was the most common, not legally constituted associations were found. Another situation found is that the work force associated with the production system panela, is made up of 80% of male and 20% female, where 75% have more than 50 years of age, 17.5% in the range of 40-20 years and 9.5 are under 20 years.

These results do not differ much from those reported for Colombia as a whole and it is thought that thanks to these characteristics, the cultivation of sugarcane for panela production has a very heavy socioeconomic weight in the country. It is considered an important rural agro-industry, based on the number of production centers, planted area, the size of the associated workforce and, most importantly, its relationship with the small farmer and family industry (FAO, 2004; M.A.D.R, 2005; Rodríguez, 2005; Osorio, 2007; Tarazona, 2008). These systems are the most common throughout the country, but there are some exceptions which have been termed food industries (Castellanos *et al.*, 2010).

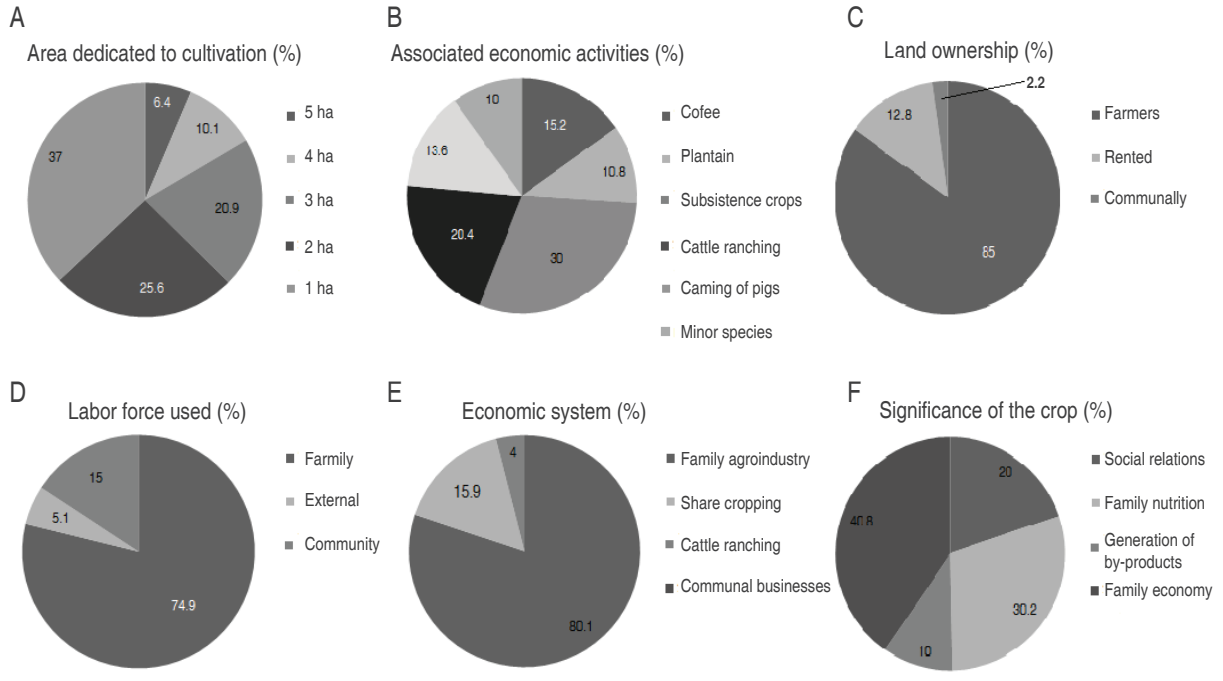

Figure 1. Features associated with socio-economic component sugarcane for panela production in the study area. The values are represented by the relative frequency.

In regard to the significance this activity represents for the farmer (Figure 1F), the results obtained show the importance of this agro-industry in aspects associated with the family economy, nutrition, generation of by-products and, particularly, social relations within the community ($P<0.05$).

An aspect that is just as important as panela's economic contribution is its role in nutrition in the country. The benefits are not limited to the farmers, but are also experienced by the consumers, since Colombia is the biggest *per-capita* consumer of panela at a global level (FAO, 2004; Osorio, 2007; Osorio and Restrepo, 2007; Tarazona, 2008; Castellanos *et al.*, 2010). Moreover, as the M.A.D.R. (2006) notes, panela is a low-cost sweetener that forms part of the family food basket in low-income sectors, due to its caloric and nutritional value. Another important contribution is the by-products produced, which are utilized in animal and human nutrition, and which provide multiple benefits for the family economy by decreasing the need to buy high-cost nutritional supplements at the market (Osorio and Restrepo, 2007; Tarazona, 2008).

The social impact of panela is also invaluable, given the relationships that are generated and sustained through its production chain. The FAO (2004) and Rodríguez (2005) propose that these can be economic in nature, but that family and community relationships and nonmonetary exchanges are still prevalent. These elements help increase circulation and the access of various production agents to resources such as a labor force, land and capital, all of which are important factors for the subsistence of the family unit and social and economic stability in the local and regional sphere.

Planting, management, harvest and transport

The planting of sugarcane for the production of panela in the area of study was characterized by the adaptation of the land through the removal of soil and the formation of furrows. The planting distances ranged between 1-1.2 m, and the seed was produced at the same farm through plant reproduction, and planted in a system of regular intervals or in furrows. During the growth and management stage, agronomic labors were greatly reduced, with weed control the principal activity ($P<0.05$) (Figure 2A). Harvesting is performed using a system of selective cutting or suckering,

using visual parameters including changes in the color of the bark of the sugar cane stalk and the shortening of the internodes of the interior of the plant, which occurs over distinct periods of time, usually in periods greater than 20 months (P<0.05) (Figure 2B). This late harvest largely affects the quality of the final product (Mosquera *et*

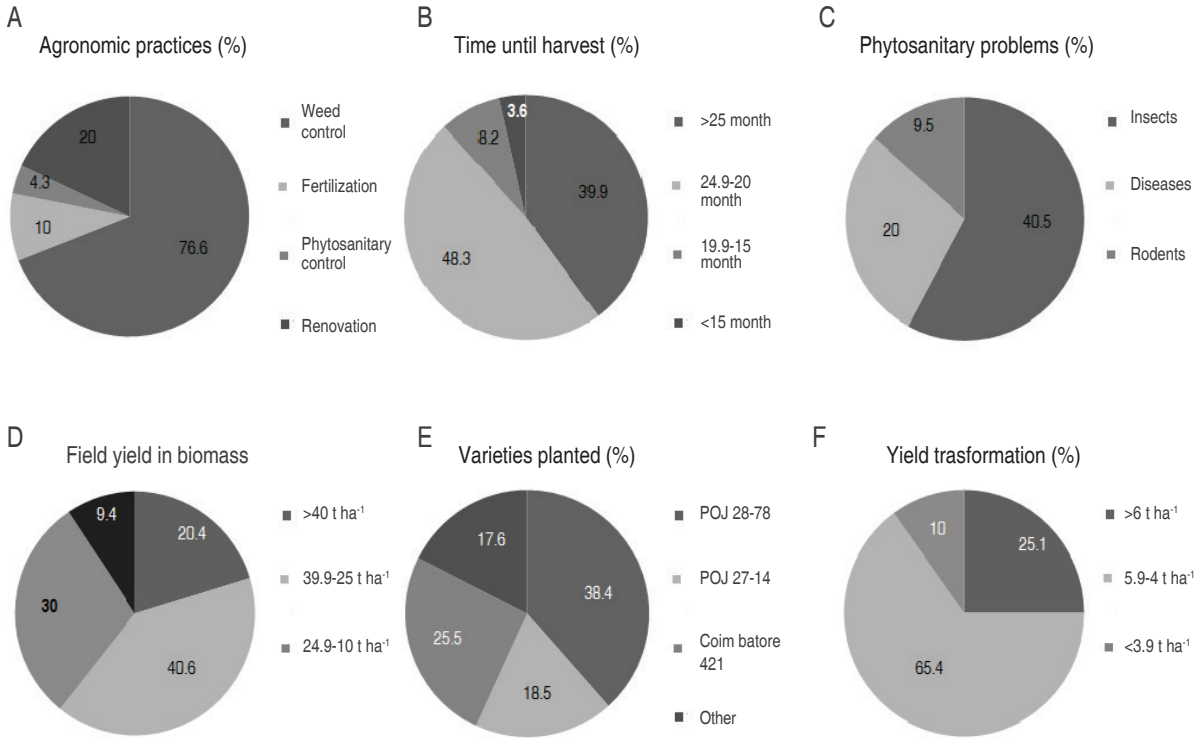

Figure 2. Features associated with cycle sugarcane for panela production in the study area. A: importance for each one. Insects**:** *Diatrea* sp. (60%), *Paratrechina fulva* (Mayr) (20.3%), *Aeneolamia* sp. (10.5) and *Mahanarva* sp. (9.2). Diseases: *Puccinia melanocephala* H. Sydow y P. Sydow (64.6 %), and *Ustilago scitaminea* Sydow (35.4 %). Rodents:*Mus musculus* L. (55.3 %) and *Sciurus vulgaris* L. (44.7%). The values are represented by the relative frequency.

al., 2007). None of the labors associated with this stage are performed using agricultural machinery, and transport to agro-industrial processing centers is completed using mules. The low number of agronomic practices performed (Figure 2A) results in multiple agronomic problems, among which are a high incidence of insects (*Diatrea* sp., *Paratrechina fulva*, *Aeneolamia* sp. And *Mahanarva* sp.), diseases (*Ustilago scitaminea* and *Pucciniam elanocephala*) and rodents (*Mus musculu s*and *Sciurus vulgaris)* (Figure 2C).

This situation coincides with that found in this and other production systems, where technological lags are common, leading to multiple agronomic and sustainability problems. Are common where high incidences of pests and diseases and their inadequate management, which is based almost exclusively on chemicals (Mosquera *et al.*, 2007; Martínez, 2013; Ramirez *et al.,* 2014).

These lead to low yield in the field (P<0.05) (Figure 2D), where the varieties planted (P.O.J. 27-14, P.O.J. 28-78 and Co 421) are also very old (Figure 1E), and, according to Osorio (2007) are not the best option, given that currently there are other varieties that perform better in terms of agronomic factors and yield (Garcia *et al.,* 2007). These situations affect the performance of the final product (P<0.05) (Figure 1F).

For many of the problems identified above, there is report of technological tools that could improve crop productivity (Lopez, 2015), but exist a problem, which is associated with the unknown of technology or your using, which

implies a lack of coordination between the generation of technological processes and those conducting their transference.

Agro-industrial processing and marketing system

In 90% of cases ($P<0.05$), the agro-industrial processing of sugarcane is performed with artisanal sugar mills, and consists in the extraction of juices, pre-cleaning, clarification and liming, evaporation of the water and concentration of the honeys, tapping and shaking, molding, cooling, packaging and baling (Figure 3B, C, D and F). The other 10% ($P<0.05$) is performed with more technologically advanced sugar mills, which comply with the requirements listed in Resolution 779 of 2006 (FEDEPANELA, 2008; INVIMA, 2009).

In regard to installations, artisanal sugar mills have dirt floors, no walls, are not divided into rooms, do not have sanitary services, the utensils used in the process are made of copper, the channels for conduction and storage and the pre-cleaning containers for the juices are made of cement, the water is taken from natural sources and is not pretreated, the facilities lack adequate infrastructure for the final disposal of the waste, and the employees do not use the appropriate attire. In regard to the mechanical infrastructure, the grinder has an internal combustion engine (diesel), with a grinding capacity of less than 0.5 t h^{-1}, the capacity of the furnaces is between 41-49 kg of panela $hour^{-1}$, and the percentage of juice extraction by weight of the machines varies between 47% and 50%. This value is considered low, since the minimum value should be 58% (Osorio, 2007). The fuel necessary for the furnaces is supplied almost entirely with sugarcane pulp, but on some occasions it is necessary to utilize firewood, a circumstance which, according to Velásquez *et al.* (2006) is due to the inefficiency of these artisanal furnaces, and has a negative impact on the environment.

Figure 3. Sugarcane for panela production and agro-processing. A: typical agroecosystem B: area planted to sugarcane for the production of panela. C, D, E y F: basic infrastructure for the processing of panela.

The above description does not comply with the requirements put forth in Resolution 779 of 2006 by the Ministry of Social Protection, which establishes technical regulations regarding the sanitary requirements that should be complied with in the production and sale of panela for human consumption (FEDEPANELA, 2008; INVIMA, 2009). Moreover, Velásquez *et al.* (2006) propose that the infrastructural conditions and machinery described

are obsolete and inefficient, coinciding with that found in other regions production systems (Mosquera *et al*., 2007). The inefficient practices used in agro-industrial processing, in addition to the harvesting of overripe sugar cane and the long time lapse between cutting and processing (Figure 2B) leads to major losses due to the reversion of glucose (Larrahondo and Torres 1990; Mosquera *et al*., 2007). When combined with low levels of extraction by obsolete machinery (Figure 3D), this results in a low yield of panela (Figure 2F). The marketing stage is completed via multiple intermediaries, as shown in Figure 4A. This phenomenon will be discussed further below.

Problems identified by small farmers of sugarcane in regard to the panela production system

For all of the farmers analyzed in the region of study, there was a consensus that fluctuations in sales prices (P<0.05) (Figure 4B and 4C) was one of the problems that had the most negative impact on sugarcane farming. These direct relationship is observed as increased purchase prices lead to increases in production, leading causes saturation in the market, which reacts and lowers purchase prices. They identified this as the cause of the low profit margins that currently exist (Table 1). This phenomenon is a direct consequence of the inefficient sales process, little incorporation of aggregated value, lack of homogeneity in quality, low levels of associativity and very poor crop planning. Osorio (2007), Rodríguez (2005), M.A.D.R. (2006) and Castellanos *et al*. (2010) have noted that the panela market involves innumerable intermediaries, which generates significant dispersion and inefficiency, as well as additional transaction costs. This situation favors the wholesalers who store the merchandise in urban settings, to the detriment of the farmer.

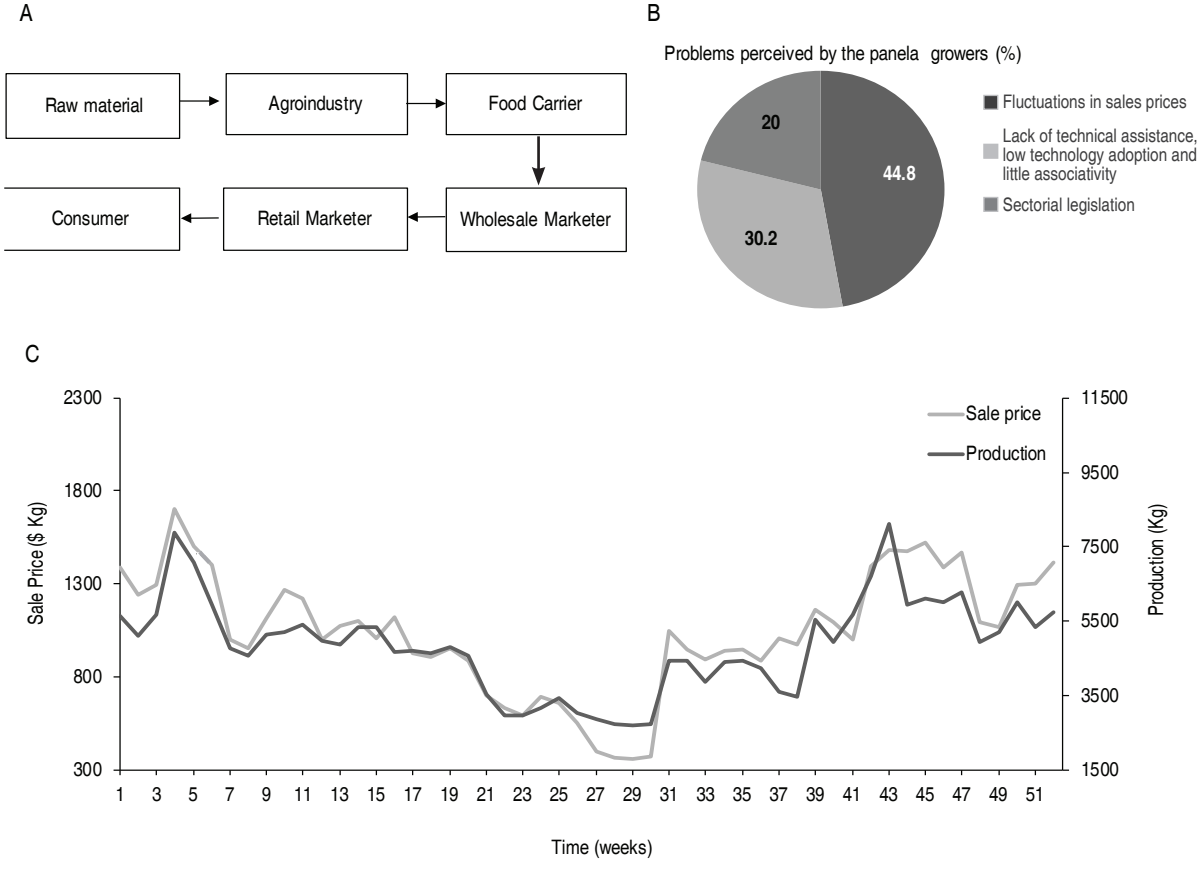

Figure 4. Marketing chain and main problems identified system of sugarcane for panela production in the study area. A: Marketing chain of panela sugarcane production. B: Problems perceived by the panela growers. The values are represented by the relative frequency. C: Dynamics of the sales price and production of panela systems evaluated.

Meanwhile, Rodríguez and Gottret (1998), M.A.D.R. (2005), Osorio (2007), FEDEPANELA (2009) and Castellanos *et al*. (2010) attribute the fluctuations in sales prices in the panela sector to multiple factors. These include: the commercial schemes of the merchants; the existence, in some cases, of long sales chains that lead to a rise in prices for the consumer; the use of sugar in the manufacturing of panela by illegal producers, a practice that is performed particularly when the price of panela is high and that of sugar low, and which leads to excess supply and price decreases; deficiencies in the packaging, transport and storage systems of the panela, which lead to considerable losses due to the deterioration of the product; and the false expectations of resolving these problems by constructing, in some panela producing regions, distilling plants for ethanol and honey plants that use up some of the sugarcane planted. Those long chains of marketing are identified in Figure 4 A, in which the large number of processes and links involved observed is evaluated production systems. Production costs are increased each and are part of a link off the previous one and that is part of groups of different people, resulting in an expensive product to the consumer, but with very low profit margin for the producer raw material or farmer.

A lack of technical assistance was the second problem identified in the present study (Figure 4B). None of the farms evaluated had received such assistance for the production stage, while in the case of agro-industrial processing, only the growers with contacts in the technologically advanced sugar mills had received it. This situation is reflected in the scarcity of agronomic labors performed (Figure 2A) and the underdevelopment of this crop in the production stage, as well as at the level of agro-industrial processing for the growers-transformers who use traditional sugar mills (Figures 3B, C, D and E). This is a common problem not onlyin the Antioquia department, but in most of the country, where low productivity is constant for this production system as a direct consequence of various problems, among them a lack of integral technical assistance (Osorio and Restrepo, 2007; Anuario Estadistico de Antioquia, 2010, 2014). It is believed that this production system has only made some advancements in regards to the modernization of all links in the production chain, rather than just the processing end, in a few regions of the country (FAO, 2004; M.A.D.R 2006; Osorio, 2007).

A third situation that the producers evaluated identified as limiting (Figure 4B) was the sectorial legislation. This refers to the issuing of Resolution 003462 of 2008, which sets forth the minimum requirements for the agro-industrial processing of panela, based on compliance with the sanitary conditions mentioned in Article 9, Number 1, Letters C and D; Number 2, Letters A and B; Number 8, Letter B; and Number 9, Letters A, B and C of Resolution 779 of 2006 (FEDEPANELA, 2008; INVIMA, 2009). The above situation is of significant concern, due to the high number of sugar mills within the regional and national production system that do not comply with the current technical requirements described in the legislation for the honey and sugar sectors (Tarazona, 2008). This problem does not appear to have a quick solution given the current dynamics of the sector, with the producers lacking the economic capacity to assume the high construction costs of facilities that comply with the regulations mentioned.

Economic aspects and financial indicators associated with two farms

Table 1 shows the results for two farms with different agro-industrial processing technologies. The results indicate that the traditional sugar mill has a 1.1 cost-benefit relationship, meaningthat the earnings are low but allow the farmer to assume the costs of production and have some margin of profit. This was not the case with the producer that had transformed his sugar mill into a more technologically advanced one, and was working for a loss. These results are supported by the indicators G.P.M and O.P.M. While for the farmer who uses a traditional sugar mill, both of these are positive, for the technologically advanced sugar mill, both are negative. These differences can be clearly explained by observing the indicator R.A, which is positive for the artisanal sugar mill and negative for the technologically advanced one. This is due to the fact that the fixed costs for the latter are very high, because the installed capacity is very superior to the capacity used. This means that the small amount of production obtained assumes all of the fixed costs (Table 1). These results coincide with those found by Arango (2006), who reported that the small producers of panela in the Guatape municipality (Antioquia) had negative profit margins, indicating that they were unable to compensate for production costs with sales prices.

Table 1. Income statements and financial indicators for two sugarcane for panela production systems in the study area.

Parameter	[1,2]Traditional	[1,3]Modernized
(+)Net Sales (NS)	24,486,302	25,500,000
(-)SoldCost of production	21,500,302	32,725,456
(=) Gross Profit (G P)	2,986,000	-7,225,456
(-)Administrative Expenses	1,201,305	1,305,505
(=)Operating Profit (UO)	1,784,695	-8,530,961
(+)OtherIncome	0	0
(=)Income Before Taxation and Interest (IBTI)	1,784,695	-8,530,961
(-)Interest	0	0
(=)Income Before Taxation (IBT)	1,784,695	-8,530,961
(-)Taxation	535,408	0
(=)Net Income(NI)	1,249,287	-8,530,961
Capacity of the Infrastructure Used (%)	92	10
Cost-benefit relationship	1.1	0.77
Gross Profit Margin (%)	12.1	-28.3
Operating Profit Margin (%)	7.28	-33.5
Return on Assets (%)	26.3	56.8

[1]Values are in constant Colombian money for 2012**.** [2]Infrastructure without modernization, the cost of infrastructure use and the costs associated with production and processing stage is assumed. [3]Infrastructure that meets regulated in resolution 779 of 2006, the cost of infrastructure use and the costs associated with production and processing stage is assumed.

The precarious and underdeveloped primary production system contrasts with the high levels of investment in agro-industrial processing facilities on the part of the state, generating an negative returnssince in many cases not even 10% of productive capacity is used (Table 1). This situation is the result of low levels of associativity, the small number of farms in the areas where processing units are constructed (Figure 1A) and the low yields per hectare (Figure 2D).

The low levels of technology, negative profit margins, inefficient marketing processes, lack of business or union organizations and misplaced state assistance, among many other problems, have relegated this economic sector to a second tier in the region of study. This has caused the land dedicated to panela cane production to be reallocated to other production systems, with a preference for monoculture dedicated to the production of animal feed. In the worst of cases, it has led to migration towards the cities, a phenomenon that is not foreign for the country (M.A.D.R., 2005; Osorio, 2007; Tarazona, 2008). Faced with this dynamic, the production of panela by small farmers is seriously threatened.

The above situation points to the need to redefine the strategies and programs financed by the state, with a look towards improving the production systems for panela sugarcane in order to increase the productivity and profitability of the entire production chain. At the same time, the farmers are in large part responsible for this situation, since they have very little culture of adopting preexisting technologies for this crop, including the use of more productive varieties and more appropriate agronomic practices (García *et al.,* 2007).

Strategies to improve the sustainability of small sugarcane production systems for the production of panela

Many of the problems listed above have been associated with small producer of panela throughout the country **(**Castellanos *et al*., 2010) and there are alternatives to improve many of these technological delays and it is used in some production systems with good results. (Lopez, 2015). This could be due to multiple causes, where the most potential are given as a result of a break between who makes the technological developments and those in

charge of the transfer or non-adoption by the producer.This implies the first strategy that could pose to improve system productivity, which should be associated to establish direct communication mechanisms among all players in the chain with goals and targets for each one, where the researcher can generate alternatives adapted to the conditions and needs of the producer and there is a dynamic and effective mechanism to ensure the transfer of these technologies. Meanwhile the producer is willing to make changes leading to improve their sustainability. In addition to making innovative advertising tools to improve the perception by farmers to system more sustainable, which involves the use of information technologies are included to achieve an educational revolution in which we look for farmers to access new technologies. An important aspect that should be sought is the generation of technologies that meet specific needs of different production systems and not from the premise of general technological packages, this mechanism would involve a great effort from the entire chain, but generate strategies more integrated into territory where the community perceive them closer and there may be a greater appropriation.

As a second strategy should be avoided migration of the young population to the cities and the loss the generational change, put at serious risk the production of panela. This situation could counteract by an entire rural restructuring associated with greater safety, better infrastructure, health centers, sports and cultural scenarios, better wages and working conditions, should also ensure access to quality education and to new information technologies.

As a third component is necessary to strengthen the social base and to enhance and create associative processes that could help improve the production stage of the crop, as well as profits and marketing. This involves combining the available technology with a permanent integrated technical accompaniment that would improve the competitivity and sustainability of this subsector in the country. In this regard, the M.A.D.R (2006), Osorio (2007) and Tarazona (2008) have noted that the panela farmers' lack of business or union organizations is one of the major problems hindering and preventing the adequate development of the sector. From this can be generated cooperative production systems, involving greater ability to offer products on the market and access systems technical assistance, credit, among others. Under these production systems can generate greater value added, seeking to offer other kinds of products whose raw material is the panela (Castellanos *et al.*, 2010). Where this alternative is considered the most appropriate to avoid the drop in sales prices for periods of over-supply (Álvarez and Sánchez, 2010). This part should identify successful cases and associated factors, in addition to identifying errors in unsuccessful processes. Moreover in partner process to generate added value must be integrated to the final consumer, which you may be charged on the production cycle of the same, where the strategy to implement serious that you can know who and how the food they consume is produced, this could create direct links between urban and rural.

As fourth aspect arises that is fundamental to keep in mind when making a private or public investment that this should not be limited only to one link in the chain, but rather, should take into account the entire production model in order to avoid using technology in an isolated and disjointed way. As this study clearly showed, setting up infrastructure just for the processing of the sugarcane failed to resolve the problems of the panela growers.The fifth and final aspect that arises is the need of a product of excellent quality, which should be based on good manufacturing practice, this implies the need to adapt the small plants, so it is proposed that no infrastructure become very large and expensive, but small functional units according to the production capacity of raw materials in the area. This require technological adaptations inexpensive and easy and fast implementation, which would generate a high impact because much of the production of panela is made by small producers.

CONCLUSIONS

The traditional panela production system evaluated showed great technological lags in each of the stages of its production system. This production system requires a restructuring, which must come from the producer's needs as fundamental to the productivity and sustainability of the system, where it manages to incorporate the technological solutions adapted to each particular situation, which would ensure that resources seen well channeled. Meanwhile is necessary the cohesion of all players in the chain of production system in order to improve their economic expectations and avoid the loss of this activity in areas of artisanal production.

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Morpho-agronomic assessment of introductions of butternut squash (*Cucurbita moschata* Duch.) from Central America

Evaluación morfoagronómica de introducciones centroamericanas de zapallo (*Cucurbita moschata* Duch.)

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ABSTRACT

A Central American collection of butternut squash (*Cucurbita moschata* Duch.) was characterized based on a series of morpho-agronomic descriptors and its variability assessed. Floral asynchrony ranging from 10 to 15 days was observed between staminate and pistillate flowers. Differences were also observed among introductions for all quantitative characteristics evaluated ($P<0.01$). An interaction between introduction and planting cycle ($P<0.05$) was observed for 50% of the evaluated variables: fruit weight, polar diameter of fruit, wall thickness of fruit, fruit color, days to harvest, and total fruit seed weight. Cluster analysis revealed that groups 3 and 5 gathered introductions presenting high yields and larger, heavier fruits. Group 4 gathered introductions with intermediate yields, high seed production, and thick fruit walls. In all cases, genetic improvement aiming to increase the production of fruit for fresh consumption or agro-industrial purposes as well as the production of oilseed should use introductions from groups 3 and 5 in well-planned crossings with introductions from group 4.

Key words:
Plant morphology
Agronomic characters
Germplasm
Squashes
Multivariate analysis

RESUMEN

Se caracterizó una colección centroamericana de zapallo (*Cucurbita moschata* Duch.) con base en una serie de descriptores morfo-agronómicos y luego se evaluó su variabilidad. Se evidenció una asincronía floral entre flores estaminadas y pistililadas de 10 a 15 días. Se encontraron diferencias entre las introducciones ($P<0,01$) respecto a todos los caracteres cuantitativos evaluados. Se presentó una interacción introducción x ciclo de siembra ($P<0,05$) en 50% de las variables evaluadas: peso del fruto, diámetro polar del fruto, espesor de la pared del fruto, color de la matriz del fruto, días a cosecha y peso total de la semilla del fruto. El análisis de conglomerados detectó que en los grupos 3 y 5 se ubicaron las introducciones con altos rendimientos y frutos más grandes y pesados. El grupo 4 estuvo conformado por las introducciones con rendimientos intermedios, alta producción de semillas y amplio espesor de pared del fruto. En todos los casos, el mejoramiento genético dirigido a aumentar la producción de fruto para consumo en fresco o para fines agroindustriales o la producción de semillas de tipo oleaginoso deberá utilizar las introducciones de los grupos 3 y 5 en cruzamientos bien planeados con las del grupo 4.

Palabras claves:
Morfología vegetal
Características agronómicas
Germoplasma
Ahuyama
Análisis multivariado

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The ancestral and current uses of the fruit of butternut squash (*Cucurbita moschata* Duch.) as both horticultural and edible crop not only in Colombia but throughout the Americas have been well documented by Patiño (1967), Piperno *et al*. (2000), Piperno and Stothert (2003), Piperno (2011), and Ortiz *et al.* (2013).

In the case of Colombia, butternut squash ranks first as domesticated horticultural crop, with a total planted area of 3800 hectares and an annual production of 65,000 t (FAOSTAT, 2013). Butternut squash is frequently intercropped or rotated with fruit, ornamental, and forest species as a staple crop in agro-ecosystems of rural economy. It also plays an important role as horticultural crop in the country's food security, being planted as main or transitory crop on small or intermediate farms (Jaramillo, 1980; Estrada *et al*., 2010). Its popularity can be attributed to its versatility for fresh consumption and its industrial uses (Espitia *et al*., 2005; Ortiz *et al*., 2008; Ortiz *et al*., 2013; Ordoñez *et al*., 2014).

Because of its nutritional value, butternut squash was included in the list of priority foods that guarantee a balanced human diet and, according to FAO, form part of Colombia's basic food basket (PNSAN, 2012).

This study aimed to evaluate and characterize the initial morpho-agronomic traits of a collection of butternut squash from Central America, which would serve as basis to identify superior genotypes for release to farmers.

MATERIALS AND METHODS

Location

Field trials were conducted at CEUNP, the Experimental Center of the Universidad Nacional de Colombia, Sede Palmira, which is located in Candelaria (rural community of El Carmelo), department of Valle del Cauca, Colombia (03°25'25.3"N, 76°25'47.8"S), at an altitude of 972 m above sea level, with an average annual temperature of 26 °C, 76% RH, and average annual rainfall of 1100 mm (Ortiz *et al*., 2013).

Germplasm used

Thirty-four butternut squash introductions original of Central America (Table 1) were used; of these, six disappeared during the first planting cycle due to prevalent conditions (excess rainfall or excess drought).

Nursery and planting in the field

Nurseries were established and subsequently planted in the field during two consecutive planting seasons of 2014, according to the protocol of the UNAPAL's Horticultural Program (Ortiz *et al.,* 2013), using commercial peat as substrate.

Two seeds were sown per 12-oz cup. After 20 days in the nursery, the trials were planted in the field using a randomized complete block design with three replications and five plants per replication. The experimental unit was represented by five plants, planted in 3 x 3 m arrangement in the field. Agronomic practices were coordinated by the Horticultural Program (Ortiz *et al*., 2013).

Evaluated traits

Plant- and fruit-related traits were assessed based on the descriptors proposed for butternut squash by Esquinas and Gulick (1983), Montes *et al.* (2004), and ECPGR (2008). Three plants were sampled per furrow and two fruits per plant were evaluated in the three replications. The following variables were measured: days to staminate flowering (DSF); days to pistillate flowering (DPF); days to harvest (DH); fruit weight (FW) in kg; number of fruits per plant (NFP); fruit yield (FY) in t ha^{-1}; total seed weight per fruit (TSWF) in g; 100-seed weight (100-SW) in g; polar diameter of fruit (PDF) in cm; equatorial diameter of fruit (EDF) in cm; thickness of fruit wall (TFW) in cm; diameter of placental cavity (DPC) in cm; fruit form (FF) (scale 1–14); pericarp color (PC) (scale 1–10); fruit pulp color (FPC) (scale 1–15); fruit matrix color (FMC) (scale 1–3); and pulp quality (PQ) (scale 1–2); placenta location (PL): on the wall (1), in the middle (2).

Statistical analyses

Variance analysis was conducted to detect differences between introductions and estimate the effect of the environment on the expression of variability. The SAS 3.0 statistical package was used (SAS, 2009). Means separation was based on Fisher's Least Significant Difference (LSD) test at a 5% significance level (Steel and Torrie, 1985).

Cluster analysis was used to determine the importance of variables and genetic relationships between introductions. The Ward-Modified Location Model (Ward-MLM) was used, using the algorithm proposed by Franco *et al*. (1998), where clustering occurs under the assumption of minimum variance

Table 1. Butternut squash introductions from Central America.

Entry	Introduction	Country	Locality
1	10789	Panama	David
41	12444	Panama	El Valle-Penonome
7	12043	Nicaragua	Playitas
9	11993	Nicaragua	Rivas
30	12035	Nicaragua	Diriamba
38	12054	Nicaragua	Matagalpa-Playitas
4	15715	Guatemala	Zacapa
18	11877	Guatemala	Antigua
28	16041	Guatemala	San Marcos
46	14921	Guatemala	
10	9099	El Salvador	El Guayabal
12	9060	El Salvador	Chinameca
16	9092	El Salvador	Puerto Nuevo
29	9069	El Salvador	La Unión
47	9091	El Salvador	Puerto Nuevo
3	20120	Costa Rica	Roxana, Guápiles
11	10810	Costa Rica	Garita-Central
22	6368	Costa Rica	Central
8, 24	13425, 6369	Costa Rica	Costa Rica
14	9213	Mexico	Cunduacán
42	8009	Mexico	Chiapas de Corzo
2, 17, 37, 40, 45, 48	18943, 18932, 18942, 9284, 18859, 18834	Mexico	
23	12139	Honduras	La Paz
27	11015	Honduras	Potrerillos
36	11044	Honduras	La Esperanza
39	12168	Honduras	Siguatepeque
43	12125	Honduras	Flórez
44	12088	Honduras	Ajuterique

within the group but maximum heterogeneity between groups. Heterogeneity between groups was estimated based on Mahalanobis distances and the pseudo-F statistic determined the number of selected groups (maximum value). The number of groups was decided based on the lowest value of the function. Gower's index was used to estimate genetic distances. The data matrix was standardized and the distance matrix was estimated by the Ward-Gower method (Gower, 1971), which allows the use of continuous, nominal, and binary variables. The Proc IML, Proc Cluster, and Proc Tree procedures of the SAS 9.3 statistical package (SAS, 2009) were used in all cases.

RESULTS AND DISCUSSION

Phenology and fruit and seed yield

Asynchrony between DPF and DSF was detected in most butternut squash introductions (Table 2). The results agree with those of Ortiz (2009), who evaluated the Colombian collection of butternut squash and observed physical deterioration in 10-day-old plants. These plants only produced pollen-producing flowers with no receptive pistillate flowers for fertilization and fruit formation. Therefore, if pollination is not performed and seed formation is not effective, then the fruit is not developed and aborts.

Regarding DH, plants from the Central American collection matured at least 30 days earlier as compared to those of the Colombian collection (Table 2), presenting relative precocity (Ortiz, 2009; Valdés *et al.*, 2010).

Although FW varied broadly, results indicated that 35% of the genotypes of the Central American collection presented heavy fruits, weighing more than 5 kg. These results agree with those of different studies conducted by UNAPAL's Horticultural Program (Montes *et al.*, 2004; Ortiz, 2009; Valdes *et al.*, 2014).

The NFP presented relative uniformity across introductions (Table 2), averaging 3.8. In introduction 42, however, there was a marked asymmetry, with 25 fruits per plant. The NFP of the remaining introductions of the collection did not exceed four fruits per plant. This characteristic is important from the genetic viewpoint because prolificacy is a selection factor that can be significantly affected by the environment. For example, the absence or low presence of pollinating bees can cause the early abortion of fruit due to the absence of effective pollen (Lau and Stephenson, 1993; Ortiz *et al.*, 2013). Water availability at critical times of fruit fill and limited amounts of trace minerals in the soil solution can also cause early abortion of the fruit (Lopes de Sousa, 2011).

Average fruit yield (FY) of the Central American collection was similar to that of commercial butternut squash in Valle del Cauca, Colombia, which is 14 t ha^{-1}. However, 21% of the genotypes presented yields above 20 t ha^{-1}, which opens the opportunity to select prolific genotypes with heavy fruit and superior performance (Ortiz *et al.*, 2013).

Regarding TSWF and 100-SW, 39% of the Central American introductions presented above-average values, which reflects in the presence of large, heavy seeds with added value for agro-industrial purposes (Ordoñez *et al.*, 2014). The remaining introductions presented typical values, being similar to those reported by Valdes *et al.* (2014).

Table 2. Analysis of combined means for two planting cycles of 28 butternut squash introductions from Central America in Valle del Cauca in 2014 regarding phenology, fruit yield, and seed yield.

Introduction	DSF	DPF	DH	FW	NFP	FY	TSWF	100-SW
	(days)			(kg)	(No.)	(t ha^{-1})	(g)	
Mean/Mode	35.0	44.0	116.6	4.6	3.8	14.7	50.3	11.3
Minimum	31.0	31.7	102.4	0.5	1.9	4.7	16.0	6.2
Maximum	37.2	54.6	123.9	10.0	25.6	22.5	80.7	15.0
LSD	2.5	6.5	7.7	2.0	1.5	7.6	18.9	2.1

Fruit characterization

The results of means analysis of fruit-related traits of Central American butternut squash introductions are presented in Table 3. Characterization was based on the fruit descriptors for butternut squash developed by ECPGR, 2008; Esquinas and Gulick, 1983; and Montes *et al.*, 2004 (Table 4).

Analysis results indicated that 75% of the evaluated introductions presented cylindrical, pear-, diamond-, spindle- or gourd-shaped fruits based on PDF and EDF (Table 3) and the rest, a flat, vessel-shaped fruit based on EDF (Figure 1), indicating that there are materials available that have fleshy fruits with small placental cavity, two traits that industries find attractive.

Regarding the predominant PC of the fruit, 57% of the introductions presented a cream-colored pericarp (Figure 1), which is attractive from the commercial viewpoint. However, the demand of the fresh consumption market in Colombia is for bright green fruits (Ortiz *et al.*, 2013).

The TFW, a desirable character to ensure marketable butternut squash for commercial fresh consumption, presented a relatively low average for 62% of the introductions (Table 3); however, 38% presented above-average values, with introduction 30 presenting the highest TFW value (4.1 cm) (Figures 2, 3A, and 4A). Market demand determines desirable characteristics

Table 3. Analysis of combined means for two planting cycles of 28 butternut squash introductions from Central America in Valle de Cauca in 2014 regarding fruit characterization.

Introduction	PDF	EDF	FF**	FC*	TFW	DPC	FPC	FMC
Mean/Mode	20.6	14.5	7.0	3.0	2.6	9.6	15.0	3.0
Minimum	6.1	7.5	1.0	1.0	0.9	5.8	1.0	1.0
Maximum	29.6	20.8	15.0	10.0	4.1	14.8	15.0	5.0
LSD	4.4	4.2	2.0	2.0		0.7	2.1	1.6

Polar diameter of fruit (PDF) in cm; equatorial diameter of fruit (EDF) in cm; thickness of fruit wall (TFW) in cm; diameter of placental cavity (DPC) in cm; fruit form (FF) (scale 1–14); fruit pulp color (FPC) (scale 1–15); fruit matrix color (FMC) (scale 1–3). * FC: fruit color.
** Based on indicators given by Esquinas and Gulick (1983), Montes *et al.* (2004); ECPGR (2008),

Figure 1. Predominant pericarp color of fruit, with 57% of the introductions of the Central American collection presenting a cream-colored pericarp.

Figure 2. Fruit pulp color characteristic of butternut squash cultivar Abanico 75, which is highly demanded by the animal feed industry as well as for human fresh consumption. Source: Ortiz *et al.*, 2013.

of fruit pulp quality (Figure 2) and serves to select the most appropriate model for genetic improvement (Ortiz *et al.*, 2013).

Contradictory results were obtained regarding the DPC of the fruit. If the diameter is wide, then the amount of seed it can hold is significant; however, a wide DPC could affect

the TFW, making it necessary to find an introduction that meets both characteristics (good seed production and high TFW). In any case, 57% of the introductions presented above-average values (Table 3 and Figures 4A and 4B).

Regarding FPC, 75% of the Central American introductions presented high values of bright yellow on the Roche scale. The remaining materials were pale yellow or yellow with green or bright green stripes, which reduces their commercial value for fresh consumption (Figures 3, 4A, and 4B). A green-colored pulp is an indication that the material has a marked wild or naturalized origin (Sanjur *et al.*, 2002) and, given the cross-pollination that occurs in butternut squash, advanced genotypes could be derived that would seriously impair the breeding process for FPC. Both FMC and FPC, typically yellow in butternut squash, ranged from bright yellow in several of the Central American introductions to greenish-black for 71% of the introductions assessed (Figures 3 and 4B). This finding disagrees with the bright yellow FMC that consumers found attractive in previously evaluated collections (Ortiz, 2009; Valdes *et al.*, 2010; Valdes *et al.*, 2014). However, the greenish-black color of the pulp when the fruit is opened or several hours after processed is not an indication of fruit deterioration. The fresh consumption market in Colombia does not find introductions with a green- or black-colored matrix attractive, but their nutritional or productive qualities for the animal feed industry should be further studied.

Table 4. Qualitative descriptors for the fruit of butternut squash based on indicators given by ECPGR (2008); Esquinas and Gulick (1983); Montes *et al.* (2004).

Descriptor	Scale
Fruit shape	
Globular	1
Flattened	2
Disk-shaped	3
Oblong block-shaped	4
Elliptical (oval)	5
Heart-shaped	6
Pear-shaped	7
Dumbbell-shaped	8
Elongated	9
Coil-shaped (upper)	10
Crested	11
Coil-shaped (lower)	12
Curved	13
Gooseneck-shaped	14
Secondary color of fruit	
White	1
Green	2
Blue	3
Cream	4
Yellow	5
Orange	6
Red	7
Pink	8
Texture of fruit pericarp	
Smooth	1
Slightly granular	2
Fairly granular	3
Granular	4

Descriptor	Scale
Predominant color of fruit	
Green	1
Blue	2
Cream	3
Yellow	4
Orange	5
Red	6
Pink	7
Brown	8
Gray	9
Black	10
Design of secondary color	
Absent	1
Dotted	2
Mottled	3
Striped	4
Banded	5
Bi-sectional	6
Spotted	7
Fruit rib	
Absent	1
Superficial	2
Intermediate	3
Deep	4

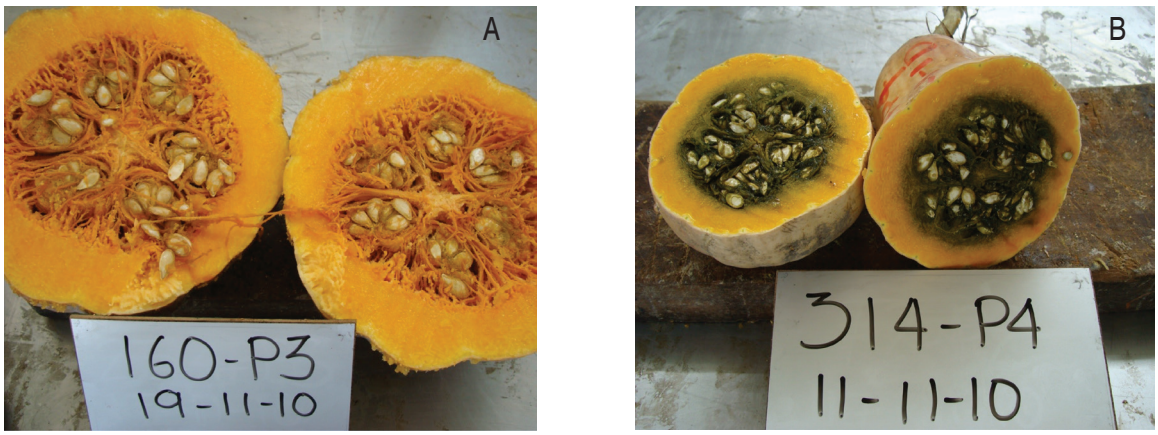

Figure 3. Fruit matrix color of two butternut squash introductions of the Central American collection, Horticultural Program, Universidad Nacional de Colombia-Palmira. A. Introduction 20120 from Costa Rica; stable, bright yellow matrix after cut. B. Introduction 9092 from El Salvador, greenish black matrix with short-term oxidation.

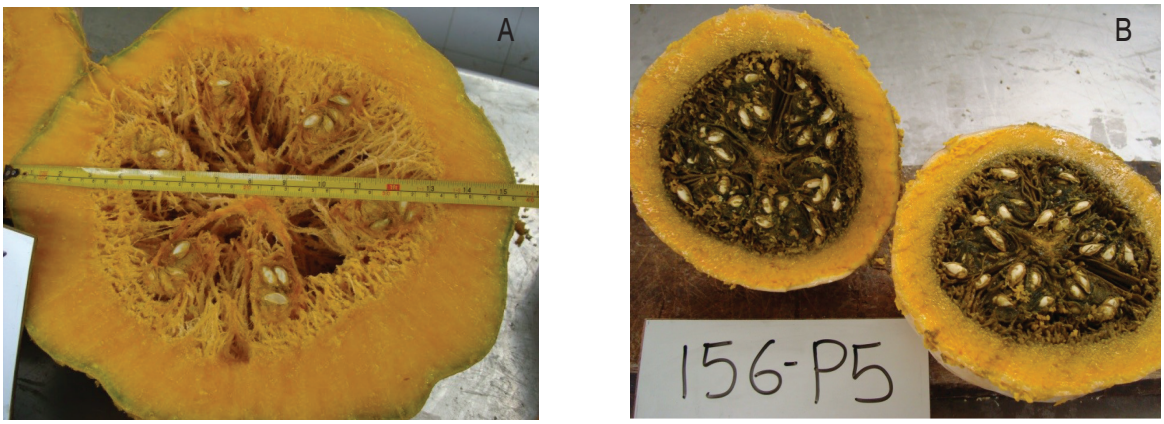

Figure 4. Thickness of fruit wall of two butternut squash introductions. A. Outstanding (5.0±2.5 cm); B. Minimal (3.0 ±1.5 cm).

Analysis of variance

The analysis of variance showed highly significant differences among introductions (P <0.01) for all study variables (Table 5). Overall, the coefficients of variation for the descriptors evaluated were low to intermediate, ranging between 5.4 and 34.2% (Table 5). This variation is considered appropriate because the assessment of traits was based on individual plants and this assumes that the variation of micro-environments has been controlled to some extent (Ceballos, 1998).

The analysis of the interaction cycle x introduction indicated no significant differences (P <0.05) for Y, NFP, DPC, FPC, PDF, PC, and 100-SW (Table 5); however, highly significant differences (P <0.01) were observed regarding FW, PDF, and FMC.

Overall, broad-sense heritability was high, ranging between 66 and 98% (Table 5). Although this indicator is usually important in plant breeding processes, in the case of this study care should be taken when quantifying broad-sense heritability as, according to Ceballos (1998), heritability is not only a property of the plant trait but also of the plant population and is affected by the environmental conditions under which the individuals develop and how the phenotype is evaluated. The changing value of this trait is associated with all components of variance and will accordingly be affected by changes in cropping conditions (Espitia, 2004; Ortiz, 2009). Heritability is expected to increase in variable environments and decrease under more favorable conditions (Falconer and Mackay, 1996). Based on the above, in the cases where the biological or statistical differences observed can be attributable to the

interaction planting cycle x introduction (Table 5), it is highly probable that adequate breeding methods or appropriate agronomic practices can trigger positive practices associated with high heritability.

Cluster analysis

Five groups were formed based on the Ward method (Figure 5 and Table 6). The graphic of the pseudo-maximum likelihood shows a sharp decline in group

Table 5. Mean squares of variance analysis, coefficients of variation, and heritability for different traits of butternut squash in two planting cycles in 2014 in Valle del Cauca, Colombia.

Variable	Unit	CMI	P	CM C x I	P	CV (%)	H^2 (%)
PY	t ha^{-1}	113.5	**	32.9	ns	34.2	71
FW	kg	20.1	**	2.2	**	22.5	89
NFP	#	54.6	**	1.3	ns	27.1	98
EDF	cm	106.9	**	10.9	*	12.1	90
PDF	cm	43.5	**	10.0	**	14.0	77
TFW	cm	2.1	**	0.3	*	14.2	87
DPC	cm	21.1	**	1.1	ns	11.6	95
FMC	Scale 1–3	0.2	**	1.4	**	10.0	0
FPC	1-15[1]	28.3	**	2.5	ns	14.1	91
PL	Scale 1–2	0.3	**	58.7	ns	16.8	84
Placenta color	Scale 1–2	0.2	**	0.1	ns	18.8	66
DH	days	317.3	**	85.4	*	5.4	61
100-SW	g	21.6	**	2.4	ns	12.0	89
TSWF	g	1247.4	**	193.2	*	21.1	85

CMI = mean squares of introduction; P = statistical significance with *= p=5%, **=p=1%, and ns=non-significant; CM C x I = mean square of interaction planting cycle x introduction; CV = coefficient of variation; H^2= broad-sense heritability.
[1]Scale of 1 to 15 of the Roche Color Fan.

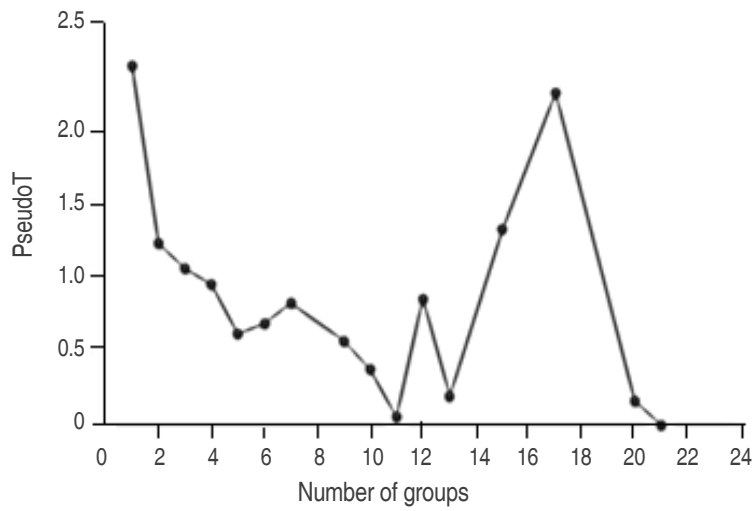

Figure 5. Comparison of pseudo-T function with number of groups estimated by Ward's method to gather 28 introductions of the Central American collection of butternut squash.

4 with a subsequent rise in group 6 and is assumed to represent the breakeven point for determining work groups. Introduction 42 was excluded from the cluster analysis because it presented an outlier value for NFP.

Figure 6 shows how the 27 introductions were grouped. Five groups were formed based on Ward's hierarchical clustering (Table 6). Group 1 was composed of four introductions, one each from Nicaragua, El Salvador, Mexico, and Honduras (accounting for 15% of the total), with an average distance of 0.57 between introductions. Group 2 was composed of seven introductions, one each from Panama, Costa Rica, and Guatemala, two from Honduras, and two from Mexico (accounting for 26%), with a distance of 0.60 between introductions and located far from groups 3 and 5. Group 3 includes seven introductions, with one introduction each from Honduras, Nicaragua, Mexico, and Costa Rica and three from El Salvador (accounting for 26%), with a distance of 0.57 between introductions and located far from groups 1 and 5. Group 4 consists of six introductions, one each from Guatemala, Salvador, and Honduras, and three from Mexico (accounting for 22%), with a distance of 0.56 between introductions. Finally, group 5 includes three introductions, one each from Costa Rica, Nicaragua, and Honduras (accounting for 11%), with a distance of 0.61 between introductions.

Table 6. Absolute, percentage, and cumulated frequency of groups of Central American introductions of butternut squash formed based on cluster analysis.

Group	Absolute frequency	Percentage frequency	Cumulated frequency	Mean distance
1	4	15	15	0.57
2	7	26	41	0.60
3	7	26	67	0.57
4	6	22	89	0.56
5	3	11	100	0.61

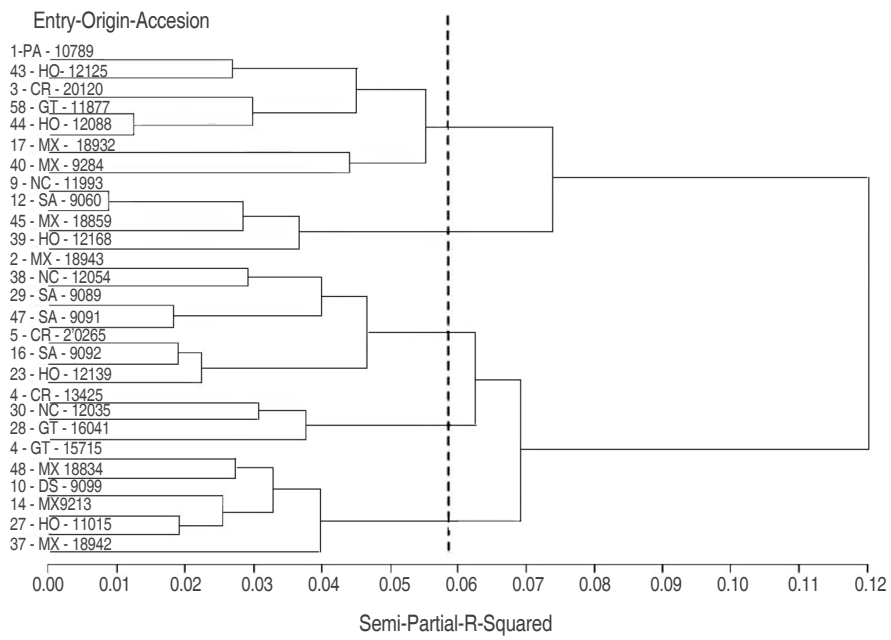

Figure 6. Cluster analysis of 28 introductions of a Central American butternut squash collection, based on Ward's hierarchical grouping.

Although phenotypic variability is evident in the Central American collection of butternut squash introductions studied, the cluster analysis failed to group introductions according to origin (country), probably because of the very active exchange of germplasm in Central America. Butternut squash, maize, and beans are the basis of Milpa agriculture and food security crops in traditional family-based farming systems in Central America (Patiño, 1967; Piperno *et al.*, 2000; Piperno and Stothert, 2003; Piperno, 2011).

Table 7 presents the results of means analysis and the coefficient of variation for study variables for the five groups, based on Ward's method. Groups 1 and 2 gathered the introductions with the lowest means for traits related to fresh fruit yield per hectare, smaller and lighter fruit, lower PDF and EDF, less TFW and DPC, fewer seeds, lower 100-SW, and lower TSWF. Fruit with green-colored pulp predominated, and introductions of group 1 matured the earliest. Therefore, the introductions

Table 7. Means analysis and coefficient of variation for variables assessed in five groups for 28 Central American introductions using Ward's method for two planting cycles in 2014.

Variable	Group 1		Group 2		Group 3		Group 4		Group 5	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
PY per ha	10.7	32	10.9	47	18.9	17	15.3	24	18.9	22
FW weight	3.2	31	3.2	50	5.5	23	5.0	26	7.8	26
NFP	3.2	15	3.2	42	3.3	9	3.0	18	2.2	16
100-SW	11.0	22	9.2	11	12.2	9	13.4	11	11.9	12
TSWF	47.7	18	33.8	16	60.0	20	65.9	19	49.7	39
Number of sedes	450.1	21	383.2	21	504.1	15	499.2	11	430.0	30
PDF	20.1	13	18.1	18	25.3	10	19.4	7	23.7	25
EDF	12.3	20	12.8	19	15.7	17	15.8	13	18.4	7
TFP	2.1	23	2.4	29	2.8	9	2.8	18	3.5	14
Diameter placental cavity	8.4	18	8.2	22	9.8	16	11.3	17	11.9	12
DH	114.5	4	119.6	3	115.7	2	115.3	6	117.7	4
Days to male flowering	34.8	4	35.1	2	34.6	2	35.3	2	36.2	2
Days to female flowering	45.9	16	46.8	15	41.0	9	42.7	8	48.3	10
Color seed border	4.0	29	3.4	23	1.9	58	1.7	62	3.7	31
Fruit rib	1.0	0	1.9	58	2.1	63	1.8	41	3.7	16
Fruit color	1.5	67	4.4	74	3.1	74	2.7	31	2.3	49
Pulp color	15.0	0	12.0	23	15.0	0	13.3	24	13.7	17
Spots on leaves	2.8	35	2.0	50	1.9	20	1.7	31	2.0	87
Peduncle insertion	4.0		2.0		2.0		2.0		2.0	
Final fruit shape	4.0		2.0		4.0		2.0		2.0	
Transversal shape of fruit	1.0		1.0		1.0		2.0		4.0	
Matrix color	3.0		3.0		3.0		3.0		3.0	
Shape of leaf lobe	1.0		1.0		1.0		1.0		1.0	
Leaf size	2.0		2.0		3.0		2.0		3.0	
Seed size	2.0		2.0		2.0		2.0		2.0	
Shape of seed margin	2.0		2.0		2.0		5.0		2.0	
Secondary color design	7.0		1.0		7.0		1.0		7.0	
Fruit peel texture	1.0		1.0		1.0		1.0		7.0	

of these groups rank low when selecting for fresh fruit yield, seed yield, and fruit pulp quality.

In the case of group 2, the only relevant trait regarding the rest of the introductions evaluated was the yellow color of the pulp (Table 7). The remaining variables presented average or below-average values.

Groups 3 and 5 gathered introductions with higher average values for traits related to PY, FW, PDF and EDF, number of seeds per fruit and 100-SW (Table 7). However, at the same time introductions maturing the latest in terms of days to harvest also took the longest in terms of DSF and DPF. Unfortunately, green-colored pulp and fruit matrix predominated in group 3, presenting rapid oxidation and blackening (Figure 3B), which disqualifies these introductions for fresh consumption.

Group 4 gathered introductions presenting higher averages regarding TSWF, 100-SW, seed size, EDF, and DPC; intermediate values for PY per hectare, FW, NFP, and DH (late). The bright yellow color of the pulp of these introductions stands out, which adds value for fresh consumption.

Group 5 gathered introductions presenting higher averages in terms of PY per hectare, FW, PDF and EDF, TFW, DPC, and FPC. The positive traits of the introductions of this group were yellow-colored pulp, TFW, TFP, and wide DPC (Figure 3A) and DH. Negative traits included a longer time to DPF and DSF, marked ribbed fruit, fruit pericarp with a rough to frogskin-like or bumpy texture, and green color matrix with rapid oxidation and blackening (Figure 3B).

If the products of cluster analysis (Figure 6) and means analysis (Table 7) are integrated, it can be inferred that the introductions that would prove useful for a breeding program that aims to develop materials that produce fruit for fresh consumption or for agro-industrial purposes are those that correspond to group 3 (introductions 2, 5, 16, 23, 29, 38, 47) and group 5 (introductions 4, 10, 14, 27, 37, 48) (Table 7). The same methodology was used by Ortiz (2009), Valdes *et al.* (2010), and Ortiz *et al.* (2013) to develop cultivars for fresh consumption and agro-industrial purposes.

Furthermore, to produce grain derived from seed for oilseed purposes (Ordoñez *et al.*, 2014), the recommendation is to use genotypes derived from group 3 (introductions 2, 5, 16, 23, 29, 38).

CONCLUSIONS

The Central American collection of butternut squash introductions evaluated in this study presents variability regarding traits of interest, indicating the availability of source material to develop a breeding program to produce squash fruit for fresh consumption or agro-industrial purposes as well as to produce oilseed.

There was no interaction between introductions regarding planting time, indicating that introductions of the Central American collection tend to be stable over time.

Breeding processes aiming to increase either the production of fruit for fresh consumption or for agro-industrial purposes or to increase oilseed production should use the introductions identified in groups 3 and 5 in well-designed crosses with introductions of group 4.

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Effect of post-harvest forestry residue management practices on the diversity of epigeal coleopterans

UNIVERSIDAD NACIONAL DE COLOMBIA

Efecto de prácticas de manejo de residuos post cosecha sobre la diversidad de coleópteros epigeos

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ABSTRACT

The aim of this work was to study the effect of post-harvest forestry residue management practices on the epigeal coleopterans diversity and abundance in a 1-yr old *Pinus radiata* plantation. Approximately 25-yr old *P. radiata* plantation was available, which was harvested by clear-cutting at the beginning of 2010 in Constitución, central Chile. Three post harvest residue management treatments were installed in a fully randomized design in blocks: 1) a control, where residues were intact left; 2) residue removed (> 2.5 cm diameter); and 3) burned. The coleopterans were sampled in spring and summer, using linear transects with pitfall traps. The abundance and richness, and diversity index of coleopterans were determined. A total of 23 species of 13 families were obtained. Both in the spring and summer, the treatment with residues left registered significantly greater abundance and species richness. In the residue management, the practices of intact leaving residue should be considered as the least impacting on diversity and abundance of epigeal coleopterans and is recommended for creating refugee areas to promote diversity of beetles in this area of study.

Key words:
Insects
Pinus radiata
Diversity
Richness
Abundance

RESUMEN

El objetivo de este trabajo fue estudiar el efecto de prácticas de manejo de residuos de post-cosecha en plantaciones de un año de *Pinus radiata* sobre la diversidad y abundancia de coleópteros epigeos. El estudio se realizó en Constitución, Chile central, donde existía una plantación de *P. radiata* de cerca de 25 años, que se cosechó con el método de tala rasa a comienzos de 2010. Se consideraron tres tratamientos de post-cosecha con un diseño completamente aleatorio en bloques: 1) uno control, con los residuos intactos; 2) con extracción de residuos (> 2,5 cm de diámetro); y 3) con quema. Los coleópteros se muestrearon en primavera y verano mediante transectos lineales con trampas de caída. Se determinó la abundancia y riqueza, y un índice de diversidad de coleópteros epigeos. Se obtuvieron un total de 23 especies de 13 familias. En primavera y verano, el tratamiento con residuos dejados intactos tuvo una abundancia y riqueza de especies significativamente mayor. Cuando se apliquen tratamientos de post-cosecha la práctica de dejar los residuos intactos debería ser considerada como la de menos impacto sobre la diversidad y abundancia de coleópteros epigeos y ser recomendada por crear áreas de refugio para promover la diversidad de coleópteros en esta área de estudio.

Palabras claves:
Insectos
Pinus radiata
Diversidad
Riqueza
Abundancia

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Insects play important role in ecosystem functioning, and act as predators, pollinators, or decomposers of organic matter, and as indicators of biodiversity capable of reflecting the level of conservation, diversity, endemism, and degree of intervention or disturbance of the ecosystem (Coddington *et al*., 1991; Colwell and Coddington, 1994). Also most insects possess the ability of detecting changes in the functioning of forest ecosystems (Didham, 1997; Langor and Spence, 2006), and are very vulnerable to fragmentation and habitat loss. Similarly, habitat loss has strong effects on biodiversity, and is considered the most important factor in the extinction of populations and species (Fahrig, 2003). The negative effects of habitat loss refer not only to attributes of biodiversity, such as species richness (Steffan-Dewenter *et al*., 2002; Steffan-Dewenter, 2003), the abundance and distribution of populations (Best *et al*., 2001), and genetic diversity, but also to patterns or ecological processes that depend of biodiversity (Keller *et al*., 2005). The loss of biodiversity due to human activities has become one of the major environmental problems of global concern. Anthropogenic activities like habitat overuse, deforestation, pollution, and introduction of exotic species, among others, are the main forces of changes in community diversity and composition throughout the world (Spooner, 2005; Coates *et al*., 2006; Roe *et al*., 2006). Thus, the replacement of natural vegetation by forest plantations as those *Pinus radiata* D. Don, can be considered a building process of "green deserts" in which biodiversity is scarce when compared with those harboring native forests of the same geographic areas (Bonham *et al*., 2002).

Human endeavors, such as harvesting, modify forest composition and dynamics, affecting biodiversity. Different forest management systems influence the edaphic microarthropod fauna, which in turn is important to maintain the properties of the soil subsystem (Covarrubias and Contreras, 2004). Forest practices may have profound effects on population levels and species composition of diverse organism groups (Jactél *et al*., 2005). Also, managing vegetation allows to control forest pests and to keep groups of undesired insects below damaging levels (Smith, 1990). Currently, in Chile, there are about 1,600,000 ha to *P. radiata* (INFOR, 2011). This large area represents a high risk for the propagation of pests and diseases, even though at world level, *P. radiata* is one of the forest species most resistant to sanitary problems (Clapp, 2001). Despite the extensive territory covered by forest plantations in the country, the effect of forest residue managements has been documented scarcely (Briones and Jerez, 2007). Most insects have the ability to detect changes in the functioning of forest ecosystems. This is evident when observing alterations in the distribution, abundance and composition in the communities of these organisms (Langor and Spence, 2006). Among the organisms most potentially affected by plantations of exotic species are epigeal (i.e. ground-dwelling) insects such as beetles and ants, given their high sensitivity to changes in ecosystems (Sackman *et al*., 2008). The epigeal coleopteran constitutes a taxonomically and ecologically diverse group, and hold documented potential as model organisms or indicators for ecological impact research (Paritsis and Aizen, 2008; Koivula, 2011; Roberge and Stenbacka, 2014). The hypothesis of this investigation was that the post-harvest residue managements affect the diversity of epigeal coleopterans by season. In this study we aim to examine if the post harvest residue management of *P. radiata* plantations affect the diversity of epigeal coleopterans in the Maule region of Chile.

MATERIALS AND METHODS

The survey area is located in an approximately 25-yr old plantation of *P. radiata* (3 ha) (35°43' - 35°47'S; 72°29' - 72°31'W), which was harvested by clear-cutting at the beginning of 2010 in Constitución, Maule Region, central Chile. This area belongs to the Experiment Center Dr. Justo Pastor León of the Faculty of Forestry Sciences and Nature Conservation, University of Chile. The area is located in the dry coastal Talca Province in the district of Agroclimatic Empedrado-Coronel, which is characterized by an average annual rainfall of 897 mm, with minimum and maximum temperature of 6 °C and 24.4 °C, respectively. The soils belong to the Constitución series, originating from metamorphic-granitic (Peralta, 1976). The study area is between coastal *Nothofagus glauca* and *Azara petiolaris*, and *N. glauca* and *Persea lingue* Mediterranean deciduous forests, being the first forest dominated by *N. glauca*, *Gevuina avellana*, *Lomatia hirsuta*, and *A. petiolaris* as major tree species, and the second forest dominated by *N. glauca*, *Nothofagus obliqua*, *G. avellana*, and *P. lingue* (Luebert and Pliscoff, 2006). In the study area original sclerophyllous forests and shrubs have been subjected to a profound transformation so that in some deeply disturbed places the original

vegetation has been completely transformed into ruderal shrubs associated with the introduction of allochthonous species *Pinus radiata* plantations (Amigo *et al*., 2000; Luebert and Pliscoff, 2006).

In three different sites three post-harvest treatments (plots of 30m x 30m) were applied in a fully randomized design: i) a control in which harvest residues were left intact, ii) a in which harvest residues greater than 2.5 cm in diameter were removed, and iii) a in which all the harvest residues were burned (letting the fire to grow against the breeze or down the slope) (Julio, 2005). These three sites are separated around 20 m between them. Three linear transect were randomly selected in each one the three post harvest residue management treatments (experimental unit). In each linear transect of 20 m was delimited placing three pitfall traps within it at 0, 10 and 20 m of distance. Thus, a total of 27 pitfall traps (3 traps x 3 transects x 3 treatments) were finally disposed to examine the variation in epigeal coleopterans. The pitfall traps have a capacity of 750 mL being filled with 250 mL of 75% ethanol to preserve the specimens and 500 mL of distiller water with traces of detergent to decrease surface tension. The beetles were sampled in October 2010 (spring) and January 2011 (summer) due to temporal variations. It was considered that each pitfall trap had an activity radius of 5 m (Péfuar and Pérez, 1995), and then each transect was transformed to an area of 0.0236 ha. All the pitfall traps were removed 15 days after installation (Briones and Jerez, 2007).

The insects were stored in plastic vials with 70% ethanol and later counted and identified using taxonomic keys and by comparison with specimens in entomological collections in the Forest Entomology Laboratory, Faculty of Forestry Sciences and Nature Conservation, University of Chile, and the Entomology Institute, Metropolitan University of Education Sciences, in Santiago, Chile (Escobar, 2000).

The alpha diversity index of the epigeal coleopterans was determined for each treatment and season by calculation of means of relative abundance and specific richness (Moreno, 2001a). To determine the diversity of taxonomic groups the Shannon-Wiener (H') index was calculated (Magurran, 1988). In addition, a factorial ANOVA was used to determine statistical differences in species abundance and richness between residue treatments and season as factors. Finally, the HSD Tukey tests (P<0.05) *a posteriori* was executed.

RESULTS AND DISCUSSION

With 27 traps we obtained 176 specimens that represent 23 species of 13 families of Coleoptera. Five most abundant species were *Enneboeus* sp. (Archaeocrypticidae), *Homalotrichus striatus*, *Cyanotarsus foveolatus* (Staphylinidae), *Lathrobium dimidiatum* (Staphylinidae) and *Hylurgus ligniperda* (Curculionidae). Some species are represented in nearly all treatments, as *Hylurgus liniperda* and *Homalotrichus striatus*. Few species are present only in one treatment and scarce abundance, as *Acanthinodera cumingii* (Cerambycidae), *Germarostes posticus* (Hybosoridae), *Deromecus vulgaris* (Elateridae) and *Afrasida propensa* (Tenebrionidae) (Table 1).

The treatment is the only factor affecting abundance variations ($F_{2,12}$=61.02, P<0.001) while season factor and the "treatment by season" interaction term are not statistically significant ($F_{1,12}$=1.06, P=0.32 and $F_{2,12}$=0.82, P=0.45, respectively), showing that effect of the different treatments seem to not depend of the season. During spring, the treatment with residues left registered significantly greater species abundance than the other two (87% greater than the treatments with residues burned, and 73% more than that with the residues extracted). This trend also occurred during the summer, when the treatment with residues left continued to have a significantly greater abundance (79% greater than the treatments with residues burned or extracted). When comparing the abundance in each treatment between spring and summer no differences occurred between the treatment with residues burned, extracted or left (Table 1).

Similar results can be observed in the case of species richness. The species richness of epigeal coleopterans significantly differ between treatments ($F_{2,12}$=23.56, P<0.001), but no when the two seasons are compared ($F_{1,12}$=2,97, P=0.11). During the spring, the treatment with residues left had a significantly greater richness than the other two treatments (64 and 56% greater than in the treatment with residues burned and extracted, respectively). During the summer, the treatment varied slightly, and the treatment with residues left continued having greater species richness, 47% greater than the treatment with residues burned and 58%, a significantly

Table 1. Abundance (number of individuals/transect) (transect equivalent to 0.0236 ha) of epigeal coleopteran by post-harvest residue management treatment and season and mean abundance and species richness by transect and Shannon's (H') diversity index.

Families / Species	Spring 2010			Summer 2011		
	Residues burned	Residues left	Residues removed	Residues burned	Residues left	Residues removed
ARCHAEOCRYPTICIDAE						
Enneboeus sp.	0.0	324.9	70.6	28.2	42.4	0.0
CARABIDAE						
Cnemalobus obscurus (Brulle, 1834)	0.0	14.1	0.0	0.0	0.0	14.1
Cyanotarsus foveolatus (Chaudoir, 1873)	14.1	169.5	0.0	0.0	42.4	0.0
Pachyteles gracilis (Chaudoir, 1868)	0.0	0.0	0.0	14.1	28.2	0.0
CERAMBYCIDAE						
Acanthinodera cumingii (Hope, 1833)	0.0	0.0	14.1	0.0	0.0	0.0
CURCULIONIDAE						
Acalles sp.	0.0	14.1	14.1	0.0	0.0	0.0
Hylastes ater (Paykull, 1800)	0.0	0.0	0.0	28.2	28.2	28.2
Hylurgus ligniperda (Fabricius, 1787)	28.2	155.4	56.5	56.5	56.5	0.0
Rhyephenes humeralis (Guérin-Méneville, 1830)	0.0	0.0	28.2	0.0	0.0	0.0
Xyleborinus saxeseni (Ratzeburg, 1837)	0.0	28.2	28.2	0.0	0.0	0.0
ELATERIDAE						
Deromecus vulgaris (Solier, 1851)	0.0	0.0	0.0	14.1	0.0	0.0
HYBOSORIDAE						
Germarostes posticus (Germar, 1843)	0.0	14.1	0.0	0.0	0.0	0.0
LEIODIDAE						
Neopelatops sp.	0.0	14.1	14.1	0.0	14.1	0.0
MELYRIDAE						
Astylus trifasciatus (Guérin, 1844)	42.4	0.0	0.0	0.0	0.0	0.0
PTINIDAE						
Ptinus sp.	0.0	14.1	0.0	0.0	0.0	0.0
SCARABAEIDAE						
Antarctia sp.	0.0	98.9	0.0	0.0	0.0	0.0
Glypholoma sp.	0.0	14.1	14.1	0.0	0.0	14.1
Megathopa villosa (Eschscholtz, 1822)	28.2	0.0	0.0	0.0	0.0	0.0
STAPHYLINIDAE						
Homalotrichus striatus (Solier, 1849)	14.1	28.3	14.2	0.0	296.6	14.1
Lathrobium dimidiatum (Say, 1830)	0.0	70.6	0.0	28.2	169.5	98.9
TENEBRIONIDAE						
Afrasida propensa (Wilke, 1921)	0.0	0.0	0.0	0.0	14.1	0.0
Nycterinus sp.	0.0	0.0	0.0	0.0	28.2	0.0
Mean number of individuals / transect	127.10 ± 42 a	960.50 ± 37 a	254.20 ± 65 a	169.50 ± 42 a	805.1 ± 149 b	169.5 ± 49 a
Mean number of species / transect	127.10 ± 42 a	353.10 ± 28 b	155.40 ± 28 a	141.20 ± 14 a	268.4 ± 14 b	113.0 ± 51 a
Shannon's (H') diversity index	1.52	1.97	1.98	1.68	1.92	1.23

Different letters in the row indicates significant differences bettween treatments by season, HSD Tukey tests, $P<0.05$. ± Deviation Standard.

greater level than the treatment with the residues extracted, respectively. Again, the "treatment by season" interaction is not statistically significant ($F_{2,12}=1.74$, $P=0.21$) (Table 1). Shannon's diversity index (H') did not register significant differences between species of epigeal coleopterans. The greatest abundance of epigeal coleopterans occurred

in the treatment with residues left in both seasons, maybe because many species found there better habitat conditions and trophic resources necessary for survival (Grez *et al*., 2003; Correa and Roa, 2005). Work *et al*. (2013), evaluate the initial changes in the abundance, species richness and community composition of rove (Staphylinidae) and ground beetles (Carabidae), immediately following 1) stem-only harvesting, in which logging debris (e.i. tree tops and branches) are retained on site, and 2) whole-tree harvesting, in which stems, tops and branches are removed in mature balsam fir stands in Quebec, Canada. Catch rates in whole-tree harvesting were greater than stem-only harvesting sites; however, they were attributable to increased catches of only three species of Staphylinidae.

The greatest richness of epigeal coleopterans occurred in the treatment with residues left in both seasons, which may be due to the greater availability of food, favorable habitat and resource conditions preserved in this treatment, allowing for their establishment and survival, and resulting in an increased attraction for diverse species (Moreno, 2001b; Grez *et al*., 2003). The treatment with residues burned had the least species richness, probably because burning had a greater effect on natural habitat of insects affecting the ground and vegetation, thus the insects did not find the adequate conditions for establishment. Besides, insects are indirectly affected by fire given soil warming, increased metabolic activity and CO_2 emissions from litter and soil (Price *et al*., 2011).

The variation in abundance and species richness of epigeal coleopterans in both seasons could be caused by some species being univoltine while others may develop several life cycles in a year; an example is *Hylurgus ligniperda*, one of the most abundant species with not a seasonal cycle, as the females begin galleries any time of the year, although more frequently in the warmer months, with 10-12 wk cycles (Artigas, 1994).

The results obtained with the post-harvest treatments indicate that the effects of management of forest residues in harvest impacts on biodiversity and that different treatments influence biodiversity in diverse ways. The production process should consider preserving fragments of biodiversity reservoirs for preservation (Fisher and Lindenmayer, 2002; Tscharntke *et al*., 2002). Also, it is known that vegetation with residues possess a specific fauna and provides refugees for many sensitive species, and it is necessary to keep this in mind when anthropogenic changes of landscape occur. The preservation of biodiversity depends progressively on the protection of small areas and biota outside protected wild areas (Simonetti, 1998). This may be part of the strategies to follow to maintain biodiversity, particular in the region studied, that presents a high degree of endemism (Simonetti *et al*., 2002; Simonetti, 2006).

CONCLUSIONS

Both in the spring and summer, the treatment with residues left registered significantly greater abundance and species richness of epigeal coleopterans. This treatment is then recommended in this area of study as it increases food availability and creates refugee site for beetles because of its greater vegetation cover, an important factor to promote their diversity. Then, when applying post-harvest residue management practices in this area of study is important to consider the treatment with the least alterations of the site, to generate adequate refugee areas to promote epigeal coleopteran diversity.

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Production and composition of buffalo milk supplemented with agro industrial byproducts of the african palm

Producción y composición de leche de búfala bajo suplementación con residuos agroindustriales de palma africana

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ABSTRACT

The goal for this applied research was to assess the production and composition of buffalo milk when Oil Palm kernel flour and Oil Palm kernel cake are supplemented to their diet. Thirty buffaloes from the grasslands of the Andes valleys in Colombia with 1 to 3 lactations and an average weight of 575 kg were selected for the experimental. The animals were randomly assigned to one of three experimental groups: (T1 Control Group) with no diet supplements, (T2) 1000 g·day^{-1} of oil palm kernel cake and 350 g·day^{-1} of molasses diet supplement, and (T3) 1150 g·day^{-1} of oil palm kernel flour. During the first 100 days of lactation, the milk livestock were individually weighed and milked. Milking was scheduled every 15 days, for a total of seven samples. The supplement consumption was recorded and a bromatological analysis of grasses was performed. The chemical composition of the milk was determined using an ultrasonic Ekomilk analyzer and a fatty acid full profile analysis was made using High Liquid Pressure Chromatography (HLPC). A 7 x 3 multiple variable statistical analysis was performed by sampling seven fifteen day periods and three types of diet. The average values of milk components observed were: 3.54% protein; 7.4% fat; total solids 16.9%; non-fat solids 9.5%; 2.1 fats to protein ratio. The profile of fatty acids showed 2.34% of polyunsaturated; 33.1% of monounsaturated; 64.6% of unsaturated fatty acids; and 0.96% of Omega 6 acids. In conclusion was observed partial effect to fat supplementation in the buffaloes milk production.

Key words:
Buffalo milk
Fatty acids
Milk chemical composition
Oleaginous plants

RESUMEN

El objetivo del presente trabajo constituyó la evaluación de la producción y composición química de la leche de búfalas sometidas a suplementación con derivados de la industria de la palma de aceite. El presente trabajo se realizó en la época de transición de lluvias en un sistema bufalino ubicado en el Magdalena Medio colombiano. Se utilizaron 30 búfalas de dos o tres partos, con pesos promedio de 575 kg y una condición corporal de 3.5 al inicio del experimento. La composición de los subproductos torta y harina de palmiste, y la de los forrajes fueron determinadas mediante análisis bromatológico. Los animales fueron dispuestos en forma aleatoria en tres grupos, control y dos suplementados con residuos agroindustriales. Los tratamientos fueron: T1 = Sin suplementación (control), T2 = torta de palmiste y, T3 = harina de palmiste. En los primeros 100 días de lactancia y cada 15 días se realizó pesaje individual, medición de la producción, perfil de ácidos grasos por HLPC y composición química de la leche por análisis ultrasónico. Se hizo análisis multivariado con arreglo factorial 7 x 3 (periodos x tratamientos). En leche la media de sólidos totales fue 16.9% y 9.5% de sólidos no-grasos; El perfil de ácidos grasos presentó porcentajes totales de 2.34 de poli-insaturados, 33.1 de mono-insaturados, 64.6 de insaturados, y 0.96 de ácidos omega 6. En conclusión se observó efecto de la suplementación sobre la producción de leche de búfala y efecto parcial sobre la composición grasa.

Palabras claves:
Leche de búfala
Ácidos grasos
Composición química de la leche
Plantas oleaginosas

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High production, protein quality and fat contents of buffalo (*Bubalus bubalis*) milk, justifies a selection of ecologically conditioned outstanding specimens from this bovine breed found in the valleys of the central mountain range of the Andes in Colombia. According to the FAO (2010) the worldwide production of buffalo milk grows at an annual rate of 248%, for various reasons apart from its high content of total solids (TS) compared to other milk cattle breeds. During the last 42 years Colombia's buffalo population has been increasing due to its capacity to adapt to different eco-systems and to the quality of its milk (Bolívar *et al.,* 2010).

Chemical composition of buffalo milk varies widely as a consequence of numerous factors such as breeds, climate and lactation period. The Murrah is the main milk production in the buffaloes breed (Malhado *et al*., 2013).

The production of milk with buffaloes is actually an alternative for large, medium and small milk producers in Latin American and Caribbean countries. It is therefore of utmost importance for producers, technicians and the milk industry in general to know the chemical composition of this type of milk compared to other milk cattle breeds and the factors that affect it.

In areas where agricultural activities produce residues with high potential use for animal nutrition, it is important to assess its effects during the Buffalo first lactation phase in which milk production coincides with nutritional unbalance of buffaloes, a problem still unresolved by research in animal production (Terramoccia *et al.,* 2005).

The African Palm produces 200 million tons of biomass per year worldwide; of which only 10% is used. An important part of this by-product is burned, which causes CO_2 contamination. It is estimated that every hectare of this crop produces 25 tons of plant residue (Pauli, 1999), that could be used to produce energy as biomass and in animal nutrition; with which an environmentally friendlier palm cultivation could be achieved (Abdalla *et al.,* 2008).

In various countries the oil palm subproducts are use in ruminant nutrition (Castro *et al.,* 2016; Hassim *et al.,* 2010). This agro-industry produces two different by-products depending on the extraction processes: the oil palm kernel flour and the oil palm kernel cake. The first is obtained by applying mechanical pressure to the seeds after they have been mashed with help of chemicals solvents, while the palm kernel cake is made by desolventizing the oils once obtained by chemical lixiviation. This last residue contains less fat, higher amount of fiber (NDF), more protein and raw energy. The chemical composition and the digestibility of the palm cake vary in content of the tegument of the seed, and the amount of residual oil. The palm flour is 20% more digestible for its low tegument residue content (FEDNA, 2003).

The greater percentage of milk fat of the buffalo is related to the presence and composition of the polyunsaturated fatty acids (FA) (Patiño *et al*., 2008), which are important in the development of nutrients that have a preventive action on certain pathogens; this is the main reason why the present study is oriented towards obtaining nutraceuticals rich in special fatty acids (Hernández and Serna, 2003; Valdés, 2011).

The primary objective of this study was to evaluate the effects of the supply of palm kernel meal and palm cake expeller in the production and composition of the milk produced by the buffaloes during the first 100 days of lactation, grazing in the mid-grass lands of the Andes Valley in Colombia.

MATERIALS AND METHODS

The study was conducted at a specialized buffalo milk production system located in the fertile soil of the Mid-Magdalena River Valley (6°19'N and 73°57'O). Thirty female Murrah breed buffaloes with two or three calvings; between 550 and 600 kg in weight and with at least a 3.5 body condition score on a scale of one to five (Alapati *et al.,* 2010). The selected buffaloes were pregnant with an estimated calving date due for the first week of September. The assessment period was programmed for the 1st stage of lactation (0 - 100 days). The study was approved for the committee of Animal experimental and ethics of the Facultad de Ciencias Agrarias.

The selected livestock were previously conditioned during 15 days by being fed with one kg of palm kernel flour and 1.15 kg of oil palm kernel cake. The supplement was offered once a day after milking and its consumption was total. The buffaloes were milked by hand once a day and the scientific measuring and analysis of the chemical

compositions of the milk was taken every 15 days. The livestock grazed in rotation system on "Angleton" (*Dichatium aristatum* Benth), "Climacuna" (*Dichantium annulatum*), "German" (*Echhynochloa polystachya*) and "Puntero" (*Hyparrhenia rufa*) grass, with mineralized salt (8% phosphorus) and water *ad libitum*. Openly available to groups of 10 animals each as follows:

T1: Control group, no diet supplement; T2: Diet supplement (1 kg oil palm kernel cake with 350 g of molasses per animal per day) and T3: Diet supplement (1.15 kg of oil palm kernel flour without molasses). The groups were considered to be isoproteic and isonergetic. The qualities of the oil palm kernel cake and flour as well as the pastures were assessed with help of bromatological analysis (Table 1).

Table 1. Bromatological analysis* for oil palm kernel cake and oil palm kernel flour.

Determination (%)	Oil palm kernel flour	Oil palm kernel cake
Dry matter	91.28	94.39
Ash	3.59	3.26
Protein	16.20	14.03
Fat (ether extract)	5.59	19.90
NDF	68.57	53.77
Carbohydrates	6.07	9.04
Energy (Mcal·k^{-1})	4600.10	5376.29

* Weende method

The milk samples for measurement and analysis (n=210) were taken on milking days 1, 16, 31, 46, 61, 76, and 91 after calving. Protein, fat and Total solids analysis was made using the Ekomilk Fast automated machine. A profile of fatty acids (FA) was made by using a Shimadzu GC-14A gas chromatograph, equipped with flame detector (260 °C), flow gas carrier to 15 psi and reverse phase capillary column.

The statistical model used for the analysis of the data was multivariate analysis with factorial arrangement 7 x 3 (periods x treatments):

$$Yijk = \mu + ti + Bj + Cijk + eijk$$

Where:

Yijk = value of the dependent variable for j that received the ijk diet supplement
μ = overall average
ti = fixed diet effect
Bj = j fixed time effect
Cijk = fixed interaction effect between diet and time
Eijk = random error of j that received the ijk diet

The level of probability was $P<0.05$; the variation analysis was made with the statistics program SAS 9.2 (SAS Institute, 2007).

RESULTS AND DISCUSSION

Milk Production

Significant differences for daily milk production were found among the treatments ($P<0.001$). The average production in the T1, T2 and T3 were 2.69, 3.82 and 3.40 L·day^{-1}, respectively, with an average value of 3.3 L·day^{-1} which is similar to the production of 3.21 obtained by Bolívar *et al.* (2010) with buffaloes in pasture grazing and slightly higher than 3.1 L·day^{-1} found by Hurtado *et al.* (2005) in conditions of extensive pastures in the same region of the present study. Medina and García (2008) supplemented the diet of a commercial herd of Water Buffalo with yeast in the department of Sucre (Colombia) and found a daily average milk production of 4.49 and 3.72 L per animal in supplemented diet and non-supplemented diet, respectively; this greater production probably was due to the increased consumption of metabolic energy as is suggested by Mahyuddin and Praharani (2010). When water buffalo had a diet supplement of corn and sorghum silage (Barile *et al.,* 2007) or with sugar cane (Franzolin and Alves, 2010), the milk production was not significantly different to that of grazing buffaloes. (Quintero *et al.,* 2008) found that milk production is affected by the number of calvings and climate change.

During the first calving, the average maximum daily milk production per animal was of 3.81 L during rainy season

as compared to 3.76 L during the dry season. On the fifth calving, milk production during the rainy season was 4.52 L and 4.37 L during of dry season. This information shows that when the buffalo reaches its adult weight, the production of milk is higher during the rainy season.

For this experiment, the first 100 days of the milking period, correspond to a transitional climate period between the wet and dry seasons.

Total Solids (TS) in buffalo milk

The TS in the milk varied between the dietary treatments 16.6%, 16.7% and 17.2% for T1, T2 and T3, respectively ($P<0.001$). Differences were found between the results of T1 and T2 groups and T3 superior to T2, in the TS content. The percentage differences of T3 were of 2.55 versus T2 and of 3.19 in respect to T1. These differences were affected by the amount of fat in the milk, while the percentage of protein showed the least variation. The amounts of TS found in the present study are similar to those obtained by Hurtado *et al*. (2005) (16.9%) and greater to those found by Patiño et al. (2002), Patiño (2004) (15.7%) and Bastos (2005) (16.6%).

Milk composition

No differences were found among the dietary treatments ($P>0.05$) for the protein contents in buffalo milk. The average amounts found were 3.56% for the control group, 3.54% for the group with a diet supplement of oil palm kernel cake and molasses; and 3.50%, for the group with the diet supplement of oil palm kernel flour. The protein average for all the experiments was 3.53%, similar to 3.58% found by Medina and García (2008) in buffaloes supplemented with *Saccharomyces cerevisiae* and 3.66% by (Patiño, 2004) in a study of buffaloes fed on grass without dietary supplementation. However, the protein content found in this study was less than that reported by (Cervantes *et al.,* 2010) in a study on the adaptability of the buffalo in Colombia, where milk proteins range between 4.07% and 4.77 % for an average of 4.40%.

It has be pointed out that for this research the female buffaloes used were in their second or third quarter of lactation, therefore, in their first 100 days of lactation; a time span during which low percentages of protein are normally registered.

The average percentage of fat in buffalo milk was 7.16%, 7.25% and 7.74% for dietary treatments T1, T2 and T3, respectively ($P>0.01$), with an average percentage value for all the supplements of 7.38%. The absence of statistical differences between the different diet supplements was probably due to the fact that the oil palm kernel cake and the oil palm kernel flour contain low fat remnants. The fat value found coincides with the larger amount (7.36%) reported by Hurtado *et al.* (2005) while working with buffaloes under extensive grazing and supplemented with molasses and with the amount of 7.22% found by Patiño (2004) in a study carried out in Argentina with buffaloes fed on natural grasslands without diet supplementation. The percentage of fat in buffalo milk was 8.19% in a range between 6.89% and 9.34 similar to that found by Cervantes *et al.* (2010) in buffalo milk in a study for nine places of the Magdalena river valley in Colombia. Among the factors that could have caused differences in the fat readings are analytical methods, genetic factors, nutritional and physiological factors; and the environmental and sanitary conditions of the animals (Walker *et al.,* 2004; Patiño *et al.,* 1999).

As a result, fat in this research exceeds 4.16% and 5.19% reported by Medina and García (2008) in buffaloes supplemented and not supplemented with yeast in the humid region of Colombia; as is different to 6.78% found by Patiño *et al.* (2002) and 3.56% and 4.35% in studies made by Bastos (2005). The lactose average (5.24%) did not vary ($P>0.05$) no statistical effect was found for diet supplement or time period.

Determination of the fatty acids types in buffalo milk

The average percentages for short chain FA (Table 2) found were: undecilic (0.91%), and lauric (1.83%), and long chain FA were: mirístic (8.6%); miristoleic (0.62%); pentadecanoic (1.91%); palmitic (30.6%); palmitoleic (0.77%); heptadecanoic (1.04%); estearic (19.6%); vaccenic (5.47%); oleic (26.2%); linoleic (0.95%); and conjugated linoleic acid (CLA) at (1.38%). The FA profile did not show any significant differences between the dietary treatments in the FA content of monounsaturated (MUFA), nor in the FA polyunsaturated (PUFAs) and the FA saturated (SFAs). The content of saturated FA in the T1 group was 63.8%, and 36.2% in unsaturated FA (33.8% mono-unsaturated and 2.4% polyunsaturated). For T2, 63.9% of the FA content was saturated and 36.1%

unsaturated, of which 33.7% were monounsaturated and 2.33% polyunsaturated. In T3 the results showed that 65.9% of the FA were saturated and 34.1% were unsaturated of which 31.8 % were monounsaturated and 2.28% polyunsaturated.

Patiño *et al.* (2008) working with Murrah buffaloes and crossbreeds between Murrah and Mediterranean breeds in natural grasslands, found the content of saturated FA in the milk was 56.9%, 6.88% less than that found in the present study; while the content of the unsaturated FA was 43.7%, 7.47% more than the amount found in T1. The animals whose diet was supplemented with corn showed that 57.1% of FA in the milk were saturates, which represents 6.84% less of the amount than T2 and 42.9% of unsaturated FA, exceeding T2 by 6.84%.

Buffalo milk contains high amounts of unsaturated long chain FA as: palmitoleic and linoleic; while the amounts of miristoleic and oleic FA are low contributing to a better nutrition in the FA profile of milk (Verruma and Salgado, 1994).

Table 2. Fatty Acids in Buffalo Milk in grazing and receiving Oil Palm residues as a Diet Supplement (in two periods beginning –day 15- of lactation and at the end –day 100- of the lactation.

Fat Acid	Abbreviation	Diet supplement					
		Control		Oil palm kernel flour		Oil palm kernel cake + molasses	
		Beginning [a] *	End [b] **	Beginning [a] *	End [b] **	Beginning [a] *	End [b]
Undecilic	C11:0	0.92	0.82	1.00	0.91	1.01	0.82
Láuric	C12:0	1.49	1.20	2.03	3.01	1.53	1.73
Miristic	C14:0	8.23	7.91	9.35	9.99	8.26	8.28
Miristoleic	C14:1	0.23	0.41	0.36	0.52	1.82	0.43
Pentadecanoic	C15:0	1.52	1.67	1.77	1.61	3.04	1.88
Palmitic	C16:0	30.5	30.4	32.90	31.1	28.90	29.60
Palmitoleic	C16:1	0.45	0.82	0.40	0.78	1.32	0.87
Heptadecanoic	C17:0	1.42	1.15	0.31	0.92	1.31	1.17
Estearic	C18:0	20.80	19.5	19.70	17.3	21.30	19.00
Vaccenic	C18:1t-11	5.13	6.05	4.83	5.37	5.29	6.33
Oleic	C18:1n-9c	26.90	27.7	25.10	26.1	24.20	27.20
Linoleic	C18:2n-6c	1.35	0.83	1.10	0.74	0.92	0.81
CLA	C18:2-9cis-11t	1.06	1.56	1.16	1.57	1.05	1.89
	—	100	100	100	100	100	100
Polyunsaturated	Pufas	2.41	2.39	2.26	2.31	1.97	2.70
Monounsaturated	Mufas	32.70	34.90	30.70	32.80	32.60	34.80
Total Saturated	Sfas	64.90	62.70	67.00	64.90	65.40	62.50
Total Omega 3	n - 3	0.00	0.00	0.00	0.00	0.00	0.00
Total Omega 6	n - 6	1.35	0.83	1.10	0.74	0.92	0.81
Total n - 6/n - 3	n - 6/n - 3	0.00	0.00	0.00	0.00	0.00	0.00

* [a] Average of 10 measurements at the beginning of the trial (day 15)
** [b] Average of 10 observations at the end of the trial (day 100)

CONCLUSIONS

The diet supplements of the Oil Palm kernel cake with molasses increased the production of the milk by 29.2% when compared to the control group. The type of diet supplement did not affect protein percentage in milk. The type of diet supplement affected fat percentage in

milk. It increased in group (T3) more than that of group (T2). The diet supplement of the oil palm kernel flour improved the total milk solids of the buffalo milk.

Only contents of the myristic acid and the palmitoleic were affected by diet. Medium chain acids: undecilic, lauric were unchanged. no differences were found in the long chain acids: miristoleic, pentadecanoic, palmitoleic, heptadecanoic, estearic, vaccenic, oleic and linoleic.

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Spatial distribution of the exchangeable base ratios in the soils of the R.U.T. irrigation district

UNIVERSIDAD NACIONAL DE COLOMBIA

Distribución espacial de las relaciones entre las bases de cambio en suelos del distrito de riego R.U.T.

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ABSTRACT

Key words:
Soil
Fertility
Nutrients
Percentage of saturation

One of the more important agricultural production centers in Colombia is the R.U.T. Irrigation District, located in the Valle de Cauca. This study evaluated the spatial distribution of ratios of Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}, along with the percentage of saturation of these bases in the cation exchange capacity. 100 samples were taken at two depths to determine the EC, pH, Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}. The interpolations were made using the Geostatistical Analyst extension of ArcGis 10.3.1. The best fit semivariogram model was used, obtaining a raster surface with values of each chemical property, with which the plans were generated. The central area of the (La Union) irrigation district was more affected, with percentages of exchangeable magnesium between 40% and 75%, an inverted Ca^{2+}/Mg^{2+} ratio, and a low calcium saturation percentage. The ratios were high for Mg^{2+}/K^{+}, normal for K^{+}/Mg^{2+}, high for Ca^{2+}/K^{+}, and broad for $(Ca^{2+} + Mg^{2+})/K^{+}$, indicating a probable potassium deficiency that affected fertility. An ionic imbalance in the exchange complex was evident in the main bases of change, which may indicate degradation processes for fertility.

RESUMEN

Palabras claves:
Suelos
Fertilidad
Nutrientes
Porcentajes de saturación

Uno de los más importantes centros productivos agrícolas de Colombia es el Distrito de Riego R.U.T. localizado en el Valle del Cauca. El presente estudio evaluó la distribución espacial de las relaciones entre Ca^{2+}, $Mg^{2+,}$ Na^{+} y K^{+}, además del porcentaje de saturación de estas bases en la capacidad de intercambio de cationes. Se tomaron 100 muestras a dos profundidades, a las cuales se les determinó la CE, pH, Ca^{2+}, Mg^{2+}, Na^{+} y K^{+}. Las interpolaciones se realizaron mediante el uso de la extensión Geostatistical Analyst del ArcGis 10.3.1 Se utilizó el modelo de semivariograma de mejor ajuste, obteniendo una superficie raster con valores de cada propiedad química, con los cuales se generaron los planos. La zona centro (La Unión) del distrito de Riego presentó mayor afectación con porcentajes de magnesio intercambiable entre 40% y 75%, relación Ca^{2+}/Mg^{2+} invertida y porcentaje de saturación de calcio bajo. Las relaciones entre las bases de cambio Mg^{2+}/K^{+} es alta, K^{+}/Mg^{2+} normal, Ca^{2+}/K^{+} alta, $(Ca^{2+} + Mg^{2+})/K^{+}$ amplia, indicando probable deficiencia de potasio afectando la fertilidad. Se evidenció en las principales bases de cambio un desequilibrio iónico en el complejo de cambio, lo que puede estar indicando procesos de degradación en la fertilidad.

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The soils in the north of Valle del Cauca are very fertile, making this area a productive zone of great importance for the agribusiness sector. The R.U.T irrigation district, located between the municipalities of Roldanillo, La Union and Toro, produces about 50% of the food for the Valle del Cauca and 12% of the food nationally (Urrutia, 2001). This makes it one of the more important production centers for fruit and vegetable in the country. Soils that are dedicated to large-scale agriculture require constant tilling, adjustment activities, preparation, fertilization and irrigation, among others; in this sense, they see permanent changes in their physical, chemical and biological properties (García, 2013).

An important factor for success in agricultural production is that the soil used for the cultivation has excellent chemical properties, with exchangeable bases that are at suitable levels during development because high concentrations have ionic imbalances and limitations in the water uptake needed for normal growth (Castro and Gómez, 2010). An imbalanced nutrient ratio can cause inadequate development in crops, along with fungal problems, Aguirre *et al*. (2006) found that, when there is an imbalance of N, P and S (*Sclerotium cepivorum*) is present.

Well-nourished plants are the product of proper fertilization in the phenological stages of the crop, providing not only increases in production but also cultivation health (Marschner, 1995). Sadeghian *et al.* (2014) argued that the availability of elements in the soil is determined by the contents in the exchangeable phase and their relative proportion. A percentage can arise in relation to others in terms of the ability to remain in the CEC or ECEC, considering the competition that they exert on each other in the exchange complex or active sites of absorption in the plant roots.

In a study on soil fertility, the level of concentration of the elements available to plants is important, but their ratios are interesting because they affect the mineral nutrition of plants, which can promote or affect growth and performance (Puentes *et al.,* 2016), but this type of research is rarely carried out.

Research has not conducted on the soils of the R.U.T irrigation district that would allow farmers to evaluate the behavior and relationship of exchangeable bases on scaled maps, considering that ideal contents may exist and an imbalance between them could affect production; therefore, the present work was carried out with the aim of evaluating the spatial distribution of the exchangeable bases, their ratios and their percentages of saturation in the CEC of soils that are very important to Colombia.

MATERIALS AND METHODS

The research was conducted in La Union, Roldanillo, Toro (R.U.T), irrigation district, in northern Valle del Cauca, Colombia, 4°27'15" North latitude and 76°07'27" west longitude, with an area of 11,025 ha and an altitude of 965 m, an average temperature of 26 °C, average rainfall of 1015 mm year^{-1} and average evaporation of 1145 mm year^{-1}.

The sampling points were selected based on the study by IGAC-CVC (2004), considering the percentage distribution of the five taxonomic orders and 25 cartographic units that represent the area. Samples were taken at two depths (0-25 and 25-50 cm) and each sampled point was georeferenced (Figure 1). The evaluated properties in each sample were pH (potentiometric, 1:1 soil-water ratio), Ca^{2+}, Mg^{2+}, K^{+}, Na^{+} and CEC in cmol^{+} kg^{-1}. (1N ammonium acetate, pH 7.0, with absorption atomic spectrophotometry -EAA) (IGAC, 2006).

With the use of the Gamma Design (version 10) software, four semivariogram models (Linear, Gaussian, Exponential, Spherical) were built for the laboratory data obtained at the georeferenced sampling sites (pH, EC, Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}). In order to select the best fit model, the parameters Residual Sum Square (RSS) and proportion of explained variance (C / Co +C) were used, where C was the explained variance or sill and C_o was the variance that was not explained or nugget; the selected semivariogram model with best fit was the one with the lowest RSS value and a high proportion value of explained variance (Table 1).

The selection of the interpolation method was done by taking into account the proportion of explained variance because, according to Oliver and Webster (2014), values greater than 75% indicate the existence of high geo-statistical correlation between the data; however, lower values indicate a lower correlation. For the former, geo-statistical methods are recommended and, for the latter, deterministic methods are

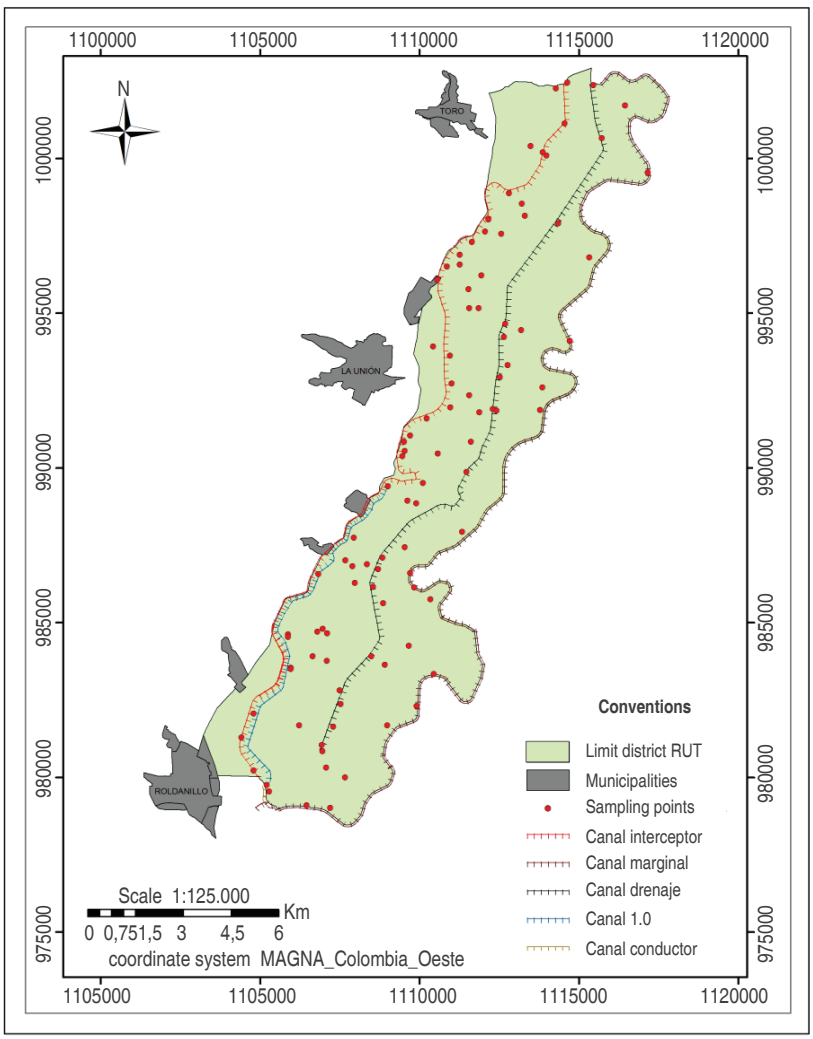

Figure 1. Sampling site locations in the R.U.T. irrigation district.

Table 1. Semivariogram models and methods selected interpolation

Parameter	Depth	Semivariogram	C_o	$C_o + C$	C	Residual SS	r^2	$C/C_o + C$	Interpolador	Power
K^+	0- 25 cm	Spherical	0.05	0.64	058	0.16	0.24	0.90	Ordinary Kriging	
Na^+		Spherical	0.02	0.20	0.18	0.01	0.22	0.89		
Ca^{++}		Linear	165.56	165.56	0	10333	0.06	0	Inverse Distance Weighting - IDW	1.22
Mg^{++}		Exponential	63.50	189.40	125.90	3405	0.80	0.66		2.26
K^+	25-50 cm	Spherical	0.04	0.48	0.44	0.18	0.04	0.91	Ordinary Kriging	
Na^+		Spherical	0.02	0.31	0.28	0.04	0.13	0.92		
Ca^{++}		Linear	125.68	125.68	0	1571	0.01	0	Inverse Distance Weighting - IDW	1.66
Mg^{++}		Spherical	97.80	204.70	106.90	4000	0.80	0.52		1.41

used. The raster surface obtained with the Cross Validation Tool of ArcGis 10.3.1 Geostatistical Analyst was validated in order to contrast the model obtained with the actual data generated in this research.

RESULTS AND DISCUSSION

Ca^{2+}/Mg^{2+} Ratio

Figure 2 shows the spatial distribution of the Ca^{2+}/Mg^{2+} ratio in the soils of the irrigation district. The behavior at depth was similar in the south and north, presenting critical levels with levels lower than two (SCCS, 2013) in an area of 9,690 ha. The central zone had an inverted Ca^{2+}/Mg^{2+} ratio in 1214 ha. This behavior was similar to the spatial distribution seen in Figure 3, where the percentages of exchangeable magnesium are high.

The results obtained agree with those of Madero *et al.* (2004), with the effect from magnesium seen in all of the flat areas of the Valle del Cauca for the Ca^{2+}/Mg^{2+} ratios under two. Values under two in this base ratio could negatively affect crops, such as vegetables, oil palm, coffee and potatoes, among others (Castro and Gomez, 2013). This causes farmer to apply Ca^{2+} in their fertilization plans, seeking to establish a ratio with adequate levels for the crop, which could be in a range of 3-5 $cmol^{+}\ kg^{-1}$ (SCCS, 2013).

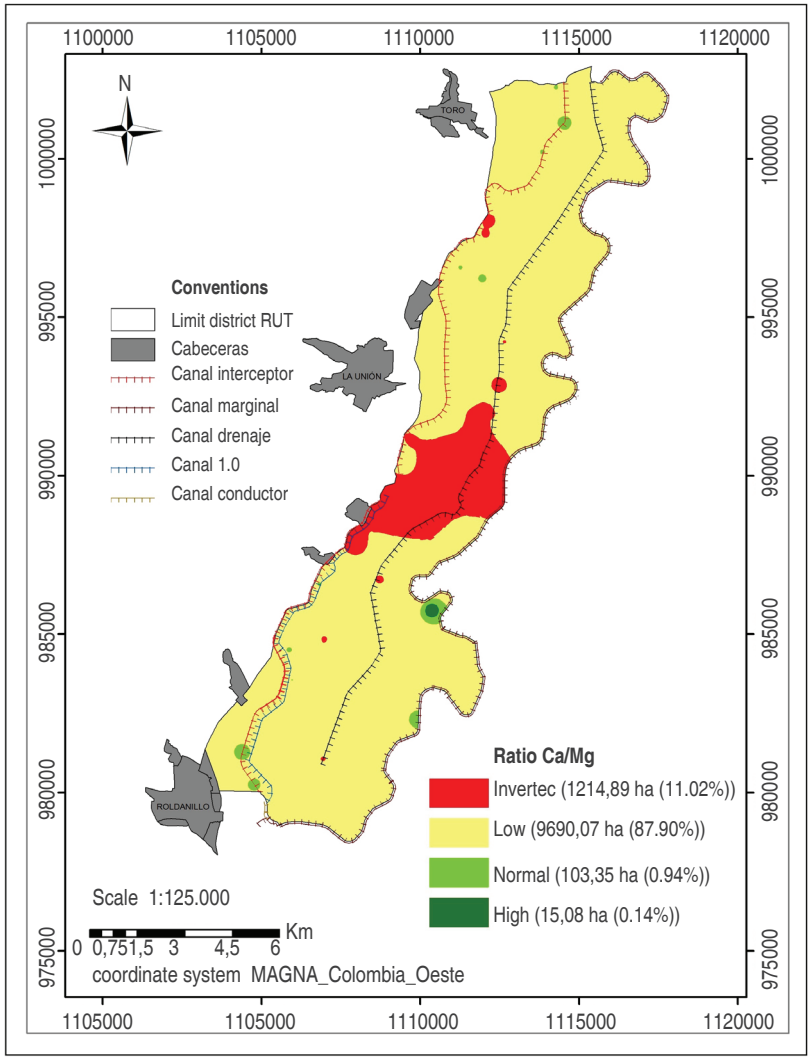

Figure 2. Distribution of the Ca^{2+}/Mg^{2+} ratio in the soils of the R.U.T. irrigation district.

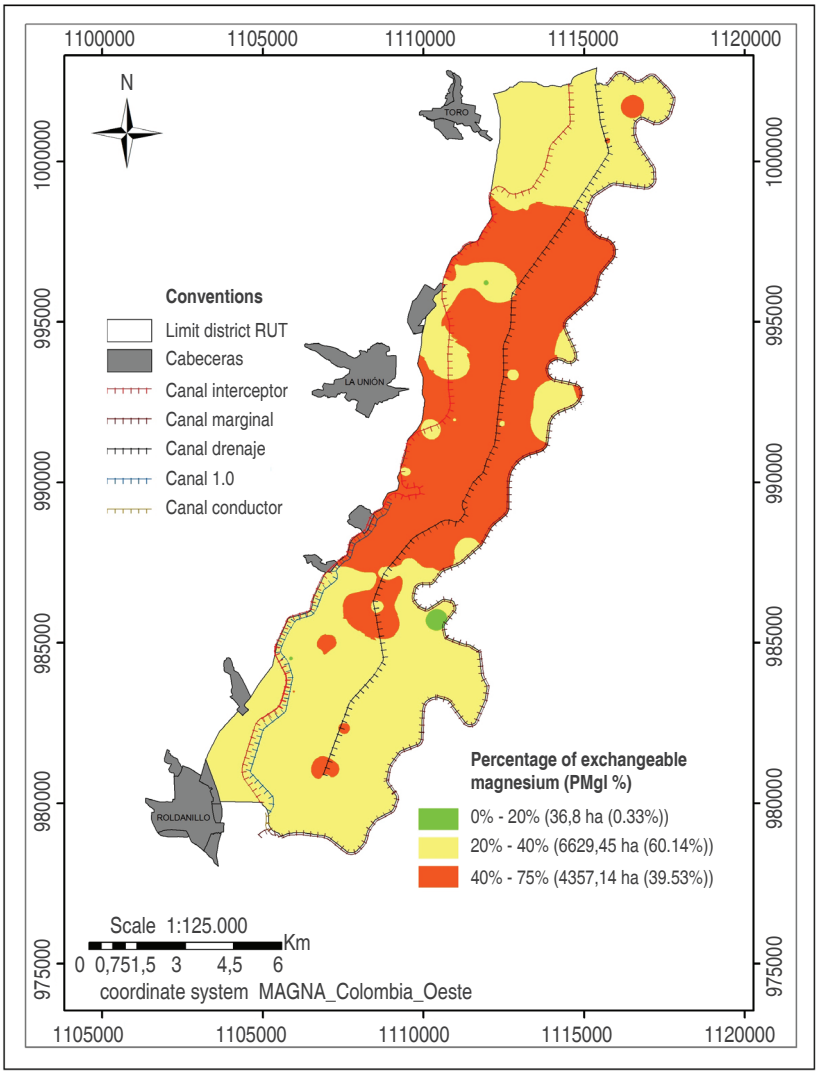

Figure 3. Distribution of PMgI in the soils of the R.U.T.

Mg^{2+}/K^{+}, K^{+}/Mg^{2+}, Ca^{2+}/K^{+}, $(Ca^{2+} + Mg^{2+})/K^{+}$ Ratios

Table 1 shows the ratios of the exchangeable bases in the soils of the irrigation district, where Mg^{2+}/K^{+} had values over ten, K^{+}/Mg^{2+} had values under two, Ca^{2+}/K^{+} had values over thirty, and $(Ca^{2+} + Mg^{2+})/K^{+}$ had values over forty, indicating high levels of Ca^{2+} and Mg^{2+} in the exchange complex (Microfertiza, 2012), levels that could be affecting the availability of potassium in the soils. Hirzel (2008) reported on the induced lack of potassium for levels of K^{+}/Mg^{2+} less than 0.1. Navarro and Navarro (2003) stated that adequate proportions of Ca^{2+}, Mg^{2+} and K^{+} in base ratios do not have antagonisms; however, if the K^{+}/Mg^{2+} ratio is less than unity, the Ca^{2+}/K^{+} ratio is greater than thirty, and the Ca^{2+}/K^{+} ratio is higher than ten, antagonisms are likely to appear. Castro and Gomez, (2013) suggested that, for soils with agricultural purposes, the ideal ratios would be 6:8 Mg^{2+}/K^{+}, 0.2:0.3 K^{+}/Mg^{2+}, 12:18 Ca^{2+}/K^{+} and 12:20 $(Ca^{2+} + Mg^{2+})/K^{+}$.

Undoubtedly, the ratios of the base balance presented a potassium deficiency in the soils of the irrigation district, which was probably attached to the clays (Borrero, 2005). Also, there may have been potassium absorption processes in the crops, without being replenished at adequate quantities to satisfy the nutritional requirements of the crop (Puerto *et al.,* 2014) and the

additional contributions to the soil. This suggests that studies should be conducted with scientific rigor in the R.U.T soils concerning the ratio between the availability of cations and the ion exchange phase (Lince *et al.,* 2015) for nutritional plans for each crop, such as those in the northern part of the valley for growing peppers (*Capsicum* spp.) (Rodriguez *et al.,* 2010) and for the Isabella fox grape (*Vitis labrusca*) (Puerto *et al.,* 2014).

Percentage of base saturation

Table 3 shows the levels of base saturation in the horizons; high Mg^{2+} saturation percentages are evident (Microfertiza, 2012), leading to an imbalance in the ratios of the other bases. In order to avoid antagonisms, the soil exchange complex should be balanced with values of 55% - 65% Ca^{2+}, 15% - 20% Mg^{2+} and 3% - 5% Potassium (SCCS, 2013). High saturations for elements, such as Al (>25%), Na (>10%), and Mg (>30%), generate a high chance of blockages and ionic imbalances (Castro and Gomez, 2013). Moreira *et al.* (2005) evaluated the behavior of exchangeable bases in an Oxisol cultivated with alfalfa (*Medicago sativa* L.) by applying different doses of lime, finding that the saturation of Ca^{2+}, Mg^{2+}, and K^{+} correlated directly with the production.

Table 2. Ratios of the exchangeable bases in the soils of the R.U.T. irrigation district.

	Mg^{2+}/K^{+}	K^{+}/Mg^{2+}	Ca^{2+}/K^{+}	$(Ca^{2+} + Mg^{2+})/K^{+}$
First horizon				
Mean	54.01	0.04	80.17	134.18
Standard deviation	35.59	0.08	49.92	78.00
Second horizon				
Mean	73.09	0.03	80.17	106.81
Standard deviation	44.76	0.05	49.92	47.78

Table 3. Base saturation percentages levels at two depths.

Bases	25 cm depth	Level	50 cm depth	Level
Ca^{2+}	56.51	Medium	52.52	Medium
Mg^{2+}	34.69	High	35.90	High
K^{+}	1.32	Deficient	0.93	Deficient
Na^{+}	0.55	Low	0.81	Low

The high percentages of saturation of Mg^{2+} in the soils of the R.U.T irrigation district produced an effect on the physical properties of the soils (Torrente *et al.,* 2003), influencing the efficient delivery of water, preventing proper retention of available water for crops, moderate stability, and hydraulic conductivity with average performance (Sanchez, 2016). This should draw the attention of farmers in the district since this behavior must be affecting the normal development of crops and, therefore, the quality and final production.

Figure 4 shows the spatial distribution of the calcium saturation percentage, being similar for depth. The medium level had the largest area with 6991 ha; the central area of the district had deficient levels in an area of 1809 ha; its spatial location was related to that seen in the maps of percentage of exchangeable magnesium, with high levels and an inverted Ca^{2+}/Mg^{2+} ratio. Ideal levels of Ca^{2+} were seen in small zones distributed in the south and north, with less intensity in the central zone. The saturation percentage of Mg^{2+} was high in 98% of the soils, in an area of 10,857 ha, and the saturation of potassium was deficient in 98.3% of the area (10,834 ha). The sodium saturation percentage was low in 100% of the soil.

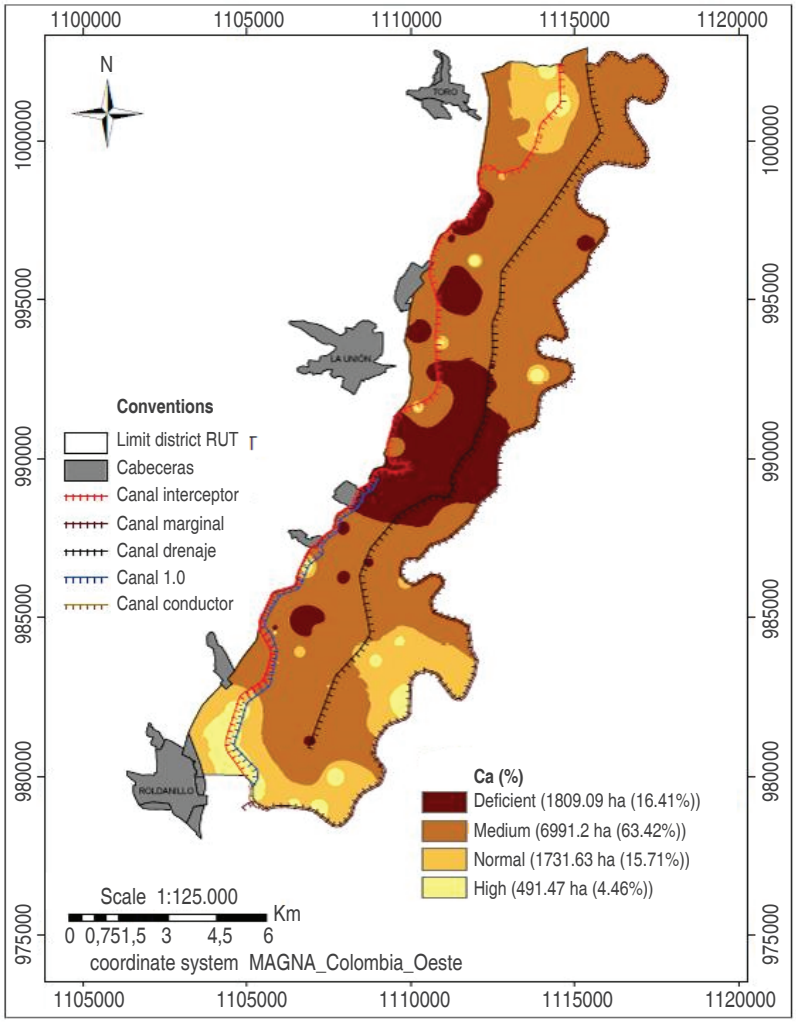

Figure 4. Distribution of the saturation of the Ca^{2+} bases in the R.U.T soils.

The soils of the north part of the valley, including those of the R.U.T irrigation district, have been observed with good fertility (Madero *et al.,* 2011), but the evaluated results had different chemical conditions that may be detrimental to nutrient availability; high saturation percentages of magnesium in the soil make it necessary to apply calcium to reach a suitable Ca^{2+}/Mg^{2+} ratio, which could be caused by increases in Ca^{2+} in the soil, passing from 17.5 $cmol^{+}\ kg^{-1}$ (CVC, 2004) to 35 $cmol^{+}\ kg^{-1}$, as seen in the results of this research. This may also occur due to lack of information that the farmers in the area have on how to mitigate the damage that may result from magnesium in large quantities in soils; however, the use of chemical fertilizers per se does not necessarily lead to degradation, but careless and non-technical management do (García, 2013). Studies should be conducted in the area to establish suitable management for the saturation of magnesium, whether or not by washing the magnesium ion to improve the physical conditions and chemical ratios. It is important to note that the availability of exchangeable bases for plants depends not only on the content in the exchange complex and in the solution, but also on the competition that can occur between these elements; this behavior can vary according to the cationic selectivity of soils (Sadeghian, 2014).

CONCLUSIONS

The spatial distribution of the evaluated bases of change showed that the central area of the R.U.T irrigation district had greater degradation problems; in addition, the

saturation percentages were unbalanced, which was seen in the following ratios: Mg^{2+}/K^{+}, K^{+}/Mg^{2+}, Ca^{2+}/K^{+}, $(Ca^{2+} + Mg^{2+})/K^{+}$ and Ca^{2+}/Mg^{2+} , affecting the soil fertility and crop development.

Further research is needed to approach the current problems of possible degradation in soil fertility from a scientific and technical viewpoint, especially focusing on the central area, which had the highest Percentage of exchangeable magnesium (PMgl) level and where the physical properties of the soils could be most affected.

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Methicillin resistant *Staphylococcus aureus* isolated from meat raw in Cartagena, Colombia

UNIVERSIDAD NACIONAL DE COLOMBIA

Staphylococcus aureus resistente a meticilina aislado de productos cárnicos crudos en Cartagena, Colombia

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ABSTRACT

To determine the prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) isolated in establishments that commercialize raw ground beef and pork chops in Cartagena- Colombia. 160 samples were analyzed through microbiological cultures in Baire Parcker agar, and it was determined the presence of mecA gen that codifies the methicillin resistance and the pvl that codifies the Panton- Valentine leukocidin toxin (PVL) by the multiplex PCR technique. The antibiotic susceptibility profile for MRSA strains was realized by automatized methods and for MSSA strains it was used Kirby Bauver. 66 samples were confirmed as *S. aureus* by PCR. The prevalence of MRSA was 7.5% and 33.8% of MSSA. The 66% of the strains were isolated from raw ground beef and the 34% of pork chop meat. The isolations presented about 2 – 12% of multi-resistance to the antibiotics used. The MRSA showed resistance to amoxicillin- clavulanate (57%), ampicillin-sulbactam and cefazolin (85%), erythromycin and clindamycin (7%), tetracycline (35%). The 10% of the isolated strains had the gen of PVL toxin and the 71% of those were identified in samples of raw pork meat and the 28% in raw ground beef. This study reports for the first time, how meat raw products commercialized in the city of Cartagena could build a dissemination source of MRSA carrier of PVL toxin that could generate a public health disease.

Key words:
MRSA
MSSA
PCR
Panton Valentine
Leukocidin toxin (PVL)
Meat products
Bacterial resistance

RESUMEN

Se determinó la prevalencia de *Staphylococcus aureus* resistente a Meticilina (SARM) aislado en expendios que comercializaban carne cruda molida de res y chuleta de cerdo en Cartagena - Colombia. Fueron analizadas 160 muestras a través de cultivo microbiológico en agar Baire Parcker y se determinó la presencia del gen *mecA* que codifica la resistencia a la meticilina y de *pvl* que codifica la toxina leucocidina de Panton-Valentine (PVL) mediante la técnica de PCR múltiple. El perfil de susceptibilidad antibiótica para las cepas SARM fue realizado por métodos automatizados y para cepas SASM se utilizó Kirby Bauver. 66 muestras fueron confirmadas como *S. aureus* por PCR, la prevalencia de SARM fue de 7.5% y 33.8% de SASM. El 66% de las cepas fueron aisladas de carne molida de res y 34% de carne de chuleta de cerdo. Los aislamientos presentaron entre 2 a 12% de multirresistencia a los antibióticos utilizados. Los SARM mostraron resistencia a amoxacilina-clavulonato (57%), ampicilina – sulbatam y cefazolina (85%), eritromicina y clindamicina (7%), tetraciclina (35%). El 10% de las cepas aisladas tenían el gen de la toxina PVL y el 71% de estos fue identificado en muestras de carne cruda de cerdo y el 28% en carne cruda molida de res. Este estudio reporta por primera vez, como productos cárnicos crudos comercializados en la ciudad de Cartagena pueden constituir una fuente de diseminación de cepas SARM portadoras de la toxina PVL, lo cual podría generar un problema de salud pública.

Palabras claves:
SARM
SASM
PCR
Toxina Panton Valentin
Leucocidina (PVL)
Productos cárnicos
Resistencia bacteriana

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Since its isolation in 1961 until now, methicillin resistant *Staphylococcus aureus* (MRSA) has been considerated one of the main pathogens healthcare-associated infections at hospitals, nevertheless, in the last two decades the scene of infections caused by this bacteria has been changing due to the emergency and dissemination of productive strains of community acquired infections, known as CA-MRSA (Bustos *et al*., 2006). This strains defer from the traditional hospitalarie ones, not only in their epidemiological behaviour but also in their susceptibility to antibiotics and virulence (Zetola *et al*., 2005). The CA-MRSA strains has been showed virulent features, and have caused serious outbreaks in gated communities as family, millitary groups, recluses, kindergartners and athletes (Ho *et al*., 2007). Most of these strains produce the toxin Panton-Valentine leukocidin (PVL), which has been associated with necrotizing pneumonia in all age groups; containing the chromosomal cassette SCCmec type IV or V, which confers methicillin resistance which is encoded in the *mecA* gene that is located in this cassette (McClure *et al*., 2006; Hiramatsu *et al*., 2001). This genetic vehicle can carry other genes as Tn554 gene that confers macrolid, clindamycin and streptogramins resistance, the pT181 gene, confers tetracycline resistance. The same SSCmec can carry virulence genes enterotoxin B and C and toxic shock toxin (Barbara-Wysockib *et al*., 2010; Zuo *et al*., 2008). Additionally, MRSA strains have the ability to produce intermittent colonizations (children, 10-40% and adults, 30%) being the most common site, the nasal cavity, which can lead to infections with severity varying degrees as infections of the skin and tissues through osteomyelitis and rapidly evolving and high mortality necrotizing pneumonia (Quiroga *et al.,* 2013).

Regarding to multi-resistant *Staphylococcus aureus* strains related with food industry it has been shown that these has predominantly colonized people who works in the production of animal origin food. It also has been reported high prevalence of *S. aureus* on pigs of different parts of the world (Smith and Pearson, 2010; Rijen *et al*., 2007). By the way, studies such as Gilchrist *et al*. (2007), have shown contamination of meat with multiresistant *S. aureus* to clinically important antibiotics as Ciprofloxacin, quinupristin / dalfopristin, clindamycin, erythromycin, oxacillin, and daptomycin. Also in a study made in Colombia, they found that the prevalence of resistance to erythromycin and clindamycin in *S. aureus*, was around 57 and 58%, respectively (Reyes *et al*., 2007). In the city of Cartagena they have been studies that report the presence of CA-MRSA in individuals with no clinical antecedents. Álvarez *et al*. (2006), in patients with nasal pathology treated in the Otolaryngology service at the Universitary Hospital of the Caribbean in Cartagena, reported a prevalence of *S. aureus* 22.8% and 5.26% for MRSA; another investigation by Bettin *et al*. (2012), reported that the 16% of the Medical students carry at least one of the MRSA strains in their nostrils for one or two years of their stay in clinics, where they perform their professional practices. The purpose of this study was to determine the prevalence of *S. aureus* CA-MRSA and antimicrobial susceptibility profile from samples of ground beef and raw pork meat sold in the city of Cartagena, in order to meet potential sources of spread of such pathogenic microorganisms.

MATERIALS AND METHODS

Obtaining the samples

An observational descriptive study was conducted in 40 establishments which commercialize beef and raw pork, distributed in three locations in the city of Cartagena: 19 (47.5%) in the localidad histórica y del Caribe (LHC); 16 (40%) in the localidad industrial de la bahía (LIB) and 5 (12.5%) in the localidad turística (LT), where the statistical criteria for the selection of the shops that marketed the two types of meat was: estimated variance of 0.19 according to preliminary investigation; a 5% of error and a minimum probability of 75%. The establishments (sampling units) were randomly selected and in each one dispensing samples between 100-300 g of ground beef (CM) and raw pork chop meat (CC) were taken. The sampling was conducted from July 2012 to May 2013, taking into account the guidelines set out in the Norma Técnica Colombiana NTC 4491-2 (2004).

Microbiological analysis

Once the samples were taken, these were labeled, refrigarated to 4 °C and transported to the science laboratory of food at the Universidad del Sinú-Seccional Cartagena. Microbiological cultures were performed for the count of *S. aureus* through the count in Baird Parcker® agar plate technique. The procedure consisted on performing three samples of serial dilutions (10^{-1}, 10^{-2} and 10^{-3}), and plated in duplicates, incubated it for 48 hours at 35 °C,

according to INVIMA (1998), and the NTC 4779 (2007). The sample reading was realized according to the NTC 1325 (2008) that establishes as an aceptable microbiological requirement for *S. aureus* 100-300 CFU g^{-1} of meat raw procesed, cool and/ or frozen products. Samples were the growment was not showed, were reported as <100 CFU g^{-1}. The specificity and sensibility controls of the culture medium realizated with the *S. aureus* sample ATCC 25923.

Molecular Identification

The genomic DNA in each isolation was reached according to the protocole described by Bettin *et al*. (2012). Were each presumptive isolation was cultured on a Plate Count® agar and incubated for 24 h at 37 °C. Five colonies were suspended in 0.5 mL of TE buffer and centrifugated at 13,000 rpm x 5 min, heated for 30 min and finally centrifuged at 13,000 rpm for 15 min. The supernatant that contains the bacterial DNA was stored at -20 °C until a subsequent amplification through the multiplex polimerase chain reaction (M-PCR).

Polymerase chain reaction (PCR) test

Every isolated MRSA were tipifyed using a multiple PCR essay according with the protocole described by (Zhang *et al*., 2005). The recolected DNA was subjected to a M-PCR to amplify the *mecA* gene (who determine the methicillin resistance), that amplifies a 147 pb fragment, the *nuc* gene of the specific *S.aureus* thermostable nuclease that amplifies a 300 pb fragment and the 437 pb of the *Luk*-PVL of the PVL toxin. The control strains were *S. aureus* ATCC 33591 (*mecA* +; *nuc* +; PVL -) and ATCC 25923 (*mecA* -; *nuc* +; PVL +) and water for negative control of the PCR assays. The DNA was amplified in a reaction volume of 25uL containing 12.5 uL of PCR mix (PCR master Mix; Promega), 0.2 uM of each primer and 5 uL of template DNA. The reaction was conducted in an Axigen® thermal cycler under the following conditions: an initial cycle of denaturation at 94 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C 2 min with a final extension cycle at 72 °C for 10 min. All products were visualized on agarose gel 1.5% with ethidium bromide (0.5 ug mL^{-1}), using a UV transilluminator.

Antibiotic susceptibility profile. The method used to identify MRSA strains was the MicroScan automated system, Siemens®, using panels for dehydrated Gram possitive, supplied by Siemens. For the MSSA strains was used the diffusion method on Kirby Bauver disc. The MSSA strains were determined the following antimicrobials susceptibility: gentamicin 10 mg, 15 mg erythromycin, trimethoprim / sulfamethoxazole 23.75 mg and 1.25 mg, 2 mg clindamycin and rifampin 5mg, those antibiotics were supplied by DIFCO. For oxacillin resistance mediated by the *mecA* gene, was performed as a screening test the microdilution agar technique and a disk diffusion with 30 ng cefoxitin (DIFCO) in a Mueller-Hinton agar. The plates were incubated at 35 °C between 16 to 20 hours. Reading and interpretation was performed according to the guide lines of CLSI, (2013). The possitive *mecA* control strain was *S. aureus* ATCC 43300.

RESULTS AND DISCUSSION

Emergence of methicillin resistant *Staphylococcus aureus* strains in the community with different characteristics from MRSA hospital strains, has caused an alert among health centers and organizations dedicated to the study and control of this microorganism. CA-MRSA infections are an emerging problem in many parts of the world. The injury level yet known, the epidemiological change, clinical manifestations and control can become into a significant public health problem in the near future. Establishing health measures it's neccesary to control the re-emergence of this pathogen and, as possible, to eliminate it in time for preventing it to become a serious threat to the community. In total 160 samples were taken in 40 establishment that comercialized raw meat products, 80 samples of ground beef and 80 samples of pork chop meat. 47.5% (76/160) of the samples was taken in the LHC, 40% in the industrial bay place (LIB) and 12.5% in the tourist place (LT).

From the 160 samples analyzed, in 46% of the samples (75/160) were obtained presumptive counts os *S. aureus* greater than 100 CFU g^{-1} in 54% (85/160) no microbial growth was obtained, which is reported as counts <100 CFU g^{-1}. At the 75th samples counted > 100 CFU g^{-1} were subjected to the technique of polymerase chain reaction (PCR) to confirm the identification of the bacterial genus and in 66 samples (88%) the presence of *S. aureus* was confirmed and in 9 (12%) was not possible to determine the genus. From the 66 samples confirmed by PCR, 18% (12/66) were positive for the *mecA* gene indicating that they were MRSA strains, and 54 (82%) did not have the gene, confirming them as methicillin sensitive or MSSA strains. As for its origin, 66% were isolated from ground

beef meat and 34% pork chop, regardless if they were MRSA or MSSA. Regarding to it provinance, the 66% were isolated from ground beef and 34% from pork chop meat regardless if they were MSRA or MSSA.

From the 66 confirmed samples, 23 (35%) had acceptable microbiological counts of food quality (100-300 CFU g^{-1}) according to the Norma Técnica Colombiana NTC 1325 (2008); and 43 samples (65%) presented higher counts than 300 CFU g^{-1}. From the 12 possitive samples for MRAS, just 4 of them had acceptable quality counts, while 8 had counts above the accepted standard. The results of the isolated global prevalence of MRAS and MSSA are presented in the Table 1. Furthermore, in Figure 1 the results of PCR amplified products for the presence of *mecA* in positive samples are shown.

Table 1. Prevalence of Methicillin resistant *Staphylococcus aureus* (MRSA) and sensible (MSSA) in beef and pork samples

Count (FCU g^{-1})	Samples (n)	MRSA	MSSA	Other Genus
		(%)		
< 100	85*	0	0	0
100 – 300	32	4 (125)	19 (594)	9 (281)
> 300	43	8 (186)	35 (814)	0
Total	160	12 (75)	54 (338)	9 (56)

* Number of samples detected was not microbial growth.

Most animals can be colonized by *S. aureus*, various researches reports isolated MRSA strains from pigs, cows, chicken and others (De Neeling *et al.*, 2007; Huijsdens *et al.*, 2006), especially reported colonization cases by this strain in pigs, farmers and their families. In Netherland people who are in contact with pigs it's recognized as a risk factor for MRSA (Van Duijkeren *et al.*, 2007), according to this, it has been suggested the relation to the emergence of MRSA strains in pigs and the antibiotic use in agriculture (Wulf and Voss, 2008). Results of this research reports the evidence of MRSA in meat products comercialized in the city of Cartagena, and the increase of this organism in relation to the virulence and pathogenicity, coinciding with other researches were the identification and development of new cases in different geographical locations has been confirmed by the implications on public health in both developed and developing countries (Lim *et al.*, 2012; Deurenberg *et al.*, 2006).

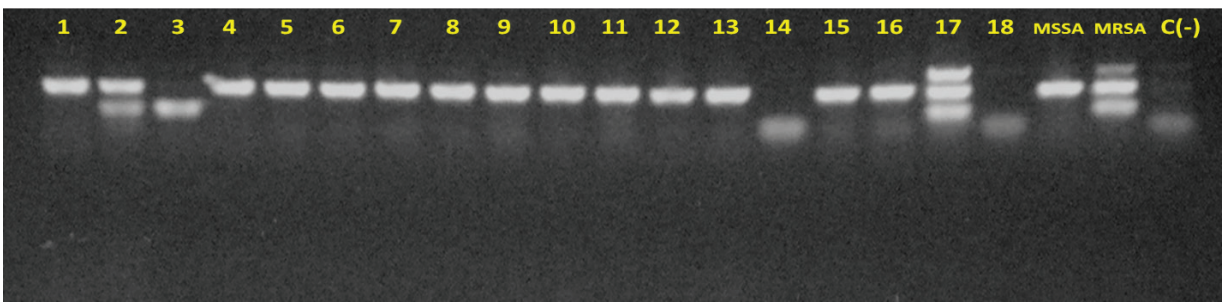

Figure 1. Electrophoresis in Agarose gel of the products of multiplex PCR, indicating possitive detection from the *nuc* gene (MSSA) and *mecA* (MRSA) in the analized strains. Lanes 1, 4-13, 15 and 16 indicate confirmation MSSA strains. Lanes 3, 14 and 18 indicate that there was not *nuc* detection, they do not belong to the Staphylococcus genus. Lanes 2 and 17 indicate the presence of MRSA and additionally to the presence of PVL gene in strain of the lane 17. C (-) negative control.

For the distribution of MRSA strains it was observed that there was the presence of these strains in the three locations, being LIB and LT the localities that contributed with four isolates each one, and the localidad del caribe

norte contributed with two. There were 10 establishments in total which the presence of the *mecA* gene in the analyzed samples was there. The presence of the PVL gene was found in 7 (10.6%) of the 66 strains confirmed as *S. aureus* by PCR, of which six samples, two of ground beef and 4 pork meat were identified as MRSA and only one of the pork meat as MSSA. This gene was isolated in the (71%) of the pork chop meat samples and in the (28%) of the ground beef samples, one of the pork isolated strains was identified as MSSA. Studies show the prevalence of MRSA in ground raw meat (Fontes *et al*., 2013; Boer *et al*., 2009; Hanson *et al*., 2011) in agreement with our results. The presence of this strain in food may be due to the contamination of the slaughterhouse ducts or by the deficient hygiene practice of the operator, inasmuch as nasal area is considered the primary colonized site by *S. aureus,* who is also founded in the intestinal tract (Bhalla *et al*., 2007).

Two of the samples presented the encoding gene for the PVL toxin, they were isolated from meat samples with colony counts that were between the reference parameters: 100-300 CFU g^{-1} (Table 2) which is considered suitable for human consumption, five of the samples with the presence of this gene were rejected by showing higher counts than 300 CFU g^{-1}, according to the Norma Técnica Colombiana NTC 1325 (2008). The establishments were the toxin genes were found were distributed in the three localities, and corresponds to two establishments per locality. Presence of MRSA strains that carriers PVL toxin in establishments in the three localities in the city Cartagena constitutes a risk to the population because it could be presented pathologies associated to this toxin that has the ability of destroying white blood cells and also necrotizing injuries in lungs and soft tissues (Rossney *et al*., 2007). Because of the invasive and virulent ability of this toxin, it has reached a great importance and concern in society, which currently conducted numerous studies in different countries about the isolation of MRSA's capable of producing the toxin (Witte *et al*., 2005). According to this Hanson *et al.* (2011) reported two isolated strains from pork meat, with a global prevalence of 1.2%; instead just one isolated MRSA was possitive to the PVL gene. A recent study in Japan identified MSSA t034 in pigs (Asai *et al*., 2012). Another study in China described T189, associated with ST188, as one of the more common clones responsible for bacteremia (Yu *et al*., 2012). Therefore, food handlers carrying *S. aureus* T189 may present higher risk of infection and food transmission.

Table 2. Colony count (CFU g^{-1}) vs presence of the encoding gene for the PVL toxin.

Code Samples	Type of sample	count FCU g^{-1}	present / absent MRSA	gen PVL
5CM1	Beef	1500	positive	positive
27CM1		600	positive	positive
5CC1	Pork chop	500	positive	positive
27CC1		600	positive	positive
31CC1		200	positive	positive
55CC1		1700	positive	positive
23CC2		200	negative	positive

Figure 2 shows the percentage results of multiresistant strains of *S. aureus* isolated from meat products. Antibiotic multidrug-resistance were observed in the 66 isolated strains. One of the isolated strain showed resistance to 8 antibiotics (Amoxicillin-clavulanate, erythromycin, clindamycin, tetracyclines and cefazolin, penicillin, rifampin and ampicillin sulbatam), but even so it was identified as MSSA. MRSA strains were resistant to the following antibiotics: 57% amoxacilln-clavulonate, ampicillin - sulbactam and cefazolin, 85% penicillin, 7% erythromycin and clindamycin, 35% to tetracycline.

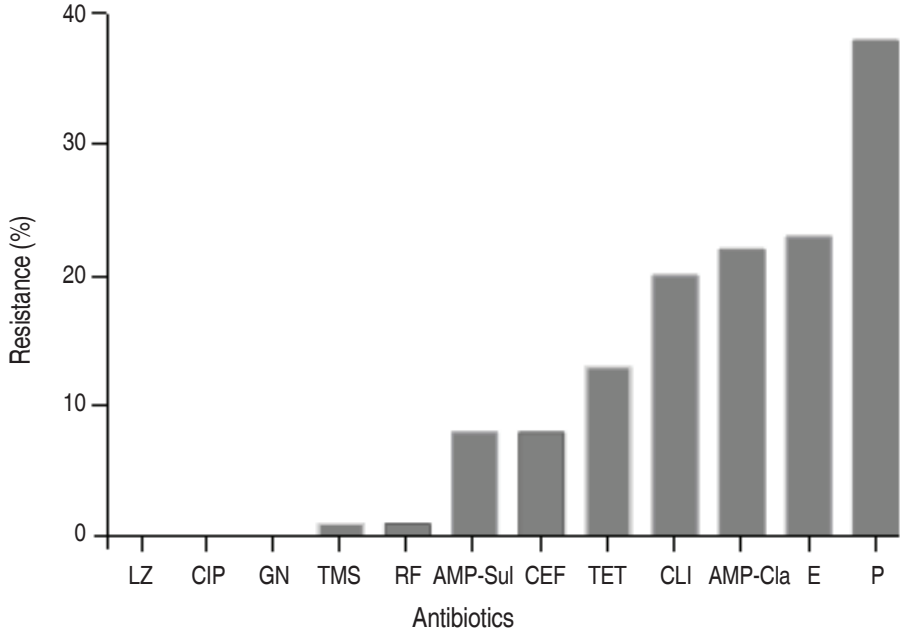

Figure 2. Strains percentage of multirresistant *S. aureus* isolated from ground meat products (n=66). Antibiotics: LZ: linezolid; CIP: ciprofloxacin, GN: gentamicin, TMS: Timetrophrim- sulfametoxazol, RF: rifampicin, AMP-Sul: ampicillin-sulbactam, CEF: cefazoline, TET. Tetraciclyn, CLI: clindamycin, AMP-Cla: ampicillin- Clavulanic acid, E: eritromycin, P: penicillin.

Regarding the antibiotic susceptibility of *S. aureus* isolated in food, the study of Gilchrist *et al*. (2007) where 5136 samples of meat and poultry were analyzed, founding that 47% of the samples were contaminated with *S. aureus*, which 52% of isolates showed multidrug resistance to antibiotics, which allows to state that the widespread use of antimicrobials in food production of animal origin, could be one of the causes of the emergence of multidrug resistance. A study by Ho *et al.* (2014) in food handlers made in six food companies, showed that people exposed to raw meat have higher risk of being colonized with *S. aureus* strains, it was also reported strains resistant to tetracycline (20%) and erythromycin (16%). So it is possible that multidrug-resistant strains come from food handlers.

Another study conducted in five cities of USA, founding in beef and poultry, multiple resistance to clinically important antibiotics like ciprofloxacin, quinupristin / dalfopristin, clindamycin, erythromycin, oxacillin, and daptomycin (Marty *et al.*, 2012). This study is consistent with our results, as it was found highly resistant to antibiotics commonly used to treat infections caused by this bacterium. MRSA has apparently responded well to therapies based on clindamycin and even with trimethoprim sulfametazole in communities with high prevalence of Methicillin Resistant Staphylococcus for handling soft tissues minor infections (Jorgenser, 2000; Agnoletti *et al.*, 2014). In the present study it was found 7% of MRSA strains resistant to clindamycin, indicating the risk of acquiring these strains through food.

CONCLUSIONS

7.5% of *Staphylococcus aureus* reported were MRSA and 33.8% were MSSA, in a total of 25% of the sampled establishments. The type of analyzed meat that obtained the highest percentage of MRSA was ground beef with 66%. MRSA strains are distribute in the three locations in the city of Cartagena, 40% in the localidad Virgen y Turística, 40% in the localidad Industrial de la Bahía and 20% in the localidad Histórica y del Caribe. It was found a marked antibiotic resistance in the methicillin resistant *Staphylococcus aureus* strains and in strains that carried the PVL toxin, obtaining a antibiotic multidrug resistance. It was confirmed circulation and possible spread of MRSA strains capable of producing genes encoding PVL toxin in meat products, which represents a risk to meat consumers in the city of Cartagena.

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Preliminary characterization of the enzyme polyphenol oxidase and rheological behavior from *Averrhoa carambola* juice

Caracterización preliminar de la enzima polifenol oxidasa y comportamiento reológico del jugo de *Averrhoa carambola*

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Key words:
Carambolo
Enzymatic browning
Rheology
Tropical fruits
Agroindustry
Peroxidase enzyme

ABSTRACT

Averrhoa carambola fruit represents a potential as an agro-industrial production line. A restriction on the use of this exotic fruit is the susceptibility to enzymatic browning, affecting nutritional and visual acceptance. The aim of this study was to determine the physical and chemical composition of *A. carambola* at three stages of maturity. The polyphenol oxidase enzyme has also been characterized on the fruits and juices. Also, the enzymatic effect on the ripening stages and the fruit juices flow behavior were equally determined using different rheological models. The increasing in degrees Brix (unripe (UR) 6.63 ± 0.25, intermediate (IN) 6.8 ± 0.10 and ripe (R) 8.26 ± 0.37) and the decreasing of the pectinic content (UR 4.35% ± 0.98, IN 3.6% ± 1.26 and R 2.25% ± 0.76) could be considered as indicators of fruit ripening. The protein content and levels of organic acids decreased during the ripening of fruit was observed, indicate a high metabolic rate during this process. For all stages, the polyphenol oxidase exhibited a maximum activity at pH 8 and 40 °C. In the fruit aqueous fraction, the enzyme would be an appropriate indicator in industrial handling (temperatures below 20 °C and pH less than 7), which would control enzymatic browning. According to the rheological study, the viscosity variation (η: UR (13.4 to 1.1), IN (15.4 to 1.4), R (69.6 to 2.9)) with temperature changes (10 to 50 °C) is adjusted to the Arrhenius equation, whereas the effect of soluble solids content on the samples viscosity was accurately described by an exponential equation. This appears to be the first work to study the rheological properties and polyphenol oxidase enzyme of *A. carambola* fruit in our country, the information provided from this study could be helpful to the successful development of new food functional products.

Palabras claves:
Carambolo
Pardeamiento enzimático
Reología
Frutos tropicales
Agroindustria
Enzima peroxidasa

RESUMEN

Averrhoa carambola representa un potencial como una línea de producción agroindustrial. Una restricción en el uso de esta fruta exótica es la susceptibilidad al pardeamiento enzimático, que afecta su aceptación nutricional y visual. En este estudio se determinó la composición física y química de *A. carambola* en tres estados de madurez; además se caracterizó la enzima polifenol oxidasa de fruta y zumos. De igual manera, se evaluó el efecto enzimático y el comportamiento al flujo del jugo en las etapas de maduración de la fruta utilizando diferentes modelos reológicos. El incremento en los grados Brix (verde (V) 6,63 ± 0,25, intermedio (IN) 6,8 ± 0,10 y maduro (M) 8,26 ± 0,37) y la disminución del contenido pectínico (V 4,35% ± 0,98, IN 3,6% ± 1,26 y M 2,25% ± 0,76) podrían considerarse como indicadores de la maduración del fruto. Se observó que el contenido de proteína y los niveles de ácidos orgánicos disminuyeron durante la maduración de la fruta, indicando una alta tasa metabólica durante este proceso. Para todas las etapas, la máxima actividad de la polifenol oxidasa se encontró a pH 8 y 40 °C. En la fracción acuosa de la fruta, la enzima es un indicador apropiado en el manejo industrial (temperaturas por debajo de 20 °C y pH inferior a 7), lo que controlaría el pardeamiento enzimático. El estudio reológico mostró que la variación de la viscosidad (η: V (13,4 a 1,1), IN (15,4 a 1,4), M (69,6 a 2,9)) con los cambios de temperatura (10 a 50 °C) se ajusta a la ecuación de Arrhenius, mientras que el efecto del contenido de sólidos solubles sobre la viscosidad de las muestras fue descrito con precisión por una ecuación exponencial. Este parece ser el primer trabajo para estudiar las propiedades reológicas y la enzima polifenol oxidasa de *A. carambola* en nuestro país; la información proporcionada aquí podría ser de utilidad para el desarrollo exitoso de nuevos productos funcionales alimentarios.

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The fruit world market is mainly supplied by developed northern hemisphere countries (USA, Canada and some European countries), with species such as apple, pear, plum, and cherry, among others, or subtropical regions by producers of orange, tangerine, lemon, etc.; there are some southern hemisphere countries included such as Argentina, Chile and Brazil, which export grape, pear, apple, cherry, among others (Giacinti, 2001). The only tropical fruit with which Colombia competes in that market is banana, which has a prominent place in its production (Orduz-Rodríguez and Mendoza, 2002).

However, the geostrategic position of the Colombian Andes confers them different climatic zones and land availability, nominating them as an area with potential for developing agricultural business with a diversity of fruit products, among which, carambolo (*Averrhoa carambola* L.), cape gooseberry (*Physalis peruviana* L.), small pineapple (*Bromelia karatas*) and the purple mangosteen (*Garcinia mangostana*) may be mentioned, just to name a few examples. Nevertheless, the main restriction on the use of exotic Colombian fruits is the lack of technical information on many of them, underestimating their potential. Another limitation is the fruit susceptibility to enzymatic browning, which affects both nutritional and visual acceptance (Ma *et al*., 2010).

The carambolo (*Averrhoa carambola* L., Oxalidaceae), is a small tree that grows best in the hot humid tropic. This species is established in smallholder farms in the foothill region of Meta (Colombia) for household consumption and sale in small supermarkets. Owns a fruit with good nutritional content and multiple uses both fresh and processed. There exist the acids and sweets types, in which it finds a lot of varieties. The acid type is smaller, very sour, richly flavored and more oxalic acid; the sweet type is larger, mild-flavored, rather bland and with less oxalic acid (Manda *et al*., 2012). Like most fruit, carambolo has deterioration problems during storage or when processed. Cutting and other mechanical procedures damage the walls of the fruit and allow oxygen to penetrate, resulting in darkening, which is better known as enzymatic browning reaction (Márquez *et al*., 2015). The color change that leads to golden brown is a consequence of enzymatic reactions involving the polyphenolic compounds (Sellés-Marchart, 2007), specifically caused by browning enzymes, generically known as polyphenoloxidase (Fang, 2007).

Moreover, in both the processing and the storage stages, juices suffer continuous changes in soluble solid contents, because they undergo a water removal stage through evaporation, where they are subjected to different temperatures. They continuously change their properties such as viscosity, given that the conditions depend on concentration and temperature. Knowledge of these changes can be of prime importance in the manipulation, calculation and design of all the unit operations involved in the juice processing, mainly those based on heat transfer and motion.

Some reports are available on the physical (Bezerra *et al*., 1989), physical chemical characterization of the ripening (Mitcham and McDonald, 1991) and chemical composition (Herrman, 1994) of carambolo fruit. The mineral, amino acids, volatile flavors and carotenoid compositions of the fruit have been reported (Becerra *et al*., 1992). Manda *et al*. (2012), reviewed data on the taxonomy, botanical description of the plant, its distribution and ecological requirement of this fruit. Also, the phytochemical and pharmacological profile were reviewed by Thomas *et al*. (2008) and Gheewala *et al*. (2012), whereas Saghir *et al*. (2013), investigated on traditional uses to pharmacological activities from star fruit and Dasgupta *et al*. (2013), reviewed and compile all the updated information on botany, phytochemical and pharmacological properties, drug interaction, contraindication and toxicity studies of *A. carambola*.

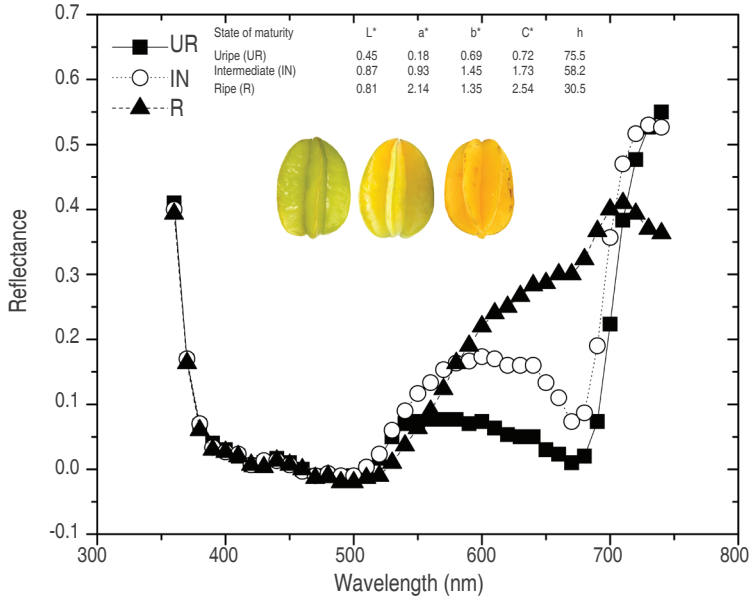
In Colombia, Grajales-Agudelo *et al*. (2011) studied the effect of freezing and heating rates on rehydration of lyophilizated fruit; and Mateus-Cagua and Orduz-Rodríguez (2015) presented a brief description of the principal zones where *A. carambola* crop has been established. Also, Gonzalez *et al*. (2001), characterized an acid variety of carambolo adapted to the Amazonian foothills conditions, through physical, chemical and physiological analyses from the setting to the commercial maturity, in function of study of fruits growth and development and to determine the appropriates parameters to indicate the harvest moment. Nevertheless, processed carambolo products are not available in our market and very little work has been done on the processing of carambolo in Colombia. To the best of our knowledge, from literature survey, no work has been carried out on the chemical composition, the characterization of the enzyme polyphenol oxidase

and rheological behavior from *A. carambola* juice in our country.

The aim of this study was to determine the physical and chemical composition, assessment of cell wall content and make the phytochemical screening on carambolo fruit at three stages of maturity. Similarly, characterizing the polyphenol oxidase enzyme and its effect on the ripening stages of *A. carambola* and flow behavior of fruit juices was also studied using different types of rheological models, all of which will help to a better understanding of fruit oxidative processes and will contribute, at least in part, to preserve good organoleptic properties of the plant material.

Materials and Methods

Harvesting of carambolo fruits was held at municipality of Lleida-Tolima (366 m, 26 °C and 100 mm rainfall per year). A plant specimen was collected with leaves, fruits and flowers, and was taxonomically determined at TOLI Herbarium in the University of Tolima, Colombia. The collected material was taken to the laboratory, where it was washed and classified depending on its ripening degree and plant health, according to the following criteria: Unripe stage (UR): completely green epicarp, intermediate stage (IN): whitish epicarp, accepting green edges, ripe stage (R): completely yellow or orange epicarp (Figure 1).



Indice	Color	Descripción
1	Yellow-Green 1	Something yellow light green color
2	Yellow-Green 2	Greenish yellow
3	Brown-orange 1	Opaque yellow
4	Brown-orange 2	Little intense orange opaque
5	Brown-orange 3	Intense orange opaque, completely colored fruit

(Gonzalez *et al.*, 2001)

Figure 1. Color table of *A. carambola* fruit at three stages of maturity (Using the standard illuminant D65 and 10^0).

From each stage (± 500 g), and through a juice extractor (Black and Decker, USA), the respective aqueous fraction was obtained, which was clarified by centrifugation (1613 g, 15 min), and from now on is called "juice;" another part of the plant material was dried in an industrial stove (70 to 80 °C for three days); nuts were ground to reduce particle size and stored in amber labeled bottles under refrigeration (4 °C) until use.

Physical and chemical composition of *A. carambola* fruits and juice at three stages of maturity

Twenty-five fruits of each maturity group were individually analyzed for physical characteristics. Length and diameter were measured with a Vernier caliper. The measurement of length was made between apex and stem. The maximum width of the fruit, measured in the direction perpendicular to the polar axis, was denominated as diameter. The measurements for the ridges were made for length and its maximum width. On the other hand, moisture, ash, lipid, total (TC) and reducing (RC) carbohydrates, hexoses, pentoses, Vitamins C, A and E; mineral elements (major and minor) contents were estimated according to the methods described by determining the nutritional content of raw plant material (AOAC, 2005). The study is complemented with the information from the cell wall content and preliminary phytochemical screening of star

fruit. The physical characterization of the juice was made by determination of density, pH, total solids, firmness (Bertuzzi penetrometer, Italia), refractive index, degrees Brix and total acids (AOAC, 2005); the pectin content was also determined (Ismail *et al*., 2012).

Assay of polyphenol oxidase (PPO) activity from Carambolo

PPO enzyme activity was performed with a Helyos UV/VIS spectrophotometer (Thermo Fisher Scientific, UK) by measuring the initial rate of quinone formation, by an increase in absorbance at 420 nm (20 min intervals for one hour at 27 °C) by using catechol as substrate. The assay reaction contained catechol (2.5 mL, 0.5 M in a sodium citrate buffer 0.1 M/sodium phosphate 0.1 M, pH 7) and 1 mL of freshly prepared crude enzyme extract (Falguera *et al*., 2012). A unit (U) of PPO activity was defined as the amount of the enzyme that increased the absorbance by 1.00 min^{-1} (U min^{-1}), under the conditions of the assay.

pH and temperature optimums

To find the best activity conditions of the PPO enzyme, pH scan (range of 7 to 8.5, room temperature) was performed using catechol in two buffer solutions: sodium citrate buffer (0.1M, pH range of 4.5 to 6), and sodium phosphate buffer 0.1 M, pH 7. The optimum temperature obtained from this study was used in other determinations. The effect of temperature on enzyme activity was evaluated changing the variable from 10 to 60 °C (intervals of 10 °C for 60 min, at optimum pH). The reaction mixture contained 3.9 mL of phosphate buffer (pH 7.0), 1.0 mL of 50 mM substrate and 0.1mL of crude PPO extract (Calan *et al*., 2010). The optimum temperature obtained from this study was used in other determinations

Quantification of protein

The quantification of protein by the method of Lowry (Pavel *et al*., 2013), complements the information. Under alkaline conditions the divalent copper ion forms a complex with peptide nitrogen in which it is reduced to a monovalent ion. Monovalent copper ion and the radical groups of tyrosine, tryptophan, and cysteine react with Folin reagent to produce an unstable product that becomes reduced to molybdenum/tungsten blue. Absorbance readings were interpolated on the curve made with bovine serum albumin (BSA) at different concentrations (200 to 800 µg mL^{-1}).

Peroxidase enzyme activity (POD)

POD activity was estimated by the Andrade-Cuvi *et al.* (2010) method, with some modifications. The measuring cell, to a final volume of 2 mL, contained juice (200 µL) and a guaiacol mixture 1.8 µL (60 mM:H_2O_2 100 mM) in phosphate buffer 20 mM, pH 8.0; this mixture was kept at 25 °C. The blank solution was previously boiled in water bath (92 °C, 10 min), which ensures the enzyme denaturation and the not reaction with the substrate. One unit of enzyme activity was defined as the change in absorbance per min. G fresh tissue.

Rheological measurements

The rheological measurements of the samples tested were carried out with a Rheometer Haake RS 80 RheoStress, equipped with a concentric cylinder measuring system Z40-DIN (1.0847 radius ratio). For temperature control, there was used a programmable thermostat Thermo Haake C25 P, which allows setting temperature with a variation of ± 0.2 °C. To evaluate rheological behavior, samples were sheared at a constant rate (100 s^{-1}), and measured the variation over time. In the study of the flow behavior, the samples were previously sheared at 400 s^{-1} (3 min), followed by a ramp shear rate values of ascending and descending. To study the flow behavior of the carambolo juice, there were used different rheological models at different temperatures (10, 20, 30, 40, 50 °C):

Newton equation: $\sigma = \eta \cdot \dot{\gamma}$ (1)

Bingham equation: $\sigma = \sigma_0 + \eta' \cdot \dot{\gamma}$ (2)

Power law (Ostwald de Waele equation): $\sigma = K \cdot (\dot{\gamma})^n$ (3)

Herschel-Bulkley equation: $\sigma = \sigma_0 + K \cdot (\dot{\gamma})^n$ (4)

Where, σ is shear stress (Pa), σ_0 threshold stress (Pa), $\dot{\gamma}$ shear rate (s^{-1}), η viscosity (Pa s), *K* consistency index (Pa s^n), and *n* flow behavior index (dimensionless).

Statistical Analysis

Each of the characterization tests of the PPO was individually analyzed and data was reported as the means of three determinations (n=3x3) ± SD. For the analysis of enzyme kinetics, a multivariate analysis of variance MANOVA was applied, consisting of three factors: enzyme kinetics, ripening stage, and pH-temperature. Additionally, multiple comparisons Fisher's

LSD post hoc tests were then applied to identify noted differences among ripening stages. A P value of less than 0.05 was considered statistically significant. The statistical program InfoStat/Professional® Version1.2, was used for these analysis. Experimental results of carambolo juice were adjusted to different rheological models using the statistical data processing software Statgraphics (STSC Inc. Rockville, Md, USA, version Plus 5.1). Both the settings and estimates of the parameters were calculated at a significance level of 95%.

RESULTS AND DISCUSSION

Physical and chemical composition of fresh fruit and juice characterization at three stages of maturity

The plant of interest in this paper was identified at Toli Herbarium of the Tolima University with the name of *Averrhoa carambola* Linnaeus (COL 10121), and it was described as belonging to the Oxalidaceae family. This is a very cold-susceptible plant species, its ideal temperature is between 26 and 28 °C. It is not very demanding on soil conditions, but it grows best in soils with plenty of organic matter. The pH can range from 5.5 to 6.5 (Mateus-Cagua and Orduz-Rodríguez, 2015).

The current study reveals a large variation in the physical and chemical composition of the carambolo fruit during maturation from green mature stage to ripe fruits. Table 1 presents some certain physical parameters of the star fruit juices obtained, which complement the physical and chemical characteristics about the fruit of this plant species. It reveals that fruits become more turgid as ripening progresses, substituting biomass for water content. In relation to the decrease in firmness with ripening progress in carambolo, this behavior is typical of fleshy berries, in which the parenchymal tissue accumulates large amounts of water, making it a very succulent fruit. Similar results were reported by other authors (Narain *et al*., 2001; Navarro, 2011). On the other hand, the fruit equatorial and longitudinal diameters suggest that growth occurs initially by longitudinal cell division and later by cell elongation mainly in the mesocarp. While the accumulation of water improves physical fruit appearance and decreases its acid concentration, also makes it prone to pathogen attack, just as its mechanical strength decreases, exposing it to physical deterioration from bruises.

Also, the values specified in the table show that the refractive index, density and pH do not seem to be related to the maturation stages, which is possible to see through the values of total solids, degrees Brix and the content of pectic substances. Furthermore, organic acids are a direct respiratory power source both in animal and plant cells. The final product of ß-oxidation (Acetyl-CoA) is converted into CO_2 and H_2O, preferably via the Krebs cycle (Cheftel *et al*., 1989); thus, the fruit cells are able to use them as a respiratory substrate and convert them into sugars. This explains, at least in part, that the levels of organic acids descend during the ripening of carambolo: UR 0.234%, IN 0.174% and R 0.099%; indicate a high metabolic rate during this process, and that the fruit cells are capable of using them as a source of breathing energy during the high respiratory rate required with ripening advance. Many of them are really contributing to the flavor (Da Conceicao-Neta *et al*., 2007). The increasing in degrees Brix and the decreasing of the pectinic content could be considered as indicators of fruit ripening. The progressive decrease of the pectinic contents could be a response to the decrease in fruit firmness and in the acidity. During ripening of the fruit, protopectins are depolymerized and solubilized strongly as a result of the action of pectolytic enzymes, pectin methylesterases, polygalacturonases and glycosidases located in the middle lamella of the cell wall (Cheftel *et al*., 1989).

The titratable acidity, reducing sugars, vitamin A and tannin contents of the fruits were significantly different in fruits among all stages of maturity, and calcium and sodium contents of the fruits were higher in the rape stage than the fruits at green mature or half-ripe stages. The presence of polyphenols, flavonoids, saponins, phenylpropanoids and terpenoids was also observed.

Assay of polyphenol oxidase (PPO) activity from carambolo

Effect of pH and temperature. As seen in Figure 2, optimal pH for carambolo PPO was found from 7.5 to 8.0 for catechol substrate. The peak for all stages was reached at pH 8. There is dependence between enzyme activity and the increase of pH. The increased activity is observed in the UR, but is not significantly different to the other (P>0.05). Similar results were achieved in apple (Manohan and Wai, 2012). Nevertheless, the common range of pH for PPO activity in other fruits is between pH 6.0 and 7.0 (Fang, 2007).

Table 1. Physical parameters of carambolo fruit juice in three stages of maturity.

	Parameters	Ripening stage		
		Unripe	Intermediate	Ripe
Fruit	Whole fruit weight (g)	71.65 ± 2.68	44.76 ± 1.22	22.21 ± 4.22
	Edible part - pulp (%)	95.67 ± 1.08	93.23 ± 0.95	87.04 ± 1.01
	Seed (%)	0.86 ± 0.01	1.75 ± 0.01	3.08 ± 0.01
	Equatorial diameter (cm)	3.48 ± 0.02	4.09 ± 0.02	5.17 ± 0.01
	Length diameter (cm)	5.98 ± 0.01	6.99 ± 0.03	8.04 ± 0.02
	Fruit firmness (kg cm^{-1})	8.56 ± 0.30	7.20 ± 0.35	4.50 ± 0.25
	Maturity index (°Brix/acid)	28.30 ± 0.00	39.10 ± 0.00	83.40 ± 0.00
	Humidity (%)	89.56 ± 0.00	91.51 ± 0.00	96.08 ± 0.00
	Dry meterial (%)	10.44 ± 0.00	8.49 ± 0.00	3.92 ± 0.00
	Fiber (%)	8.37 ± 0.00	7.29 ± 0.00	7.80 ± 0.00
	Protein (%)	6.23 ± 0.00	5.47 ± 0.00	6.71 ± 0.00
	Lipids (%)	5.48 ± 0.00	6.01 ± 0.00	4.11 ± 0.00
	Ash (%)	3.80 ± 0.00	1.80 ± 0.00	4.50 ± 0.00
	Total carbohydrates (mgEG/g)	162.83 ± 0.00	169.90 ± 0.00	180.97 ± 0.00
	Reducing carbohydrates (mgEG/g)	53.52 ± 0.00	66.43 ± 0.00	72.38 ± 0.00
	Hexoses (mgEG/g)	44.82 ± 0.00	57.10 ± 0.00	61.18 ± 0.00
	Pentoses (mgER/g)	8.69 ± 0.00	9.32 ± 0.00	11.19 ± 0.00
	Vitamin C (mgEAA/100g)	14.83 ± 0.01	13.61 ± 0.01	8.35 ± 0.01
	Vitamin A (mgEβC/100g)	11.65 ± 0.01	5.08 ± 0.01	4.25 ± 0.01
	Vitamin E (mg/αT100g)	139.29 ± 0.01	44.76 ± 1.23	42.59 ± 0.01
	Major mineral elements (%)	Ca K Mg Na* P	Ca K Mg Na* P	Ca K Mg Na* P
		0.31 1.39 0.24 221.6 0.02	0.39 1.34 0.13 54.40 0.07	0.36 0.61 0.12 28.50 0.02
	Minor mineral elements (mg kg^{-1})	Fe Zn Cu Mn S** B	Fe Zn Cu Mn S** B	Fe Zn Cu Mn S** B
		119.00 37.20 1.59 5.80 0.18 434	72.33 22.66 2.22 2.35 0.21 371	208.33 15.33 1.91 N.D 0.30 139.50
	NDF	20.11 ± 0.00	22.54 ± 0.00	21.90 ± 0.00
	ADF	19.95 ± 0.00	18.31 ± 0.00	16.31 ± 0.00
	LDF	9.83 ± 0.00	12.20 ± 0.00	12.72 ± 0.00
	Cellulose	3.34 ± 0.00	6.23 ± 0.00	2.73 ± 0.00
	Lignin	8.98 ± 0.00	11.57 ± 0.00	12.24 ± 0.00
	Hemicellulose	6.94 ± 0.00	4.11 ± 0.00	6.45 ± 0.00
	Sílica	0.85 ± 0.00	0.63 ± 0.00	0.48 ± 0.00
	Cell content	79.89 ± 0.00	77.46 ± 0.00	78.10 ± 0.00
Juice	Density (g cm^{-3})	0.35± 0.00	0.35 ± 0.00	0.36 ± 0.01
	pH	3.75 ± 0.01	3.71 ± 0.00	3.68 ± 0.00
	Titratable acidity (% Oxalic acid)	0.23 ± 0.10	0.17 ± 0.05	0.10 ± 0.10
	Refractive index	1.34 ± 0.00	1.34 ± 0.00	1.34 ± 0.00
	Total Solids (%)	6.00 ± 7.30	6.12 ± 7.37	5.08 ± 1.32
	°Brix	6.63 ± 0.25	6.80 ± 0.10	8.26 ± 0.37
	Pectin (%)	4.35 ± 0.98	3.60 ± 1.26	2.25 ± 0.76
	Carbohydrates	+++	++++	++++
Phytochemistry screening (Extract Pulp)	Reducing carbohydrates	++	+++	++++
	Carbohydrates nonreducing	++++	++	++
	Polyphenols	++++	+++	+++
	Tannines	+++	+	+
	Flavonoids	+++	+	++
	Alkaloids	N.D.	N.D.	N.D.
	Anthocyanins	N.D.	N.D.	N.D.
	Saponins	+	+	N.D.
	Phenylpropanoids	++	+	++
	Iridoids	N.D.	N.D.	N.D.
	Lactones	+++	+	++
	Cardiac glycosides	N.D.	N.D.	N.D.
	Terpenes / steroids	+++	++	++
	Anthraquinone	N.D.	N.D.	N.D.

* mg kg^{-1}

** %

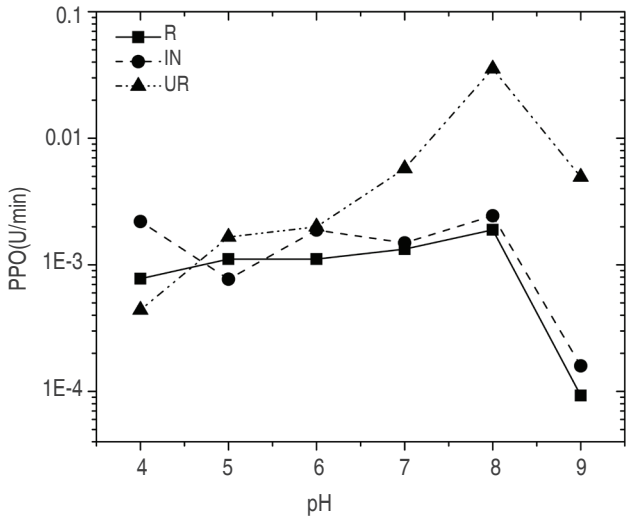


Figure 2. Effect of pH vs. activity of the PPO enzyme in three stages of carambolo ripening.

It was found that optimum pH value of carambolo PPO was higher than that reported in the literature using catechol as a substrate (Dogan *et al*., 2013). Variations in the optimum pH in diverse vegetables can be explained because of the different substrates used in the activity evaluation and the location of the enzyme in the plant or in the cell (Assis *et al*., 2006). The temperature influence on the activity of the PPO of *A. carambola* for three ripening stage is shown in Figure 3. The maximum enzyme activity for all stages was revealed at 40 °C. However, there was no significant difference (P>0.05) between the evaluated temperature (10 to 60 °C), but between stages (P<0.05).

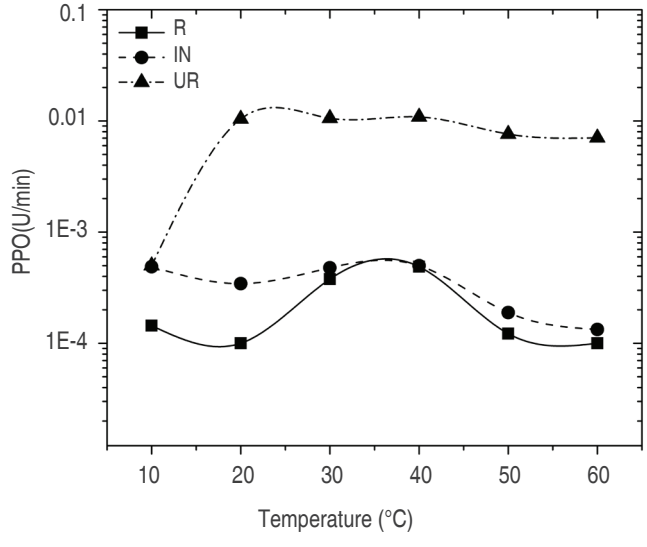


Figure 3. Effect of temperature vs. the PPO enzyme activity in all three carambolo stages of ripening.

All juices showed a possible inhibition from 40 °C and low activity at 10 °C, similar to the reported by Zhang and Shao (2015). In general, PPO exposure to temperatures between 70 to 90 °C destroys its catalytic activity, but the time required for the inactivation depends on the vegetable product. Chutintrasri and Noomhorm (2006) found that the pineapple PPO reduces its activity by 60% after heat exposure between 40 to 60 °C (30 min).

Polyphenoloxidase is an oxide reductase copper-dependent, also known as phenolase or catechol oxidase. It participates as a catalyst in the reaction between catechol and oxygen, resulting in the formation of quinones, which are able to react with cell components leading to formation and precipitation of a dark polymer similar to melanin, a phenomenon known as enzymatic browning (Mejía-Doria *et al.*, 2014). Thus, PPO properties are also of interest, just as the information obtained in these studies can be of use to drawn important conclusions for food chemistry.

Quantification of protein concentration (enzyme)

Protein content in carambolo decreased with ripening: 390.2 mg g^{-1}, 75.9 mg g^{-1} and 39.4 mg g^{-1}, for the UR, IN and R, respectively; which is lower than earlier reported for the same fruit (Edem *et al.*, 2008). Differences between our data and the literature could be explained by soil nutrients deficiency, for example, with low organic matter.

Peroxidase enzyme activity (POD)

The activity of this enzyme during the carambolo ripening was similar to that shown by the PPO, it means a decreasing tendency from the unripe fruit (0.0076 UPOD h^{-1}), intermediate (0.0045 UPOD h^{-1}) and ripe fruit (0.0028 UPOD h^{-1}). This implies that when peroxidase shows low activity, the increase of hydrogen peroxide is allowed and promotes contact between phenols and polyphenoloxidase, leading to browning process (Mizobutsi *et al.*, 2010).

The peroxidase is an enzyme that controls the physiological growth, differentiation and development of the plants. POD activity in the carambolo could also be a result of the participation of the enzyme in the last step of polymerization of cinnamyl alcohols to form lignin, showing the process of lignification in the darkening of tissues subjected to mechanical damage. It could also be noticed that in senescence, H_2O_2 levels increase and lysis of vacuoles containing phenols occurs (Cheftel *et al.*, 1989), which would provide suitable conditions for PPO activity to increase significantly, and allow the observed enzymatic browning in *A. carambola* fruits.

Rheological behavior of carambolo

The study showed that there was no variation of shear thixotropic time. The variation of shear strength concerning the strain rate was adjusted to different rheological models (power law, Herschel-Bulkley equation, Bingham equation and Newton equation).

Figure 4 shows the rheograms corresponding to the ripe state of carambolo juices (10 °C), for different soluble solid contents. For the other states of ripening and different temperatures, there are analog rheograms obtained to those illustrated in Figure 4 (data not shown). Depending on the sample, the best adjustments were obtained with the Bingham and Newton equations. Results of these adjustments for the three states of ripening studied are shown in Tables 2, 3 and 4. Is well known, that for a determined

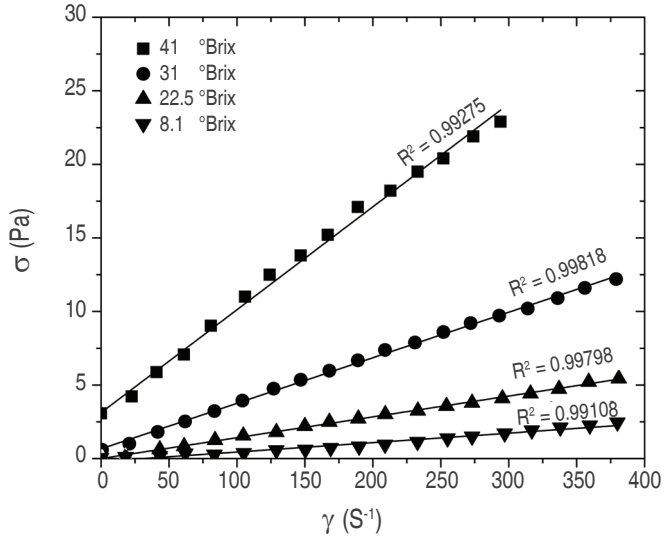


Figure 4. Rheogram of mature stage carambolo juice at 10 °C with different soluble solids.

Table 2. Rheological flow behavior of ripening stage carambolo juice, depending on temperature and soluble solids

Soluble solids (°Brix)	T (°C)	σ_0 (Pa)	η (mPa·s)	R^2
41.6	10	3.06	69.60	0.992
	20	2.33	47.30	0.994
	30	1.57	36.30	0.996
	40	1.13	28.80	0.998
	50	0.89	25.10	0.999
31.5	10	0.87	29.30	0.998
	20	0.54	21.70	0.999
	30	0.36	16.50	0.999
	40	0.25	13.10	0.999
	50	0.18	10.90	1.000
22.5	10	0.22	13.90	0.999
	20	0.14	10.60	0.999
	30	0.08	8.30	0.999
	40	0.06	6.80	0.999
	50	0.04	5.70	0.997
8.1	10	0.13	5.30	0.997
	20	0.09	4.40	0.994
	30	0.08	3.70	0.997
	40	0.06	3.30	0.990
	50	0.05	2.90	0.992

Table 3. Rheological flow behavior of intermediate maturation stage carambolo juice, depending on temperature and soluble solids.

Soluble solids (°Brix)	T (°C)	σ_0 (Pa)	η (mPa·s)	R^2
40.1	10	0.07	15.10	0.999
	20	0.03	11.30	1.000
	30	0.01	8.40	0.999
	40	0.00	6.90	0.997
	50	0.00	6.10	0.985
31.6	10	0.02	10.90	0.999
	20	0.01	8.10	0.999
	30	0.00	6.30	0.999
	40	0.01	5.00	0.999
	50	0.00	4.60	0.981
21.3	10	0.00	6.50	0.999
	20	0.00	4.90	0.999
	30	0.00	3.80	0.999
	40	0.00	3.20	0.994
	50	0.00	2.70	0.994
7.2	10	0.00	2.80	0.999
	20	0.00	2.20	0.999
	30	0.00	1.80	0.999
	40	0.00	1.50	0.999
	50	0.00	1.40	0.998

state of ripening and soluble solids content, the viscosity of the samples decreases as the temperature increases (Tables 2, 3 and 4). In the same way, for a given state of ripening and a certain temperature, the viscosity decreases as well as soluble solids content. It is evident that the threshold stress responses are very small and only in the ripe state samples have non-zero values.

Moreover, threshold effort values less than unity can be neglected (Syang-Peng and Mei-Sia, 2013). It can be said that the only samples with some plastic behavior are juices from ripe fruit with a soluble solids content of 41.6 °Brix, although their values are close to unity. Therefore, it can be considered that the samples tested have an almost Newtonian flow behavior.

Regarding the influence of the ripe stage, it is observed that for a given concentration of soluble solids and the same temperature, the samples have a higher viscosity as the ripening state increases.

Tabla 4. Rheological flow behavior of unripe stage carambolo juice, depending on temperature and soluble solids.

Soluble solids (°Brix)	T (°C)	σ_0 (Pa)	η (mPa·s)	R^2
42.5	10	0.03	13.40	1.000
	20	0.00	10.00	0.999
	30	0.00	7.40	0.999
	40	0.00	6.20	0.989
	50	0.00	5.60	0.975
33.0	10	0.00	10.80	1.000
	20	0.00	7.90	0.999
	30	0.00	6.60	0.982
	40	0.00	5.20	0.984
	50	0.00	4.20	0.984
22.0	10	0.00	6.70	0.997
	20	0.00	5.20	0.987
	30	0.00	4.20	0.979
	40	0.00	3.60	0.969
	50	0.00	3.10	0.968
5.2	10	0.00	2.70	0.973
	20	0.00	2.20	0.965
	30	0.00	1.70	0.979
	40	0.00	1.30	0.997
	50	0.00	1.10	0.997

Effect of temperature

In Tables 2, 3 and 4 it is observed that the viscosity of any of the studied samples depends on temperature, so it is important to quantify the effect that temperature has on the viscosity of those samples. The variation of viscosity with temperature can be described by an Arrhenius type equation (Keshani *et al*., 2012; Saghir *et al*., 2013).

$$\eta = K_0 \exp\left(\frac{E_a}{RT}\right) \qquad (5)$$

Where η is the viscosity, K_0 is a constant, E_a is the activation energy of flow, R is the gas constant and T is the absolute temperature in Kelvin.

The values of viscosity shown in Tables 2, 3 and 4 are set to the linearized form of Equation 5 by linear regression. These settings allow obtaining the values of K_0 and E_a for each concentration and ripeness (Table 5). Both the settings as estimates of the parameters have been significant at 95% probability. Figure 5 shows data for juice made from ripe fruit, showing that the data is properly adjusted to the

linearized form of the equation 5. For the other ripening states similar figures were obtained (data not shown).

Table 5 shows the parameters of the Arrhenius equation obtained in the fittings. In the case of the ripening state it is observed that the values of the activation energy (*Ea*) and the parameter K_0 tend to increase with the soluble solids content. This trend is similar to that shown by other types of juices (Guerrero and Alzamora, 1997, 1998; Ibarz *et al*., 2009), although the values of the activation energy of flow is lower, which is attributed to the presence of pulp in the carambola juice studied.

Table 5. Parameters of the Arrhenius equation for different carambolo juice soluble solids content and ripening stages.

State	C (°Brix)	K_0 (mPa·s)	E_a (kJ mol^{-1})	R^2
Mature	41.60	17.3x10^{-3}	19.39	0.981
	31.50	9.3x10^{-3}	18.92	0.997
	22.50	10.1x10^{-3}	16.97	0.998
	8.10	4.13x10^{-3}	11.39	0.995
Intermediate	40.10	8.05x10^{-3}	16.88	0.980
	31.60	8.17x10^{-3}	17.60	0.984
	21.30	5.33x10^{-3}	16.65	0.993
	7.20	8.64x10^{-3}	13.52	0.980
Green	42.50	9.32x10^{-3}	17.01	0.975
	33.00	6.10x10^{-3}	17.55	0.996
	22.00	13.38x10^{-3}	14.56	0.993
	5.20	11.52x10^{-3}	17.65	0.995

It is observed that the activation energy of flow varies from 19.39 to 11.39 kJ/mol, for juices of 41.6 °Brix and 8.1 °Brix, respectively, indicating that for more concentrated juices one variation of temperature will affect more the viscosity variation. To the intermediate ripening state is observed a similar trend, although the range of variation of the activation energy of flow values is lower. In the case of juice obtained from unripe carambola, there is not obtained a definite trend of the variation in activation energy with the soluble solids content, with values close to 17 kJ/mol.

This trend is similar to that shown by other types of juices (Diamante and Umemoto, 2015), although the values of the activation energy of flow are lower, which is attributed to the presence of pulp in the carambola juice studied.

Figure 5 shows data for juice made from ripe fruit; it is properly adjusted to the linearized form of the equation 5. For the other ripening states, similar figures were obtained (data not shown).

It is observed that the activation energy of flow varies from 19.39 to 11.39 kJ mol^{-1}, for juices of 41.6 °Brix and 8.1 °Brix, respectively. For the intermediate ripening state a similar trend is observed, although the range of variation of the activation energy of flow values is lower. In the case of juice obtained from unripe carambola, a definite trend of the variation of activation energy with the soluble solids content, with values close to 17 kJ mol^{-1} was not obtained.

Effect of soluble solids

The data shown in Tables 2, 3 and 4 are set to an exponential equation, allowing to predict the viscosity of the samples in function of the soluble solids content:

$$\eta = \eta_0 \exp(bC) \qquad (6)$$

where η is the viscosity, η_0 is the viscosity when the soluble solids content is 0 °Brix, b is a constant and C is the concentration expressed in °Brix.

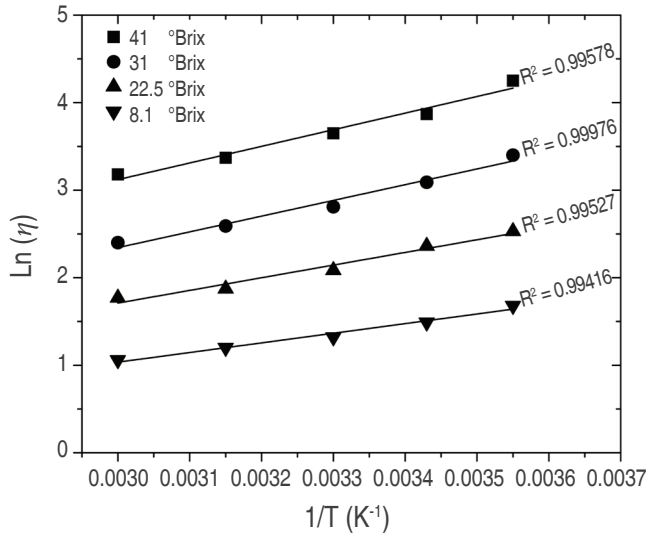


Figure 5. Effect of temperature on mature state carambolo juice viscosity in function of soluble solids.

Table 6 shows the parameter values of the settings and the correlation coefficients for each temperature tested for the three ripening stages. In the case of juice from ripe fruits and intermediate, the trend of the parameters η_0 and *b* is to decrease when temperature increases. Similar trends have been observed in other works (Ibarz *et al*., 2009).

Table 6. Exponential equation parameters of the effect of soluble solids in the carambolo juice viscosity at different temperatures and maturation states.

State	T (°C)	η_0 (mPa·s)	b (°Brix^{-1})	R^2
Mature	10	2.68	0.077	0.996
	20	2.34	0.071	0.996
	30	1.99	0.068	0.993
	40	1.80	0.065	0.988
	50	1.55	0.064	0.981
Intermediate	10	2.03	0.052	0.992
	20	1.60	0.050	0.994
	30	1.33	0.048	0.993
	40	1.12	0.047	0.994
	50	1.02	0.046	0.995
Green	10	2.32	0.044	0.977
	20	1.91	0.041	0.980
	30	1.52	0.041	0.954
	40	1.18	0.043	0.948
	50	0.98	0.043	0.959

The parameter *b* is a measure of the influence that the soluble solids content exerted on the viscosity, and since its value decreases with increasing temperature, it indicates that higher temperatures influence less on the change in viscosity when soluble solids content varies, (considered statistically insignificant). In the case of juice from unripe fruits, the parameter *b* hardly varies with the temperature change, indicating that the changes in soluble solids content similarly influenced, independently of the working temperature considered.

Combined effect temperature-soluble solids content

From the engineering point of view, it is useful to have a single equation that describes the combined effect of temperature and soluble solids content on the viscosity of the carambola juice. Different authors have used diverse equations (Assis *et al*., 2006; Bozdogan, 2015; Guerrero and Alzamora, 1997, 1998; Ibarz *et al*., 2009). Since the influence on the viscosity of the temperature and the soluble solids is described by exponential equations (Eq. 5 and 6), in this work it has been used the following equation:

$$\eta = a\exp\left(\frac{E_a}{RT} + bC\right) \qquad (7)$$

in which a is a pre-exponential factor, E_a is the activation energy of flow, b is a constant, R is the gas constant, T is the absolute temperature, and C is the concentration expressed in °Brix.

In both cases, both the fittings and the estimates of the parameters prove significant at a probability level of 95%. Table 7 shows the parameters values obtained in the adjustment analyses. It is observed that the values of the activation energy of flow are similar for the three ripening states, so that the temperature effect on the carambola juice viscosity is analogous and independent of the ripening state. For the parameter *b*, it is noted that its value increases with the ripening state increasing, indicating a greater effect of concentration on the viscosity as fruit ripening increases.

Table 7. Combined effect temperature-soluble solid content on viscosity carambola juice with different state of maturation.

Estado	*a* (mPa·s)	E_a (kJ/mol)	*b* ($^oBrix^{-1}$)	R^2
Mature	$2.526x10^{-3}$	16.83	0.069	0.988
Intermediate	$2.213x10^{-3}$	16.17	0.048	0.990
Green	$1.967x10^{-3}$	16.69	0.042	0.970

CONCLUSIONS

The study describes the characterization, not reported before, of the fruits and juices of *Averrhoa carambola* in three ripening stages. The maximum activity of carambolo polyphenoloxidase, for all stages, allowed to classify as basic type; the maximum temperature of the enzyme activity was observed at 40 °C, with a potential inhibition from 50 °C and low activity at 10 °C. The characterization of the polyphenoloxidase enzyme of carambolo fruits can be performed at temperatures below 20 °C and pH less than 7, which would slightly control enzymatic browning.

The rheological study of carambolo juice allowed to establish that the viscosity variation with temperature is adjusted to the Arrhenius equation, whereas the effect of soluble solids content on the viscosity of the samples was adequately described by an exponential equation. This appears to be the first work to study the rheological properties and polyphenol oxidase enzyme of *A. carambola* fruit in our country; the information provided here could be helpful to the successful development of new food functional products.

All authors have no conflict of interest to declare.

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Development of mango (*Mangifera indica* L.) energy drinks

Desarrollo de bebidas energizantes de mango (*Mangifera indica* L.)

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ABSTRACT

The effect of two hydrocolloids, pectin and carboxymethyl cellulose (CMC), was evaluated in mango beverage stability (*Mangifera indica* L.) formulated and developed with caffeine at a concentration of 30 mg/100 mL. The physico-chemical and sensory characteristics of color, acidity, viscosity, total soluble solids, pH, flavor, aroma and texture were studied every three days over a 12-day period. The beverages were packaged in high-density polyethylene containers with a 250 mL capacity and were stored at 5 °C and 90% RH for the duration of the experimentation period. The drinks with added pectin showed greater stability and lower acidity values than the control, but higher values than those prepared with CMC. The drinks made with CMC had a significantly higher viscosity at a 95% confidence level than those made with pectin or the control beverages. The treatment that showed the lowest browning index was the one added with pectin. Concerning the sensory evaluation, the drinks showed significant differences at a 95% confidence level; the drink made with pectin was the most widely accepted. It was concluded that the most stable drinks were those made with pectin because they presented the lowest height in millimeters of precipitate solids over the storage period. No off-flavors in beverages were perceived by the judges.

Key words:
Agroindustry
Caffeine
Pectin
Carboxymethyl cellulose

RESUMEN

Se evaluó el efecto de dos hidrocoloides, pectina y carboximetil celulosa (CMC) en la estabilidad de bebidas de mango (*Mangifera indica* L.) adicionadas con cafeína en concentración de 30 mg/100 mL. Se estudiaron las características físico-químicas y sensoriales; color, acidez, viscosidad, sólidos solubles totales, pH, sabor, aroma y textura bucal, cada tres días durante doce días. Los refrescos fueron empacados en envases de polietileno de alta densidad con capacidad para 250 mL y se almacenaron a 5 °C y 90% de HR durante el tiempo de experimentación. Las bebidas adicionadas con pectina mostraron mayor estabilidad y valores de acidez más bajos que él control, pero superiores a las preparadas con carboximetilcelulosa (CMC). Las bebidas con CMC presentaron una mayor viscosidad con un nivel de confianza del 95% respecto a las elaboradas con pectina y el control. El tratamiento que mejor color presentó fue el adicionado con pectina. Las bebidas en cuanto a su evaluación sensorial presentaron diferencias significativas con un nivel de confianza del 95%, siendo la de mayor aceptación de acuerdo a la evaluación sensorial la elaborada con pectina. Se pudo concluir que las bebidas con pectina presentaron menor altura de sólidos precipitados expresados en milímetros durante el tiempo de almacenamiento. No se percibieron sabores extraños en las bebidas por parte de los jueces.

Palabras claves:
Agroindustria
Cafeína
Pectina
Carboximetil celulosa

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The food hydrocolloids are high molecular weight polysaccharides, which are water soluble, used in a variety of functions in food systems for increasing viscosity, forming gel structures and films, controlling crystallization, inhibiting syneresis, improving texture, encapsulating flavors and increasing physical stability, among others. Due to its functional properties such as water retention capacity, balance of rheological properties and ionization of aqueous solutions, it is used to control the instability of food and the suspension of insoluble particles (Figueroa, 2015).

In food industry, polysaccharides are commonly used as emulsifiers to stabilize beverages. The typical beverage emulsion usually contains an oil phase and aqueous phase. The oil phase is comprised of flavor (such as orange oil, lemon oil, among others) while the aqueous phase that includes emulsifiers, acids and antimicrobial agents, which need to be stabilized (Zhao *et al*., 2015).

The beverages should be stable, homogeneous and without particle aggregation. However, there are three physical phenomena that cause instability of a suspension: sedimentation, aggregation and coalescence. Sedimentation is the result of a density difference between the dispersed phase and the continuous phase, and produces two separate phases with different concentrations and viscosities. The suspended particles settle differently, depending on their characteristics and concentration. The discrete particles are those whose characteristics do not change (shape, size and density). In the free sedimentation (i.e., without interference between particles), the particles are held by hydraulic forces and the fall can be described by Stokes' law (Figueroa, 2015).

The technological problem of these juices is to stabilize particles during storage due to the presence of pectin methyl esterase (PME). The result is an unattractive beverage in its sensory properties. This separation is a serious quality defect, which reduces the attractiveness and marketability of the product. Usually, the heat treatment used for PME inhibition stabilizes the colloids and the suspended particles. However, hydrocolloids negative charge (like sodium alginate, CMC and Arabic gum) in concentrations as low as 0.05% completely inhibits juice clarification. Furthermore, gum absorption on particles may give rise to steric repulsion stabilizing the beverages (Ibrahim *et al*., 2011).

Energy drinks first appeared in the 1960s in Europe and Asia. However, the introduction and aggressive marketing of Red Bull in Austria in 1987, and in the U.S. in 1997, created a trend towards the consumption of energy drinks with high caffeine content. Energy drink production has seen exponential growth since its inception, with nearly 500 new brands reportedly launched around the world in 2006 and 200 brands launched in the US over a 12-month period ending in July 2007 (Reissig *et al*., 2009). A study by Aranda *et al*. (2006) found that the main active ingredient in energy drinks was caffeine, although the authors also found that substances including taurine, riboflavin, pyridoxine, nicotinamide and other B vitamins were present, along with various phytochemical derivatives.

The population that consumes energy drinks is varied; it includes athletes, students and executives. Energy drinks are attractive to consumers because they provide the temporary benefits of increased alertness, improved mood and increased mental and physical energy (Costa *et al*., 2014). Consumers expect the consumption of these drinks to provide a stimulating effect, particularly of a physiological nature (Reissig *et al*., 2009). A study by researchers at the Pontificia Universidad Javeriana defined attention as a central processing control mechanism that acts in accordance with the objectives of the organism by activating and inhibiting processes; it can be directed towards the senses, knowledge structures in memory, and response systems. Within this framework, the researchers found that energy drinks make participants to react faster. This type of energizing beverage can also have an effect mainly on the organism's peripheral nervous system, leading to increased tonic components in attentional processes, which are related to processes such as waking (arousal or mental), physical endurance and preparing the organism to respond faster (Aguilar *et al*., 2008).

Studies have shown that consumption of fruit drinks among teenagers declined from 1999 to 2008, while consumption of energy drinks tripled. Energy drinks contain added sugars and high amounts of caffeine, which is often supplemented with other natural stimulants to enhance its effects. They may also contain vitamins, minerals and proteins intended to increase concentration and physical performance (Larson *et al*., 2014).

Mango (*Mangifera indica* L.) is cultivated in 16 Colombian departments, of which Tolima is one of the main producers. It is a species of Indian origin planted at altitudes ranging from sea level to 1650 m (Corpoica, 2013). The color of mangoes varies depending on their stage of maturation and variety; some go from being green to purple and red, while others are yellow in their mature stage. Sixteen mango varieties exist in the Colombian market. These are classified into two groups: mangoes of creole origin and table mangoes, the latter of which are improved varieties. The grade Brix of mangoes depends on the altitude where they are cultivated (Garcia, 2009).

Mango is a commercial fruit widely consumed at a national and global scale. Consumers also accept processed products. These include fruit drinks, which are prepared from fresh mango pulp or reconstituted dried pulp, added with water, sweeteners, flavorings and other additives (according to national legislation). These are subjected to the preservation treatment deemed appropriate for the manufacture of these products. It should be kept in mind that the Colombian Institute for Technical Standards and Certification (ICONTEC) is responsible for defining the standards and quality parameters for products (ICONTEC, 1999).

In order to develop an energy drink from mango, it is necessary to consider the resolutions that regulate the ingredients used in commercial products. These include NTC 3549, regarding fruit drink processing, and resolution 4150 of 2009, which establishes the technical regulations on the requirements that energy drinks must meet for human consumption (ICONTEC, 1999).

The objective of the present study was to evaluate the stability of mango energy drinks with caffeine, added with hydrocolloids such as pectin and CMC.

MATERIALS AND METHODS

Materials and equipment

The energy drinks were prepared using unripe mangoes with a maturity index of 0 according to NTC 5210. The fruit was acquired from the Central Minorista market (Jose Maria Villa) of Medellin, Colombia. Mangoes that showed no signs of mechanical damage or deterioration and were homogeneous in size and color were selected and standardized. To characterize the pulp of the fruit, an Ohaus balance with a precision of 0.01 g and a Leica Abbe auto refractometer with a 0-32% scale were used by applying the AOAC 932.12/90 method. The acidity was determined by acid-base titration with 0.1N NaOH, and was expressed as a percentage of citric acid, by applying AOAC 942.05/90 method. The pH was measured with a CG-840B Schott digital potentiometer, and viscosity was measured with an Ostwald viscometer (Bernal, 1993).

Pulp preparation

The fruits were washed and sanitized by immersion in sodium hypochlorite (50 ppm) for 15 min. They were then rinsed, peeled, and processed, with a small amount of water added to facilitate this process. They were subsequently homogenized using an Oster blender and sieved to separate the pulp.

Production of the drinks

According to previous essays and NTC 3549, there were selected two stabilizers for the treatments: one with 0.1% CMC and the other one with 0.1% pectin. The energy drinks were prepared with a share of 25% pulp, 12° Brix total soluble solids, and a caffeine content of 30 mg per 100 mL. A third treatment without a stabilizer was used as control. To produce the beverages, sucrose and pulp were added to water and the mixture was then gently stirred until it became homogenized. After the hydrocolloids were added, the solution was homogenized again for five minutes. The products obtained were packaged in sterile 250 mL polyethylene bottles and refrigerated (5 °C) for 12 days which it is normal for fruit beverages without heat treatment time. Each beverage was manufactured six times (repetitions), and all of the analyses were performed on the six samples in each treatment for day of evaluation. The test were destructive analyzes for each sample and for each day. The drinks were evaluated for total soluble solids, pH, acidity, viscosity, color, and precipitate height in plastic bottles after 3, 6, 9 and 12 days of storage. Sensory quality was evaluated after the 12-day storage period. The effect of stabilizing agents in beverages was measured with the height of precipitate in centimeters using a calibrator, something that could be related to turbidity obtaining supernatant phase.

Browning index

The effect of the stabilizers on the browning rate in mango drinks with caffeine added was determined by measuring

color using a sphere spectrophotometer with a D65 illuminant and a 10° observation angle. The equipment used was a Model SP64 sphere spectrophotometer, X-RITE Inc., MI, USA. The evaluation was performed every 3 days during a 12-day period. The readings were obtained in the CIE-L*a*b* (Commission Internationale d'Eclairage) color coordinates, where "L" stands for brightness, scaled from 0 to 100, "a" represents the range of colors from green to red, and "b" represents the range of colors from blue to yellow. Measurements were taken for the six experimental units (EU) in each treatment. The browning index (BI) was calculated based on Equations 1 and 2 (Maskan, 2001).

$$BI = \frac{[100(X - 0{,}31)]}{0{,}17} \quad (1)$$

$$x = \frac{(a* + 1{,}75L*)}{(5{,}645L* + a* - 3{,}012b*)} \quad (2)$$

Sensory analysis

The beverages were subjected to sensory evaluation by 15 panelists knowledgeable about the sensory characteristics of mango drinks. The panelists received approximately 20 mL of each sample in plastic cups coded with random three-digit numbers, at temperatures of 7 °C - 8 °C. They were also offered water to clean their palates. The panelists quantified the perceived intensities of each attribute using a scale unstructured without neutral, with a ranging from 1 (low) to 5 (high). The attributes evaluated were the following: appearance (color and consistency), aroma, flavor (sweetness and acidity) and texture in the mouth (viscosity) (Márquez, 2015).

Statistical analysis

The experimental design consisted in a randomized complete block, with three treatments and six replications, for 18 experimental units per day of evaluation, measuring were performed for days 0, 3, 6, 9 and 12. Statistical analysis of the mean, standard error and anova were applied, using the statistical program Statgraphics plus.

RESULTS AND DISCUSSION

The physicochemical measurements for the mango pulp used in the production of the energy drinks showed the following characteristic values: °Brix = 7 ± 0.1; pH = 2.29 ± 0,13; and acidity = 1.17% ± 0.15.

Figure 1A shows that the pectin and CMC treatments had higher soluble solid concentrations than the control; this was likely due to the action of these two substances as stabilizers that facilitated moisture retention and sugar generation in the energizing drink. Another relevant factor could be the temperature of the medium. Figure 1B shows that viscosity changed depending on the compound used as stabilizer; the drink containing CMC had the highest viscosity (19 to 23 cp), while the formulation containing pectin was less viscous, but had a higher viscosity than the control. This is because the CMC molecules generate negative charges due to the carboxyl groups, leading to electrostatic repulsion between the chains, which was manifested in the increased viscosity of the beverage (Fennema, 1993).

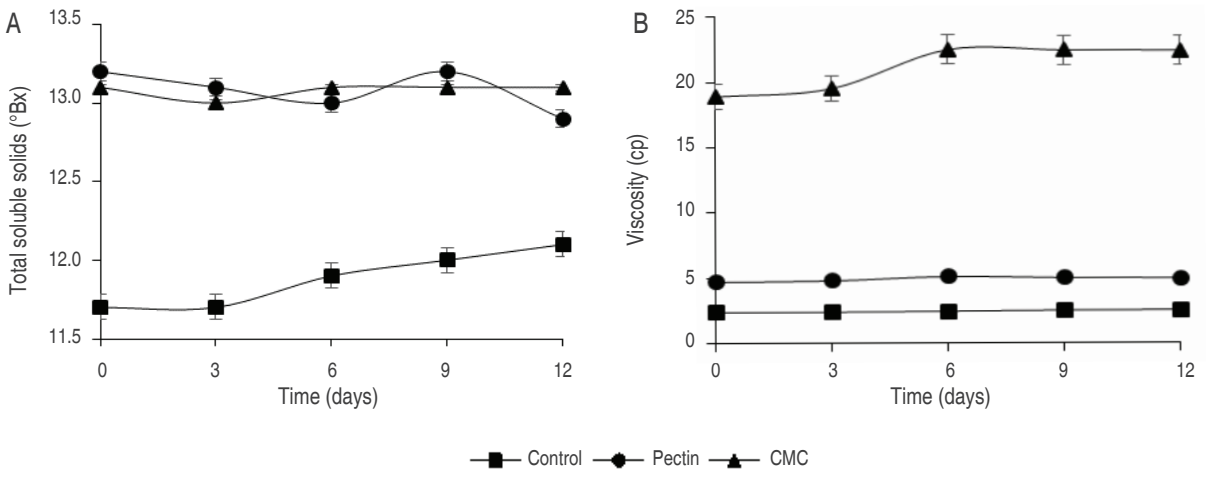

Figure 1. Effect of hydrocolloids on ºBrix (A) and viscosity (B).

The acid content was found to increase for all treatments during the storage period. This was due to the acid synthesis produced by the reactions of phytochemical substances. As shown in Figure 2A, this change was for the treatment without stabilizer. The samples with the CMC treatment maintained the most stable acid content during the storage period. Figure 2B shows that the drinks with CMC as a stabilizer had the highest pH values (the least acidic drinks), followed by those containing pectin. The drinks with the lowest pH (highest acidity) were those containing no stabilizer; thus, the most acidic drinks had the lowest pH.

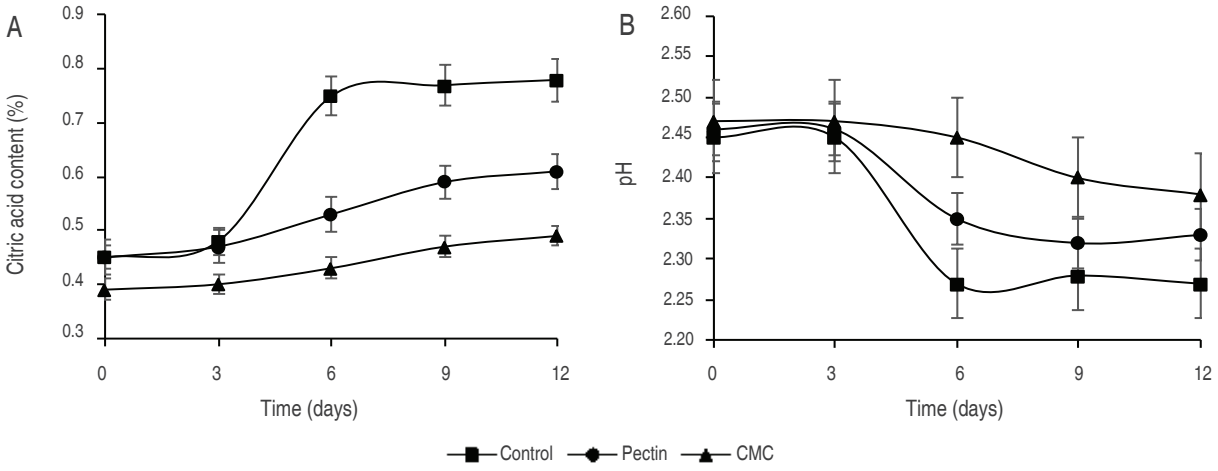

Figure 2. Effect of hydrocolloids on acidity (A) and pH (B).

Figure 3A shows the browning index, which is a function of the CIEL*a*b* parameters measured with the spectrophotometer. The shelf life of food is determined by color variation, respiration rate, pulp firmness, among others (Vignoni *et al.,* 2006). The browning index in the energy drinks was analyzed as a factor of product lifetime. Temperature and light degrade components associated with the color of the drink, usually flavonoids. The drinks previously made were stored in a cool, dark room, which is why the browning index decreased, improving the visual appearance of the beverages. Figure 3B shows the stability of the beverages as a function of the level of precipitates. It can be observed that the pectin samples presented the best hydrocolloid action, showing significant differences from the CMC samples and the control, with a 95% confidence level. This is due to L-rhamnose and D-xylose functional groups, which are capable of reacting with the solutes in the beverages to cause stabilization (Fennema, 2010).

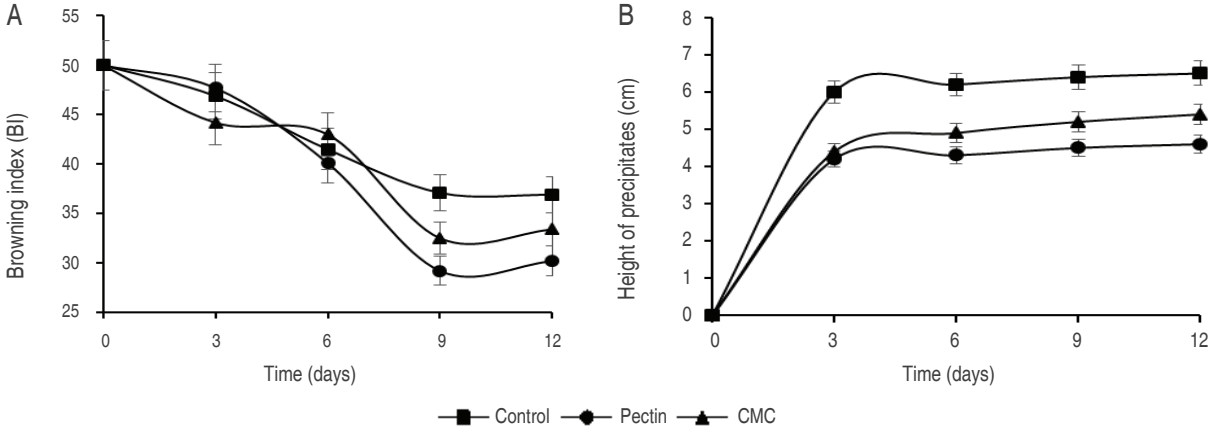

Figure 3. Effect of hydrocolloids on browning index (A) and stability of beverages with height of precipitates.

The sensory analysis established that the drinks with pectin had the highest acceptance level, with 95 % significance, compared to the control and the CMC beverages, because the latter had a high viscosity. This parameter is directly related to the attribute of texture in the mouth, which was less acceptable for the panelists in the CMC beverages.

The sensory evaluation showed that the drink with added pectin was the most widely accepted, with a 95% confidence level, for the attributes of flavor, color, and texture in the mouth. For aroma, no significant difference was found between the treatments. In the sensory evaluation by judges they found no extraneous flavors therefore considered that caffeine did not affect drinks.

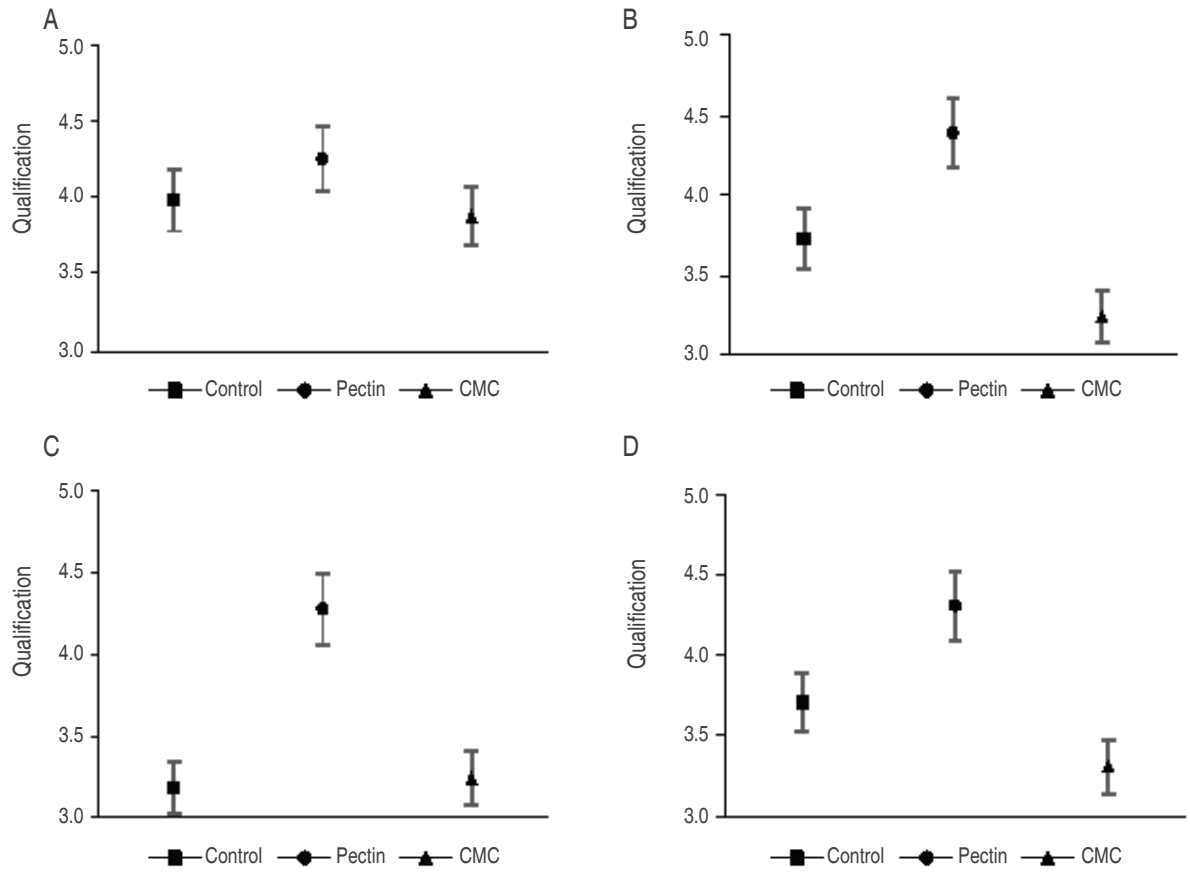

Figure 4. Sensory evaluation of aroma (A), flavor (B), color (C) and texture in the mouth (D)

CONCLUSIONS

The best stabilizer for the mango drinks added was with pectin since the beverages manufactured with this stabilizer presented less precipitate solids with a height of 4.4 cm over the storage period. They also were the best formulation according to sensory characteristics and they can be consumed as energy drinks.

The browning index decreased for all treatments, which is related to the loss of brightness in the drinks.

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ÍNDICE DE AUTORES

POLÍTICA EDITORIAL

REVISTA FACULTAD NACIONAL DE AGRONOMÍA

La Revista Facultad Nacional de Agronomía (RFNA), es una publicación de la Facultad de Ciencias Agrarias de la Universidad Nacional de Colombia - Sede Medellín. Esta orientada a profesores, investigadores, estudiantes, extensionistas y a todos aquellos profesionales que crean conocimiento y articulan la ciencia y la tecnología para hacer más productivo el campo a nivel empresarial y de economía campesina.

La periodicidad de la Revista es semestral, con circulación nacional e internacional y tiene como objetivo divulgar artículos escritos en inglés, originales e inéditos de carácter científico que respondan a preguntas específicas y que proporcionen soporte y pruebas a una hipótesis, en aspectos relacionados con las Ciencias Agronómicas, Zootecnia, Ciencias Forestales e Ingeniería Agrícola y de Alimentos y otras afines que contribuyan a la solución de los limitantes del agro en el trópico.

Teniendo en cuenta los criterios considerados por Colciencias, la revista acoge documentos de las siguientes tipologías:

Artículos de investigación científica y tecnológica: Documento que presenta, de manera detallada, los resultados originales de proyectos terminados de investigación. La estructura generalmente utilizada contiene cuatro partes fundamentales: Introducción, metodología (materiales y métodos), resultados y discusión, y conclusiones.

Artículos de revisión: Documentos producto de una investigación terminada donde se analizan, sistematizan e integran los resultados de investigaciones publicadas o no publicadas, sobre un campo en ciencia o tecnología, con el fin de dar cuenta de los avances y las tendencias de desarrollo. Se caracteriza por presentar una cuidadosa revisión bibliográfica de por lo menos 50 referencias.

Artículos de reflexión: Documento que presenta resultados de investigación terminada desde una perspectiva analítica, interpretativa o crítica del autor, sobre los temas específicos ya citados, recurriendo a fuentes originales.

Artículos cortos: Documento breve que presenta resultados originales preliminares o parciales de una investigación científica o tecnológica, que por lo general requieren de una pronta difusión. Para todos los casos el 60% de las citas debe provenir de artículos publicados en los últimos diez años.

Los artículos deben ser presentados de acuerdo a los lineamientos establecidos en las “Instrucciones a los Autores”; quienes incumplan las normas básicas no iniciarán el proceso editorial. Se debe diligenciar el formato “Autorización para Publicación de Obras y Cesión de Derechos Patrimoniales”, el cual será suministrado por la Revista. Dicho documento es explícito en mencionar que todos los autores están informados y de acuerdo con someter el artículo a consideración de la Revista, que no hay conflictos de interés entre ellos y expresa que el contenido del manuscrito no ha sido ni será enviado para su publicación a otra Revista.

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Los artículos pueden ser enviados al correo electrónico: rfnagron_med@unal.edu.co, o a través del Open Journal System en el Portalde Revistas de la Universidad Nacional de Colombia http://www revistas.unal.edu.co/. Sólo serán considerados artículos escritos en inglés. Adjunto se debe remitir el formato "Autorización para Publicación de Obras y Cesión de Derechos Patrimoniales", en el cual se acepta la no postulación simultánea del artículo a otras revistas u órganos editoriales y se ceden los derechos a la Revista para su difusión. Las formas de publicación son: artículos de investigación científica y tecnológica, artículos de revisión, artículos de reflexión y artículos cortos. Los artículos pueden ser elaborados por profesores y/o investigadores de la Universidad Nacional de Colombia, o cualquier otra institución afín, nacional o internacional, en los temas Agropecuarios, Forestales y de Ingeniería Agrícola y de Alimentos. La extensión no debe exceder de 5.200 palabras, las hojas deben ser tamaño carta, escritas a interlineado sencillo, letra o fuente Times New Roman o Verdana, tamaño 12 puntos, márgenes de 3 cm en la parte superior, 2 cm en la inferior y 2,5 cm en las márgenes laterales derecha e izquierda. Las tablas y figuras (es decir, los gráficos, dibujos, esquemas, diagramas de flujo, fotografías y mapas) se deben mostrar incorporadas en el texto y con numeración consecutiva (Tabla 1... Tabla n; Figura 1... Figura n, etc.). Los textos y tablas se deben presentar en el procesador de palabras MS-Word®;las tablas y los diagramas de frecuencia (barras y tortas) originales se deben suministrar en el archivo del documento y también en su original de MS-Excel®; otras figuras, como fotografías sobre papel y dibujos, se pueden enviar en original o escaneadas y ser remitidas en el formato digital de compresión JPG (o JPEG) preferiblemente con una resolución de 600 x 600 dpi (mínimo 300 dpi); es deseable que las fotografías originales sean enviadas como diapositivas. Como norma general, las tablas y figuras sólo se aceptan en blanco y negro; excepcionalmente se incluirán en color cuando sea estrictamente necesario y a juicio del Comité Editorial.

Unidades, abreviaturas y estilo

Se debe utilizar el Sistema Internacional de Unidades (SIU), y aquellas unidades específicas de mayor uso por parte de la comunidad científica. Cuando se requiera se debe usar la forma exponencial. Ejemplo: kg ha^{-1}. El significado de las abreviaturas debe citarse por extenso cuando se mencionan por primera vez en el manuscrito. El estilo de escritura debe ser absolutamente impersonal, en tiempo gramatical pasado para la introducción, los procedimientos y los resultados y presente para la discusión, evitando la conjugación de verbos en primera o tercera persona del singular o el plural.

Los números del uno al nueve se escriben en palabras, excepto cuando incluyen unidades de medida o se mencionan varios números. Ejemplo: "ocho tratamientos", "3, 7 y 9 lecturas", "15 kg". Use cero antes del punto decimal. Para separar números en intervalos de uno o más años, use la letra "a", y guión para temporadas de crecimiento. Ejemplo: Periodo 2002 a 2005; temporadas de crecimiento 1999-2000, 2000-2001.

Título y autores

El título del artículo no debe incluir abreviaturas y es obligatoria su respectiva traducción al idioma español. En lo posible, el título no debe exceder de 15 palabras y debe reflejar con precisión el contenido del documento. Cuando contenga nombres científicos de especies vegetales o animales, éstos se deben escribir con letra cursiva (itálica) en minúsculas, sólo con mayúsculas la primera letra del género y del clasificador. Debajo del título en inglés se escribe el nombre(s) y apellido(s) de los autores, sin sus respectivos títulos académicos, ni cargos laborales, en una línea horizontal y de acuerdo con su contribución en la investigación y/o preparación del artículo.

Como nota al pie de la primera página, se escribe el título de pregrado, el cargo laboral de los autores, el nombre y la ciudad de ubicación de la entidad a la cual prestan sus servicios o del patrocinador para la realización del trabajo y su respectiva dirección de correo electrónico, indicando el autor de correspondencia. Además, se debe adjuntar un resumen de la hoja de vida de los autores, donde se mencionen los artículos publicados en otras revistas.

Resumen, abstract y palabras claves

El resumen no debe exceder de 250 palabras escritas en un único párrafo. Se debe escribir en inglés y español. Debe contener en forma breve la justificación, los objetivos, los métodos utilizados, los resultados obtenidos más relevantes y las conclusiones. Es obligatorio acompañar el resumen con un máximo de seis palabras clave distintas a las utilizadas en el título. Se aceptan como palabras clave no sólo las palabras simples, sino también términos compuestos hasta de tres palabras. Deben ir escritas en minúsculas y separadas por comas.

Introducción

Puede tener o no título. Define el problema e informa sobre el estado del arte respecto al tema principal del artículo; además, señala las razones que justifican la investigación y plantea los objetivos de la misma. Es obligatorio acompañar los nombres vulgares con el nombre(s) científico(s) y la abreviatura(s) del clasificador en la primera mención dentro del texto. No se deben mencionar marcas de productos, sino su nombre genérico o químico

Materiales y métodos

En este apartado se deben describir en forma clara, concisa y secuencial, los materiales (vegetales, animales, implementos agrícolas o de laboratorio) utilizados en el desarrollo del trabajo; además, se mencionan los aspectos relacionados con la ubicación, preparación y ejecución de los experimentos. Se debe indicar el diseño seleccionado, las variables registradas, las transformaciones hechas a los datos, los modelos estadísticos usados y el nivel de significancia empleado. Evitar detallar procedimientos previamente publicados.

Resultados y discusión

Son la parte central del artículo, deben estar respaldados por métodos y análisis estadísticos apropiados. Se deben presentar de manera lógica, objetiva y secuencial mediante textos, tablas y figuras; estos dos últimos apoyos deben ser fáciles de leer, autoexplicativos y estar siempre citados en el texto. Las tablas se deben elaborar con pocas columnas y renglones. Se debe tener

la precaución de incluir el nivel de significancia estadística representado por letras minúsculas del comienzo del alfabeto (a, b, c, d,...), un asterisco simple (*) para *P*<0,05, doble asterisco (**) para P<0,01 o triple asterisco (***) para *P*<0,001. Las investigaciones que no siguen un diseño estadístico, deben mostrar la información de manera descriptiva. Use subíndices para modificaciones, reserve superíndices para potencias o notas al pie en tablas y figuras.

La discusión Se refiere al análisis e interpretación objetiva de los resultados, confrontándolos con los obtenidos en otras investigaciones, o con los hechos o teorías conocidos sobre el tema. Explica los resultados en particular cuando difieren de la hipótesis planteada. Destaca la aplicación práctica o teórica de los resultados obtenidos y las limitaciones encontradas. Resalta la contribución que se hace a una determinada área del conocimiento y el aporte a la solución del problema que justifica la investigación. Finalmente, proporciona elementos que permitan proponer recomendaciones o lanzar nuevas hipótesis. No se deben hacer afirmaciones que van más allá de lo que los resultados pueden apoyar.

Conclusiones

Son las afirmaciones originadas a partir de los resultados obtenidos, deben ser coherentes con los objetivos planteados y la metodología empleada; además, expresar el aporte al conocimiento en el área temática estudiada y proponer directrices para nuevas investigaciones.

Agradecimientos

Si se considera necesario, se incluyen los agradecimientos o reconocimientos a personas, instituciones, fondos y becas de investigación, que hicieron contribuciones importantes en la concepción, financiación o realización de la investigación.

Literatura citada

Sólo se listan las referencias bibliográficas mencionadas en el texto. No se aceptan notas de clase, artículos en preparación o en prensa, o cualquier otra publicación de circulación limitada. Se debe evitar el exceso de autocitas.

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En cada referencia para todos los autores cite primero el apellido seguido de la inicial del nombre sin puntos, separando autores con coma y espacio.

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Para libros: Autor (es). Año. Título del libro, edición, ciudad de su sede, casa editora y, páginas consultadas (pp. # - #) o páginas totales (# p.). Ejemplo: Robinson A, Morrison J, Muehrcke P, Kimerling AJ and Guptill S. 1995. Elements of Cartography. Sixth edition. John Wiley and Sons, Inc., New York. 674 p.

Para capítulos de libros: Autor (es). Año. Título del capítulo, páginas consultadas (pp. # - #). En: Apellidos y nombres de los compiladores o editores (eds.), título del libro, edición, casa editora y ciudad de su sede, páginas totales (# p.). Ejemplo: Bernal H. 1996. Capítulo 6: Evapotranspiración. pp. 112-125. En: Agrios G. (ed.). Fitopatología. Segunda edición. Editorial Limusa, México D.F. 400 p.

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Ponencias en memorias de congresos, seminarios, simposios: García M. 1998. La ingeniería geotécnica y la protección del medio ambiente. pp. 65-94. En: Memorias IX Congreso Colombiano de la Ciencia del Suelo. Sociedad Colombiana de la Ciencia del Suelo. Santa Fé de Bogotá.

Tesis, trabajos de grado. Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae). Tesis Magister en Bosques y Conservación Ambiental. Facultad de Ciencias Agropecuarias. Universidad Nacional de Colombia. Medellín. 78 p.

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Para citas de internet: Autor (es). Año. Título del artículo. En: Nombre(s) de la publicación electrónica, de la página web, portal o página y su URL, páginas consultadas (pp. # - #) o páginas totales (# p.); fecha de consulta. Ejemplo: Arafat Y. 1996. Siembra de olivos en el desierto palestino. En: Agricultura Tropical, http://agrotropical.edunet.es. 25 p.; consulta: noviembre 2003.

PUBLISHING POLICY

REVISTA FACULTAD NACIONAL DE AGRONOMÍA

The National Faculty of Agronomy Journal (RFNA) is published by the Faculty of Agricultural Sciences of Universidad Nacional de Colombia – Medellín. It is aimed at teachers, researchers and students in agronomy, animal, and forestry sciences, food and agricultural engineering, agricultural advisers and at all those professionals who create knowledge and articulate science and technology to make the field more productive at business and rural economy levels.

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Critical reflection articles: A document presenting completed research results from an analytical, interpretive or critical author's point of view, on the specific issues already mentioned, using original sources.

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Units, abbreviations and style

International System of Units (SI), and those specific units of greater use by the scientific community must be used. When required must be used the exponential form. Example: kg ha^{-1}. The meaning of abbreviations should be cited in full when first mentioned in the manuscript. The writing style should be totally impersonal. Introduction, procedures and results should be written in grammatical past tense. Discussion should be written in grammatical present tense, avoiding the conjugation of verbs in first or third person singular or plural.

The numbers from 1 to 9 are written in words, except when they include units of measure or several numbers are listed. Example: "eight treatments", "3,7 and 9 readings", "15 kg". Use zero before the decimal point. To separate numbers in intervals of one to two years, use the letter "a" and hyphen for growing seasons. Example period 2002a2005, growing seasons 1999-2000, 2000-2001.

Title and authors

The article should not include abbreviations and its translation into English is required. As far as possible, the title should not exceed 15 words and must accurately reflect the paper content. When the article contains scientific names of plants or animals, they should be written in italics in lower case, only the first letter of gender and classifier should be capital. Under the title in English the author or authors' name (s) and surname (s) is /are written, without academic degrees or job positions, in a horizontal line according to the contribution to research and / or preparation of the article.

As a footnote on the first page, write the title of undergraduate, authors' job positions, the name and city location of the entity to which they serve, or the sponsors for the research work and their respective email address. In addition, a summarized authors' résumé including reference to the articles published in other magazines should be attached.

Abstract and key words

The abstract should not exceed 250 words written in a single paragraph. It must be written in English, Spanish or Portuguese. It should contain in brief the justification, aims, methods used, the most relevant results, and conclusions. It is required to accompany the abstract with a maximum of six key words, translated into English, different from those used in the title. Single words as well as compound terms of up to three words are accepted as key words. They must be written in lowercase, separated by commas.

Introduction

It may or not have a title. It defines the problem and reports on the state of the art on the main subject of the article, it also points out the reasons for the research and sets out its aims. It is required to accompany common names with the corresponding scientific name (s) name and abbreviation (s) of the classifier at the first mention in the text. Brands must not be mentioned but the generic or chemical name.

Materials and methods

In this section, materials (crops, livestock, agricultural or laboratory implements) used in the development of work should be clearly, concisely and sequentially described. Aspects related to the location, preparation and execution of experiments should also be mentioned. The selected design, the recorded variables, the changes made to data, the statistical models used and the significance level used should be indicated. Authors must avoid detailing procedures previously published.

Results

They are the central part of the article and must be supported by appropriate statistical methods and analysis. They should be presented in a logical, objective and sequential way through texts, tables and figures; the latter two supports should be easy to read, self- explanatory and always quoted in the text. The tables should be composed by few columns and rows. Care should be taken to include the statistical significance level represented by lowercase letters of the beginning of the alphabet (a, b, c, d,…), a single asterisk (*) for $P<0.05$, double asterisk (**) for $P<0.01$ or triple asterisk (***) for $P<0.001$. Researches that do not follow a statistical design should display the information in a descriptive way. Use subscripts to modifications, reserve superscripts for potencials or footnotes in tables and figures.

Discussion

It refers to the analysis and objective interpretation of results, comparing them with those obtained in other researches, or with known facts or theories on the subject. It explains the results, especially when they differ from the stated hypothesis. It emphasizes the practical or theoretical application of the obtained results and constraints encountered. Discussion also highlights the contribution that is made to a particular area of knowledge and to the solution of the problem that justifies the research. Finally, it provides elements that allow making recommendations or launching new hypotheses. Statements that go beyond what the results may support should be avoided.

Conclusions

Conclusions are assertions arising from the obtained results. They should be consistent with the objectives stated and the methodology used. They should also express the contribution to knowledge in the studied subject area and propose guidelines for further researches.

Acknowledgements

If necessary, acknowledgements or recognitions to individuals, institutions, funds and research grants that made important contributions in the design, financing or carrying out of the research are included.

Cited Literature

Only bibliographical references cited in the text are listed. Lecture notes, articles in preparation or in press, or any other publication with limited circulation are not accepted. Excessive self-citation should be avoided.

The bibliography should be included at the end of the text, containing only the references cited in it, including the doi number. Citations in the text should include author's surname and year, with comma between author and year. Example: Pérez, 1995. They should also keep the following citation order:

- If more than one date, they are separated by commas: Example: Pérez, 1995, 1998, 2001.

- If there are two authors, they will be separated by the conjunction and. Example: Gil and Ortega, 1993.

If there are several works by an author, published in the same year, they will be cited with a letter in alphabetical sequence of titles, adjacent to year. Example: Gómez, 2000a, 2000b, 2000c.

For citations with three or more authors, it is necessary to mention in the text the surname of the first and replace the others by the Latin expression *et al.,* which means and others. All authors should be mentioned in the bibliography.

Personal communications should be cited at the bottom of the page and not included in the bibliography.

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The references should be arranged alphabetically by first author, without numbers and without indentation. To cite several publications by the same author, follow the chronological up order, if they are of the same year, follow the alphabetical order of the titles.

References should contain all the data allowing to its easy location.

Examples:

For books: Author (s), year. Book title, edition, place of publication, publisher and pages consulted (pp. # - #) or total pages (# p.). Example: Robinson A, Morrison J, Muehrcke P, Kimerling AJ and Guptill S. 1995. Elements of Cartography. Sixth edition. John Wiley and Sons, Inc., New York. 674 p.

For book chapters: Author (s), year. Chapter title, pages consulted (pp. # - #). En: Surnames and names of the editors or publishers (eds.), book title, edition, publisher and place of publication, total pages (# p.). Example: Bernal H. 1996. Chapter 6: Evapotranspiración. pp. 112-125. In: Agrios G. (ed.). Fitopatología. Second Edition. Editorial Limusa, México D.F. 400 p.

For journals: Author (s), year. Article title, journal full name volume(number): page-page. Example: García S, Clinton W, Arreaza L and Thibaud R. 2004. Inhibitory effect of flowering and early fruit growth on leaf photosynthesis in mango. Tree Physiology 24(3): 387-399. doi: 10.1093/treephys/24.4.387

Presentations in Memoirs of Congresses, seminars and symposia: García M. 1998. Geotechnical engineering and environmental protection. p. 65-94. In: Memorias IX Colombian Congress of Soil Science. Colombian Society of Soil Science. Santa Fé de Bogotá.

Theses and dissertations: Gómez C. 2004. Autoecología de mortiño (*Vaccinium meridionale* Swartz Ericaceae). Master's Thesis in Forestry and Environmental Conservation. Faculty of Agricultural Sciences. Universidad Nacional de Colombia. Medellín. 78 p.

Abril G. 2002. Biogeografía y descripción de las especies del género *Collaria* sp. en seis zonas lecheras del departamento de Antioquia, Dissertation. Faculty of Agricultural Sciences. Universidad Nacional de Colombia. Medellín. 49 p.

Citation of a citation: Magalhaes LM e da Cruz AJ. 1979. Phenology do pau-rosa (*Aniba duckei* Kostermans), Lauraceae, em floresta primária na Amazônia Central. Acta Amazónica 9(2): 227-232. Cited by: Gomez CP. 2004. Autoecología de mortiño (*Vaccinium meriodinale* Swartz Ericaceae). Master's Thesis in Forestry and Environmental Conservation. Faculty of Agricultural Sciences, Universidad Nacional de Colombia. Medellín. 46 p.

Journal Supplement: Silva AM y Carrillo NN. 2004. El manglar de piruja, Golfito, Costa Rica: un modelo para su manejo. Journal of Tropical Biology 52 Suppl. 2: 195-201.

For internet citations: Author (s), year. Article. In: electronic publishing Name (s), the web page, portal or page name and its URL, pages consulted (pp. #) or total pages (# p.), date of consultation. Example: Arafat Y. 1996. Siembra de olivos en el desierto palestino. In: Tropical Agriculture, http://agrotropical. edunet.es. 25 p.; accessed: November 2003.

POLÍTICA EDITORIAL

REVISTA FACULTAD NACIONAL DE AGRONOMÍA

A Revista Facultad Nacional de Agronomía é uma publicação da Facultad de Ciencias Agrarias da Universidad Nacional de Colombia – Sede Medellín. Orienta-se a professores, pesquisadores, estudantes e a todos os profissionais que criam conhecimento e articulam a ciência e a tecnologia para fazer o campo mais produtivo no âmbito empresarial e da economia camponesa.

A periodicidade da Revista é semestral, com circulação nacional e internacional e seu objetivo é divulgar artigos originais e inéditos de caráter científico que respondam perguntas específicas e forneçam suporte e provas a uma hipótese, em aspectos relacionados com das Ciências Agronômicas, Zootecnia, Ciências Florestais e Engenharia Agrícola e de Alimentos e disciplinas afins que contribuam à solução dos limitantes do agro no trópico.

Levando em conta os critérios considerados por Colciencias, a revista considera documentos das seguintes tipologias:

Artigos de pesquisa científica e tecnológica: Documentos que apresentam, de forma detalhada, os resultados originais de projetos de pesquisa concluídos. A estrutura utilizada contém, geralmente, quatro partes fundamentais: introdução, metodologia (materiais e métodos), resultados e discussão, e conclusões.

Artigos de revisão: Documentos produto de uma pesquisa concluída onde são analisados, sistematizados e integrados os resultados de pesquisas publicadas ou não publicadas, sobre um campo em ciência e tecnologia, a fim de dar conta dos avanços e tendências de desenvolvimento. Caracteriza-se por apresentar uma cuidadosa revisão bibliográfica de pelo menos 50 referências.

Artigos de reflexão: Documentos que apresentam resultados de pesquisa concluída com uma perspectiva analítica, interpretativa ou crítica do autor, sobre os temas específicos antes mencionados, recorrendo a fontes originais.

Artigos curtos: Documentos breves que apresentam resultados originais preliminares ou parciais de uma pesquisa cientifica ou tecnológica, e que geralmente precisam de uma rápida difusão. Para todos os casos o 60% das citações deve provir de artigos publicados nos últimos dez anos.

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Os artigos podem ser enviados ao endereço eletrônico: rfnagron_med@unal.edu.co ou também ingressando no site das Revistas da Universidad Nacional de Colombia usando o programa Open Journal System http://www.revistas.unal.edu.co/. Serão considerados apenas os artigos escritos em Inglés. Junto com o trabalho deverá encaminhar o formulário "Autorização para Publicação de Obras e Sessão de direitos" no qual se aceita a não postulação simultânea do artigo a outras revistas ou órgãos editoriais e cedem-se à Revista os direitos de difusão. As formas de publicação são: artigos de pesquisa científica e tecnológica, artigos de revisão, artigos de reflexão e artigos curtos. Os artigos podem ser elaborados por professores e/ou pesquisadores da Universidad Nacional de Colombia, ou qualquer outra instituição afim, nacional ou internacional, nos temas agropecuários, florestais, e de engenharia agrícola e de alimentos. A extensão não deve superar as 5.200 palavras, as folhas devem ser tamanho carta, escritas a duplo espaço, letra ou fonte Times New Roman ou Verdana, tamanho 12 pontos, margens de 3 cm na parte superior, 2 cm na inferior e 2,5 cm nas margens laterais direita e esquerda. As tabelas e figuras (isto é, gráficos, desenhos, esquemas, diagramas de fluxo, fotos e mapas) devem aparecer em folhas independentes e com numeração consecutiva (Tabela 1... Tabela n; Figura 1... Figura n. etc.). Os textos e tabelas devem ser apresentados no processador de palavras MS-Word®; as tabelas e diagramas de freqüência (gráficos de barras e de pizzas) originais devem aparecer tanto no arquivo do manuscrito quanto no original de MS-Excel®; outras figuras, como fotos sobre papel e desenhos, podem ser enviadas em original ou digitalizadas, e remitidas no formato digital de compressão JPG (ou JPEG) preferivelmente com uma resolução de 600 x 600 dpi (mínimo 300 dpi); é desejável que as fotos originais sejam enviadas como slides. Como norma geral, só serão aceitas tabelas e figuras em preto e branco; imagens coloridas serão incluídas só em caso estritamente necessário e a juízo do Comitê Editorial.

Unidades, abreviaturas e estilo

Deve utilizar-se o Sistema Internacional de Unidades (SIU), e aquelas unidades específicas de maior uso por parte da comunidade científica. Quando seja necesario debe-se usar a forma exponencial Exemplo: kg ha^{-1}. O significado das abreviaturas deve ser citado por extenso quando mencionadas por primeira vez no manuscrito. O estilo da escrita deve ser absolutamente impessoal, em tempo gramatical pretérito na introdução, procedimentos e resultados, e presente na discussão, evitando a conjugação de verbos em primeira ou terceira pessoa do singular ou do plural.

Os números de um a nove devem-se escrever em palavras, exceto quando refletem ou indicam unidades de medida ou se colocam vários números consecutivamente Exemplo: "oito tratamentos", "3, 7 y 9 leituras", "15 kg". Deve-se utilizar o zero antes do ponto decimal. Para separar intervalos de um o mais anos, deve-se usar a letra "a", e hífen para períodos de crescimento (safras). Exemplo. Período 2002 a 2005, safras 1999-2000, 2000-2001.

Título e autores

O título do artigo não deve incluir abreviaturas e é obrigatória sua tradução ao inglês. Sempre que possível, o título não deve superar as 15 palavras e deve refletir com precisão o conteúdo do documento. Em caso de conter nomes científicos de espécies vegetais ou animais, estes devem ir em itálica minúscula, com maiúscula somente a primeira letra do gênero e do classificador. Embaixo do título em inglês escreve-se o nome(s) e sobrenome(s) dos autores, sem seus títulos acadêmicos, nem cargos laborais, numa linha horizontal e conforme a sua contribuição à pesquisa e/ou preparação do artigo.

Na parte inferior da primeira página, como nota ao rodapé, escreve-se o cargo laboral dos autores, o nome e a cidade onde se localiza a entidade para a qual trabalham ou do patrocinador para a realização do trabalho e o correspondente endereço eletrônico. Adicionalmente, deve anexar-se um resumo do currículo dos autores, onde se mencionem os artigos publicados em outras revistas.

Resumo, abstract e palavras-chave

O resumo não deve superar as 250 palavras escritas num único parágrafo. Deve ser redigido em espanhol, inglês ou português. Deve conter em forma breve justificação, objetivos, métodos utilizados, resultados obtidos mais relevantes e conclusões. É obrigatório acompanhar o resumo com um máximo de seis palavras-chave, traduzidas ao inglês (key words), diferentes às utilizadas no título. Aceitam-se como palavras-chave não somente palavras simples, mas também termos compostos por até três palavras. Estas devem ir escritas em minúscula e separadas por vírgulas.

Introdução

O título não é obrigatório. Define o problema e informa sobre o estado da arte a respeito do tema principal do artigo, além disso, indica as razões que justificam a pesquisa e propõe os objetivos da mesma. É obrigatório acompanhar os nomes vulgares com o nome(s) científico(s) e a abreviatura(s) do classificador na primeira menção dentro do texto. Não mencionar marcas de produtos, mas nomes genéricos ou químicos.

Materiais e métodos

Aqui devem ser descritos em forma clara, concisa e seqüencial, os materiais (vegetais, animais, implementos agrícolas ou de laboratório) utilizados no desenvolvimento do trabalho, assim mesmo mencionam-se os aspectos relacionados com a localização, preparação e execução dos experimentos. Devem indicar-se o desenho escolhido, as variáveis registradas, as transformações feitas aos dados, os modelos estatísticos usados e o nível de significância empregado. Evitar detalhar procedimentos previamente publicados.

Resultados

São a parte central do artigo, devem ir respaldados por métodos e análises estatísticas apropriadas. Devem apresentar-se de maneira lógica, objetiva e seqüencial mediante textos, tabelas e figuras; estes dois últimos apoios devem ser de fácil leitura, interpretáveis de forma autônoma e ir citados sempre no texto. As tabelas devem conter poucas colunas e linhas. É preciso incluir o nível de significância estatística representado por letras minúsculas do começo do alfabeto (a, b, c, d,...), asterisco simples (*) para $P< 0,05$, duplo asterisco (**) para $P< 0,01$ ou três asteriscos (***) para $P<0,001$. As pesquisas que não obedecem um

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Discussão

Refere-se à análise e interpretação objetiva dos resultados, confrontando-os com os resultados obtidos em outras pesquisas, ou com os fatos ou teorias conhecidas sobre o tema. Explica os resultados, particularmente quando diferem da hipótese proposta. Destaca a aplicação prática ou teórica dos resultados obtidos e as limitações encontradas. Ressalta a contribuição a uma determinada área do conhecimento e o aporte à solução do problema que justifica a pesquisa. Finalmente, proporciona elementos que permitem propor recomendações ou lançar novas hipóteses. Não devem ser feitas afirmações que vão além do que os resultados podem apoiar.

Conclusões

São as afirmações originadas a partir dos resultados obtidos, devem ser coerentes com os objetivos propostos e a metodologia empregada; adicionalmente, expressar a contribuição ao conhecimento na área temática estudada e propor diretrizes para novas pesquisas.

Agradecimentos

Caso for necessário, incluir-se-ão os agradecimentos ou reconhecimentos a pessoas, instituições, fundos ou bolsas de pesquisa que fizeram contribuições importantes na concepção, financiamento ou realização da pesquisa.

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Devem aparecer somente as referências bibliográficas mencionadas no texto. Não se aceitam notas de aula, artigos em construção ou no prelo, ou qualquer outra publicação de circulação limitada. Evitar o excesso de auto-citas.

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-Se houver vários trabalhos de um autor publicados no mesmo ano, estes se citam com uma letra em seqüência alfabética dos títulos, do lado do ano. Exemplo: Gómez, 2000a, 2000b, 2000c.

-Em caso de citações com três ou mais autores, é preciso mencionar no texto os sobrenomes do primeiro e substituir os outros pela expressão latina abreviada *et al.* que significa y outros; já na bibliografia devem aparecer citados todos os autores.

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Exemplos:

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Para revistas: Autor(es), ano. Título do artigo, nome completo da revista (volume)número: página-página. Exemplo: García S, Clinton W, Arreaza L and Thibaud R. 2004. Inhibitory effect of flowering and early fruit growth on leaf photosynthesis in mango. Tree Physiology 24(3): 387-399. http://dx.doi.org/10.1093/treephys/24.4.38

Participações em memórias de congressos, seminários, simpósios: García M. 1998. La ingeniería geotécnica y la protección del medio ambiente. p. 65-94. Em: Memorias. IX Congreso Colombiano de la Ciencia del Suelo. Sociedad Colombiana de la Ciencia del Suelo. Santa Fé de Bogotá.

Teses, trabalhos de formatura. Gómez C. 2004. Autoecología del mortiño (*Vaccinium meriodinale* Swartz Ericaceae). Tese Mestrado em Bosques e Conservação Ambiental. Facultad de Ciencias Agropecuarias. Universidad Nacional de Colombia. Medellín. 78 p.

Abril G. 2002. Biogeografía y descripción de las especies del género *Collaria* sp. en seis zonas lecheras del Departamento de Antioquia, Trabalho de formatura. Facultad de Ciencias Agropecuarias. Universidad Nacional de Colombia. Medellín. 49 p.

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Para citas de internet: Autor(es), ano. Título do artigo. Em: Nome(s) da publicação eletrônica, da página web, portal ou página e sua URL, páginas consultadas (pp.#) ou páginas totais (# p.); data de consulta. Exemplo: Arafat Y. 1996. Siembra de olivos en el desierto palestino. Em: Agricultura Tropical, http://agrotropical. edunet.es. 25 p.; consulta: novembro 2003.

ÉTICA EN LA PUBLICACIÓN CIENTÍFICA Y ACUERDO SOBRE POSIBLES MALAS PRÁCTICAS

La revista Facultad Nacional de Agronomía espera y verificará que los autores, revisores, editores y en general la comunidad académica y científica involucrada en nuestro proceso editorial, sigan estrictamente las normas éticas internacionales requeridas en el proceso de edición.

La revista Facultad Nacional de Agronomía sigue las normas éticas presentes en el COPE Best Practice Guidelines for Journal Editors y por el International Standars for Editors and Authors publicado por Committee on Publication Ethics.

Los autores deben evitar incurrir al plagio de la información. La revista define los siguientes lineamientos, criterios y recomendaciones sobre la ética en la publicación científica:

1. Criterios generales[1]

1.1. Los artículos deben contener suficiente detalle y referencias que permitan replicar o rebatir el estudio.

1.2.Declaraciones fraudulentas o deliberadamente inexactas constituyen un comportamiento poco ético.

1.3. Si el estudio incluye productos químicos, procedimientos o equipos que tienen cualquier riesgo inusual inherente a su uso, el autor debe identificar claramente estos en el artículo.

1.4. Si el estudio implica el uso de animales o de seres humanos, el autor debe asegurarse que el artículo contenga una declaración que haga explícito que se realizaron todos los procedimientos de conformidad con las leyes y directrices institucionales.

1.5. Se deben respetar los derechos de privacidad de los seres humanos.

2. Autoría[2]

Criterios:

2.1. Un "autor" es la persona que ha hecho una contribución intelectual significativa al artículo, por lo tanto, todas las personas nombradas como autores deben reunir los requisitos de autoría, y todos aquellos que los reúnan deben ser mencionados de forma explícita.

2.2. Se deben cumplir colectivamente tres criterios básicos para ser reconocido como autor:

a) Contribución sustancial a la concepción y diseño, adquisición de datos, análisis e interpretación del estudio.

b) Redacción o revisión del contenido intelectual.

c) Aprobación de la versión final.

2.3. El orden de la autoría debe ser una decisión conjunta de los coautores.

2.4. Las personas que participen en un estudio pero que no se ajusten a los criterios de autoría deben aparecer como "Colaboradores" o "Personas reconocidas".

2.5. Hay tres tipos de autorías que se consideran inaceptables: autores "fantasma", que contribuyen sustancialmente pero no son reconocidos (a menudo pagados por promotores comerciales); autores "invitados", que no hacen ninguna contribución discernible pero se nombran para aumentar las posibilidades de publicación; y autorías "honorarias", que se basan únicamente en una afiliación tenue con un estudio.

Recomendaciones:

2.6. Antes de iniciar la investigación se recomienda documentar la función y la forma como se reconocerá la autoría de cada investigador.

2.7. No se debe mentir sobre la participación de una persona en la investigación o publicación, si su contribución se considerada "sustancial" se justifica la autoría, bien sea como coautor o colaborador.

2.8. No se debe asignar una autoría sin contar con el consentimiento de la persona.

2.9. Todas las personas nombradas como autores deben reunir los requisitos de autoría, y todos aquellos que reúnan los requisitos deben aparecer como autores o contribuidores.

2.10. Algunos grupos colocan los autores por orden alfabético, a veces con una nota para explicar que todos los autores hicieron contribuciones iguales al estudio y la publicación.

3. Cambios en la autoría[3]

Criterios:

3.1. Hace referencia a la adición, supresión o reorganización de los nombres de autor en la autoría de un artículo aceptado.

3.2. Las peticiones de añadir o eliminar un autor, o para reorganizar los nombres de los autores, deben ser enviados por el autor correspondiente del artículo aceptado, y deben incluir:

a) La razón por la cual debe ser añadido o eliminado, o los nombres de los autores reorganizado.

b) La confirmación por escrito (e-mail) de todos los autores que están de acuerdo con la adición, supresión o reorganización. En el caso de adición o eliminación de los autores,esto incluye la confirmación de que el autor sea añadido o eliminado.

4. Conflicto de intereses[4]

Criterios:

4.1. Cuando un investigador o autor, editor tenga alguna opinión o interés financiero/personal que pueda afectar su objetividad o influir de manera inapropiada en sus actos, existe un posible conflicto de intereses. Este tipo de conflictos pueden ser reales o potenciales.

4.2. Los conflictos de intereses más evidentes son las relaciones financieras, como:

a) Directas: empleo, propiedad de acciones, becas, patentes.

b) Indirectas: honorarios, asesorías a organizaciones promotoras, la propiedad de fondos de inversión, testimonio experto pagado.

4.3. Los conflictos también pueden existir como resultado de relaciones personales, la competencia académica y la pasión intelectual. Por ejemplo, un investigador que tenga:

a) Algún tipo de interés personal en los resultados de la investigación.

b) Opiniones personales que están en conflicto directo con el tema que esté investigando.

Recomendaciones:

4.4. Revelar si se está en algún conflicto real o potencial de intereses que influya de forma inapropiada en los hallazgoso resultados del trabajo presentado, dentro de los tres (3) años de haber empezado el trabajo presentado que podría influir indebidamente (sesgo) el trabajo.

4.5. Revelar el papel de un promotor (o promotores) del estudio, si los hubiere, en el diseño del estudio, en la recopilación, análisis e interpretación de los datos, en la redacción del informe y en la decisión de presentar el documento para su publicación.

4.6. Los investigadores no deben entrar en acuerdos que interfieran con su acceso a todos los datos y su capacidad de analizarlos de forma independiente, y de preparar y publicar los manuscritos.

4.7. Al presentar un documento, se debe hacer una declaración (con el encabezamiento "Papel que ha tenido la fuente de financiación") en una sección separada del texto y colocarse antes de la sección "Referencias".

4.8. Algunos ejemplos de posibles conflictos de intereses que deben ser revelados, incluyen: empleo, consultoría, propiedad de acciones, honorarios, testimonio experto remunerado, las solicitudes de patentes / registros y subvenciones u otras financiaciones.

4.9. Todas las fuentes de apoyo financiero para el proyecto deben ser revelados.

4.10. Se debe describir el papel del patrocinador del estudio.

5. Publicación duplicada[5]

Criterios:

5.1. Los autores tienen la obligación de comprobar que su artículo sea basado en una investigación original (nunca publicada anteriormente). El envío o reenvío intencional de su trabajo para una publicación duplicada se considera un incumplimiento de la ética editorial.

5.2. Se produce una publicación duplicada o múltiple cuando dos o más artículos, sin hacerse referencias entre sí, comparten esencialmente las

mismas hipótesis, datos, puntos de discusión y/o conclusiones. Esto puede ocurrir en diferentes grados: Duplicación literal, duplicación parcial pero sustancial o incluso duplicación mediante parafraseo.
5.3. Uno de los principales motivos por los que la publicación duplicada de investigaciones originales se considera no ético es porque puede dar lugar a una "ponderación inadecuada o a un doble recuento involuntario" de los resultados de un estudio único, lo que distorsiona las pruebas disponibles.

Recomendaciones:

5.4. Los artículos enviados para su publicación deberán ser originales y no deberán haberse enviado a otra editorial. En el momento del envío, los autores deberán revelar los detalles de los artículos relacionados (también cuando estén en otro idioma), artículos similares en prensa y traducciones.
5.5. Aunque un artículo enviado esté siendo revisado y no conozca el estado, espere a que la editorial le diga algo antes de ponerse en contacto con otra revista, y sólo si la otra editorial no publicará el artículo.
5.6. Evite enviar un artículo previamente publicado a otra revista.
5.7. Evite enviar artículos que describan esencialmente la misma investigación a más de una revista.
5.8. Indique siempre los envíos anteriores (incluidas las presentaciones de reuniones y la inclusión de resultados en registros) que pudieran considerarse una publicación duplicada.
5.9. Evite escribir sobre su propia investigación en dos o más artículos desde diferentes ángulos o sobre diferentes aspectos de la investigación sin mencionar el artículo original.
5.10. Se considera manipulador crear varias publicaciones a raíz de la misma investigación.
5.11. Si desea enviar su artículo a una revista que se publica en un país diferente o en un idioma diferente, pregúntaselo a la editorial si se puede hacer esto.
5.12. En el momento del envío, indique todos los detalles de artículos relacionados en un idioma diferente y las traducciones existentes.

6. Reconocimiento de las fuentes

Criterios:

6.1. Los autores deben citar las publicaciones que han sido influyentes en la determinación de la naturaleza del trabajo presentado.
6.2. Información obtenida de forma privada, no debe ser usada sin explícito permiso escrito de la fuente.
6.3. La reutilización de las tablas y / o figuras requiere del permiso del autor y editor, y debe mencionarse de manera adecuada en la leyenda de la tabla o figura.
6.4. La información obtenida en el transcurso de servicios confidenciales, tales como manuscritos arbitrales o las solicitudes de subvención, no debe ser utilizada sin el permiso explícito y por escrito del autor de la obra involucrada en dichos servicios.

7. Fraude científico[6]

Criterios:

7.1. El fraude en la publicación científica hace referencia a la presentación de datos o conclusiones falsas que no fueron generados a través de un proceso riguroso de investigación.
7.2. Existen los siguientes tipos de fraude en la publicación de resultados de investigación:
a) Fabricación de datos. Inventar datos y resultados de investigación para después comunicarlos.
b) Falsificación de datos. La manipulación de materiales de investigación, imágenes, datos, equipo o procesos.
La falsificación incluye la modificación u omisión de datos o resultados de tal forma que la investigación no se representa de manera precisa. Una persona podría falsificar datos para adecuarla al resultado final deseado de un estudio.

Recomendaciones:

7.3. Antes de enviar un artículo, lea cuidadosamente las políticas editoriales y de datos de la revista.
7.4. Nunca modifique, cambie u omita datos de forma intencional. Esto incluye materiales de investigación, procesos, equipos, tablas, citas y referencias bibliográficas.
7.5. Tanto la fabricación como la falsificación de datos son formas de conducta incorrecta graves porque ambas resultan en publicaciones científicas que no reflejan con precisión la verdad observada.
7.6. El autor debe hacer una gestión adecuada de los datos que soportan la investigación, teniendo especial cuidado en la recopilación, producción, conservación, análisis y comunicación de los datos.
7.7. Mantenga registros minuciosos de los datos en bruto, los cuales deberán ser accesibles en caso de que un editor los solicite incluso después de publicado el artículo.

8. Plagio[7]

Criterios:

8.1. El plagio es una de las formas más comunes de conducta incorrecta en las publicaciones, sucede cuando uno de los autores hace pasar como propio el trabajo de otros sin permiso, mención o reconocimiento. El plagio se presenta bajo formas diferentes, desde la copia literal hasta el parafraseado del trabajo de otra persona, incluyendo: datos, ideas, conceptos, palabras y frases.
8.2. El plagio tiene diferentes niveles de gravedad, como por ejemplo:
a) Qué cantidad del trabajo de otra persona se tomó (varias líneas, párrafos, páginas, todo el artículo)
b) Qué es lo que se copió (resultados, métodos o sección de introducción).
8.3. El plagio en todas sus formas constituye una conducta no ética editorial y es inaceptable.
8.4. La copia literal solo es aceptable si indica la fuente e incluye el texto copiado entre comillas.

Recomendaciones:

8.5. Recuerde siempre que es esencial reconocer el trabajo de otros (incluidos el trabajo de su asesor o su propio trabajo previo) como parte del proceso.
8.6. No reproduzca un trabajo palabra por palabra, en su totalidad o en parte, sin permiso y mención de la fuente original.
8.7. Mantenga un registro de las fuentes que utiliza al investigar y dónde las utilizó en su artículo.
8.8. Asegúrese de reconocer completamente y citar de forma adecuada la fuente original en su artículo.
8.9. Incluso cuando haga referencia a la fuente, evite utilizar el trabajo de otras personas palabra por palabra salvo que lo haga entre comillas.
8.10. El parafraseado solo es aceptable si indica correctamente la fuente y se asegura de no cambiar el significado de la intención de la fuente.
8.11. Incluya entre comillas y cite todo el contenido que haya tomado de una fuente publicada anteriormente, incluso si lo está diciendo con sus propias palabras.

9. Fragmentación[8]

Criterios:

9.1. La fragmentación consiste en dividir o segmentar un estudio grande en dos o más publicaciones.
9.2. Como norma general, con tal de que los "fragmentos" de un estudio dividido compartan las mismas hipótesis, población y métodos, no se considera una práctica aceptable.
9.3. El mismo "fragmento" no se debe publicar nunca másde una vez. El motivo es que la fragmentación puede dar lugar a una distorsión de la literatura haciendo creer equivocadamente a los lectores que los datos presentados en cada fragmento (es decir, artículo de revista) se derivan de una muestra de sujetos diferente. Esto no solamente sesga la "base de datos científica", sino que crea repetición que hace perder el tiempo de los editores y revisores, que deben ocuparse de cada trabajo por separado. Además, se infla injustamente el número de referencias donde aparece citado el autor.

Recomendaciones:

9.4. Evite dividir inapropiadamente los datos de un solo estudio en dos o más trabajos.
9.5. Cuando presente un trabajo, sea transparente. Envíe copias de los manuscritos estrechamente relacionados al manuscrito en

cuestión. Esto incluye manuscritos publicados, enviados recientemente o ya aceptados.

10. Consentimiento informado

Criterios:

10.1. Los estudios sobre pacientes o voluntarios requieren la aprobación de un comité de ética.

10.2. El consentimiento informado debe estar debidamente documentado.

10.3. Los permisos y las liberaciones deben ser obtenidos, cuando un autor desea incluir detalles de caso u otra información personal o imágenes de los pacientes y cualquier otra persona.

10.4. Especial cuidado debe tenerse con la obtención del consentimiento respecto a los niños (en particular cuando un niño tiene necesidades especiales o problemas de aprendizaje), donde aparece la cabeza o la cara de una persona, o cuando se hace referencia al nombre de un individuo u otros datos personales.

11. Corrección de artículos publicados[9]

Criterio:

Cuando un autor descubre un error o inexactitud significativa en el trabajo publicado, es obligación del autor notificar de inmediato a la revista y cooperar en el proceso de corrección.

Referencias

Black, William, Rodolfo Russo, y David Turton. «The Supergravity Fields for a D-Brane with a Travelling Wave from String Amplitudes». Physics Letters B 694, n.º 3 (noviembre de 2010): 246-51.

Elsevier. «Autoría. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0010/183394/ETHICS_ES_AUTH01a_updatedURL.pdf.

———. «Conflicto de intereses. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0006/183399/ETHICS_ES_COI01a_updatedURL.pdf.

———. «Envío simultáneo/múltiple, publicación duplicada. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0019/183403/ETHICS_ES_SSUB01a_updatedURL.pdf.

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———. «Plagio. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0016/183400/ETHICS_ES_PLA01a_updatedURL.pdf.

[1] Elsevier, «Ethics. Conducting research», accedido 8 de agosto de 2014, http://www.elsevier.com/journal-authors/ethics#conducting-research.

[2] Elsevier, «Autoría. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0010/183394/ETHICS_ES_AUTH01a_updatedURL.pdf.

[3] William Black, Rodolfo Russo, y David Turton, «The Supergravity Fields for a D-Brane with a Travelling Wave from String Amplitudes», Physics Letters B 694, n.º 3 (noviembre de 2010): 246-51.

[4] Elsevier, «Conflicto de intereses. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0006/183399/ETHICS_ES_COI01a_updatedURL.pdf.

[5] Elsevier, «Envío simultáneo/múltiple, publicación duplicada. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0019/183403/ETHICS_ES_SSUB01a_updatedURL.pdf.

[6] Elsevier, «Fraude en investigación. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0017/183401/ETHICS_ES_RF01a_updatedURL.pdf.

[7] Elsevier, «Plagio. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0016/183400/ETHICS_ES_PLA01a_updatedURL.pdf.

[8] Elsevier, «Fragmentación. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0018/183402/ETHICS_ES_SS01a_updated updatedURL.pdf.

[9] Elsevier, «Ethics. Writing an article», accedido 8 de agosto de 2014, http://www.elsevier.com/journal-authors/ethics#writing-an-article.

PUBLICATION ETHICS AND PUBLICATION MALPRACTICE STATEMENT

The journal Revista Facultad Nacional de Agronomia follows the COPE Code of Conduct and Best Practice Guidelines for Journal Editors and the International Standards For Editors and Authors, published by Committe on Publication Ethics.
The journal puts forth the following criteria and recommendations for ethical scientific publications:

1. General criteria[1]

1.1. Articles must contain sufficient details and references that allow the study to be replicable or refutable.
1.2. Fraudulent or deliberately inexact statements constitute unethical behavior.
1.3. If a study includes the use of chemical products, procedures, or equipment that presents an inherent risk, the author must state so in the article.
1.4. If the study involves the use of animals or human beings, the article must contain a clear statement that all of the procedures were carried out in strict compliance with laws and institutional directives.
1.5. The privacy of the human beings must be respected.

2. Authorship[2]

Criteria:
2.1. An "author" is a person that has made a significant intellectual contribution to an article; all of the individuals that are named as authors must fulfill the requirements for authorship and all of those individuals that do so must be explicitly named.
2.2. Three basic criteria must be met in order to be considered an author:
a) Substantial contribution to the study concept, design, and data collection, analysis and interpretation.
b) Revision of the intellectual content.
c) Approval of the final version.
2.3. The order of the author list must be a joint decision of the coauthors.
2.4. The individuals that participate in a study but that do not meet the criteria for authorship must be listed as an "Assistant" or "recognized person."
2.5. There are three types of unacceptable authorship: "ghost" authors, who make a substantial contribution but are not recognized (often paid by commercial promoters); "guest" authors, who do not make a discernable contribution but are named in order to increase the probability of publication; and "honorary" authors, who only have a tenuous connection to the study.
Recommendations:
2.6. Before starting the research, establish the function of each researcher and the manner in which they will be recognized.
2.7. It is not necessary to mention an individual's participation in a study or publication, but if their contribution is substantial, than authorship would be justified, either as an author or assistant.
2.8. Authorship cannot be bestowed on an individual without their consent.
2.9. All of the individuals that are named as authors must meet the requirements for authorship and all of those that meet the requirements must appear as authors or assistants.
2.10. Some groups list the authors alphabetically, sometimes with a notation that indicates that all of the authors contributed equally to the study and the publication.

3. Changes in the authorship[3]

Criteria:
3.1. Additions to, removals from, and reorganization of the author names in accepted articles must be noted.
3.2. Petitions to add to, remove from, or reorganize the authors must be sent by the corresponding author of the accepted articles and must include:
a) The reason for the addition, elimination, or reorganization.
b) A written statement (e-mail) from all of the authors that confirms their agreement with the addition, elimination, or reorganization. In the case of an addition or elimination, a confirmation is also required from the author to be added or removed.

4. Conflict of interest[4]

Criteria:
4.1. When a researcher or author has a financial/personal opinion or interest that could affect their objectivity or improperly influence their actions, there exists a possible conflict of interest. Conflicts can be actual or potential.
4.2. The most evident conflicts of interest are financial, such as:
a) Direct: employment, stocks, scholarships, patents.
b) Indirect: assistantship to promoting organizations, investment funds, paid expert testimony.
4.3. Conflicts can also arise from personal relationships, academic competition, and intellectual passion. For example, an author could have:
a) Some personal interest in the results of the research.
b) Personal opinions that are in direct conflict with the research topic.
Recommendations:
4.4. Disclose all conflicts of interest, actual or potential, that inappropriately influence the findings or results of a study, including any that arise within the three (3) years after the start of said study if they could unduly (bias) influence the study.
4.5. Disclose the role of any promoter (or promoters) in the study, if any, in the design, in the collection, analysis or interpretation of the data, in the document review, or in the decision to present the document for publication.
4.6. The researchers must not enter into agreements that interfere with their access to all of the data or with their ability to independently analyze the data or to prepare and publish the manuscript.
4.7. The document must contain a statement (with the heading "Role of the financial source") in a section that is separate from the text and before the References section.
4.8. Some examples of conflicts of interest that must be revealed include: employment, consulting, stocks, honorariums, paid expert testimony, patent requests or registration, and subsidies or other financing.
4.9. All of the sources of financial support for the project must be revealed.
4.10. The role of any study sponsors must be described.

5. Duplicate publication[5]

Criteria:
5.1. Authors have the obligation of proving that their article is based on original research (never before published). The intentional submission or resubmission of a manuscript for duplicate publication is considered a breach of editorial ethics.
5.2. A duplication publication, or multiple publication, results when two or more articles, without any reference to each other, essentially share the same hypothesis, data, discussion points, and/or conclusions. This can occur to different degrees: literal duplication, partial but substantial duplication or paraphrasal duplication.
5.3. One of the main reasons that duplicate publications are considered unethical is that they can result in the "inappropriate weighting or unwitting double counting" of results from just one study, which distorts the available evidence.
Recommendations:
5.4. Articles sent for publication must be original and not sent to other editors. When sent, the authors must reveal the details of related articles (even when in another language) and similar articles being printed or translated.

5.5. Even though a submitted article is being reviewed and the final decision is not known, wait to receive notification from the editors before contacting other journals and then only do so if the editors decline to publish the article.
5.6. Avoid submitting a previously published article to another journal.
5.7. Avoid submitting articles that essentially describe the same research to more than one journal.
5.8. Always indicate previous submissions (including presentations and recorded results) that could be considered duplicate results.
5.9. Avoid writing about your research in two or more articles from different angles or on different aspects of the research without mentioning the original article.
5.10. Creating various publications based on the same research is considered a type of manipulation.
5.11. If an author wishes to send an article to a journal that is published in a different country or a different language, ask for permission from the editors first.
5.12. When submitting an article, indicate all of the details of the article that were presented in a different language along with the relevant translations.

6. Acknowledging sources

Criteria:

6.1. Authors must cite the publications that had an influence on the determination of the nature of the offered study.
6.2. Privately obtained information cannot be used without the express written consent of the source.
6.3. Republishing tables or figures requires the permission of the author or editor, who must be appropriately cited in the table or figure legend.
6.4. Information obtained through confidential services, such as arbitration articles or subsidy applications, cannot be used without the express written consent of the author of the work involved in said services.

7. Scientific fraud[6]

Criteria:

7.1. Fraud in scientific publications refers to the presentation of false data or conclusions that were not obtained through a rigorous research process.
7.2. The following types of fraud exist for the publication of research results:
a) Fabricating data. Inventing research data and results for later dissemination.
b) Falsification of data. The manipulation of research material, images, data, equipment or processes. Falsification includes the modification or omission of data or results in such a way that the research is not represented in a precise manner. A person may falsify data in order to obtain the desired final results of a study.

Recommendations:

7.3. Before submitting an article, carefully read the editorial and data policies of the journal.
7.4. Never modify, change or omit data intentionally. This includes research material, processes, equipment, tables, citations, and bibliographical references.
7.5. Fabricating and falsifying data constitute grave misconduct because both result in scientific publications that do not precisely reflect the actual observations.
7.6. Authors must appropriately manage the data that supports the research, taking special care in the compilation, production, preservation, analysis and presentation of the data.
7.7. Maintain precise records of the raw data, which must be assessable in case the editors request them after publication of the article.

8. Plagiarism[7]

Criteria:

8.1. Plagiarism is one of the more common types of misconduct in publications; it occurs when an author passes the work of others off as their own without permission, citations, or acknowledgment. Plagiarism can occur in different forms, from literally copying to paraphrasing the work of another person, including data, ideas, concepts, paragraphs, and phrases.
8.2. Plagiarism has different degrees of severity; for example:
a) The quantity of work taken from another person (various lines, paragraphs, pages, or the entire article).
b) What is copied (results, methods, or introduction section).
8.3. Plagiarism, in all of its forms, constitutes unethical behavior and is unacceptable.
8.4. Literal copying is acceptable if the source is indicated and the text is placed in quotation marks.

Recommendations:

8.5. Always remember that it is vital to recognize the work of others (including the work of your assistants or your previous studies).
8.6. Do not reproduce the work of others word for word, in totality or partially, without the permission and recognition of the original source.
8.7. Maintain a record of the sources that are used in the research and where they are used in the article.
8.8. Be sure to accurately acknowledge and cite the original source in your article.
8.9. Even when referencing the source, avoid using the work of others word for word unless it is placed in quotations.
8.10. Paraphrasing is only acceptable if the source is correctly indicated and the source's intended meaning is not changed.
8.11. Use quotations, and cite all of the content that is taken from a previously published source even when using your own words.

9. Fragmentation[8]

Criteria:

9.1.Fragmentation occurs when a large study is divided or segmented into two or more publications.
9.2. As a general rule, as long as the "fragments" of a divided study share the same hypothesis, populations, and methods, this not considered an acceptable practice.
9.3. The same "fragment" can never be published more than one time. Fragmentation can result in distortion of the literature, creating the mistaken belief in readers that the data presented in each fragment (i.e. journal article) are derived from different subject samplings. This not only distorts the "scientific database", but creates repetition that results in a loss of time for editors and evaluators that must work on each article separately. Furthermore, the cited author receives an unfair increase in their number of references.

Recommendations:

9.4. Avoid inappropriately dividing the data of one study into two or more articles.
9.5. When presenting your work, be transparent. Send copies of the manuscripts that are closely related to the manuscript in question, including published, recently submitted and accepted manuscripts.

10. Informed consent

Criteria:

10.1. Studies on patients and volunteers require the approval of the ethics committee.
10.2. The informed consent must be duly documented.
10.3. Permission and waivers must be obtained when an author wishes to include details of a case or other personal information or images of the patients or any other person.
10.4. Special care should be taken when obtaining the consent

of children (especially when a child has special needs or learning disabilities) when their head or face is displayed or when reference is made to the name of an individual or other personal data.

11. Correction of published articles[9]

Criterion:

When an author discovers a significant inexactitude or error in a published article, they must immediately notify the journal and cooperate in the correction process.

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