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EVALUADORES

El Comité Editorial dentro de sus políticas, envía los artículos a especialistas, con el fin de que sean revisados. Sus observaciones en adición a las que hacen los editores, contribuyen a la obtención de una publicación de reconocida calidad en el ámbito de las Ciencias Agrarias. Sus nombres son mencionados como una expresión de agradecimiento.

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Women and the agri-food system

According to a recent study published by the FAO, "*Estimating global and country-level employment in agrifood systems"*, around 1.23 billion people are employed in the world's agrifood systems, and more than three times that figure, or almost half the world's population, live in households linked to agrifood systems. Of these 1.23 billion people, 857 million worked in primary agricultural production and 375 million in the off-farm segments of agrifood systems. The research focused on entire agrifood systems rather than only on direct agricultural sectors, reflecting the increasing importance of off-farm activities in feeding the world's population, currently 8 billion and growing, and the evolution of rural and food system transformation. In relation to this study, and another one launched more recently, also by the FAO, "*The status of women in agrifood systems*", where three important issues are discussed: women, first of all, but also youth and poverty; issues that have been gaining importance in discussions on public policies, mainly those related to empowerment and equality, education, competences for employment, training and the labour market, topics in which it finds ourselves immersed on the occasion of the structural reforms currently underway in the Universidad Nacional de Colombia. More than an analytical exercise on these topics, what is sought here is to enrich the discussion based on what has been found in these studies.

Agrifood system - AFS is a major employer of women globally and constitute a more important source of livelihood for women than for men in many countries. The data obtained indicate that women represent around half of the workforce making up the majority in the food processing and services sector. Globally, 36 percent of working women and 38 percent of working men work in AFS. For both women and men, this represents a decline of about 10 percentage points since 2005, driven almost exclusively by a reduction in employment in primary agricultural production. Some additional numerical highlights tell us that, globally, women engaged in wage employment in agriculture earn 82 cents for every dollar that men earn, however, female workers are significantly more likely than male workers to work part-time or in other vulnerable positions. While 75 percent of policy documents relating to agriculture and rural development from 68 countries recognize women's roles and/or women's challenges in agriculture and rural development, only 19 percent included policy goals related to gender.

Women and youth were heavily impacted by the pandemic because they represent a large share of low-skilled and poorly-educated workers and, as such, are more likely to be self-employed or casual workers and thus more likely to lose their jobs and to experience income disruptions. 22 percent of women lost their jobs in the off-farm segment of AFS in the first year of the COVID-19 pandemic, compared with only 2 percent of men. Women in a situation of poverty and with the presence of children in the home were the most affected. The COVID-19 crisis deepened the structural knots of gender inequality in the different regions of the world and threatened the autonomy of women. What is concluded from these studies is that, in general, women account for a greater share of agricultural employment at lower levels of economic development, as inadequate education, limited access to basic infrastructure and markets, high unpaid work burden and poor rural employment opportunities outside agriculture severely limit women's opportunities for off-farm work; but also that increasing women's empowerment is essential for women's wellbeing and has a positive impact on agricultural production, food security, diets and child nutrition.

On the other hand, youth (defined as people aged 15–35), in most of the countries, represent half of the workers engaged specifically in agriculture and more than half of the people engaged in food processing and services. In terms of poverty, consistent with the process of structural transformation, as GDP increases, the share of poor individuals engaged in AFS declines, meaning that the largest proportion of youth participating in AFS is particularly from poor countries. In almost all countries, the share of individuals in poverty in AFS outside of agriculture is almost uniformly lower than the share of individuals in poverty engaged in agriculture. This is evident at both high and low levels of per capita GDP. Regarding the socioeconomic and demographic patterns of engagement in AFS, it was found that as countries develop, the relative proportion of people engaged in the non-agriculture segments of AFS – which are more likely to be located in peri-urban or urban areas, increases. This is consistent with structural transformation processes whereby urban and rural areas become increasingly connected through more complex value chains. This pattern is related to the youth bulge in these countries, with a large youth population located in rural and peri-urban areas who have few other options than to rely on AFS for their livelihoods.

It is clear then that as in other areas of the world of work, inequality is also endemic in the AFS and that something urgent needs to be done to accelerate change and that something involves empowerment. Reducing gender inequalities in livelihoods, access to resources and resilience in AFS is a critical pathway towards gender equality and women's empowerment and towards more just and sustainable AFS. But and why is this important? It is important because women's empowerment can be transformative. Closing the gender gap in farm productivity and the wage gap in AFS employment would increase global gross domestic product by 1 percent (or nearly USD 1 trillion). This would reduce global food insecurity by about 2 percentage points, reducing the number of food-insecure people by 45 million, and finally, if half of small-scale producers benefited from development interventions which focused on empowering women, it would significantly raise the incomes of an additional 58 million people and increase the resilience of an additional 235 million people.

Women's empowerment and gender equality are not only a key part of achieving the Sustainable Development Goals (SDGs) by 2030 but are also intrinsically important for women's and men's well-being and should represent a greater commitment to AFS since it is a more important source of livelihood for women than for men in many countries. An efficient, inclusive, resilient, and sustainable AFS depend on the empowerment of all women and gender equality. In short, an effective women empowerment, including better access to resources and assets and enhancing their decision-making power, will help increase incomes and resilience for them, their households, and communities – particularly in rural areas. SDG 5 calls on us to achieve gender equality and empowerment for all women and girls by 2030 – a deadline that is fast approaching. Let´s to work for it.

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Research article

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Morphological diversity of the UN Cotové papaya (*Carica papaya* L.) variety grown under tropical dry forest conditions

UNIVERSIDAD NACIONAL DE COLOMBIA

Diversidad morfológica de la variedad UN Cotové (*Carica papaya* L.) bajo las condiciones del bosque seco tropical

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ABSTRACT

This work aimed to describe the morphological diversity of the UN Cotové papaya variety to identify outstanding plants that can be used to obtain a new local cultivar. 18 individuals were selected, and the multivariate non-hierarchical cluster technique analyzed data. A Polynomial regression model was carried out to describe growth as a function of growing degree days. The ANOVA identified significant differences between plants for most morphological variables. The Pearson correlation showed linear dependence; all the variables had a high correlation (higher than 0.82) with plant height. The Hopkins and Gap statistic determined two clusterings: Gropu "D" with higher development and group "A" with less development for all parameters. Second-order polynomial model were the best fit for the plant height, and third-order models were the best fit for the others. The non-linear functional models were not significant for the evaluated variables, presenting "Lack of Fit" values greater than 0.05. The results provided information for selecting plants with outstanding characteristics that can be used in the papaya breeding program.

Keywords:
Morphological adaptations
Physiological breeding
Plant growth

RESUMEN

Este trabajo tuvo como objetivo describir la diversidad morfológica de la variedad de papaya UN Cotové para identificar plantas sobresalientes que puedan ser utilizadas como parte de un programa de mejoramiento para obtener un nuevo cultivar local. Se seleccionaron 18 individuos y se analizaron los datos mediante la técnica de conglomerados no jerárquicos multivariados. Se llevó a cabo un modelo de regresión polinomial para describir el crecimiento en función de los grados dias acumulado. El ANOVA identificó diferencias significativas entre plantas para la mayoría de las variables morfológicas. La correlación de Pearson mostró dependencia lineal; y todas las variables presentaron una alta correlación (superior a 0,82) con la altura de la planta. Los estadisticos de Hopkins y Gap determinaron dos agrupamientos: Grupo "D" con plantas de mayor desarrollo y grupo "A" plantas con menor desarrollo para todos los parámetros. Los modelos polinomiales de segundo orden fueron los que mejor se ajustaron a la altura de la planta, y los modelos de tercer orden con mejor ajuste para el resto de variables. Los modelos funcionales no lineales no fueron significativos para las variables evaluadas, presentando valores de "falta de ajuste" superiores a 0,05. Los resultados brindan información para la selección de plantas con características sobresalientes que pueden ser utilizadas en el programa de mejoramiento de papaya.

Palabras clave:
Adaptaciones morfológicas
Fitomejoramiento fisiológico
Crecimiento vegetal

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Papaya (*Carica papaya* L.) is among the most cultivated and consumed tropical fruits globally. This crop is characterized by accelerated annual increases in production, even among commercial produce (Altendorf 2017). The main papaya producers are India, Brazil, México, Nigeria, Indonesia, and the Dominican Republic; Colombia ranks thirteenth, with 146,186 t in 6,944 ha. Although papaya production in Colombia has increased by 30% since 2000 (FAO 2020), the existing cultivars are continuously exposed to various environmental stresses (biotic and abiotic), affecting the fruit yield potential and limiting the local fruit growers. Genetic breeding programs are essential to overcoming these limitations and ensuring greater competitiveness and aim to develop hybrids, insert genes of interest, molecular selection, and micropropagation (An et al. 2020). However, these approaches do not consider interactions between the environmental conditions and the plant's physiological responses (Tardieu 2012). Therefore, genetic breeding processes need to understand how plants respond to environmental stress conditions to advance understanding of causes that influence yield, responses to environmental variations, and morphological adaptations (Catarina et al. 2020). Several studies have focused on physiological breeding in papaya to obtain genotypes highly tolerant to water deficits and excess, high CO_2 fixation, precocity, and adaptation to different environmental conditions (Reynolds et al. 2013). In this sense, Girón et al. (2021) pointed out that high cuticular wax contents contribute to greater tolerance to water deficit stress. Vincent et al. (2018) found a correlation between tolerance to water deficit and light stress; adapting plants to water deficits helps mitigate radiation excesses. Peçanha et al. (2017) indicated that high electrical conductivity in soil affects gas exchange rates, reducing plant growth. The plant's physiological breeding provides new approaches for investigating the selection of new papaya materials; hence, regarding the papaya species, according to Jiménez et al. (2014), the ideal plant morphological characteristics for high yield (70-90 fruits per plant) is that plant less than 2 m in height, with approximately 24 adult leaves and 5 to 10 cm stem diameters. The UN Cotové variety was obtained by crossing a local creole variety (Cuban origin) and a Cariflora variety from Florida (Reyes 1996). UN Cotové is cultivated in Antioquia, Risaralda, and north of Valle del Cauca. It stands out for its tolerance to viruses, with a yield of around 131 t ha^{-1}, adapted to tropical dry forests (T-df) conditions, and has a savoriness (Vallejo 1999). However, the dioecious nature of the UN Cotové variety is the main reason for the loss of purity of this genetic material. Therefore, in response to achieving the genetic identity of this cultivar, this study aimed to characterize the morphology of the UN Cotové variety under topical dry forest (T-df) environmental conditions in Santa Fe de Antioquia, Colombia.

MATERIALS AND METHODS

Experiment location

This study was carried out at the Cotové Agrarian Station (AS) of the Universidad Nacional de Colombia, Medellín. The Cotové AS is located at 6° 31' 57´´N and 75° 49' 40' W, and 507 masl, in the El Espinal village, Santa Fe de Antioquia, Colombia.

Characterization of the agroecosystem

In the study period (May to November 2018), the weather conditions of the site of the investigation were an average temperature of 27 °C, with a maximum of 42.5 °C and a minimum of 16.8 °C; the average relative humidity was 71%, the average rainfall was 815 mm. The agroecosystem corresponds to the tropical dry forest life zone (T-df) according to Holdridge's (1978) classification. With Inceptisol soil type, clayey texture, pH=6.7 and concentrations of N (56 mg kg^{-1}), P (36 mg kg^{-1}), B (1.3 mg kg^{-1}), K (0.44 cmol kg^{-1}), Ca (20.4 cmol kg^{-1}) and Mg (9.6 cmol kg^{-1}).

Growing conditions of plant material

The papaya variety UN Cotové was used. Planting was carried out at 2.5 m between plants and 3 m between rows, distributed in a triangle (1,333 plants ha^{-1}). The fertilization involved applying 2 kg of organic matter (chicken manure), and 120 g of a mixture of 25 kg of $CO(NH_2)$, 25 kg of KCL, and 500 g of H_3BO_3 per plant.

Morphological traits

Study variables

The experimental unit consisted of 18 plants, taken randomly within the nine central rows, two plants per row. In each of the experimental plants, six morphological variables were evaluated monthly according to IBPGR (1989): plant height (PH) (cm); stem diameter - SD (mm);

internode length - IL (cm); the number of leaves – LN; canopy length (North-South and East-West) (cm); and the area occupied by the plant - AOP (cm^2). The height (cm) was quantified, taken from the base of the plant to the apical meristem, the diameter of the stem (mm) at 15 cm from the soil, the number of leaves according to the descriptors of the IBPGR (1989); the canopy length in the North-South (cm) and East-West (cm) directions; and the area occupied by the plant - AOP (cm^2), the latter considering that the papaya canopy has a circular distribution as proposed by Wang et al. (2014).

Thermal time

The heat sum method was used to consider the temperature effect on plant growth and development as accumulated growing degree days (GDD) according to Equations (1) and (2). Riaño et al. (2005) suggested the simple sine method between the appropriate physiological temperature thresholds (lower limit L_l and upper limit U_l) because a symmetric daily temperature behavior is assumed for the maximum temperature with equal minimum temperatures typical of tropical conditions. For papaya, these limits are 15 to 32 °C, respectively (Allan 2002).

$$GDD = \frac{1}{\pi}\left\{\left(\frac{T_{max}+T_{min}}{2}-L_l\right)\left(\theta_2+\frac{\pi}{2}\right)+(U_l-L_l)\left(\frac{\pi}{2}-\theta_2\right)-(\alpha * \cos(\theta_2))\right\} \quad (1)$$

$$\theta_2 = \sin^{-1}\left[\left(U_l-\frac{T_{max}+T_{min}}{2}\right)/\alpha\right] \quad (2)$$

Where: U_l = Upper limit, L_l = Lower limit; Tmax = Maximum temperature; Tmin = minimum temperature, and α = (Tmax-Tmin) / 2. The temperatures are expressed in degree celsius (°C).

Statistical analysis

A Pearson correlation matrix carried out the degree of correlation between the different variables evaluated. A non-hierarchical cluster analysis (k-means) was carried out in a multivariate structure. The Hopkins and Gap statistic determined the clustering trend and the appropriate number of clusters.

Statistical differences between groups were evaluated through a repeated-measures analysis of variance (RM ANOVA). Shapiro-Wilk and Bartlett tests and post-hoc comparisons from the Tukey test (P<0.05%) were performed to qualify the statistical hypotheses of normality and homoscedasticity of variances. In addition, the plant growth was described as a function of the GDD by adjusting polynomial regression models up to the third degree. The models were selected with R^2 - adjusted, RMSE (Root mean squared error), and Akaike information criterion. The non-linear functional models were: Logistic (L), Log-logistic (LL), Log-Normal (LN), Gompertz (G), and Weibull (W). Using the R Studio, the models were estimated with the Analysis of Dose-Response Curves “drc” library, and the “mselect” function. This facilitates selection between non-linear models based on Log-likelihood value and IC value, which summarize the Akaike criterion and Lack of Fit tests, using P<0.05 as a guideline to accept the null hypothesis.

All processes were developed using the R Studio statistical software (R Development Studio Team 2020).

RESULTS AND DISCUSSION

Pearson Correlation

The Pearson correlation helped identify variables that can be used to model data according to the degree of correlation (Figure 1). After data exploration, a high correlation was observed as a function of GDD for all variables except for IL. Temperature is one of the main climatic factors that condition the development of papaya (Almeida et al. 2003), and knowledge of the thermal requirements measured in GDD is essential to predict growth and harvest (Salinas et al. 2019). The highest correlation (0.99) was presented between the NS and EW. As Wang et al. (2014) stated, these results confirm that the papaya plant shows a symmetrical position of the leaves in a spiral arrangement around the stem. All the variables had a high correlation (higher than 0.82) with plant height, except for the IL. The IL did not correlate with any variable, contrary to those exposed by Lim and Hawa (2005) in a study on early flowering, who concluded that IL has a strong correlation with PH. The differences found for this variable can be generated by high sensitivity in IL to environmental changes and competition generated by adjacent plants, which promotes the development of longer internodes (Jiménez et al. 2014).

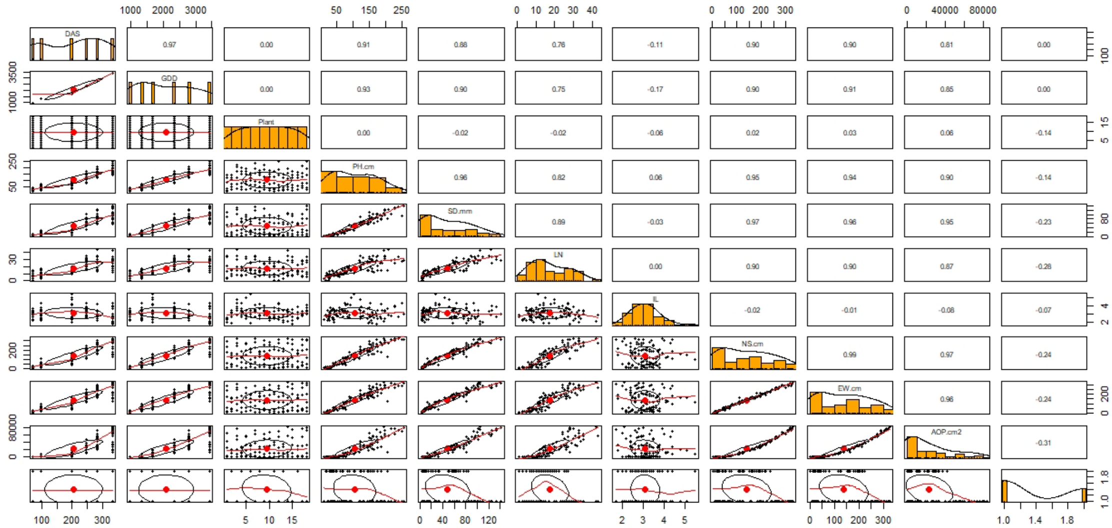

Figure 1. Pearson correlation matrix for the variables plant height (PH), stem diameter (SD), number of leaves (LN), internode length (IL), canopy length N-S and E-W, and the area occupied by the plant (AOP) in papaya plants variety UN Cotové. Cotove SA. Santa Fe de Antioquia, Colombia.

Figure 1 shows the behavior of the variables PH, SD, LN, and AOP as a function of the accumulated GDD for each measurement. In general, from the fifth measurement, two groups of plants were differentiated; for SD, the separation occurred from the fourth measurement. However, at the end of plant development, there four groups were identified (Figures 2A and 2B). In the third measurement, a stem growth reduction was observed near 2330 GDD, similar to those found by Almeida et al. (2003), which shows that changes in growth and development occur regardless of the location or sowing season. However, the behavior of the variables depends on the accumulation of GDD or thermal temperature.

An accelerated development was evidenced for LN between the second and fourth measurements (Figure 2C). Between the fourth and fifth measurements, LN decreased, coinciding with the flowering stage (90% of the plants) at 2067 GDD. Flowering is a phenological stage where photo-assimilates are used to provide the energy required for floral differentiation. The stem and leaf growth rate decreases due to the indeterminate growth habit of papaya with simultaneous vegetative growth, flowering, and fruiting (Singh et al. 2010). Thus, presenting competition between vegetative and reproductive sinks (Zhou et al. 2000). Conversely, for AOP, all individuals showed very similar values in the first stages of growth. However, some changes separated two groups between the fourth and fifth measurements (Figure 2D).

Grouping

The data analysis showed that the grouping occurred between the fourth and fifth measurements depending on the variables. According to Qiu and Cao (2016), the data will be more uniform when the Hopkins statistic is close to 0.5. Therefore, once the non-uniformity of the data had been defined with the Gap statistic (Figure 3), the formation of four homogeneous groups presented differences.

Based on the k-means procedure, all observations were divided into four groups (Figure 4), in which the internal variance between the data was the smallest (Na et al. 2010). Group D had the plants with higher average values for all variables, followed by groups A and B, and finally, group C, which grouped the plants with the lower values (Table 1).

One of the difficulties in this first grouping into four clusters was that separating the groups did not discriminate the individuals or the states of specific development in the experimental units (Table 2). On the contrary, the groupings were made from the magnitude

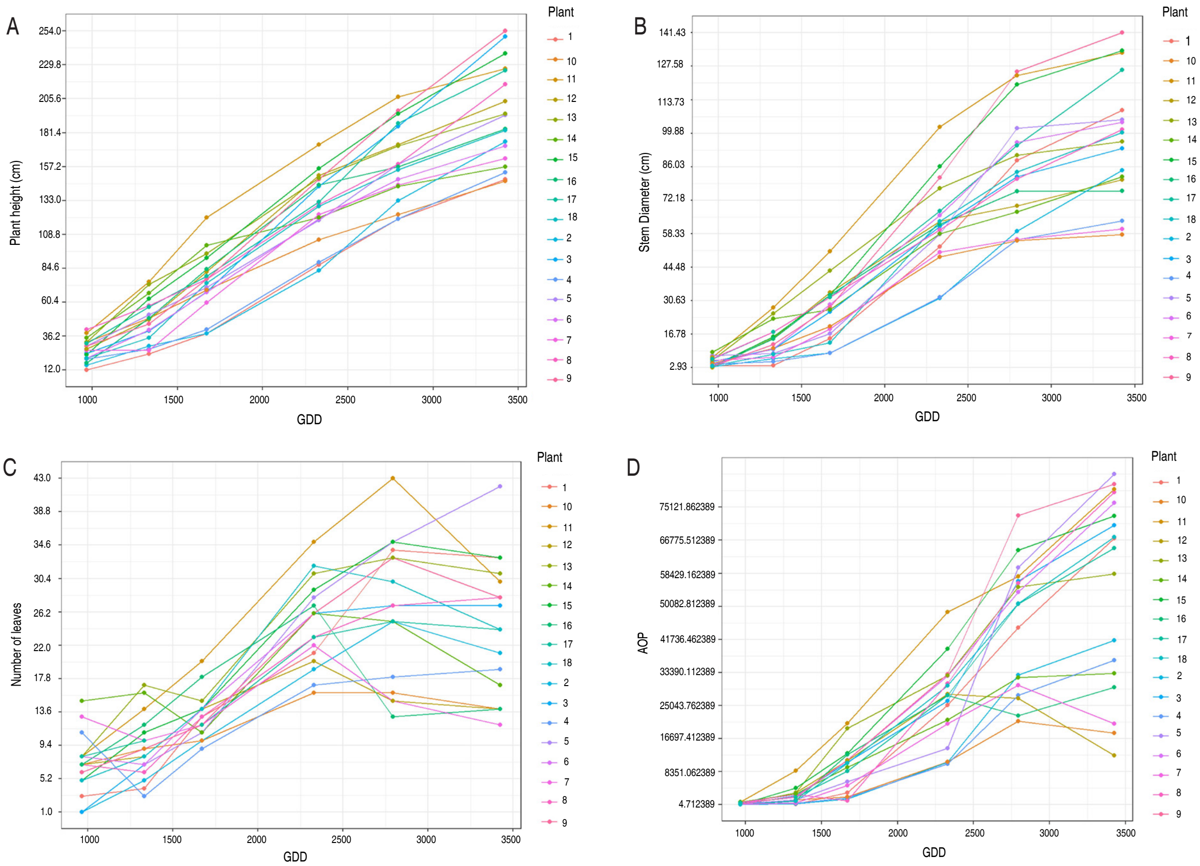

Figure 2. Allometric variables in the UN Cotové papaya variety as a function of the accumulated degree days.

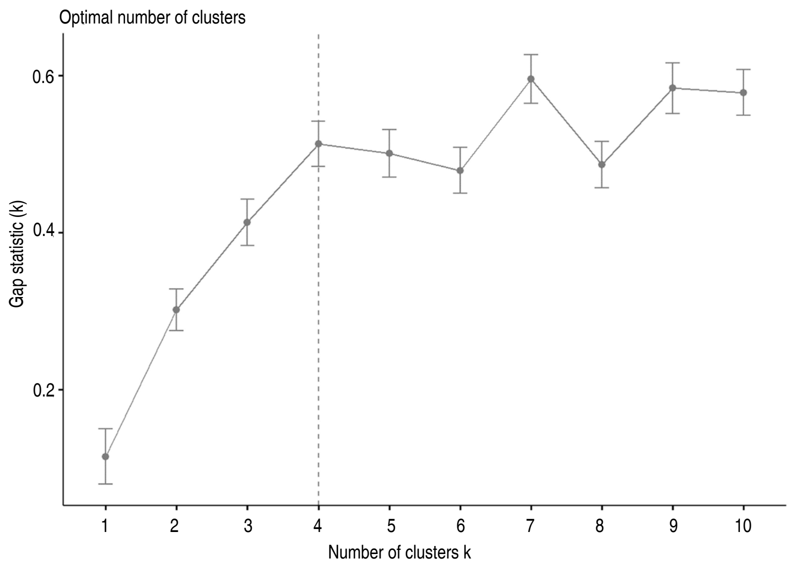

Figure 3. The optimal number of clusters with the Gap method for all data recorded during the growth of the UN Cotové papaya variety.

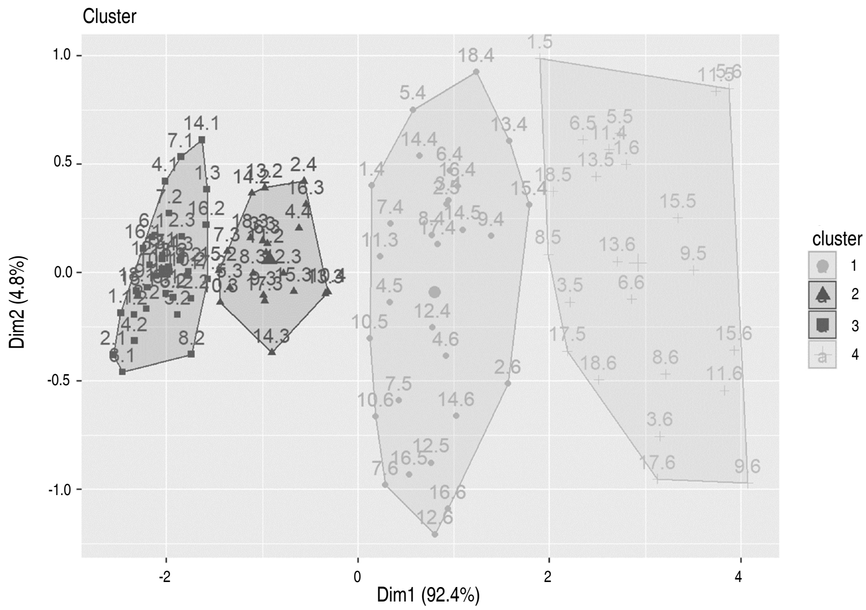

Figure 4. Clusters were determined with all data recorded during the growth of the UN Cotové papaya variety, according to the k-means methodology.

Table 1. Values of plant height (PH), stem diameter (SD), number of leaves (LN), NS length (NSL), EW length (EWL), and the area occupied by the plant (AOP) determined in four clusters of UN Cotové papaya variety plants.

Cluster	PH (cm)	SD (mm)	LN	NSL (cm)	EWL (cm)	AOP (cm^2)
	$\bar{X}$	$\bar{X}$	$\bar{X}$	$\bar{X}$	$\bar{X}$	$\bar{X}$
A	143.1±23.6	65.7±10.5	21.3±5.8	185.4±27.7	185.3±23.4	27439.1±6918.3
B	79.5±12.6	28.4±9.0	13.8±2.5	101.2±30.3	102.9±31.0	8864.1±4493.4
C	35.4±14.3	8.9±5.8	8.0±3.6	33.1±23.0	31.5±22.6	1210.7±1877.3
D	189.7±34.6	105.9±17.6	31.2±5.0	286.0±27.0	282.7±25.3	63992.2±1428.4

of each variable vector, determined by the plant age, associated with the evaluation time (measurement). Cluster C grouped the lowest values, with 100% of the data recorded in the first, 83% in the second, and 22% in the third. The benefit of this first grouping was identifying when the plants presented differences, the essential information in a plant breeding program for recognizing outstanding quality parameters from selecting individuals with characteristics of interest.

All plant were homogeneous in their characteristics, grouped in cluster C in the first measurement. In the second measurement, plants 11, 14, and 15 were differentiated based on greater development. Plant 11 had superior growth for the third measurement, but plants 1, 2, 3, and 4 were the least developed. In the fourth measurement, the groups were separated, where plant 11 continued to stand out until reaching the highest height at the end. For this plant, this greater development did not imply early flowering as expected, with the report by Kumar et al. (2015), who stated a high correlation between these two variables. In the fifth and sixth measurements, the separation of the plants into two large groups was evident: cluster A, which includes plants with lower development, and cluster D, made up of the plants with higher values. A new analysis was carried out based on the Gap statistic. Only the values obtained for each variable in measurements 3, 4, and 5 were considered, forming two groups with different characteristics (Figure 5). The first group (A) was made up of individuals 2, 4, 7, 10, 12, and 14; while the second group was made up of 1, 3, 5, 6, 8, 9, 11, 13, 15, 16, 17 and 18.

Table 2. Clustering for the UN Cotové papaya variety plants according to the group assigned by the k-means methodology.

Accession number	Measurement number/clustering					
	1st	2nd	3rd	4th	5th	6th
1	C	C	C	A	D	D
2	C	C	C	B	A	A
3	C	C	C	A	D	D
4	C	C	C	B	A	A
5	C	C	B	A	D	D
6	C	C	B	A	D	D
7	C	C	B	A	A	A
8	C	C	B	A	D	D
9	C	C	B	A	D	D
10	C	C	B	B	A	A
11	C	B	A	D	D	D
12	C	C	B	A	A	A
13	C	C	B	A	D	D
14	C	B	B	A	A	A
15	C	B	B	A	D	D
16	C	C	B	A	D	D
17	C	C	B	A	D	D
18	C	C	B	A	D	D

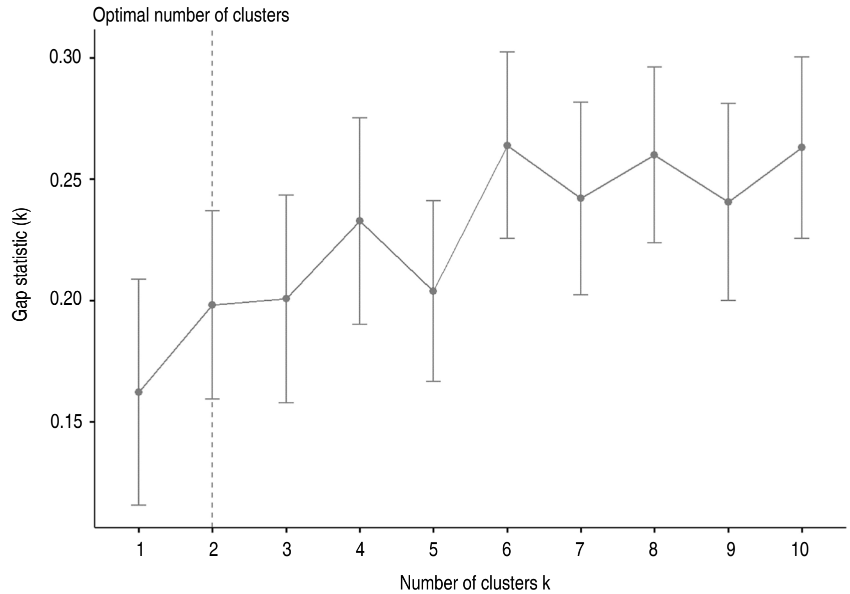

Figure 5. The optimal number of clusters was obtained with the Gap method and data from the last three measurements taken during the growth of UN Cotové papaya variety plants.

Comparison between selected groups

The assumptions of normality were checked inside all groups, and an analysis of variance was carried out with repeated measurements over time (measures). Statistical differences were observed between the groups for PH, SD, LN, and AOP (Table 3). All the morphological variables in group D (plants: 1, 3, 5, 6, 8, 9, 11, 13, 15, 16, 17, and 18) presented a higher mean value than in group A (plants: 2, 4, 7, 10, 12 and 14).

Ocampo et al. (2006) used cluster separation to evaluate geographical differences in papaya germplasm in Venezuela. The authors found two clusters, with a group representing the genotypes Venezuela, Trinidad, and Barbados and another for the genotypes Guadalupe, Martinique, and Granada. Similar to the four groups found in this study (Figure 3) using the Gap method, which separates data based on the lowest variance, optimizing the size of the groups, Asudi et al. (2010) compared the morphological diversity of germplasm in Kenya; they established four groups of plants, in which they identified vital traits to develop varieties adapted to different conditions.

Table 3. Analysis of variance between groups with repeated measurements over time forplant height (PH), stem diameter (SD), number of leaves (LN), and the area occupied by the plant (AOP) determined in four clusters of UN Cotové papaya variety plants.

Cluster	PH (cm)	SD(mm)	LN	AOP (cm^2)
	$\overline{X}$	$\overline{X}$	$\overline{X}$	$\overline{X}$
D	189.7±34.6 a*	105.9±1.76 a	31±5.0 a	63,992.2±11,428.4 a
A	143.9±23.7 b	66.2±1.0 b	21±5.9 b	27,682.9±6,917.2 b

*Values with a different letter in each column indicate significant differences according to the Tukey test (*P*<0.05%). Data are means ± s.d. (n=5).

The results obtained made it possible to identify the development stage of the papaya, where the plants show the most differences in the evaluated attributes, which supports breedings processes in the search for new progeny. Despite the plant height difference, the two groups of the UN Cotové papaya variety were short, with average heights of 189.7 cm (D) and 143.9 cm (A). According to Jiménez et al. (2014), papaya plants can reach up to 10 m, although hybrids and commercial varieties only grow up to 5 or 6 m. Almeida et al. (2003) found that the highest values for plant height and stem diameter corresponded to the highest productivity. However, plants with excessive heights make agronomic management more complex, whereas short plants are productive for a longer time. For stem diameter, Jiménez et al. (2014) pointed out that, in adult plants, it varies from 10 to 30 cm at the base and from 5 to 10 cm at the canopy. The stem diameter for the variety in this study was 10.59 cm in group D and 6.62 cm in group A. The stem provides structural support, storage capacity for defense substances (latex), transports water, nutrients, and various organic compounds, and is the site where fruits develop (Nabors 2006). Balakrishan et al. (1988) obtained a significant positive correlation between dry fruit weight, plant height, and stem circumference in 10 papaya cultivars, which suggests that plants from group D are preferred because stem diameter is a highly heritable trait and is highly influenced by the environment. The UN Cotové papaya variety presented 31 leaves for group D and 21 for group A. According to García (2010), reasonable values for a papaya cultivar in the adult stage are 30 leaves, requiring a minimum of 15 for an accepted fruit yield. Stem is relevant in production since a papaya leaf can support the development of three to four fruits (Jiménez et al. 2014). Plants with a large leaf area have a greater photosynthetic capacity, influencing the accumulation of carbohydrates in fruits (Zhou et al. 2000).

Growth modeling

Constructing a mathematical model for the two groups of plants allowed us to identify plant characteristics and predict behaviors using mathematical language through equations. The plant's development depends strongly on temperature, and this effect can be quantified using thermal time or GDD. Consequently, describing the growth dynamics of plants with equations based on thermal requirements for each phenological stage could help forecast the harvest date more accurately by reducing the observed variability, counting calendar days, predicting yield, and improving fruit quality (Salinas et al. 2019).

The proposed models fulfilled the assumptions of normality based on the Shapiro-Wilk test and homoscedasticity using the Bartlett test for all variables. The appropriate degree for the different polynomials tested was selected with the adjusted R^2 value, the mean squared error (RMSE), and the corrected Akaike index (Table 4). For plant height, the best model was a two-degree polynomial in both groups; for the other variables, a three-degree polynomial best explained the data, which corroborates the findings of Almeida et al. (2003). The second and third-order polynomial models best fit the relationships of GDD versus plant height, stem diameter, canopy diameter, and number of leaves for the different environmental conditions (Table 4).

Table 4. Adjusted R^2, mean square error (RMSE), and corrected Akaike index used to select the degree of the most appropriate polynomial for plant height (PH), stem diameter (SD), number of leaves (LN), and the area occupied by the plant (AOP) in UN Cotové papaya variety plants.

	Group D			Group A		
Criterion	**R^2 (adjusted)**	**RMSE**	**Akaike index (corrected)**	**R^2 (adjusted)**	**RMSE**	**Akaike index (corrected)**
			Plant height			
y=x	0.81	34.34	42.45	0.89	18.66	371.66
y=x^2	0.90	22.12	13.29	0.89	18.30	372.50
y=x^3	0.90	21.71	14.44	0.89	18.01	373.69
Equation	$y = e^{(-3.334^{-7}x^2+2.282^{-3}x+1.402)}$			$y = -6.221^{-6}x^2 + 8.870^{-2}x - 59.76$		
			Stem diameter			
y=x	0.90	13.58	538.04	0.88	9.45	314.53
y=x^2	0.90	13.50	539.51	0.88	9.25	315.14
y=x^3	0.92	12.17	528.13	0.90	8.66	312.20
Equation	$y = -1.471^{-8}x^3 + 9.496^{-5}x^2 - 0.1382x + 63.93$			$y = -8.167^{-9}x^3 + 5.078^{-5}x^2 - 6.523^{-2}x + 27.81$		
			Number of leaves			
y=x	0.78	5.18	410.34	0.33	4.88	258.37
y=x^2	0.84	4.46	392.70	0.48	4.32	250.10
y=x^3	0.88	3.78	372.93	0.53	4.09	247.60
Equation	$y = -5.948^{-9}x^3 + 3.494^{-5}x^2 - 4.880^{-2}x + 25.9$			$y = -3.465^{-9}x^3 + 1.909^{-5}x^2 - 2.554^{-2}x + 18.16$		
			The area occupied by the plant			
y=x	0.92	7681.4	1374.6	0.77	6292.8	860.6
y=x^2	0.94	7014.5	1364.9	0.77	6191.2	861.7
y=x^3	0.95	6221.3	1351.4	0.81	5608.2	855.9
Equation	$y = -8.149^{-6}x^3 + 5.933^{-2}x^2 - 100.9x + 50320$			$y = -6.595^{-6}x^3 + 4.178^{-2}x^2 - 67.06x + 32040$		

The graphical representation of the models is presented in Figure 6, where the group D plants show greater plant heights towards the end of the study (Figure 6A), with a very similar growth up to 1800 GDD. This result confirmed those obtained in the clustering, which indicated that the greatest morphological differences appeared after the fourth measurement. SD and the AOP exhibited a similar behavior; in the sixth measurement, the SD in group D was 64% greater than in group A, while the AOP was 35% greater in D. As for the LN, group A presented the highest value at the beginning of the plant development. However, after the fourth measurement, group D exceeded the foliar emission rate by 50% to group A.

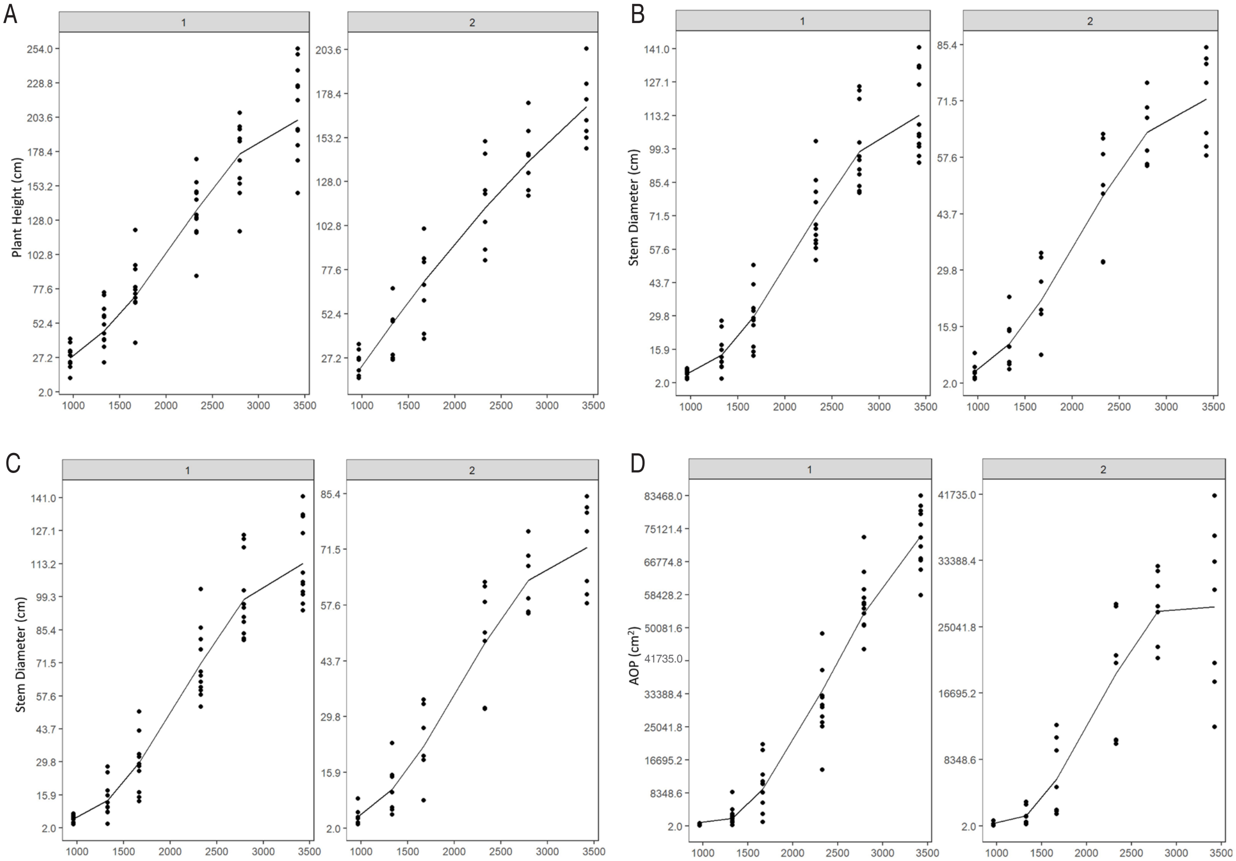

Figure 6. Representation of second and third-order models that explain growth for the variables plant height (A), stem diameter (B), number of leaves (C), and the area occupied by the plant (D) as a function of the accumulated degree days (GDD) in UN Cotové papaya variety plants.

Dos Santos et al. (2021) used mathematical models to estimate papaya fruits weight in the Alian cultivar. Salinas et al. (2019), using non-linear models in papaya, concluded that cultivars with smaller fruits need less time than cultivars with larger fruits to reach harvest. In this sense, selecting the appropriate polynomial degree to fit the data can be a problem since data can be underestimated by not ordering them correctly in the equation if a too-low polynomial order is used. Thus, data can be overestimated if a high-degree polynomial is used, especially at the ends of the curve. Hughes and Freeman (1967) suggest using third-degree polynomials to describe plant growth as a function of dry weight and leaf area.

For PH and SD, the sigmoid or non-linear models (Logistic model: L, Log-logistic model: LL, Log-Normal model: LN, Gompertz model: G, Weibull model: W) were not significant since the “Lack of Fit” values were greater than 0.05. For LN, it was observed that many of the non-linear models were statistically significant; however, the significant models did not achieve higher "loglik" values nor lower AICc values (differing by at least three units). For AOP, group D only had one model (W1.4) that was significant; however, it was impossible to obtain a lower AIC than the simple polynomial regression model; in group two, no model was statistically significant.

For the UN Cotové papaya variety, non-linear models are not the best option to describe the morphological variables. Simple polynomial regression models offer a good fit and simplicity in the equations. A possible explanation for why non-linear models do not fit well is that the GDD is used as an independent variable. This "standardized" or "normalized" variable can reduce non-performance, typical linear growth. A similar result was described by Salinas et al. (2019) when using the Richards and Weibull models, finding that the fit of data was not improved when using GDD instead of calendar days.

CONCLUSIONS

The UN Cotové papaya variety was composed of two morphotypes: group "A" presented the lowest plant height, stem diameter, number of leaves, and the area occupied by the plant. Group "D" had more developed plants. The best plants identified by morphological criteria in cluster "D" can be used as progenitors to cross with commercial materials for future breeding papaya programs. These plants confer desirable morphological characteristics such as low plant height and an adequate number of leaves because they provide better structural support. The high correlation between temperature and morphological variables indicated that quantifying environmental heat, expressed in GDD in each phenological stage, helps predict growth parameters with simple polynomial regression models.

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Research article

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Weed community of no-till avocado (*Persea americana* Mill.) crops in tropical highlands

UNIVERSIDAD NACIONAL DE COLOMBIA

Comunidad de arvenses en el cultivo de aguacate (*Persea americana* Mill.) sin labranza en el trópico alto

https://doi.org/10.15446/rfnam.v76n2.101345

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ABSTRACT

Keywords:
Importance value
Mechanical management
Perennial weeds
Weed composition

No-till avocado orchards in the highlands are gaining importance in eastern Antioquia, Colombia. To evaluate the influence of management, and edaphic properties on the composition of weed communities, 50 Hass avocado orchards were studied in nine municipalities of eastern Antioquia. The variables cover and presence of weeds were used to estimate relative frequency, dominance, and Importance Value (IV); weed management was characterized by a survey. The maximum moisture retention capacity and pH of the soil were also determined. The importance of species, rotation of management strategies, and herbicides were analyzed graphically. The association between weeds dominance, and factors, was evaluated by a generalized linear model. 66 dicots and 28 monocot weeds were classified. Out of the 10 most important species, three foreign species, *Melinis minutiflora* P. Beauv*,* *Digitaria horizontalis* Willd, and *Pennisetum clandestinum* Hoschst. Ex Chiov were the most representative. The municipalities are grouping the effects of similar management strategies in the territory. Chemical management favored the equilibrium of the weed composition, while mechanical management did not. Soil properties had less influence on the distribution and dominance of weeds.

RESUMEN

Palabras clave:
Valor de importancia
Manejo mecánico
Arvenses perennes
Composición de arvenses

El cultivo de aguacate cada vez cobra mayor importancia en el altiplano de la subregión Oriente del departamento de Antioquia, Colombia. Para evaluar la influencia de diferentes formas de manejo y de algunas propiedades del suelo en la composición de la comunidad de arvenses se monitorearon 50 huertos de aguacate variedad Hass distribuidos en nueve municipios, de la subregión oriente de Antioquia. Las variables evaluadas fueron la cobertura y la presencia de arvenses, lo que permitió estimar la frecuencia y dominancia relativas, así como el valor de importancia (IV por sus siglas en inglés). El manejo se caracterizó a través de una encuesta, aplicada a los productores de los predios seleccionados. También se determinó la capacidad máxima de retención de humedad y el pH del suelo. La importancia de las arvenses, la rotación de las estrategias de manejo mecánico y el uso de herbicidas se analizaron gráficamente. La asociación entre la dominancia y los factores se determinó a través de un modelo lineal generalizado. Se clasificaron 66 especies de arvenses dicotiledóneas y 28 monocotiledóneas. De las 10 especies con mayor IV, tres especies foráneas *Melinis minutiflora* P. Beauv, *Digitaria horizontalis* Willd y *Pennisetum clandestinum* Hoschst. Ex Chiov fueron las más representativas. Los municipios agruparon los efectos de estrategias de manejo similares en el territorio, el manejo químico favoreció el equilibrio de la composición de arvenses, mientras que el mecánico lo desfavoreció. Las propiedades del suelo presentaron menor influencia en la distribución y dominancia de las arvenses.

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The most common avocado varieties grown in Colombia are Antillana (*Persea americana* Mill.), Guatemalteca (*P. americana* var. *guatemalensis*), and Mexicana (*P. americana* var. *drymifolia*), and hybrids between them (Vega 2012; Crane et al. 2013). Avocado crops in the lowlands have a precarious implementation of agronomics practices; however, the domestic markets prefer the product due to its bigger size and distribute it in bulk. Conversely, the cultivated area in the Colombian Andean Mountains have been expanding in the last two decades (15,530 ha) (MADR 2018), It has been stimulated mainly by exports mostly of the Hass variety due to both its pulp quality and high productivity (Arias et al. 2021). The expanding fields come from the conversion of pastures, meadows, and fallow lands, in areas with high slope (>75%) (MADR 2018). In the arable fields of the temperate regions, several authors agree that the most determinant factors in the weed species composition are the type and preceding crop, crop rotation, sowing season, and tillage intensity. Environmental factors such as altitude and soil properties, although significant, remain less relevant (Nowak et al. 2015; Wells et al. 2014; Hanzlik and Gerowit 2011; Pinke et al. 2010; Šilk et al. 2009). However, Pinke et al. (2012) studying the influence of the environment, management, and site context on species composition of summer arable weed vegetation found that even for intensified agriculture, the effects of environmental factors were more important than management strategies. In Colombia, the hill landscape where the Hass avocado variety is increasing prevents tillage during both crop establishment and maintenance (Vega 2012); moreover, the perennial characteristic of the crop does not allow crop rotations and reduce the strategies available within an integrated weed management program.

The management of perennial weeds is the principal concern for the crop. An increase has been observed because their root system is not disturbed by management practices, it is favored by low inter-specific competition, and most herbicides are not fully efficient (Bajwa 2014). Buhler et al. (1994) suggest that management of established perennial weeds in reduced tillage fields is ineffective and may increase the spread of roots with vegetative reproduction. Hatcher (2017) reported changes in weed floras associated with reduced-tillage systems and cited, the increase of monocot and dicot species in the absence of tillage. Thereby, perennial weed dominance excludes floristically abundant annual species (Boatman et al. 2011); however, Buhler (1995) advises exercising caution before making statements of an increase of perennial weeds because the situation can vary among sites as a result of interactions of tillage with weed management practices, the environment, and initial perennial weed populations. Management practices such as selective application of systemic herbicides to tall-growing perennial weeds, row-space, and inter-row cultivation help reduce the density of perennial-species, regardless of the tillage system, to levels that do not reduce crop yields nor increase management costs (Streit et al. 2002). Exploration of weeds distribution and their interactions with other variables can help us adjust management strategies and establish ecological balances between weeds and crops (Olorunmaiye et al. 2011; Bastiaans et al. 2000).

The objective of the study was to evaluate the influence of weed management and edaphic properties on the composition of the weed communities, in no-till avocado orchards in the highlands of eastern of Antioquia, Colombia.

MATERIALS AND METHODS

Agroecological characteristics

Avocado orchards mainly Hass variety are expanding in the eastern Antioquia, in Colombia highlands (Figure 1). Table 1 shows the most representative soil type reliefs and Holdridge life zones and details the nine municipalities of the study. The eastern region has a broad diversity of environments and landscapes, with high and low hills, terraces, and alluvial plains. Its soil is formed from the weathering of igneous rocks and covered by a thin layer of volcanic ash, which give it andic properties. These soils are acid and have poor content of nutrients, high phosphorus fixation, and variable cation exchange capacity (Jaramillo 2009; IGAC 2007). According to Holdridge's life zone classification system, there are two land areas: the lower montane moist forest and the lower montane wet forest (Holdridge 2000). The study region presents an altitudinal range between 1,800 and 2,500 masl, an average annual temperature of 17 °C and rainfall of 2,000 mm (IGAC 2007).

Table 1. Description of the most representative soil taxonomic units of the study region including the Holdridge life zones (IGAC 2007).

Municipalities	Soil taxonomic units	Description	Relief	Holdridge life zones
San Vicente, El Peñol, Rionegro, Guarne, Marinilla, El Santuario, El Carmen de Viboral	Typic Hapludands Typic Fulvudands Hydric Hapludands	Deep to moderately deep soils, well drained, medium textures, strong (pH=5.1-5.5) to moderately (pH=5.6-6.0) acidic, low fertility (interchangeable bases 3.01-6.0).	Highlands in relief of hills and hills	bh-MB
El Retiro, La Ceja, El Carmen de Viboral	Typic Dystrudepts Typic Eutrodepts	Deep and well-drained soils, fine to medium textures, moderately acidic (5.6–6.0), low fertility (interchangeable bases 3.01-6.0)	Relief slightly undulating to moderately steep; long, straight or convex slopes ranging from 7 to 75%	bh-MB, bmh-MB

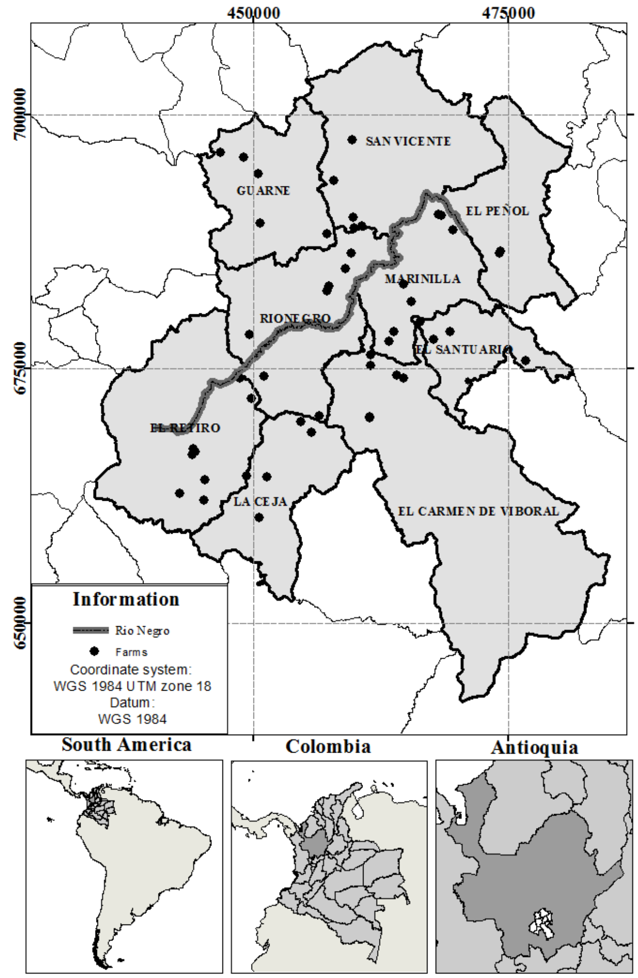

Figure 1. Map of the spatial distribution of the fifty avocado orchards, situated in the highlands of the eastern, Antioquia, Colombia.

Sampling

The fieldwork took place between 2016 and 2017 in 50 Hass avocado orchards distributed in nine municipalities (Table 1). The perimeter of the avocado orchards was established with a 62sc Garmin map GPS receiver, configured under the WGS84 UTM projection system zone 18N. The coordinates of 20 sampling points elaborated with mesh distribution on the digital map of each orchard plot were exported to the GPS (Figure 1). Each sampling site was identified in the field of the orchards surveyed using the GPS.

Evaluation and identification of weeds

The weeds were sampled among the crop grooves, avoiding the dripping of the trees and considering tree shading, the applied organic matter, and the manual handling that limits the representation of the established weeds in the system. A square frame (0.5x0.5) m was set to define each sampling point. The frequency (F%) (Equation 1) and dominance (d%) (Equation 2) were calculated based on the presence and visual cover (%) of each species identified in the frames.

$$F_{spi} = \frac{\sum_{i=1}^{n} x_{spi}}{n} * 100 \qquad (1)$$

$$d_{spi} = \frac{\sum_{i=1}^{n} c_{spi}}{n} * 100 \qquad (2)$$

Where, for *i-n* species, F_{spi}: average frequency (%), x_{spi}: frames with the specie n: total sites in the orchards, d_{spi}: average dominance (%), and c_{spi}: visual cover of specie in the frame.

Their relative composition was estimated using the expressions in the Equations (3, 4).

$$Fr_{spi} = \frac{F_{spi}}{\sum_{i=1}^{p} F_{spi}} \qquad (3)$$

$$dr_{spi} = \frac{d_{spi}}{\sum_{i=1}^{p} d_{spi}} \qquad (4)$$

The Importance Value (IV) (Equation 5), which reveals the significance of the species in the weed composition, was estimated as the sum of the relative frequency and the relative dominance.

$$IV_{spi} = dr_{spi} + F_{spi} \qquad (5)$$

The IV is a modified indicator of the phytosociological importance of weed types in a community; it compares them individually and jointly by the frequency and dominance (Bhadra and Pattanayak 2016). We classified the weed biotypes in the Medel herbarium of Universidad Nacional de Colombia.

Weed management and edaphic variables

A semi-structured survey was used to collect the information related to weed management. The categories defined to group the strategies were chemical and mechanical management. In the last group, the order of rotation of the tools was considered, such as brush cutters, machete, or manual eradication (Table 2). In the case of chemical management, it is important to ask about the types of herbicides used. In the soil, the evaluated variables were the maximum moisture retention capacity (MMRC) and pH.

Table 2. Weed management strategies evaluated in the avocado orchards of the eastern Antioquia, Colombia.

Rotations	Abbreviation	Group
Brushcutters	B	MC[1]
Brushcutters – Machete	B-Mch	MC
Brushcutters – Manual	B-Mn	MC
Brushcutters – Manual – Machete	B-Mn-Mch	MC
Machete	Mch	MC
Glyphosate	Glyphosate	QC[2]
Paraquat	Paraquat	QC

[1]Mechanical management. [2]Chemical management.

The MMRCs were determined using perforated cylindrical brass boxes of volume 22 cm^3 and known weight. The soil samples dried at 105 °C were passed through a 1 mm sieve, packed in the cylinders, and weighed. The packed samples were saturated with water and the excess was drained. Finally, the cylinder with the moist soil was weighed and the MMRC (%) was estimated. The pH was determined by the potentiometric method in the laboratory on a 20 mL sample of dry soil suspended in 50 mL of water (soil - water ratio 1:2.5 V V^{-1}) (Weil and Brady 2016). In both cases, each sampled site was evaluated by triplicated.

Statistical analysis

The statistical analysis of the data was made in three steps. 1) Selection of the ten species with the highest IV obtained as the average of all the IVs in the 50 farms and their average distribution in the territories, according to the farms monitored in each municipality. The differences between species in the IV was established by the Kruskal-Walli's test, given the non-normality of this variable. 2) Evaluation of the relationship between weeds dominance as response variable and factors: region (nine municipalities), management forms (five mechanical and two chemical), and soil (MMRC and pH) as explicative variables, and a non-linear Poisson link function through a generalized linear model. 3) Graphical description of the implemented weed management strategies. The Statistical analysis was performed using the R Project programming language (The R Core Team 2021).

RESULTS AND DISCUSSION

Weed composition

In total, 94 weed species were identified, 66 dicots and 28 monocots, within 33 botanical families. The families Asteraceae and Poaceae stand out with the highest number of species. Figure 2 shows the 10 species of weeds with the highest IV. Two perennial and one annual species of the Poaceae family occupy the first three positions of the IV ranking. *Melinis minutiflora* P. Beauv, *Digitaria horizontalis* Wild, and *Pennisetum clandestinum* Hochst ex Chiov presented the largest population in the studied territory with average dominance values higher than 0.1 (10%), thus being dominant in six, five, and three municipalities out of the nine involved respectively (Table 3). The occurrence and dominance of the species *Bidens pilosa* L., *Hypochaeris* radiata L, *Persicaria nepalense* (Meisn.) Miyabe, and Taraxacum *campylodes* G.E. Haglund were low (Dominance<0.009) throughout the region. For the other species *Pteridium aquilinum* L. Kuhn, *Oxalis corniculata* L., *Commelina diffusa* Burm.f. the dominance was above 0.1 in at least one municipality and maximum in three (Table 3); this behavior is in the middle of the two previous groups.

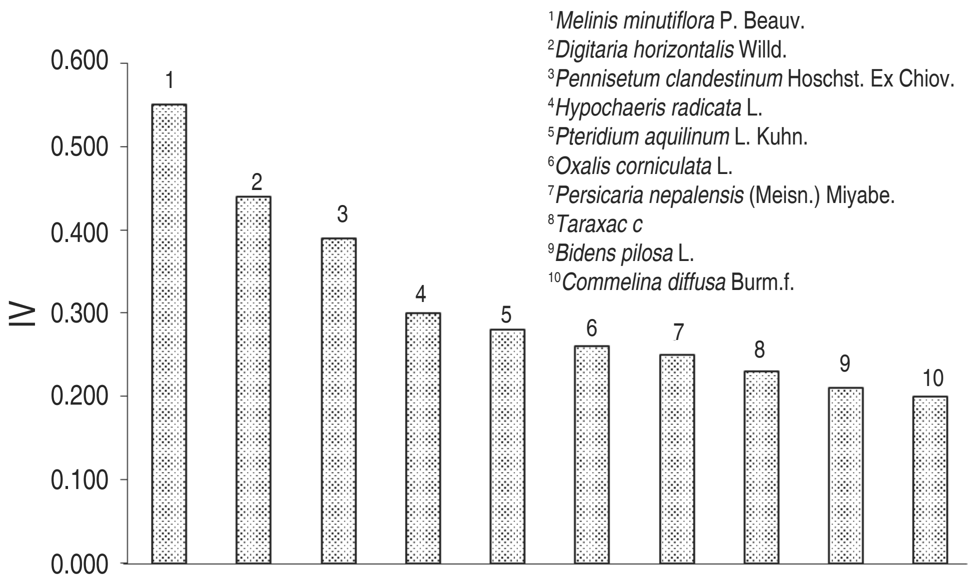

Figure 2. Importance value index of weeds in the avocado orchards in the highlands of eastern Antioquia, Colombia. Equal letters on the bars indicate no significant differences between the Importance Value of the species, based on the Kruskal-Walli's test (P<0.05).

Table 3. Average dominance of the 10 largest IV species in Hass avocado crops in the highlands of eastern Antioquia, Colombia.

Municipality	Bp[1]	Cd[2]	Dh[3]	Hr[4]	Mm[5]	Oc[6]	Pc[7]	Pn[8]	Pa[9]	Tc[10]
Carmen de V.	0.016	0.026	0.132	0.057	0.028	0.110	0.100	0.088	0.013	0.035
El Peñol	0.063	0.107	0.151	0.000	0.000	0.000	0.127	0.079	0.029	0.000
El Retiro	0.078	0.015	0.072	0.042	0.191	0.015	0.117	0.027	0.097	0.001
El Santuario	0.001	0.045	0.073	0.016	0.136	0.000	0.113	0.006	0.127	0.008
Guarne	0.024	0.004	0.080	0.082	0.120	0.010	0.106	0.007	0.109	0.027
La Ceja	0.003	0.000	0.023	0.050	0.121	0.018	0.125	0.007	0.015	0.044
Marinilla	0.061	0.076	0.082	0.029	0.020	0.031	0.041	0.049	0.021	0.001
Rionegro	0.046	0.026	0.145	0.014	0.129	0.025	0.044	0.024	0.037	0.006
San Vicente	0.053	0.027	0.034	0.058	0.081	0.009	0.059	0.069	0.073	0.026

[1]*Bidens pilosa* L. [2]*Commelina diffusa* Burm. f. [3]*Digitaria horizontalis* Willd. [4]*Hypochaeris radiata* Falk. [5]*Melinis minutiflora* P. Beauv. [6]*Oxalis corniculata* L. [7]*Pennisetum clandestinum* Hochst. ex Chiov. [8]*Persicaria nepalensis* (Meisn.) Miyabe. [9]*Pteridium aquilinum* L. Kuhn. [10]*Taraxacum campylodes* G.E. Haglund.

Within the three most significant species, *M. minutiflora* and *P. clandestinum are* perennial, rhizomatous weeds with matted roots and grass-like, herbaceous habits. They are native to the highland regions of east Africa. *D. horizontalis* is an annual weed distributed in the high and humid lands of the savannah and forests of Africa. Although those three species are considered forage, in the tropic, they grow fast and have become dominant species in the cultivated fields, replacing the native flora (Romero et al. 2004). Furthermore, *D. horizontalis* is reported with the highest aggressiveness, mainly in annual crops (Giraldo-Cañas and Baptiste 2017; Johnson and Kent 2002). In the upland rice, densities of 10 plants m^{-2} of this species reduce 40% of the production. There are few studies on the impact of this species in the perennial crops; however, Olorunmaiye et al. (2011) and Lemes et al. (2010) report intermediate to low interference in citrus and coffee, respectively.

Territory, soil, and agronomic management factors

Table 4 shows the dominance relationships of the 10 species with higher IV as a function of the factors, region, management strategies, and soil. The municipality was the most relevant characteristic related to species presence or absence in the distribution. In this sense, the orchards located in El Carmen de Viboral, had unfavorable conditions for the presence of weeds. Meanwhile, the other towns presented favorable conditions for some weeds and adverse for others. In the municipalities with less favorability producers have a better rotation of the chemical and mechanical management strategies. Figure 3 shows the management strategies implemented in different farms and compares them by municipalities. 35% of the evaluated farms managed weeds by rotating brush cutters-manual-machete (B-Mn-Mch). Rionegro was the most representative with 8%, followed by Marinilla and El Retiro, representing 6% each. The brush-cutters-manual (B-Mn) rotation practice in weed management represent 28% of participation and is the second most used procedure. The use of brush cutters (B) is in third place with 20%. The least used practices (10% and 8%) were the combination of brush-cutters-machete (B-Mch) and only the of machete (Mch), respectively.

On the other hand, chemical management is another of the strategies of great use in the territory; 80% of the 50 farms evaluated use glyphosate as one of their main management strategies, while paraquat occurred in 44% (Figure 4).

The differences in the weed composition to the municipality scale show the modulation groups by local practices according to the landscape effect. This view implies differential spatial dynamics for management, which may represent a lever to hinder, or favor weed spread and persistence in agroecosystems (Petit et al. 2013; Gabriel et al. 2009).

The dominance of *M. minutiflora* is favored significantly in five of the nine municipalities. Although the mechanical management did not show significant incidence, is

Table 4. Association of factors municipality, weeds management, and soil pH and MMRC, with the dominance of the ten species of weeds with higher IV in Hass avocado orchards using a generalized lineal model with a non-linear Poisson link function.

Factor	Species																			
	Bp[1]	Sig[2].	*Cd*[3]	Sig.	*Dh*[4]	Sig.	*Hr*[5]	Sig	*Mm*[6]	Sig.	*Oc*[7]	Sig.	*Pc*[8]	*Sig.*	*Pn*[9]	Sig.	*Pa*[10]	*Sig.*	*Tc*[11]	Sig.
El Carmen de Viboral	-6.84	*[12]	-9.96	*	-13.02	*	-9.09	*	-3.55	*	-[(13)]	-	-17.74	*	-12.58	*	-17.74	*	-8.60	*
El Peñol	1.50	*	1.21	*	2.11	*	-1.58	*	-	-	-2.49	*	-	-	-	-	-	-	-1.16	*
El Retiro	1.14	*	-	-	-0.94	*	-	-	2.43	*	-1.89	*	-	-	-1.17	*	-	-	-0.91	*
El Santuario	-	-	-	-	-1.22	*	-1.28	*	1.74	*	-2.61	*	-	-	-1.65	*	-	-	-	-
Guarne	-	-	-1.27	*	-	-	1.37	*	1.71	*	-2.23	*	-	-	-1.46	*	-	-	-	-
La Ceja	-	-	-1.55	*	-1.58	*	-	-	1.46	*	-2.11	*	-	-	-1.72	*	-	-	1.02	*
Marinilla	1.40	*	0.84	*	-	-	-	-	-	-	-1.29	*	-	-	-	-	-	-	-0.97	*
Rionegro	0.89	*	-	-	-	-	-	-	2.12	*	-1.51	*	-	-	-1.09	*	-	-	-0.67	*
San Vicente	0.98	*	-	-	-1.40	*	-	-	-	-	-1.98	*	-	-	-	-	-	-	-	-
pH	-0.62	*	-	-	0.74	*	-	-	-1.42	*	0.33	*	1.12	*	0.57	*	1.12	*	-0.36	*
MMRC[14]	-	-	-	-	-	-	-	-	-	-	-	-	0.02	*	-	-	0.02	*	-	-
B-Mch[15]	-	-	-	-	-1.36	*	1.42	*	-	-	-1.71	*	-	-	-	-	3.27	*	-	-
B-Mn[16]	-	-	-	-	-	-	-	-	-	-	-1.75	*	-	-	-	-	0.81	*	-	-
B-Mn-Mch[17]	-	-	-	-	-1.40	*	-0.97	*	-	-	-1.87	*	-	-	-	-	1.62	*	1.06	*
Mch[18]	-	-	-	-	-1.68	*	-	-	-	-	-1.56	*	2.70	*	-	-	1.39	*	-	-
Glyphosate	0.01	*	0.01	*	-0.01	*	0.00	*	-0.01	*	0.01	*	-	-	-	-	-	-	-	-
Paraquat	-	-	-	-	-	-	0.02	*	-0.02	*	-	-	-0.01	*	0.02	*	-0.01	*	-0.01	*

[1]*Bidens pilosa* L. [2]Significance. [3]*Commelina diffusa* Burm.f. [4]*Digitaria horizontalis* Willd. [5]*Hypochaeris radiata* Falk. [6]*Melinis minutiflora* P.Beauv. [7]*Oxalis corniculata* L. [8]*Pennisetum clandestinum* Hochst. ex Chiov. [9]*Persicaria nepalensis* (Meisn.) Miyabe. [10]*Pteridium aquilinum* L. Kuhn. [11]*Taraxacum campylodes* G.E. Haglund. [12]Significant coefficients in the glm model *P<0.05.* [13]*P>0.05*. [14]Maximum Moisture Retention Capacity of soil (MMRC). [15]Management of weeds with brushcutters-machete. [16]Management of weeds with brushcutters-manual. [17]Management of weeds with brushcutters-manual-machete.

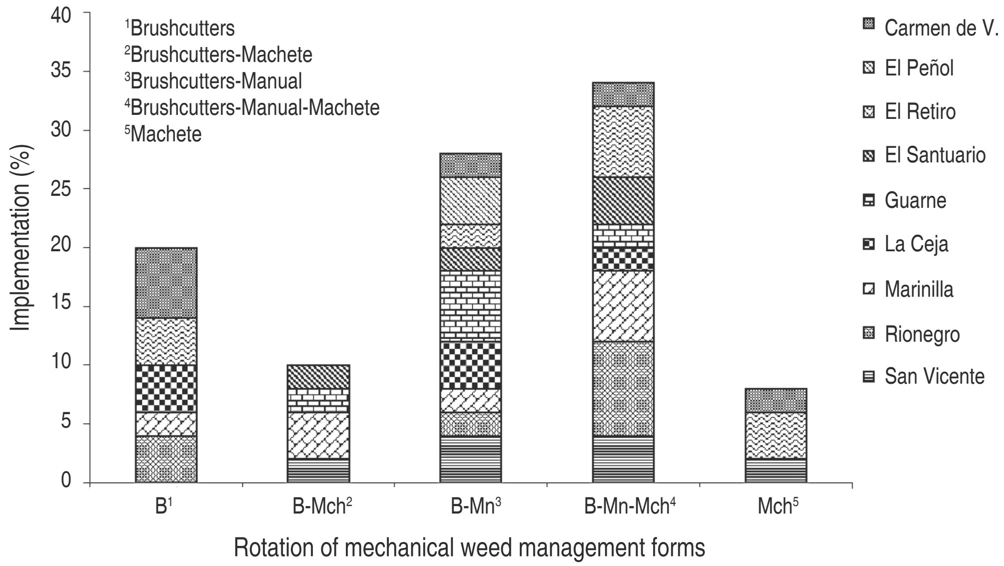

Figure 3. Rotation of mechanical handling of weeds in Hass avocado orchards in the highlands of eastern Antioquia, Colombia.

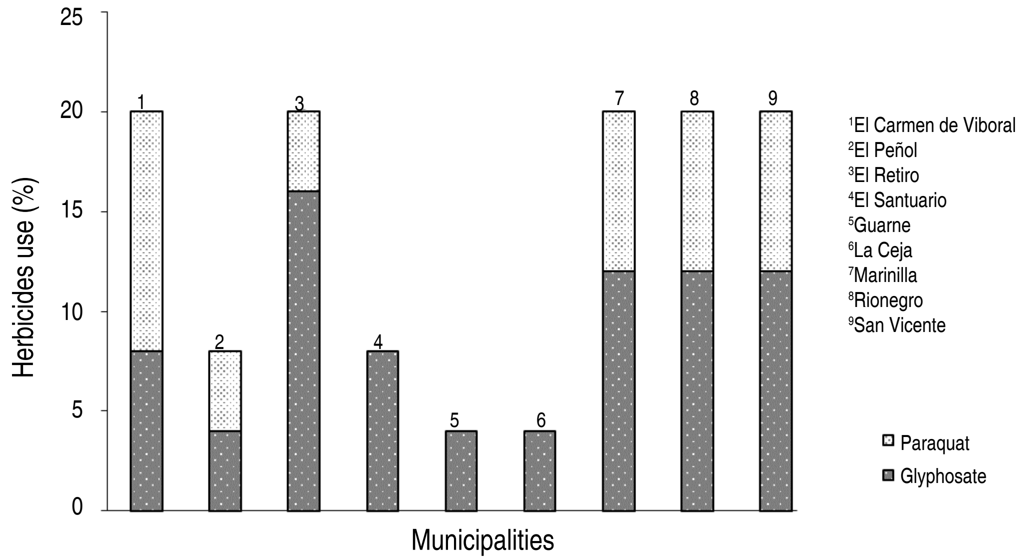

Figure 4. Type of herbicides used in Hass avocado orchards according to the municipality of the eastern region, Antioquia Colombia.

favorable for the propagation and tillering of this highly invasive species. Chemical management affects the dominance of this weed, and it is recommendable as a management strategy directed to its patches and thus favors the establishment of soft species. The municipality factor influenced the distribution of *D. horizontalis* significantly; being direct in one and inverse in five of them. Additionally, mechanical and chemical management factors impact the establishment of this species. Although most strategies work well, the brush cutter-manual-machete rotation is the most used method in the study region. The pH also had an indirect effect on *D. horizontalis,* thus indicating a positive relation with the acidic conditions of the soils.

The positive relationship of *P. clandestinum* with pH and MMRC factors evidenced a higher dominance of this species in wet soils with moderate acidity. This narrow-leaf (monocots) species presented positive relation with machete management (Mch) favors its competence and dispersion capacity (reproduction by stolon). The use of paraquat diminishes the dominance of this species and promote the germination and growth of others. Regarding *H. radicata,* the municipality factor had a positive effect in one and negative in three of the nine sampled municipalities. The chemical factor favors its establishment mainly because it eliminates the competition with the narrow-leaf weeds and enables the germination of seeds from the seed bank (Schoenfelder et al. 2010). Mechanical management strategies also have a significant impact; the brush cutters-machete rotation is positive, and the brush cutters-manual-machete rotation is negative, for the dominance of this species. Kroon et al. (1987) evaluated the population dynamics of perennial grass and *H. radicata* under different mechanical management frequencies by density-dependent simulation models. The show that the cut favors the vegetative branch and consequently the increase in the size of the rosette. Manual weeding affects the *H. radicata* dominance since the plant cannot increase the rosette. This species can be said to be sporadic in the process of succession rather than a competing weed.

The species *B. pilosa* registered a low IV in all municipalities; nevertheless, the factor was favorable for its distribution in five of the nine evaluated municipalities. The chemical management of weeds was frequent in all territories, and the main active ingredient was glyphosate. The bare ground patches allow the germination of dormant seeds in the seed bank; consequently, less competitive species such as

B. pilosa have the opportunity to grow and increase their importance. The pH of the soil also has significant adverse effect (*P<0.05*) in the dominance of the weeds.

Based on the results, the distribution of the *Commelina diffusa* Burm.f. species is regulated by the territory factor. Therefore, three municipalities reduced and two favored its dominance. The first ones made greater use of herbicides in orchards and later did the opposite. This result suggests that despite its low competitive capacity, it grows fast, and its establishment can be enhanced as a useful plant in the avocado orchards under reasonable herbicide use.

In the avocado orchards, *Oxalis corniculate* L, *Persicaria nepalensis (Meins) Miyabe* and *Taraxacum campylodes* G.E. Haglund are rare and non-aggressive species. Most of the factors have a negative impact. The positive effect of the chemical management on them is due to a decrease in the frequency and dominance of the most competitive weeds, i.e., mostly narrow-leaf species, principally. Furthermore, pH presented a direct relationship with *O. corniculata* and *P. nepalensis* species and inverse with *T. campylodes*.

CONCLUSIONS

Three foreign species dominate the weeds communities (IV>0.39) in the highlands with expanding avocado orchards in Antioquia, Colombia (*M. minutiflora, D. horizontalis, and P. clandestinum*). The high slopes restrict tillage and favor the distribution and dominance of these species in the territory. The municipalities are grouping the effects of similar management strategies and cultural practices in the region. The chemical management favored the equilibrium of the weed composition, while mechanical management did not, due to greater pressure on the most competitive species, thus favoring their establishment. Finally, the humidity and pH, although with significant effect, have less influence on weeds' distribution and dominance.

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Analysis of the effect of GE interaction on the grain yield and its related traits in rain-fed Algerian durum wheat (*Triticum turgidum* L. var. *durum*) grown in contrasting environments

Análisis del efecto de la interacción GE sobre el rendimiento de grano y sus rasgos relacionados en trigo duro argelino de secano (*Triticum turgidum* L. var. *durum*) cultivado en ambientes contrastados

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ABSTRACT

Keywords:
Environment relationship
GE interaction
Grain yield
Stability
Traits correlation

Selection for higher yield and wider adaptability are the most important tasks in crop breeding programs. (GE) interactions are commonly seen as one of the major barriers in plant breeding. The present work aims to assess the effects of GE interaction for the grain yield of 14 durum wheat varieties grown in rain-fed environments during 2014-2017 cropping seasons, and to analyze the relationships between 15 traits intra and inter-environments. Field trials were carried out in a randomized complete block design with four replicates. Grain yield data were analyzed using AMMI model. The combined analysis of variance showed that the effects of genotype, environment and their interactions were highly significant on the grain yield. Using CV and Pi index, GTA dur was the high yielding (32.5 q ha^{-1}) and most stable variety across all the environments. Based on the inter-character correlation, the indirect selection of grain yield via the number of grains per m² would be effective. Moreover, the inter-environment correlation of the studied variables confirms there was GE interaction and suggests that the best varieties should be chosen according to their specific adaptation. Cold environments differed from warm and moderate ones in the ranking of varieties. Indeed, Sétif site offers better possibilities for producing the Ofanto variety (39.9 q ha^{-1}). Whereas, GTA dur and Simeto (30.9 q ha^{-1} and 29.7 q ha^{-1}, respectively) prove to be the most efficient in terms of grain yield at Oued Smar and Khemis Miliana sites together.

RESUMEN

Palabras clave:
Relación con el entorno
Interacción GE
Rendimiento de granos
Estabilidad
Correlación de rasgos

La selección para obtener un mayor rendimiento y una mayor adaptabilidad son las tareas más importantes en los programas de mejora de cultivos. Las interacciones GE son comúnmente consideradas como una de las principales barreras en el fitomejoramiento. El presente trabajo tiene como objetivo evaluar los efectos de la interacción GE para el rendimiento de grano de 14 variedades de trigo duro cultivadas en ambientes de secano durante las temporadas de cultivo 2014-2017, y analizar las relaciones entre 15 caracteres intra e interambientes. Los ensayos de campo de 14 variedades se organizaron en un diseño de bloques completos al azar con cuatro repeticiones. Los datos de rendimiento de grano se analizaron utilizando el modelo AMMI. El análisis combinado de la varianza mostró que el efecto del genotipo, el ambiente y sus interacciones fueron altamente significativos para el rendimiento de grano. Utilizando el CV y el índice Pi, GTA dur fue la variedad de mayor rendimiento (32,5 q ha^{-1}) y más estable en todos los ambientes. Basándose en la correlación entre caracteres, la selección indirecta del rendimiento de grano a través del número de granos por m² sería efectiva. Además, la correlación interambiente de las variables estudiadas, confirman la presencia de la interacción GE y sugiere que se deben elegir las mejores variedades de acuerdo con la adaptación específica. Los entornos fríos difirieron de los cálidos y moderados en la clasificación de las variedades. En efecto, el sitio Sétif ofrece mejores posibilidades para producir la variedad Ofanto (39,9 q ha^{-1}). En cambio, GTA dur y Simeto (30,9 q ha^{-1} y 29,7 q ha^{-1}, respectivamente) demuestran ser los más eficientes en términos de rendimiento de grano en los sitios de Oued Smar y Khemis Miliana juntos.

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The production of durum wheat is highly limited by abiotic stress in semi-arid regions and biotic stress in sub-humid areas (Mansour and Hachicha 2014). Frost and desiccation damage the floral organs and developing grain, which cause yield loss (Zheng et al. 2015). Determining the traits related to grain yield (GY) is essential to improve reproductive efficiency (Mohammadi et al. 2012). In this context, variables such as grains per square meter, biomass, harvest index, and reduced plant height are positively associated with GY progress (Xiao et al. 2012). Cycle length, days to heading and anthesis can be increased when moving from the warmest and driest zones to the coldest and wettest ones, inversely to the duration of the grain filling period (Royo et al. 2014). In addition, GY genetic gains for CIMMYT material are mainly associated with flowering time, grain size and grain weight (Lopes et al. 2012; Aisawi et al. 2015).

Plant breeders are interested in the selection of varieties that have good performance in a range of environments, thus selection is complicated by the presence of Genotype by Environment interaction (GE) (Oral et al. 2018; Benkadja et al. 2022). In general, complex traits such as GY and its components show GE interaction, which may be expressed as heterogeneity in genetic variance among environments and/or in the ranking of individuals (Burgueño et al. 2008; Karimizadeh et al. 2016). Multi-environment trials (METs) are important for studying the stability and adaptation of cultivars for grain yield, as well as for predicting the performance of genotypes in different environments (Solonechnyi et al. 2015; Solonechnyi et al. 2018). In this way, both yield and stability should be considered simultaneously to reduce the effect of GE interaction (Bose et al. 2014; Mohammadi et al. 2018). Such selections are very difficult to be make. A stable genotype is defined as one having an unchanged performance irrespective of any variation in the test environments (Karimizadeh et al. 2012; Sabaghnia et al. 2012). In this context, the use of selection indices is recommended (Benmahammed et al. 2010). Several structures can be used for modeling the GE. In fact, models which separate genetic effects into common and specific components can be the superior form of increasing the accuracy of genotypic selection (Crossa et al. 2006; Burgueño et al. 2007). One of the efficient models is the additive main effects and multiplicative interaction (AMMI) (Gauch et al. 2008). It has been considered as an effective way in graphic analysis method that can be applied in breeding programs (Mladenov et al. 2012). This displays more informative results for responses of different genotypes over environments such as describing specific and non-specific adaptability of genotypes and identification of the most discriminating environments (Kendal and Sener 2015), high yielding, stable genotypes, and interrelationships among environments (Mortazavian et al. 2014; Heidari et al. 2017; Ram et al. 2020). Solomon et al. (2008) applied AMMI analysis to determine the effect of GE interaction on durum wheat. Furthermore, one of the recommended strategies for assessing specific environmental challenges is genetic correlations between locations. Therefore, selection for a specific adaptation is a good for exploiting the interaction which can result in faster genetic progress than that of the wide adaptation (Karimizadeh et al. 2016). Indeed, the environments can be grouped into sub-regions based on the similarity of the genotype performances (Annicchiarico et al. 2006).

The fundamental step that needs to be taken in a crop improvement program is to evaluate and identify the best cultivars. This study aims to (i) analyze the performance of 14 Algerian durum wheat varieties in seven contrasted environments (ii) evaluate the intra-environment correlation between traits and (iii) to determine the best varieties according to specific or wide adaptation based on inter-environments correlation by trait.

MATERIALS AND METHODS

In this study, 14 varieties of durum wheat were evaluated (Table 1). The germplasm was supplied by the Technical Institute for Field Crops (ITGC, Algiers, Algeria). The experiment was carried out at three farms belonging to ITGC, at Sétif and Khemis Miliana during two seasons (2014-2015 and 2015-2016), and at Oued Smar during three seasons (2014-2015, 2015-2016, and 2016-2017), which represent contrasting environments. The Sétif experimental station (36°9'N and 5°21'E, altitude of 1,081 m) is located 5 km southwest of Sétif. It is characterized by a semi-arid climate with cold winters, irregular rainfall, spring frosts, and very high temperature at the end of the vegetation cycle (Mekhlouf et al. in Frih et al. 2021). Khemis Miliana station is situated on Bir Ould-Khelifa

(High Cheliff), 10 km south of Khemis Miliana (Ain Defla, 36°10'N, and 2°14'E, altitude of 300 m). It is characterized by a semi-arid climatic stage with irregular rainfall and hot and drying winds. Oued Smar station is positioned on Beaulieu (Algiers, 36°43'N and 30°08'E, altitude of 24 m). It has a sub-humid climate with mild winter (Figure 1).

Table 1. Name, pedigree, and cross origin of the 14 durum wheat genotypes studied.

Name	Pedigree	Cross origin
$Bidi_{17}$	Landrace selection	INRA Algeria
Chen's	Ichwa'S'/Bit 'S'CD 26406	CIMMYT-ICARDA
GTA dur	Crane/4/PolonicumPI185309//T.glutin en/2* Tc60/3/Gll	CIMMYT-ICARDA
$Hedba_{03}$	Landrace selection	INRA Algeria
MBB	Landrace selection	INRA Algeria
Simeto	Capeiti8/Valvona	Italy
$Mexicali_{75}$	GdoVz 469/3/Jo"S"/61.130.Lds/Stk"S"CM470	CIMMYT
Vitron	Turkey77/3/Jori/Anhinga//Flamingo	CIMMYT
Waha	Plc/Ruff//Gta's/3/Rolette CM 17904	CIMMYT
Cirta	KB214-0KB-20KB-OKB-OKB-1KB-0KB	ITGC, ARS, Khroub, Algeria
Ofanto	Appulo/Adamello	Italy
Bousselam	Heider/Martes/Huevos de Oro. ICD-414	CIMMYT-ICARDA
Megress	Ofanto/Waha//MBB	ITGC, ARS, Setif, Algeria
$Amar_{06}$	ID94.0920-C-OAP.7AP	CIMMYT-ICARDA

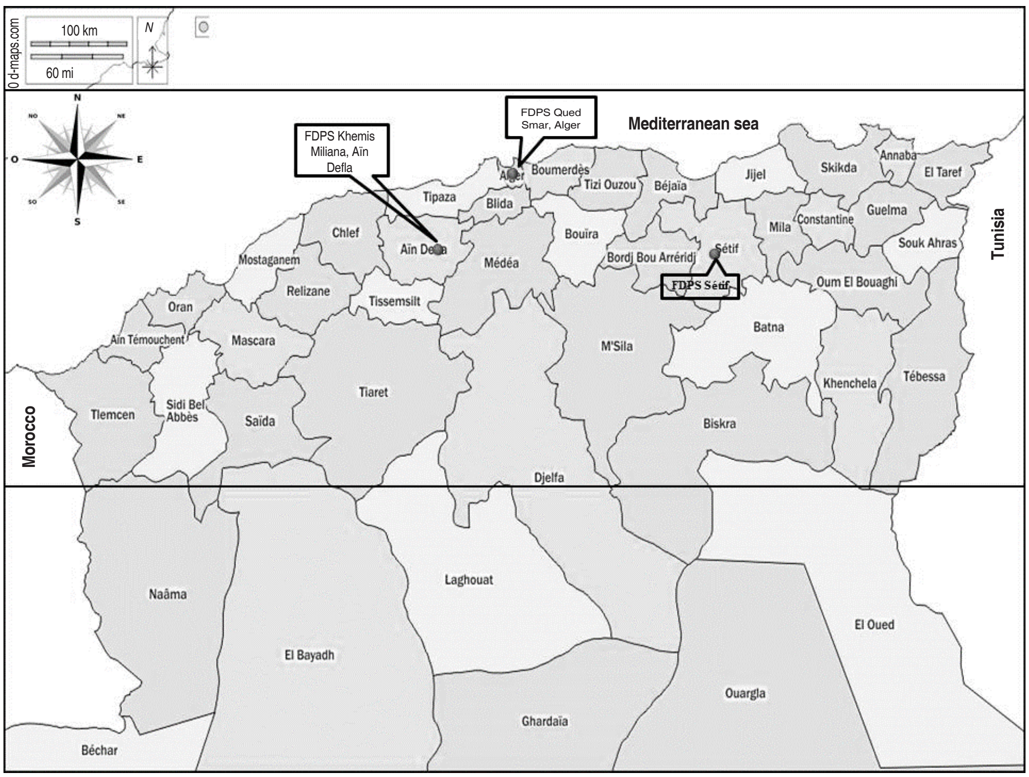

Figure 1. Geographical position of the experimental sites.

The rainfall recorded from September to June varies from 595.02 mm to 604.5 mm for the Oued Smar site, from 391.5 mm to 451 mm for Khemis Miliana, and from 339.85 mm to 340.26 mm for Sétif. The rainfall distribution presents a large monthly variability. The 2014-2015 campaign was marked by a water deficit from April to the harvesting time in all the experimental sites. Whereas the 2015-2016 campaign was characterized by a good distribution with significant amounts of rain. The temperature has a bimodal distribution, a low temperature during the vegetative stage from December to March in both sites (Oued Smar and Khemis Miliana) and from November to April in Sétif site and a high temperature at the beginning of the vegetation cycle and during the reproductive stage especially during the filling and ripening of grains.

Field trials done in seven environments (E1=Sétif 2014-2015, E2=Sétif 2015-2016, E3=Oued Smar 2014-2015, E4=Oued Smar 2015-2016, E5=Oued Smar 2016-2017, E6=Khemis Miliana 2014-2015 and E7=Khemis Miliana 2015-2016) were arranged in a randomized complete block design with four replicates. All environments were subject to the same conditions. Sowing of the 14 varieties was carried out by a plot seeder OYORD at the beginning of December using a density of 300 seeds per meter square in a micro-plot of 6 m² per variety and block. Fertilizer was applied at a rate of 100 kg ha^{-1} of superphosphate (46%) before sowing and 75 kg ha^{-1} of N during winter (Tillering to stem elongation). Several agro-morphological, physiological and biochemical parameters were analyzed such as Days to Heading DH, Plant height PH (cm), Awn Length AL (cm), Spike Length SL (cm), Number of Spikes per square meter (NSM²), Number of Grains per Spike (NGS), Number of Grains per square meter (NGM²), Thousand Kernel Weight TKW (g), Grain Yield GY (q ha^{-1}); Relative Water Content RWC (%); Chlorophyll Pigments Chla, Chlb, Chlab (µg g^{-1} of fresh matter), and soluble sugars SS (µg g^{-1} FM).

AMMI was used as a model to test the GE interaction of the 14 varieties across seven environments. Boussellam, Waha, and MBB were used as standard controls. Data were subjected to ANOVA multivariate using CropStat 7.2 (2007) software. The significance of the differences between means was determined at P<0.05 using the least significant difference (LSD) test. The correlation coefficients between pairs of characters by environment and between pairs of environments for each character were calculated by the Spearman rank using Past software version 3.2.1.The degree of stability was tested by the Lin and Binns Genotypic Superiority Index using Equation (1):

$$Pi = \left[\sum (X_i - M_j)^2\right] / 2n \qquad (1)$$

Where "X_{ij}" is the grain yield of genotype "i" in the environment "j". "M_j" is the yield of the best-performing genotype in the "j" environment. "n" is the number of environments. Stability can also be measured by the coefficient of phenotypic variation. The phenotypic coefficient of variation (CV) is obtained using Equation (2):

$$CV(\%) = 100\left(\frac{\sqrt{s^2 i}}{X_i}\right) \qquad (2)$$

Where S^2i = Environmental variance, X_i = Performance mean of genotype "i" across all environments.

RESULTS AND DISCUSSION

Yield performance and stability analyses

Analysis of variance showed high significant differences for variety and environment effects as well as their interactions in terms of yield. Relative to the LSD value at the 5% threshold which is 5.4, the additive variety effect (P<0.01, MS=460.99) indicates that GTA dur, Simeto, Chen's, Vitron, $Ammar_{06}$, Ofanto and Megress showed the best grain yields with respective means of 32.5, 31.1, 30.3, 28.8, 28.8, 27.8 and 27.2 q ha^{-1} (Table 2). However, according to the LSD value which is 1.4, E7, E2 and E4 are ranked as the most high-yielding environments (P<0.01, MS=6777.65) (Table 2). Multivariate analysis for site effect (P<0.001, MS=4361.63) showed that Sétif is the most suitable in terms of yield (33.2 q ha^{-1}. Mean value of E1 and E2) compared to Oued Smar (25.5 q ha^{-1}. Mean value of E3, E4 and E5) and Khemis Miliana (21.2 q ha^{-1}. Mean value of E6 and E7) (Table 2).

Analysis of the genotype x environment interaction (P<0.01, MS=104.00) (Table 2) indicates that the best genotype varies depending on the environment. Indeed, GTA dur, and Simeto occupied the top of the ranking in three and two among the seven environments, respectively.

Variations in yield between environments explain the variation in national cereal production, which is generally attributed to climatic conditions. This wide variation makes it difficult to create new high-yielding varieties. This kind of varietal behavior, induced by the GE interaction, has been reported by Haddad et al. (2016). It makes choosing the best genotypes difficult due to the instability of performance. Selection must therefore be made on the basis of yield performance linked to adaptability across environments. In this context, the use of selection indices is recommended (Benmahammed et al. 2010). Based on Pi index, GTA dur and Simeto are selected as stable and high-performance varieties.

Table 2. Yields means by environment, coefficient of variation CV and index Pi of the studied varieties.

	E1	E2	E3	E4	E5	E6	E7	Xi	CV%	Pi
AM6	27.6^{e}	36.3^{c}	25.5^{d}	38.6^{d}	21.8^{a}	7.6^{c}	44.5^{b}	28.8^{a}	42.8	44.6
B17	29.0^{d}	31.8^{e}	22.0^{e}	24.6^{g}	8.1^{d}	4.5^{d}	32.8^{f}	21.8^{b}	52.0	136.7
BOU	31.8^{c}	33.2^{e}	20.0^{f}	26.9^{g}	14.8^{c}	7.3^{c}	41.2^{c}	25.0^{b}	46.8	93.4
CHE	27.9^{e}	45.3^{a}	29.2^{c}	39.9^{c}	18.3^{b}	7.7^{c}	43.8^{b}	30.3^{a}	46.0	33.6
CIR	30.4^{d}	36.2^{d}	26.9^{d}	30.6^{f}	16.3^{c}	4.4^{d}	39.3^{d}	26.3^{b}	46.1	71.3
GTA	37.0^{b}	35.9^{d}	36.3^{a}	51.3^{a}	19.6^{a}	7.1^{c}	40.3^{c}	32.5^{a}	44.9	21.4
H3	22.0^{g}	26.6^{f}	18.4^{f}	21.6^{h}	7.2^{e}	2.9^{d}	22.6^{h}	17.3^{c}	50.9	223.7
MBB	39.7^{a}	31.5^{e}	21.9^{e}	26.1^{g}	10.0^{d}	1.2^{e}	29.4^{g}	22.8^{b}	57.9	133.9
MEG	36.7^{b}	33.9^{d}	22.2^{e}	32.8^{f}	22.6^{a}	5.0^{c}	37.0^{e}	27.2^{a}	42.6	71.3
MEX	24.3^{f}	37.3^{c}	25.6^{d}	34.5^{e}	16.0^{c}	11.9^{a}	37.9^{d}	26.8^{b}	38.6	69.9
OFA	40.5^{a}	39.3^{b}	24.2^{e}	33.4^{e}	17.4^{b}	8.0^{b}	32.3^{f}	27.8^{a}	43.0	69.1
SIM	33.2^{c}	35.5^{d}	27.4^{c}	41.6^{c}	19.9^{a}	7.6^{c}	52.3^{a}	31.1^{a}	46.9	26.4
VIT	30.3^{d}	36.7^{c}	31.8^{b}	46.7^{b}	19.1^{b}	13.8^{a}	23.2^{h}	28.8^{a}	38.7	77.2
WAH	26.0^{e}	32.1^{e}	28.7^{c}	35.1^{e}	16.4^{c}	8.9^{b}	18.2^{i}	23.6^{b}	39.9	138.0
X.j	31.2	35.1	25.7	34.5	16.2	7.0	35.3	26.4	42.8	86.5
LSD 5%				3.0				5.4		

MS (ENV)=6777.65**, MS (Var)=460.99**, MS (GE)=104.00**, Err=4.5. **very highly significant. E1=Sétif 2014-2015, E2=Sétif 2015-2016, E3=Oued Smar 2014-2015, E4=Oued Smar 2015-2016, E5=Oued Smar 2016-2017, E6=Khemis Miliana 2014-2015, E7=Khemis Miliana 2015-2016 GTA=Gaviota Durum, BOU=Boussellam, OFA=Ofanto, SIM=Simeto, H3=Hedba$_{03}$, CIR=Cirta, MEX=Mexicali, WAH=Waha, B17=Bidi$_{17}$, CHE=Chen's, AM6=Amar$_{06}$, MEG=Megress, MBB=Mohamed Ben Bachir, VIT=Vitron.

The grain yield gains induced by the selection of Simeto and GTA dur, relative to the average yield of the standard controls, vary from 11.15% in E2 to 74.64% in E4, for GTA dur and from 2.13% in E1 to 76.68% in E7, for Simeto. The results of the present study corroborate those of Bendjama and Solonechnyi (2018) who reported that the grain yield varies according to sites, years, genotypes, and their interaction, and the greatest variation is mostly induced by the site effect, followed by the site x years interaction effect.

A high value of CV (%) shows a high inter-environment variability, which affects the stability of varieties. The CV values vary from 38.6 to 57.9% (Table 2). Taking into consideration the smallest coefficients of variation and the highest mean values of yield, the high-performing and stable varieties, which are selected are GTA dur, Ofanto, Mexicali, Amar$_6$, Megress and Vitron (Figure 2).

Inter-character, intra environment relationships

The study of the relationships between traits is necessary for breeders to identify the effect of traits, which can be easily measured in the growing stage (DH, PH, NSM^2, SL, AL, RWC, SS…etc.), inversely to the complex characters (NGS, GY, TKW…etc.) which are measured during harvest by destructive methods. Such characters can be used in the indirect selection of complex characters as highlighted by Salmi et al. (2019).

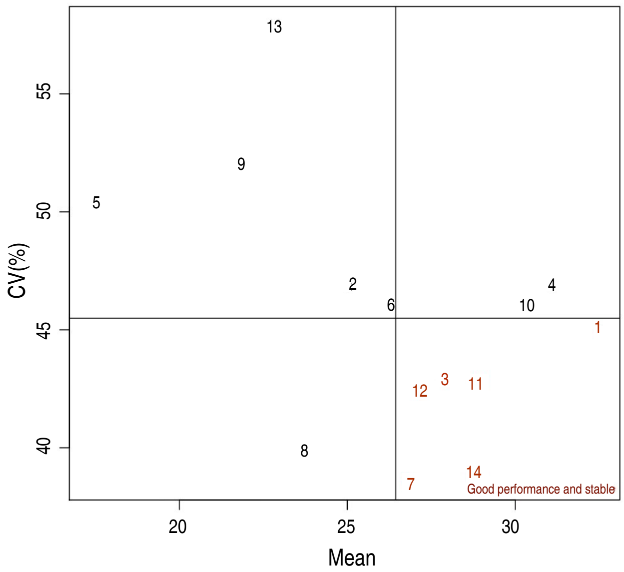

Figure 2. Performance and stability of 14 varieties of durum wheat for yield based on the coefficient of variation. 1=GTA dur, 3=Ofanto, 7=Mexicali, 11=Amar$_{06}$, 12=Megress), 14=Vitron: efficient and stable varieties.

Analysis of the correlation between the measured traits (Table 3) indicates that the DH presents variable links, depending on the environment and the target character. Under environmental conditions, similar to Oued Smar and Khemis Miliana, a longue duration of heading value is not favorable for the achievement of a high grain yield, nor the NGS and NGM^2. However, it is suitable for making a high straw yield.

These results suggest that, when the conditions of the environment discriminate clearly between genotypes tested for the DH as shown in environments belonging to Oued Smar site, selection of early varieties generates more in terms of GY, but it is accompanied by a reduction in the PH and vice versa (Table 3). Similar results were reported in other studies (Gonzalaz-Ribot et al. 2017; Mohammadi 2019; Kumar et al. 2021). The relationship between DH and physiological traits (chlorophyll content and RWC) can be explained by the difference in expression between the late genotypes that express a higher chlorophyll content and RWC than the early ones. The inverse is true for the sugar content. The late cultivars characterized by a low GY should be more resilient than the early cultivars against environmental variability.

The grain yield is significantly and positively dependent on NGM^2, NGE, NSM^2, NGE, and NSM^2, but negative with PH. The link with the SL and AL as well as with the chlorophyll content, RWC, and SS is dependent on the environment and when it is significant, it is inconsistent. Kumar et al. (2021) reported a significant and positive correlation between GY and SL at early sowing. Optimizing the grain yield of wheat is the main issue for breeders worldwide. Understanding relationships between grain yield with morphological, physiological, and biochemical traits across different environmental conditions could help plant breeders to develop wheat cultivars with improved and stable grain yield. The relationship shown between GY and PH suggests that the selection of efficient genotypes is accompanied by a reduction in the PH. Mohammadi (2019) reported that the plant height needs to be at medium level, to obtain a good grain yield. Annicchiarico et al. (2005b) found that the GY was negatively correlated with the straw yield. And the semi-dwarf varieties were top-ranking in terms of yield. Whereas, Royo et al. (2014) found that the greater plant height contributes to the good yield making under dry Mediterranean rainfed conditions. The differences SL and AL in addition to the physiological and biochemical traits do not seem to be decisive in

Table 3. Coefficients of phenotypic correlations between measured variables in seven studied environments

	DH	GY	NSM²	NGS	TKW	NGM	PH	SL	AL	CHLa	CHLb	CHLab	RWC	SS
E1	1.000	-0.187	-0.121	-0.219	0.059	-0.292	0.296	0.111	0.211	0.760**	-0.156	0.117	0.419	-0.392
E2	1.000	-0.454	-0.473	-0.321	0.137	-0.467	0.667**	0.329	0.314	0.680**	-0.640*	0.627*	-0.282	-0.107
E3	1.000	-0.581*	-0.513	-0.670**	0.239	-0.702**	0.497	0.490	0.329	-0.125	0.054	-0.187	-0.186	-0.385
E4	1.000	-0.806**	-0.342	-0.301	0.424	-0.415	0.837**	0.316	0.362	0.051	0.232	0.127	0.681**	-0.674**
E5	1.000	-0.825**	-0.659*	-0.787**	-0.083	-0.806**	0.856**	0.690**	0.582*	0.084	0.323	0.204	0.668**	-0.846**
E6	1.000	-0.785**	-0.826**	-0.590*	-0.263	-0.833**	0.370	-0.633*	-0.669**	0.108	-0.083	0.003	-0.154	-0.238
E7	1.000	-0.091	-0.577*	0.194	0.698**	-0.343	0.559*	-0.305	-0.397	0.181	0.200	0.243	-0.210	0.359
	GY	**DH**	**NSM²**	**GS**	**TKW**	**NGM**	**PH**	**SL**	**AL**	**CHLa**	**CHLb**	**CHLab**	**RWC**	**SS**
E1	1.000	-0.187	0.493	0.585*	0.559*	0.872**	-0.438	0.012	0.125	-0.013	0.405	-0.006	-0.136	0.413
E2	1.000	-0.454	0.462	0.521	0.399	0.585*	-0.734**	-0.666**	-0.514	-0.650*	0.629*	-0.595*	-0.320	0.256
E3	1.000	-0.581*	0.778**	0.537*	-0.309	0.769**	-0.218	-0.290	-0.294	0.372	0.014	0.266	0.184	0.606*
E4	1.000	-0.806**	0.253	0.640*	-0.485	0.524	-0.710**	-0.250	-0.338	-0.311	-0.554*	-0.428	-0.580*	0.697**
E5	1.000	-0.825**	0.886**	0.702**	0.209	0.940**	-0.881**	-0.606*	-0.629*	-0.258	-0.433	-0.364	-0.381	0.684**
E6	1.000	-0.785**	0.875**	0.505	0.332	0.855**	-0.072	0.703**	0.696**	-0.163	-0.046	-0.179	0.274	0.191
E7	1.000	-0.091	0.506	0.540*	0.356	0.593*	-0.354	-0.386	-0.063	0.200	-0.412	-0.332	0.236	-0.021
	NSM²	**GY**	**DPV**	**NGS**	**TKW**	**NGM**	**PH**	**SL**	**AL**	**CHLa**	**CHLb**	**CHLab**	**RWC**	**SS**
E1	1.000	0.493	-0.121	-0.267	0.297	0.412	-0.074	0.114	0.086	-0.127	0.401	0.070	-0.349	0.275
E2	1.000	0.462	-0.473	0.359	-0.385	0.820**	-0.501	-0.510	-0.524	-0.559*	0.577*	-0.502	-0.440	-0.210
E3	1.000	0.778**	-0.513	0.400	-0.409	0.770**	-0.286	-0.435	-0.421	0.500	0.193	0.406	0.017	0.584*
E4	1.000	0.253	-0.342	0.102	-0.458	0.875**	-0.600*	0.071	-0.048	-0.190	-0.321	-0.254	-0.244	0.417
E5	1.000	0.886**	-0.659*	0.486	0.150	0.932**	-0.831**	-0.342	-0.417	-0.354	-0.484	-0.448	-0.261	0.502
E6	1.000	0.875**	-0.826**	0.474	0.264	0.932**	-0.026	0.638*	0.678**	-0.201	0.126	0.036	0.273	0.018
E7	1.000	0.506	-0.577*	0.396	-0.363	0.914**	-0.650*	0.064	0.154	0.090	-0.113	-0.081	0.265	-0.150

DH=Days to Heading, GY=Grain Yield, NSM²=Number of Spikes per square meter, NGS=Number of Grains per Spike, TKW=Thousand Kernel Weight, NGM²=Number of grains per meter square, PH=Plant Height, AL=Awn Length, SL=Spike Length, CHLa, CHLb, CHLab=Chlorophyll Pigments, RWC=Relative Water Content, SS=Soluble sugars. E1=Sétif 2014-2015, E2=Sétif 2015-2016, E3=Oued Smar 2014-2015, E4=Oued Smar 2015-2016, E5=Oued Smar 2016-2017, E6=Khemis Miliana 2014-2015, E7=Khemis Miliana 2015-2016. r 5% =0.532, r 1%=0.661.

grain yield making. Therefore, the selection on the basis of these characteristics appears secondary. For the same grain yield, the choice would be made on the basis of these characteristics, and in favor of a long spike and awns and high chlorophyll content and SS. A lot of studies highlighted that using physiological traits as a complement to agronomic traits, may help in identifying selectable features that accelerate breeding for yield potential and performance under drought (Fischer 2007; Araus et al. 2008; Cattivelli et al. 2008; Mohammadi 2019). Using an indirect selection of traits, associated with greater grain yield having lower GxE interaction would make results more reliable and repeatable in most of the environments. In fact, among these traits (NGM², NSM², NGS, NGM², TKW, PH, CHL, AL, SL, RWC, and SS) only NGM² followed by NSM² can be used at an early stage to discriminate between the evaluated genotypes. Therefore, when variability exists for both variables, the selection is recommended for NGM² or NSM² and within varieties having similar NGM² or NSM². We select for other characteristics including NGS, followed by TKW and PH. Our results are in agreement with those of Laala et al. (2021), who reported a highly positive correlation between the number of spikes and the grain yield. In fact, the indirect selection via the number of spikes was the most efficient. Similar results of the relationship between grain yield and its components have been reported in previous studies (Moragues et al. 2006; Royo et al. 2006). They demonstrated that durum wheat yield grown under warm and dry Mediterranean environments is obtained mainly by the number of spikes per unit area. However, in cool and wet environments, kernel weight influences mostly the grain production. In addition, a high grain number conducive for a high yield can be achieved by the production of many small spikes (Bustos et al. 2013). Wheat yield can be affected mostly by the number of grains per m² (Slafer et al. 2014).

The NSM² is an essential determinant of NGM², on which GY is widely dependent. Consequently, this parameter could be used as a criterion in breeding plants. Slafer et al. (2014) confirmed our conclusion: they declared that large changes in NGM² are mainly related to NSM². The varieties with tall straw, which were most often late at heading, have long awns and spikes, as well as a high content of CHLa and low in CHLb and SS. Overall, these results corroborate those reported by Mansouri et al. (2018). The days to heading had significant correlations and negative signs with the weight of 1,000 grains and the grain yield. Fellahi et al. (2017) noted that the yield is more linked to the number of spikes, and remains independent of the weight of 1,000 grains which did not show a significant link with the number of spikes. Mohammadi et al. (2016) reported that higher grain yields associated with a higher grain weight resulting from early flowering and selection on the basis of the weight of 1,000 grains can further improve the grain yield.

Relations inter environments

Analysis of the correlation coefficients of ranks inter-environments indicates that the order of classification of genotypes for DH remains relatively unchanged, especially in the E2 to E7 environments (Table 4). The ranking of this variable in the E1 environment (Sétif) is not significantly linked to other environments. GY also has a significant correlation between E3 to E6 environments, inversely to other environments (E1, E2, and E7), where the varieties ranking is different. The inter-environment rank coefficients of all varieties were most often significant for PH and NGM². Whereas the inter-environment rank coefficients of NSM², NGS, TKW, RWC (data not shown), and SS (data not shown) were not significant. This means that the classification changes from one environment to another.

GE Interaction often affects the yield of cultivars. This led to the evaluation of genotypes across a large number of sites to estimate yield potential and to analyze and understand the interaction pattern, with a possibility to group locations into homogeneous recommended domains sharing the same genotypes (Annicchiarico et al. 2006).

The results induced by the relation inter environments analysis for the GY trait suggest the absence of the GE interaction between the varieties in four environments (E3, E4, E5, and E6). This means that the order of the yield performance changes a little relatively in these environments, which do not show a specific behavior. This is in contrast to environments E1 and E2 (Site of Sétif), which rank the varieties differently for grain yield performance. These results propose that the Oued Smar and Khemis Miliana sites do not require repeated trials

Table 4. Coefficients of inter-environment rank correlation by character.

P	E1	E2	E3	E4	E5	E6	E7
DH							
E1		0.009	0.046	0.160	0.205	0.089	0.141
E2	0.666**		0.003	0.006	0.001	0.002	0.005
E3	0.540*	0.727**		0.001	0.000	0.000	0.001
E4	0.397	0.694**	0.768**		0.000	0.000	0.000
E5	0.361	0.766**	0.851**	0.877**		0.000	0.000
E6	0.471	0.762**	0.845**	0.890**	0.943**		0.001
E7	0.414	0.699**	0.801**	0.816**	0.863**	0.777**	
GY	**E1**	**E2**	**E3**	**E4**	**E5**	**E6**	**E7**
NSM²							
E1		0.051	0.007	0.095	0.284	-0.227	0.143
E2	0.026		0.587*	0.626*	0.499	0.678**	0.407
E3	-0.077	0.218		0.903**	0.574*	0.581*	0.209
E4	0.064	0.330	0.420		0.758**	0.662**	0.380
E5	0.121	0.389	0.473	0.305		0.383	0.468
E6	-0.393	0.517	0.464	0.349	0.301		0.026
E7	-0.099	0.218	0.508	0.385	0.433	0.481	
NGM²	**E1**	**E2**	**E3**	**E4**	**E5**	**E6**	**E7**
PH							
E1		0.296	0.209	0.386	0.431	0.031	0.373
E2	0.616*		0.393	0.165	0.623*	0.582*	0.227
E3	0.452	0.620*		0.532*	0.441	0.741**	0.614*
E4	0.704**	0.679**	0.539*		0.378	0.497	0.579*
E5	0.541*	0.807**	0.504	0.837**		0.416	0.539*
E6	0.411	0.587*	0.860**	0.477	0.525*		0.359
E7	0.769**	0.867**	0.709**	0.713**	0.739**	0.671**	

*Significant at 1%, **Significant at 5%. *P*=Probability, DH=Days to Heading, GY=Grain Yield, NSM²=Number of Spikes per square meter, NGS=Number of Grains per Spike, TKW=Thousand Kernel Weight, NGM²=Number of grains per meter square, PH=Plant Height, AL=Awn Length, SL=Spike Length, CHLa, CHLb, CHLab=Chlorophyll Pigments, RWC=Relative Water Content, SS=Soluble sugars. E1=Sétif 2014-2015, E2=Sétif 2015-2016, E3=Oued Smar 2014-2015, E4=Oued Smar 2015-2016, E5=Oued Smar 2016-2017, E6=Khemis Miliana 2014-2015, E7=Khemis Miliana 2015-2016.

over time to identify the best performing varieties, while the Sétif site requires repeated trials over time. The data obtained for the DH suggest there is GE interaction between E1 and other environments (E2 to E7). These results indicate also that Khemis Miliana site predicts relatively well the order of varieties for the earliness at the Oued Smar site, but it is not the case for the Sétif site. Therefore, determination of the precocity can be made either on Oued Smar or Khemis Miliana site, but not necessarily on both sites at the same time. On the other hand, the order of varieties for this trait requires a specific determination on Sétif site. The divergence that appeared between environments for GY and DH traits can be explained by the fact that E1 (Sétif) is characterized by a harsh climate, especially in terms of temperatures, this makes it different from the other sites. The analysis of inter-environment rank coefficients of NSM², NGS, TKW, RWC, and SS variables was not significant, suggesting the presence of GE interaction. While the information provided by an environment for PH and NGM² can be exploited for the needs of other environments. Complex traits such as GY and its components present generally GE interaction, which affect the ranking of individuals (Burgueño et al. 2008; Karimizadeh et al. 2016). Moreover, the presence of repeatable GE interactions identifies cases of specific adaptations (Annicchiarico et al. 2006).

Based on the correlation coefficients of DH, GY, PH, and NGM², the seven environments can be classified into two sets: the site of Sétif alone and the sites of Oued Smar and Khemis Miliana together. Inside each one, the GE interaction is relatively less notable for the four characters mentioned above. Therefore, a variety of recommendations should be made based on the specific adaptation to these two sets of environments separately. Our findings corroborate partially those defined by Annicchiarico et al. (2005a) using other approaches. GTA dur and Simeto (30.9 q ha^{-1} and 29.7 q ha^{-1}, respectively) were the top-yielding over the subregion including Oued Smar and Khemis Miliani sites. Whereas, Ofanto (39.9 q ha^{-1}) was the top-yielding over the sub-region that includes Sétif site. Understanding the genetic basis of adaptation and its environmental reasons is important to understand GE interaction, to evaluate the relationship between phenotypic and genotypic values and to improve selection of performing and stable genotypes (Joshi et al. 2010). Our findings agree with those of Karimizadeh et al. (2016), who reported that selection for specific adaptation is recommended because it can speed up the genetic progress better than selecting for wide adaptation in case of different mega-environment. In this case, a genotype has the ability to better exploit the agro ecology of the specific environment (Gauch 2013). Annicchiarico et al. (2005a) reported that specific adaptation could provide 2 to 7% of gains better than wide adaptation, in stressful sub-regions.

CONCLUSIONS

Multivariate analysis showed high significant differences for variety and environment effects as well as their interactions. In terms of yield. GTA dur is selected as stable and suitable variety using both stability index (CV and Pi) and Sétif is the most high-yielding site. The study of the relationships between traits is necessary in order to identify the effect of non-destructive traits, which can be easily measured before the harvest compared to other characters, which are measured during harvest using destructive methods. Such characters can be used in the indirect selection of complex characters of the grain yield. Therefore, the number of spikes can be used to discriminate between the genotypes evaluated for the grain yield followed by other characteristics such as number of grains per spike, the weight of thousand grains and the plant height. Selection based on biochemical and physiological characters seems to be secondary. Inter-environment correlation showed that the studied varieties were classified in the same way over almost all the environments for both traits of the plant height followed by the days to heading which are less affected by the environment. Whereas, the ranking of varieties is different for the grain yield due to the complexity of this trait. This suggests there is GE interaction. Therefore, varieties should be recommended according to the specific adaptation generating two sets environments. Therefore, Ofanto is the best cultivar in the two environments belonging to Sétif site, and GTA dur is the most efficient in terms of grain yield followed by Simeto in five environments of both sites (Oued Smar and Khemis Miliana). These results could be useful in a breeding program.

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Application of a spatial risk model of the crystalline spider mite (*Oligonychus* sp.) to avocado crop damage using remote sensing

Aplicación de un modelo de riesgo espacial de la araña cristalina (*Oligonychus* sp.) al daño de cultivo de aguacate utilizando sensores remotos

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ABSTRACT

Keywords:
Avocado
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Kriging predicted and indicator
Univariate and multivariate geostatistics

The avocado is one of the most consumed foods in the world and it is affected by the mite *Oligonychus* sp., which affects the generation of chlorophyll by the plant, resulting in a decrease in productivity. Given the economic importance of the avocado, a spatial statistical methodology was used to analyze the risk of a pest in its crops. A total of 202 observations of a 1.1 ha avocado farm were used to measure the number of mites per leaf in the area of Barranca, Perú. Predictive geostatistical methods and indicators were applied. A spherical semivariogram was adjusted to estimate a univariate ordinary kriging, covariates such as vegetation indicators and geomorphometric variables were used to improve the spatial resolution of the covariates and geostatistical simulation was used and linear co-regionalization models were adjusted with which pest predictions were made with co-Kriging. Finally, the predictions were transformed into a risk model using kriging indicator. The results obtained show that the mite presents a stationary process in second order with spatial dependence of less than 10 m, in which univariante ordinary kriging was the most efficient. Despite the results, the linear co-regionalization models are consistent, but the geostatistical simulation was not enough to improve the predictions. Covariate data should be incorporated at a higher level of detail and small-scale variations should be analyzed. It is suggested to incorporate covariate data with a higher level of detail and analyze small-scale variations.

RESUMEN

Palabras clave:
Aguacate
Ácaro de cristalino
Simulación geoestadística
Kriging predicto e indicado
Geostadística univariante y multivariante

El aguacate es uno de los alimentos de mayor consumo a nivel mundial. Sus plantaciones se ven afectadas por el ácaro *Oligonychus* sp., el cual interfiere en la generación de clorofila por parte de la planta, por lo cual la productividad se ve disminuida. Dada la importancia económica de este cultivo se abordó una metodología espacial para el análisis de riesgo de esta plaga. Se dispuso de un total de 202 observaciones de una finca de aguacate de 1,1 ha en las que se midió la cantidad de ácaros por hoja en la zona de Barranca, Perú. Métodos geoestadísticos predictivos e indicadores fueron aplicados. Se ajustó un semivariograma de tipo esférico con lo que se estimó un Kriging ordinario univariante, posteriormente se utilizaron covariables como índices de vegetación y variables geomorfométricas. Para mejorar la resolución espacial de las covariables se utilizó una simulación geoestadística y se ajustaron modelos lineales de corregionalización y co-Kriging, con lo que se realizaron predicciones de la plaga. Finalmente, se transformaron las predicciones a un modelo de riesgo utilizando kriging indicador. Los resultados obtenidos manifiestan que el ácaro presenta un proceso estacionario en segundo orden con una dependencia espacial menor a 10 m, en el que kriging ordinario univariante fue el más eficiente. A pesar de los resultados, los modelos lineales de corregionalización son consistentes, pero la simulación geoestadística no fue suficiente para mejorar las predicciones. Los datos de las covariables deben incorporarse con un mayor nivel de detalle y deben analizarse las variaciones a pequeña escala.

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Avocado (*Persea americana*) consumption has increased worldwide, with a large increase in planted areas in all avocado-producing countries (Hoddle et al. 2002). This food has a wide range of possible uses such as in industrialized products (Cango et al. 2014). The phytophagous mites commonly called "spider mites" are tetranychid, cosmopolitan and highly polyphagous mites, which can develop optimally in more than 150 plant species and affect practically all crops. In avocado, the spider mites that affect this crop belong mainly to the genus *Oligonychus* (Guanilo et al. 2012; Muñoz and Rodriguez 2014). These mites develop mainly on mature leaves near the veins. They feed upon the content of the superficial cells of the leaf during in both immature and adult stages. Usually, infested leaves fall off prematurely due to cell breakdown, removal of chlorophyll and saliva injected by mites leads to leaf malfunction, such as an increased rate of perspiration, resulting in its early wilting and shedding (Cango et al. 2014).

The *Oligonychus* sp. also known as crystal mite or avocado mite, is characterized by forming colonies on the underside of the avocado leaf. The nest is covered by a dense web, under which the different stages of the mite can be observed, forming nests on the underside typically circular, between 1 and 5 mm^2 (Hoddle et al. 2002), also circular necrotic spots can be observed (Hoddle et al. 2002). The damage to the underside of leaves, leading to leaf drop in the EEUU when the percentage of the damaged area is equal to or above 7.5%-10%, corresponds to spider mite populations exceeding 100-500 individuals per leaf (Hoddle et al. 2002). In the EEUU, the presence of high densities of the mite has caused partial or total defoliation of avocado trees (Hoddle et al. 2002; Cango et al. 2014), increasing the risk of sunburn for young fruit and causing premature fruit to drop and size reduction at harvest (Aponte and McMurtry 1997). This mite is one of the most important and frequent that attack the Hass variety, it has a direct consequence on the quality of the fruit and the yields of the mentioned crop (Cango et al. 2014).

By the year 2021 the annual production of avocado in Perú totaled 62,744 metric tons according to data from the National Institute of Statistics and Informatics of Perú, being considered one of the world's leading producers of the fruit (Mendoza 2020). The threat to the economy from *Oligonychus* sp. has been mentioned in several areas of the world, such as México and Spain (Suárez et al. 2023). This pest has been widely studied in Perú and it shows a strong seasonal behavior with minimums during Autumn months and maximums at the beginning of Summer, where the highest reproductions of mite eggs occur, related to dry periods and where there is little rainfall, followed by an abrupt decline in the reproductive ranges, in early Autumn. Another factor that interferes with their reproduction is high temperatures above 27.9 °C and minimum temperatures of 22.8 °C (Chávez 2020).

The study of the population distribution of *Oligonychus* sp., during the development of the avocado in Perú was study by Cango et al. (2014), the population fluctuation of individuals (nymphs and adults) was recorded, which was obtained during the period 2013-2014, where it was found that this developed mainly between February and June, which coincides with the development of favorable climatic and agronomic factors for this pest.

Statistical risk modeling provides a holistic approach to describing and predicting the probabilities of adverse health responses based on individual exposures and other study variables. Rather than attempting to describe or simulate the detailed causal and biological processes that lead from exposure to response probabilities within an individual, statistical risk modeling relies on observed data relating exposures, covariates, and responses at the level of the whole organism or a population of organisms (Cox 2012).

The effective detection and control of diseases in humans and animals needs to consider spatial patterns of the disease's occurrence and any associated risk factors. This includes efficient data collection, management, and analysis. The integration of Geographic Information System (GIS) functionality into most modern disease information systems reflects a recognition of the importance of the spatial dimension of disease control (Pfeiffer et al. 2008). The spatial risk of pests and diseases in agricultural crops has been addressed by several authors such as Jang et al. (2006) and Nelson et al. (1999). *Oligonychus* sp. is considered a key pest, which the Hass variety is affected by fruit quality and crop yields (Hoddle et al. 2002). Considering the above, the purpose of this work is to determine an efficient method to analyze the spatial risk of *Oligonychus* sp. through spatial geostatistical methods for risk analysis using remote sensing.

MATERIALS AND METHODS

Description of the study area

The Field data from an avocado farm of 1.1 ha of avocado (*Persea americana*) var Hass planted at high density, the distance between trees is 2 m and between rows 5 m was collected (Figure 1). The study area is located in the city of Barranca, a province located 190 km northwest of the city of Lima, Perú, in the coastal desert, in the foothills of the western slope of the central Andes (Lira-Camargo et al. 2020). Given this location, an almost total absence of precipitation was perceived, as well as a high level of atmospheric humidity and persistent cloudiness. The average annual temperature at the study site is 17.5 to 19 °C, with an annual summer maximum of about 29 °C. Temperatures during the summer months, from December to April, range from 29 to 30 °C during the day and 21 to 22 °C at night (Ruiz et al. 2019).

The avocado trees have a height of 5.20 m, which were collected by the same expert. A total of 202 observations in which the measurement variable is the quantity of the crystalline mite *Oligonychus* sp. per leaf were collected with a Qfield (2019), which allows working on QGIS projects for field work equipment (QField 2019). The projection system with the European Petroleum Survey Group (EPSG) code 32718 - WGS 84/UTM zone 18S was used. Data manipulation and analysis was performed using R statistical software and QGIS software.

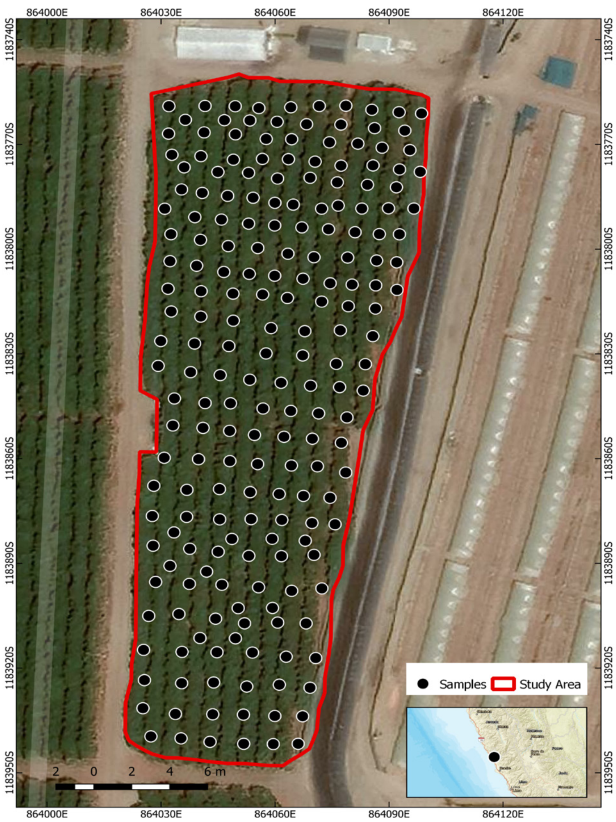

Figure 1. Location of the avocado cultivation study area.

Structural analysis of spatial dependence

For the estimation of the risk model, a geostatistical approach was applied; in this, the random variable Z(x) with spatial index varies continuously across the spatial region D (Cressie 1992). The behavior of the frequency distribution and the possibility of a transformation were evaluated, as geostatistical analysis and predictions are sensitive to outliers (Giraldo 2002).

Applied to agronomic sciences, geostatistics considers each sample value Z(x) it is associated with a position and uses this same dependence to make inferences about the distribution of the data, in this approximation a model of spatial variation that contains at least three components: first a general structure, which can be defined as a trend; a second, superimposed structure, related to spatial correlation and gradual variation; and finally a third component consisting of random variation caused by sampling errors or spatial variations at different scales sampling errors or spatial variations at scales smaller than the sample network (Cressie 1992; Li and Heap 2008).

In other words, it should be evaluated whether the process generating the data is stationary by mean or formally expressed as follows $E(Z(x_i)) = \mu$ (Giraldo 2002), to test this situation, a first-order polynomial surface was estimated and the significance of the model and the adjusted R^2 were evaluated, since this indicator essentially imposes a penalty for including additional predictors that do not contribute much to explaining the variation observed in the variable response (Diez et al. 2020).

Secondly, it is necessary to test whether the process is stationary in variance $Var(Z(x_i))=\sigma^2$, i.e., whether the data is isotropic or anisotropic (Giraldo 2002; Plant 2018), this be analyzed by defining the behavior of spatial dependence in cardinal directions (Bivand et al. 2008). Thirdly, the spatial dependence must be modeled using the experimental semivariogram, $\gamma_{(h)} = 1/2N_{(h)}\sum[Z_{(x)} - Z_{(x+h)}]^2$ where $\Upsilon_{(h)}$ is the semivariance for all samples located in space, separated by the distance interval h; $N_{(h)}$ is the total number of pairs of samples separated by a distance interval h; $Z_{(x)}$ is the value of the sample at a location x; $Z_{(x+h)}$ is the value of the sample at interval distance h from x . Variogram modelling and estimation is important for structural analysis and spatial interpolation (Oliver and Webster 2015; Li and Heap 2008).

For the adjustment of the theoretical semivariogram, the method proposed by Bivand et al. (2008) was used, which utilizes non-linear regression to fit the coefficients. For this, a weighted sum of square errors $\sum_{j=1}^{p} w_j(\gamma(h) - \hat{\gamma}(h))^2$ with $\Upsilon_{(h)}$ the value according to the parametric model is minimized. A theoretical Spherical semivariogram model was adjusted, following Equation (1).

$$\gamma(h) = \begin{cases} 0, h = 0 \\ C_0 + C_1(1.5(h/C_2) - 0.5(h/C_2), 0 < h \le C_2) \\ C_0 + C_1, h > C_2 \end{cases} \quad (1)$$

The parameter C_0 represents the nugget value induced by the spatial error when the distance is smaller than the lag distance. Whereas the variance of the process is denoted by C_1 and a combination between C_0 and C_1, where C_0 is referred to as sill. The parameter C_2 is the range in which it indicates a distance from the origin to the point of sill achieved (Moonchai and Chutsagulprom 2020).

Predictive Geostatistical Interpolation and Validation
The parameters previously analyzed, anisotropy and spatial dependence, allowed us to determine the type of geostatistical predictor interpolator most suitable to be used, since the three large families of geostatistical interpolators differ in three basic conditions: 1) Simple Kriging: the process is stationary by mean and the population mean is known; 2) Ordinary Kriging: the process is stationary by mean and the population mean is unknown; 3) Universal and Residual Kriging: the process is not stationary by mean and the population mean is unknown (Bivand et al. 2008; Li and Heap 2008; Cressie 1992; Oliver and Webster 2015).

The process of validation and quality control of the interpolation is similar to that of traditional statistical models, evaluation of the behavior of the residual semivariogram, homoscedasticity, normality of residuals (Bivand et al. 2008; Cressie 1992; Gujarati and Porter 2010). Also, Root-mean-square deviation (RMSE) and Mean Error (ME) were estimated as a validation process, this is a standardized methodology used by Andrades-Grassi et al. (2020, 2021). In addition, it was estimated the z-score, computed as: $Z_i = \frac{Z(x_i) - \hat{Z}_{[i]}(x_i)}{\sigma_{[i]}(x_i)}$; with $\hat{Z}_{[i]}(x_i)$ the cross validation prediction for x_i, and $\sigma_{[i]}(x_i)$ the corresponding kriging standard error (Bivand et al. 2008).

Generation of Spatial Risk Model and Covariate Analysis

Finally, an indicator function of risk was estimated by *Oligonychus* sp., for this the kriging predictor method selected according to the validation process was used. To convert the prediction into a risk function, the Indicator kriging was used, in this type of kriging we are interested in the event: at a given point x the value Z(x) exceeds the level Z_0. The event can be represented by a binary function, the indicator function, valued 1 if the event is true, and 0 if it is false, whose expected value is the probability of the event Z(x), exceeds Z_0 (Chilès and Delfiner 2009). In this case it must be assumed that the process of the regionalized variable $\{Z(x): x \in D \subset \mathbb{R}^d\}$ is stationary in which the following transformation is defined: $I(x_i, z_i) = \begin{cases} 1 \text{ if } Z_0 \leq Z(x) \\ 0 \text{ otherwise} \end{cases}$ (Chilès and Delfiner 2009; Giraldo 2002). Values which are much greater than a given cut-off, will receive the same indicator value as those values which are only slightly greater than that cut-off (Giraldo 2002).

Thus, indicator transformation of data is an effective way of limiting the effect of very high values. Simple or Ordinary kriging of a set of indicator-transformed values will provide a resultant value between 0 and 1 for each point estimate. This is in effect an estimate of the proportion of the values in the neighborhood which are greater than the indicator or threshold value (Chilès and Delfiner 2009). In this case, the threshold was set between 50-100 motile forms of the Hass variety of *Oligonychus* sp. per leaf which is equivalent to 40% infestation, since leaf drop occurs when the percentage of damaged area is equal to or above 7.5-10 %, which corresponds to spider populations exceeding 100-500 individuals per leaf (Hoddle et al. 2002). As can be seen, this is a wide range, so the risk function cutoff was set at five thresholds (50, 60, 70, 80 and 100 count/leaf).

As it was evidenced, up to this moment exclusively univariate geostatistical models were evaluated, therefore, it is not known if there are mappable geomorphometric causal factors that have an influence on the growth of *Oligonychus* sp. mite, the Digital Elevation Model (DEM) of ALOS Parsal of 12.5 m was used (Racoviteanu et al. 2007), as this was the free product with the best spatial resolution.

It is known that the spectral response of vegetation changes according to whether it is affected or vigorous. The typical behavior of vigorous vegetation shows reduced reflectivity in the visible spectrum, but high reflectivity in the near infrared, gradually decreasing towards the mid-infrared.

These spectral characteristics are primarily related to the action of photosynthetic pigments and water stored in the leaves. Specifically, the low reflectivity in the visible portion of the spectrum is due to the absorbing effect of the leaf pigments. As for the high reflectivity in the near infrared, it is due to the internal cellular structure of the leaf. In particular, the spongy layer of the mesophyll, with its internal air cavities, plays a leading role by scattering and dispersing most of the incident radiation in this band of the spectrum (Harris 1987). Therefore, the healthy leaf offers high reflectivity in the near infrared (between 0.7 µm and 1.3 µm), in clear contrast to the low reflectivity it offers in the visible spectrum, especially in the red range (Crippen 1990).

There are different vegetation indices that could indicate if there is a potential problem in a crop and use broad information from the electromagnetic spectrum (Crippen 1990). The Normalized Difference Vegetation Index (NDVI) is defined as the parameter calculated from reflectance values at different wavelengths and is particularly sensitive to vegetation cover. The use of this index is based on the particular radiometric behavior of vegetation, which is characterized by the contrast between the red band (between 0.6 µm and 0.7 µm), which is largely absorbed by the leaves, and the near infrared (between 0.7 µm and 1.1 µm), which is mostly reflected. The NDVI is estimated as follows NDVI = (IRC - R) / (IRC + R), where IRC is the reflectivity of the near infrared band and R is the reflectivity of the red band.

The NDVI were generated from a Sentinel-2 image taken on October 10, 2020, at 10 m spatial (Copernicus 2021) resolution, this was preprocessed and transformed to reflectance using the Qgis SPC plug-in (Congedo 2021). It was as close as possible in time to the date of field spatial data collection of *Oligonychus* sp. Information derived from vegetation indices are not causal risk factors, but rather a consequence of the effect of mite damage. Low values of vegetation indices usually indicate low vigorous vegetation, while high values indicate very vigorous vegetation (Crippen 1990). As this DEM and NDVI do not have a spatial resolution and level of detail that is sufficient for geostatistical analysis, a multi-Gaussian conditional simulation process was performed. One of the main problems of Kriging predictions is that the smoothing of the map of estimated values is smoother than the map of true values (Chilès and Delfiner 2009).

A multi-Gaussian random function Y(x), $\mathbb{R}^d$ of variogram *g*(h) was simulated at the sites x_1,... x_n in the space. The sequential simulation is performed as follows: 1) simulate a Gaussian value U_1 (mean 0, variance 2) with $Y(x_1)=U_1$. 2) for each i∈{2,…n}, with: $Y(x_i)=Y(x_i)^{SK} + \sigma_{SK}(x_i)U_i$, where $Y(x_i)^{SK}$ is the simple Kriging of $Y(x_i)$ from the previously simulated values $\{Y(x_1),\dots Y(x_i-1)\}$, $\sigma_{SK}(x_i)$ is the Kriging standard deviation U_i is a Gaussian value independent of $U_1,\dots U_{i-1}$. At each stage, the value at one site is simulated and added to the conditioning data, the set of simulated values $\{Y(x_1),\dots Y(x_i-1)\}$ has a multi-Gaussian distribution, with mean 0 and variogram *g*(h), a simulation of the random function is obtained at the sites $x_1,\dots x_n$ (Giraldo 2002).

This procedure, although complex, allows evaluating the behavior of spatial autocorrelation and forces the exploration of the NDVI and DEM covariate data. Upon completion of this procedure, the DEM issued the Topographic Wetness Index (TWI), this indicator allows the identification of potential wetland concentration sites or water accumulation zones and the Topographic Position Index (TPI) (Sörensen et al. 2006). The conditional simulation process has already been used by Andrades-Grassi et al. (2021), for the improvement of continuous random variables of low spatial quality.

The Kriging predictions equations can be simply extended to obtain multivariable prediction equations. The general idea is that multiple variables may be cross correlated, meaning that they exhibit not only autocorrelation but that the partial variability of variable A is correlated with variable B, and can therefore be used for its prediction, and vice versa (Bivand et al. 2008). This statistical technique requires the availability of information of secondary variables at all points being estimated, co-Kriging is proposed to use non-exhaustive secondary information and to explicitly account for the spatial cross correlation between the primary and secondary variables (Goovaerts 1997; Li and Heap 2008).

Co-Kriging requires that both target and co-variable be, individually, spatially autocorrelated, and in addition that they be spatially cross-correlated. To perform co-Kriging, one must first model the spatial structure of a co-variable and its covariance with the target variable, this is called a co-regionalization, and it is an extension of the theory of a single regionalized variable (Bivand et al. 2008).

The easiest way to ensure this is to fit a linear model of co-regionalization: all models (direct and cross) have the same shape and range but may have different partial sills and nuggets. The Fit uses an iterative procedure of two steps: first, each variogram model is fitted to a direct or cross variogram; next each of the partial sill coefficient matrices is approached by least squares (Bivand et al. 2008). As with the model obtained with univariate indicator kriging the RMSE and Mean Error were estimated. It should be noted that co-Kriging was evaluated by introducing a single covariate (NDVI, TWI and TPI). Finally, the best co-Kriging prediction was transformed into an indicator function, using the previously described indicator Kriging.

RESULTS AND DISCUSSION

The results of the univariate count of *Oligonychus* sp., show a double-peaked frequency distribution without the presence of additive outliers, so no transformation of the frequency distribution was required (Giraldo 2002). A stationary process in second order was identified and the condition of stationarity in first order by means was fulfilled, since the estimated parameters of the polynomial trend in first order were not significant (Table 1). The regression which shows a non-significant (R^2 adjusted = -0.008, P=0.87) process and a very low adjusted R^2 (Diez et al. 2020).

Also, the stochastic process is stationary by variance (Figure 2A). According to these two results what is being expressed is the effect of pure spatial dependence (Cressie 1992). A theoretical Spherical semivariogram model was adjusted, the parameter represents the nugget value induced by the spatial error when the distance is smaller than the lag distance. Whereas the variance of the process

Table 1. First-order polynomial model of *Oligonychus* sp. count in avocado trees.

Coefficients	Estimate	Std. Error	t value	*P*
Intercept	1.84×10^{5}	3.74×10^{5}	0.49	0.62
East	-8.8×10^{-3}	0.12	-0.06	0.94
North	-2.1×10^{-2}	0.04	-0.47	0.63

is denoted by and a combination between and is referred to as sill. The parameter is the range in which it indicates a distance from the origin to the point of sill achieved, the adjusted semivariogram has the characteristic that the spatial autocorrelation is significant at very short distances (8.75 m), in this case the sill represents the maximum variability in the absence of spatial dependence (Giraldo 2002; Oliver and Webster 2015), as shows in the Figure 2B.

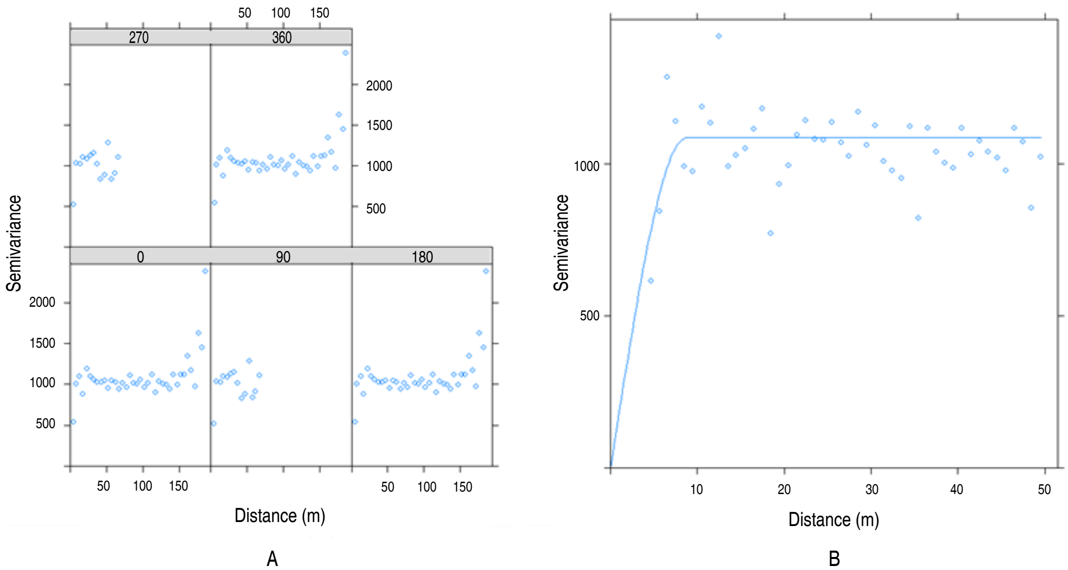

Figure 2. A. Directional semivariogram of *Oligonychus* sp. count in avocado trees; B. Adjusted semivariogram of *Oligonychus* sp. count in avocado trees.

The prediction of ordinary Kriging evidences a RMSE 32.72 counts/leaf and ME -0.16 counts/leaf, with a normal and homoscedastic distribution process of the residuals (P>0.05), ME is used for determining the degree of bias in the estimates (Hohn 1991) but it should be used cautiously as an indicator of accuracy because negative and positive estimates counteract each other and resultant ME tends to be lower than actual error (Nalder and Wein 1998), evidencing underestimation in forecasting. RMSE provides a measure of the error size, but it is sensitive to outliers as it places a lot of weight on large errors (Li and Heap 2008).

Based on this prediction, the indicator function is presented, which clearly shows a reduction of the risk zones (yellow) as the critical threshold, the incidence of *Oligonychus* sp. risk, increases. Also, the Probability As>100 mites/Leaf Figure shows the trees with the highest incidence of this mite, located to the north of the study area (Figure 3).

Two questions arise from these results, which are addressed in the following sections: 1) Which indicator function is the closest to reality? and 2) What is the influence of the covariates used in the prediction? To answer these questions, the starting point was the characterization of the spatial statistical process of the NDVI, TWI and TPI and, secondly, the co-regionalization model. The NDVI and DEM show a clearly non-stationary process by means, so it was necessary to extract the trend for the multigaussian conditional simulation process (Table 2). Additionally, the TPI shows a clear first order gradient, but the TWI does not show a gradient.

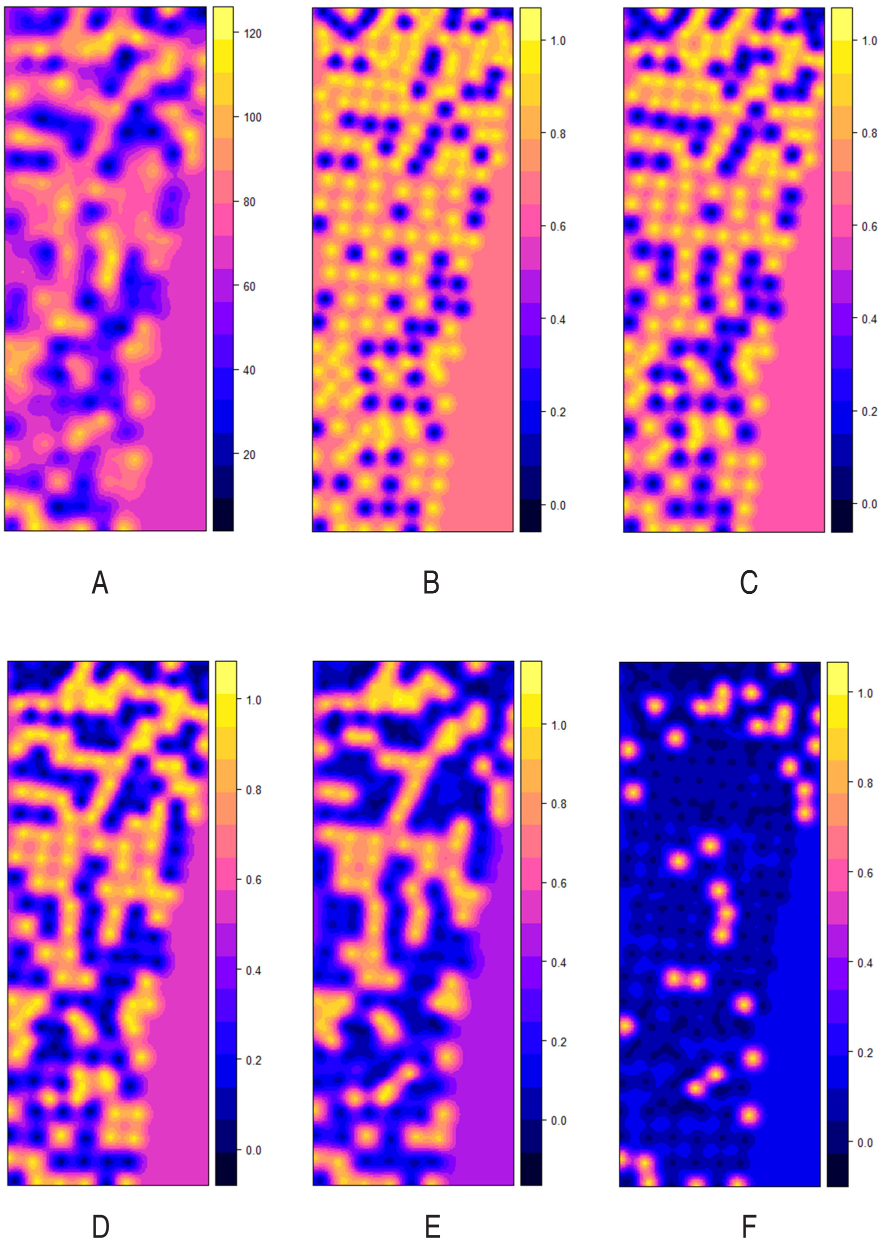

Figure 3. Univariate Predictions and risk indicator functions of *Oligonychus* sp. count in avocado trees. A. Prediction of Ordinary Kriging; B. Kriging Indicator > 50 mites/leaf; C. Kriging Indicator. >60 mites/leaf; D. Kriging Indicator > 70 mites/leaf; E. Kriging Indicator > 80 mites/leaf; F. Kriging Indicator > 100 mites/leaf.

The linear model of co-regionalization indicates that the process of the NDVI (Figure 4A) and TWI (Figure 4B) relationship are very similar in form, because they are possibly providing the same information as it is known the influence of soil moisture on the spectral response in the infrared and red bands (Bivand et al. 2008; Crippen

Table 2. First-order polynomial models of DEM, NDVI, TPI and TWI.

Variable	Coefficients	Estimate	Std. Error	t value	*P*
NDVI	Intercept	$4.09x10^{-1}$	$8.55x10^{-3}$	47.89	0***
	East	$1.57x10^{-4}$	$6.31x10^{-5}$	2.48	0.0138*
	North	$-5.73x10^{-4}$	$6.31x10^{-5}$	-9.07	0***
TPI	Intercept	$1.97x10^{-1}$	$7.56x10^{-2}$	2.60	0.00979**
	North	$-2.18x10^{-3}$	$6.45x10^{-4}$	3.38	0.00086***
TWI	Intercept	4.75	$1.95x10^{-1}$	24.32	0***
	East	$4.49x10^{-4}$	$1.44x10^{-3}$	0.31	0.755
	North	$1.95x10^{-3}$	$1.44x10^{-3}$	1.36	0.177

1990). It is possible to consider that both variables can be multicollinear (Gujarati and Porter 2010) and expressing the same information, both present a negative long-distance experimental covariance with the Count of *Oligonychus* sp.

The opposite occurs with TPI (Figure 4C), which shows a negative spatial correlation but with a short distance that allowed the inclusion of an effect similar to that found in the univariate analysis, indicating that this morphometric variable has local influence. Finally, the results of the co-regionalization model for the *Oligonychus* sp. count with the NVDI/TWI and with the NDVI/TWI/TPI are presented; they show a similar behavior to the previously described models with an apparent multicollinearity and poor fit (Figure 4D, E).

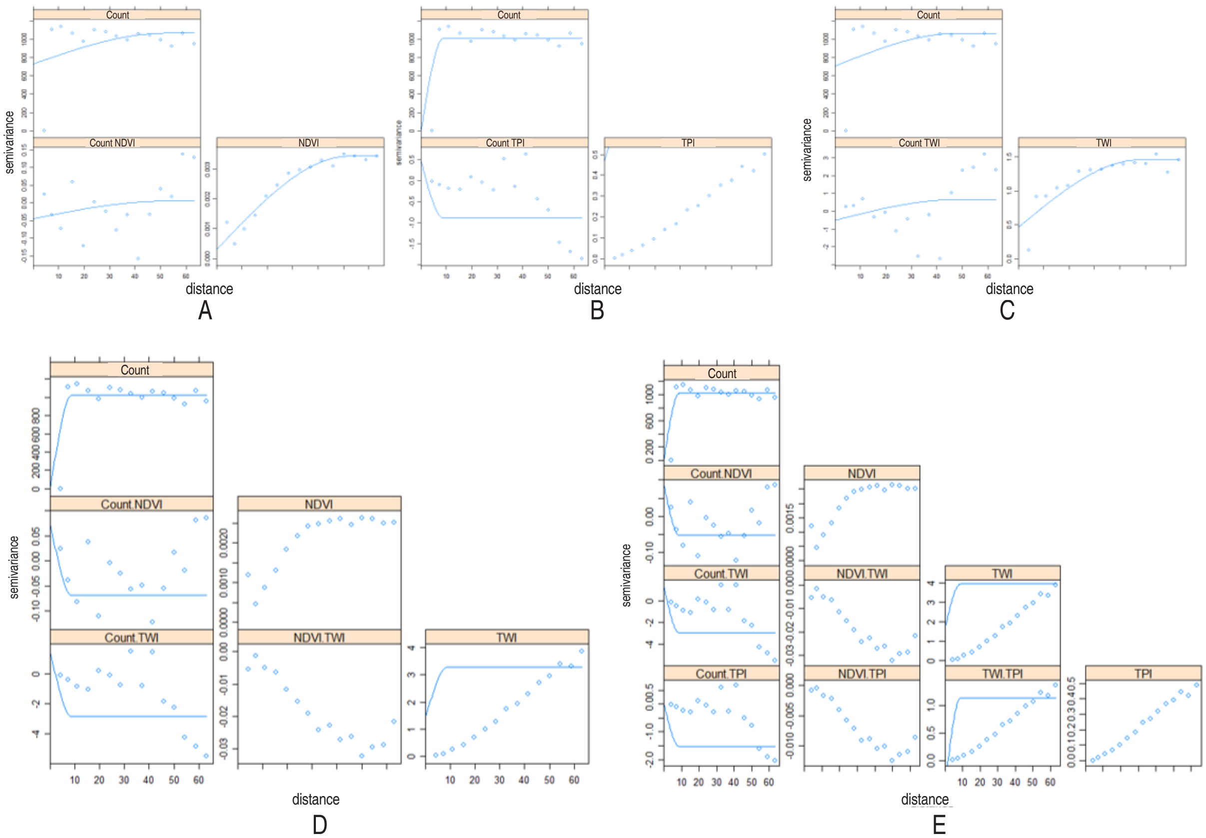

Figure 4. Linear model of co-regionalization models of count *Oligonychus* sp., A. NDVI; B. TWI; C. TPI; D. NDVI/TWI and E. NDVI/TWI/TPI.

All five cases show a poor fit of the linear model of co-regionalization, since the assumptions of the co-regionalization model were not fulfilled. The easiest to fit a linear co-regionalization model is that all models (direct and cross) have the same shape and range but may have different partial sills and nuggets (Bivand et al. 2008). This was not achieved because the ranges for all cases of the covariates are not similar to those of the response variable. The situation of the predictions of the covariates evidences it, since the predictions made by the co-Kriging with the NDVI and TWI show greater distance amplitude, while the TPI shows short distances (Figure 5). It is evident that although the conditional multigaussian simulation was not effective in improving the level of spatial detail, it is convenient to recommend for this study the use of Drone technology to obtain high spatial resolution data. This would homogenize the spatial scales and could improve the prediction.

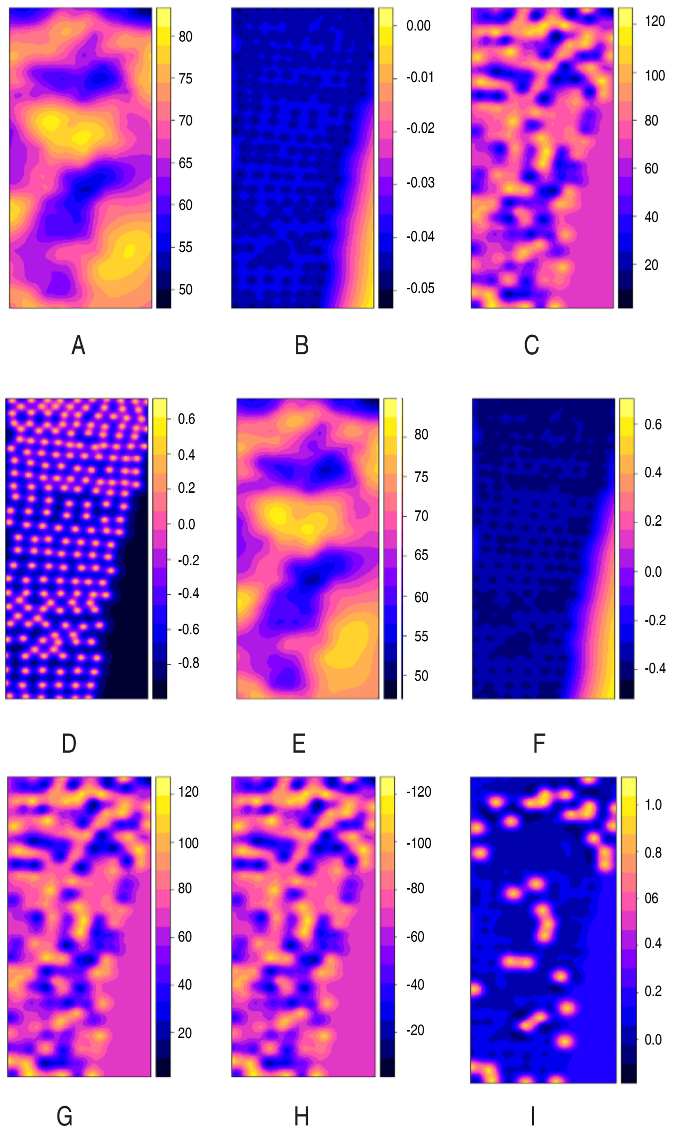

Figure 5. Co-Kriging Prediction, Covariance, and Indicator of count *Oligonychus* sp. A. co-Kriging Prediction with NDVI as covariable; B. co-Kriging Covariance with NDVI as covariable; C. co-Kriging Prediction with TPI as covariable; D. co-Kriging Covariance with TPI as covariable; E. co-Kriging Prediction with TWI as covariable; F. co-Kriging Covariance with TWI as covariable; G. co-Kriging Prediction with NDVI/TWI/TPI as covariable; H. co-Kriging Prediction with NDVI/TWI as covariable; I. co-Kriging Indicator > 100 mites/leaf NDVI/TWI/TPI as covariable.

As a selection rule, the best prediction is the one with the ME closest to zero and the lowest RMSE (Li and Heap 2008). One problem is that RMSE changes with the variability within the distribution of error magnitudes and with the square root of the number of errors (Willmott and Matsuura 2005). The RMSE is more appropriate to represent model performance than other indicators when the error distribution is expected to be Gaussian, the sensitivity of the RMSE to outliers is the most common concern with the use of this metric (Li and Heap 2008).

Considering these criteria, the best performances are those obtained by Univariate Ordinary Kriging, co-Kriging with NDVI as covariate and co-Kriging with TWI as covariate (Table 3), the co-Kriging errors are generally larger, especially near the subset sample points. This is because the co-Kriging predictions are based not only on the target variable, but also on the covariate at these points, as well as its covariance (Bivand et al. 2008). Another possible reason for these results may be that significant differences in Perú were found in the populations of the pest according to the location in the tree (Cango et al. 2014), as it was not possible to obtain data with higher spatial resolution, therefore the results are not from co-Kriging they are not entirely consistent.

The Ordinary Univariate Kriging validation is superior in all measures given that the overall precision is greater, and the extreme errors are smaller. Since the RMSE of the multivariate predictions are affected by outliers (which generate an increase in the bias of the predictions) and despite the fact that linear co-regionalization models are consistent, there are two possible scenarios for this response: 1) The spatial resolution of the covariables remains spatially insufficient to represent the detail the variability of the mite effect, and the assumptions of the co-Kriging model did not met. 2) The relationship between both the spectral response and the amount of mite per plant is nonlinear, so the multivariate Kriging model is insufficient to satisfactorily represent a spatial risk model (Bivand et al. 2008).

Table 3. Residuals per interpolation method.

Kriging type	RMSE	ME	Min.	Max.
			Error	Error
Ordinary Kriging	32.72	-0.16	-63.33	60.38
Co-Kriging with NDVI	32.76	-0.17	-101.89	102.57
Co-Kriging with TPI	36.52	-0.16	-101.81	102.88
Co-Kriging with TWI	32.76	0.16	-101.69	103.02
Co-Kriging with NDVI/TWI	36.38	-0.19	-102.08	102.92
Co-Kriging with NDVI/TWI/TPI	36.39	-0.19	-102.02	102.84

Also, the sampling density may play a key role in the performance of the spatial interpolation methods. It is often argued that if the sample size is big enough, then the effects of sample size would disappear. Apparently, this assertion is not true, in intensely sampled cases, there is still a clear pattern whereas sample size increases the performance of the spatial interpolation methods continues to improve, because the effects of sampling density is also dominated by the variation in the data (Li and Heap 2008).

In this research, the sampling scheme did not include information about the properties of the site being sampled. A reasonable size of the lag group is required to compute a good estimate of the semivariogram, so it is evident that a lot of data values are needed to adequately estimate the variogram. Authors such as Plant (2018), and Oliver and Webster (2015), state that between 100 and 200 samples should be used to generate an adequate experimental variogram.

The sampling efficiency is defined as the inverse of the sampling variance, it is desirable, from a statistical standpoint to select a configuration that minimizes the prediction error, the efficiency is calculated for all possible realizations of the variable Z by $\mathrm{Var}_{\xi}\left[Z_D^* - Z_D\right]$

using σ_K^2 , which is the geostatistical prediction error. In terms of the sampling variance, stratified random sampling is at least always equally or more accurate than random sampling; its relative efficiency is a monotone increasing function of sample size. Ideally, the density of sample points should increase in locations exhibiting greater spatial variability. When the autocorrelation is a linear decreasing function of distance, stratified random sampling has a smaller variance than a systematic design (Quenouille 1949).

If the decrease in autocorrelation is not linear, yet concave upwards, systematic sampling is more accurate than stratified random sampling. A centered systematic design, where each point falls exactly in the middle of each interval, is more efficient than a random systematic sampling configuration (Bellhouse 1977).

CONCLUSIONS

A geostatistical method for a spatial risk model of the mite *Oligonychus* sp. was presented. The univariate ordinary kriging model performs better than the multivariate ones because they present minimal error and therefore better performance (despite the linear co-regionalization models consistency). Nevertheless, it is evident that the spatial covariates do not have sufficient spatial resolution to provide enough information for model building. Although there is evidence that they are relevant variables that can be mappable at a high resolution through the use of photogrammetric techniques for the construction of the spatial risk model, a possible way forward is the use of information from Drones or Lidar surveys. It allows obtaining high resolution products at low cost, with which a more efficient model could be built. Another possible solution is the use of nonlinear spatial statistics, which allows the interaction of complex relationships.

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Bioactive compounds against *Moniliophthora roreri* (Cif & Par) identified in locally produced liquid amendments (biols)

Compuestos bioactivos contra *Moniliophthora roreri* (Cif & Par) identificados en enmiendas líquidas producidas localmente (bioles)

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ABSTRACT

Keywords:
Agriculture
Biopreparations
Fermentative process
Phytopathogens
Plaguicide

The use of liquid organic amendments (biols) is a common practice for farmers due to the multiple benefits in the management and production of their crops, including the control of pests and diseases. The present study analyzes the chemical composition of the pure compound C1 and fractions F2 and F3 of local biols produced in two provinces of Ecuador and their antifungal activity against *Moniliophthora roreri* (Cif & Par). This work incorporates the use of Gas Chromatography coupled to Mass Spectrometry (GC-MS), Nuclear Magnetic Resonance (NMR) and *in vitro* inhibition assays for sample analysis. C1 was identified as Mannitol. The percentage of inhibition against *M. roreri* in F2 and F3 was 44.37% and 8.34%, respectively; and, for C1, 28.63%. The values of the median lethal dose (LD_{50}) obtained corroborate that the F2 fraction was the one with the highest controlling activity against the pathogen. The 1,2-benzenedicarboxylic acid diisooctyl ester compound was the main compound in F2 (30.88%) and the Pentadecanoic acid, 14-methyl-, methyl ester in F3. Finally, all compounds obtained from the biol fractions were identified and it was determined that the fermentative process was suitable for producing bioactive compounds of interest to inhibit the growth of *Moniliophthora roreri*.

RESUMEN

Palabras clave:
Agricultura
Biopreparados
Proceso fermentativo
Fitopatógenos
Plaguicidas

El uso de enmiendas orgánicas líquidas (bioles) es una práctica común para los agricultores por los múltiples beneficios en el manejo y producción de sus cultivos, incluyendo el control de plagas y enfermedades. El presente estudio analiza la composición química del compuesto puro C1 y fracciones F2 y F3 de bioles locales producidos en dos provincias del Ecuador y su actividad antifúngica frente a *Moniliophthora roreri* (Cif & Par). Este trabajo incorpora el uso de Cromatografía de Gases acoplado a Espectrometría de Masas (CG-EM), Resonancia Magnética Nuclear (RMN) y ensayos de inhibición *in vitro* para el análisis de las muestras. C1 fue identificado como Manitol. El porcentaje de inhibición contra *M. roreri* en F2 y F3 fue del 44,37% y 8,34% respectivamente; y de C1, 28,63 %. Los valores de las dosis letales media (DL_{50}) obtenidos corroboraron que la fracción F2 fue la que mayor actividad controladora tuvo frente al patógeno. Los compuestos éster diisooctil del ácido 1,2-bencenicarboxílico fue el compuesto mayoritario en F2 (30,88%) y el ácido pentadecanoico, 14-metil-, éster metílico en F3. Finalmente, se identificaron todos los compuestos de las fracciones obtenidas a partir de biol, y se determinó que el proceso fermentativo era adecuado para producir compuestos bioactivos de interés para inhibir el crecimiento de *Moniliophthora roreri.*

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Organic amendments have become one of the widely used cultural strategies for disease control (Hidalgo et al. 2009; Jaramillo-Rodríguez 2015; Troncoso et al. 2015; Goyes-Campos and Monserrate-Gómez 2017; Varo-Suárez 2017) and improved crop productivity, properties attributed to the presence of micronutrients (León-Aguilar et al. 2016) and other components such as humic acids, phytohormones, enzymes, and efficient microorganisms (Quiroga et al. 2013; Troncoso et al. 2015; Serrano 2017). Besides, to incrase production, the application of organic amendments increases the values of ascorbic acid, beta-carotene, and chemical compounds in plants with high added value for human health.

The efficacy of these biopreparations in the control of plant pathogens has also been evaluated in field experiments suggesting the presence of antifungal compounds. Earlier work shows the use of organic amendments controlling important pathogens such as *Phytophthora capcisi* and *Fusarium* spp, affecting chile plants, and carnation (Uribe-Lorio et al. 2014), asparagus, and tomato crops, respectively (Melero-Vara et al. 2017). The positive effect of using organic amendments has also been documented for nematode control, including *Meloidogyne incognita*, a nematode affecting bell pepper (Revilla-Cervantes and Palomo-Herrera 2016), and some other species affecting tomatoes (Bernal 2010).

Cacao is one of the main crops cultivated in Ecuador. However, plantations continue to be severely affected by diseases, including Frosty Pod Rot or Moniliasis, caused by *Moniliophthora roreri* Cif & Par (Anzules-Toala 2021). Enríquez (2010) reports that the disease with the highest incidence in cacao cultivars in Ecuador and Colombia is Moniliasis, with an average production loss from 20% to 30%. The use of fungicides helps to fight infection. However, there are risks of contamination to farmers and the environment. Additionally, their high cost is a burden for the majority of small-scale farmers who cultivate cacao in the country. One of the alternatives for the control of this pathogen is the use of fermented products using manure and several other products referred to as biopreparations. Previous work showed that bioles could have fungistatic activity against the mycelial growth of *M. roreri in vitro* conditions (Magdama-Tobar 2016).

The composition of bioles, macro and micronutrients, the chemical identification of phytohormones, and the quantification of saponins and alkaloids have also been assessed (Jiménez 2008; Manzano et al. 2010). Several efforts have been carried out to standardize the production of bioles, as their elaboration process can be a significant variable, using quality indicators that included physical-chemical parameters (Orellana et al. 2013). Despite all the above mentioned, still there is lacking information about the active molecules or compounds present in these types of liquid amendments.

The present study aims to determine the chemical composition of bioles, assessing the activity of some fractions and pure compounds obtained by chromatographic and spectroscopic methods. The results presented in this study are of great significance for agriculture as the bioactive molecules identify in these types of products, can reinforce their use for the management of complex diseases affecting crops and food security.

MATERIALS AND METHODS

The samples processed included 10 g of lyophilized bioles elaborated at the provinces of El Oro (F2) and Guayas (F3) following anaerobic fermentation. These bioles were formulated based on internal CIBE (Biotechnological Research Center of Ecuador) protocols, containing manure, molasses, local microorganisms, and several minerals, as their main components. Further, samples were dehydrated at 60 °C in an oven with an air circulation system and subjected to constant reflux exposure to hexane, used as the main solvent, for 2 h. The hexane residues, namely F2 and F3, were later homogenized separately in a mortar with 25 g of silica without plaster with a particle size of 20-60 mesh to form a chromatography column head of 80 cm in length and 5 cm in diameter. It includes a 45 μm porous Teflon frit which was packed with 300 g of silica of 60-200 mesh. Fractioning was carried out with a mixture of solvents including, 100% ethyl acetate, ethyl acetate/methanol (80:20), and ethyl acetate/methanol (60:40). The latter was chosen for chemical characterization and antifungal evaluation against *M. roreri*. Qualitative analysis by thin-layer chromatography (TLC) was also performed on 60 F254 silica gel chromatoplate (Merck) and observed under UV light of 254 nm.

The ethyl acetate/methanol fractions (60:40) obtained from each of the bioles were rotovaporated to be reconstituted and analyzed by GC-MS, using an Agilent 7890A gas chromatograph with Agilent 5975 detector (Avondale, PA.USA), equipped with an HP 5MS column of 25 m length (0.25 mm diameter and 0.25 μm film thickness). The weight of each sample used for this process was: F2 (721 mg) and F3 (800 mg). Analytical grade helium was used as the carrier gas, and the analysis conditions were as follows: initial oven temperature: 100 °C for 3 min, increasing 8 °C min^{-1} to a final temperature of 250 °C, maintaining for 10 min. Injector and mass detector temperature: 250 °C and 300 °C, respectively. The mass detector was operated at 70 eV in scan mode with a range of 100 to 400 mass units. Assignment of F2 and F3 GC-MS structures was carried out by comparing the mass spectra of the compounds with those of the team libraries: Wiley, the 9th edition, and NIST- 2011 (Chóez-Guaranda et al. 2022).

The pure compound (C1), also obtained with ethyl acetate/methanol (60:40), was characterized by determining its melting point and analysis of the ^{1}H NMR spectra (399 MHz), ^{13}C NMR (100 MHz), and DMSO as a solvent in a VARIAN-400MHz NMR spectrometer. Chemical shifts (δ) were expressed in parts per million. The values of the coupling constants (J) are expressed in Hertz, the spectrometer temperature was 25 °C, the spin of 20 Hz for ^{1}H, ^{13}C for the rest of the experiments, and the spin goes off.

Inhibition tests against *M. roreri* strains of the pure compound and sub-fractions obtained: C1, F2, and F3, were carried out by the agar diffusion method (Montes-de-Oca-Marquez et al. 2017). The strain used in this study was obtained from a cacao infected pod showing symptoms of Frosty Pod Rot. The identity of the fungus isolated was corroborated by partial sequencing of the ITS region of the ribosomal DNA. Different concentrations of each compound, including 1, 10, 50, and 100 ppm dissolved in 1% DMSO, were mixed with papa dextrose agar (PDA) medium and poured into Petri dishes. After solidified, mycelial plugs of 5 days old growing colonies of *M. roreri* were placed at the edge of each Petri dish and incubated at 27 °C for 7 days. The radial growth of *M. roreri* mycelium was evaluated by measuring the growth on ten points around the radial growth of the colony. Three replicates per treatment were considered (n=30). The growth of *M. roreri* was also measured from three Petri dishes containing PDA mixed with DMSO as solvent (Xia-Hong 2007).

To verify significant differences between the treatments multiple and paired comparisons analyses were used, including Kruskal-Wallis, Dunn, and Wilcoxon tests for non-parametric data, respectively. Bonferroni-Holm adjusted *P* values were also considered for reducing the probability of type I and II errors. To determine the lethal dose for each compound (LD_{50}) a probit transformation method, applying a polynomial regression model with an explanation level (R^2) greater than 96%, was applied. All statistical tests were performed with a significance level of 95%. The programs R version 3.6.2 and Infostat version 2018 were used.

RESULTS AND DISCUSSION

Two fractions and one pure compound were obtained in our study, namely F2, F3, and C1. The biol F2, obtained by column separation, showed a chromatogram (Figure 1) with 11 peaks of different intensities. Table 1 lists the compounds that were structurally assigned by the database. The proposed structures for this fraction correspond mostly to hydrocarbons.

It is observed that the majority of compounds identified in this fraction, with a 30.88% relative abundance (Peak 10), were 1,2-Benzenedicarboxylic acid, diisooctyl ester with a retention time of 15.42 min (NIST 2020). With a relative abundance of 9.64%, docosane was also identified in the hexane extract of propolis (relative abundance 15.5%), this compound has been reported with antimicrobial activity (Arserim Uçar et al. 2020). Minority compounds such as tetratetracontane, dibutyl phthalate hexadecane, 1-iodo, eicosane pentadecane, heptacosane, 1-chloro, hexadecanoic acid, butyl ester, octadecane, hexadecane, 2, 6, 10, 14-tetramethyl were also identified. Literature reports that these compounds have been identified in extracts obtained from medicinal plants and microorganisms by using apolar and polar solvents, hexane and methanol, respectively, then antioxidant (Xinsong et al. 2019; Arserim Uçar et al. 2020 and Aziz et al. 2022) and antimicrobial activity (Arserim Uçar et al. 2020; Akwu et al. 2021; Albratty et al. 2023), was reported. The difference between the methodology used in that case and the one used in the present study is that the methanolic extract was fractionated in an open column with a mixture of half-apolar and polar solvents (ethyl acetate/methanol 60:40).

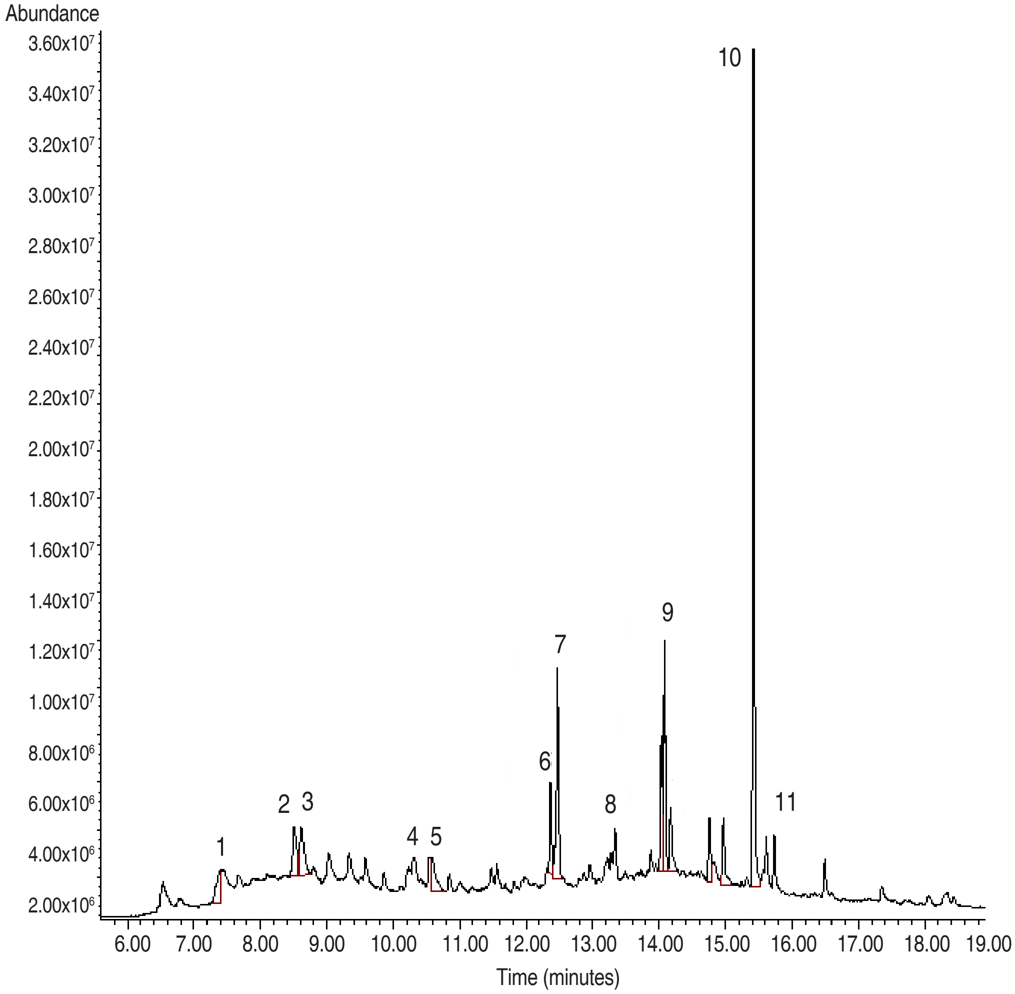

Figure 1. Analytical gas chromatogram of the F2 fraction (60:40 ethyl acetate/methanol), obtained from the biol elaborated at the El Oro province.

It is observed that the majority of compounds identified in this fraction, with a 30.88% relative abundance (Peak 10), were 1,2-Benzenedicarboxylic acid, diisooctyl ester with a retention time of 15.42 min (NIST 2020). With a relative abundance of 9.64%, docosane was also identified in the hexane extract of propolis (relative abundance 15.5%), this compound has been reported with antimicrobial activity (Arserim Uçar et al. 2020). Minority compounds such as tetratetracontane, dibutyl phthalate hexadecane, 1-iodo, eicosane pentadecane, heptacosane, 1-chloro, hexadecanoic acid, butyl ester, octadecane, hexadecane, 2, 6, 10, 14-tetramethyl were also identified. Literature reports that these compounds have been identified in extracts obtained from medicinal plants and microorganisms by using apolar and polar solvents, hexane and methanol, respectively, then antioxidant (Xinsong et al. 2019; Arserim Uçar et al. 2020 and Aziz et al. 2022) and antimicrobial activity (Arserim Uçar et al. 2020; Akwu et al. 2021; Albratty et al. 2023), was reported. The difference between the methodology used in that case and the one used in the present study is that the methanolic extract was fractionated in an open column with a mixture of half-apolar and polar solvents (ethyl acetate/methanol 60:40).

Table 1. Compounds identified in fraction F2 (ethyl acetate/methanol 60:40), obtained from biol elaborated at the El Oro province.

Peak	Retention time (min)	Compound	Abundance (%)
1	7.40	Pentadecane	3.22
2	8.51	Octadecane	4.17
3	8.62	Hexadecane, 2,6,10,14-tetramethyl-	4.16
4	10.31	Dibutyl phthalate	2.37
5	10.59	Eicosane	2.77
6	12.36	Hexadecanoic acid, butyl ester	3.90
7	12.47	Docosane	9.64
8	13.34	Tetratetracontane	2.35
9	14.17	Heptacosane, 1-chloro	3.23
10	15.42	1,2-Benzenedicarboxylic acid, diisooctyl ester	30.88
11	15.73	Hexadecane, 1-iodo-	2.60

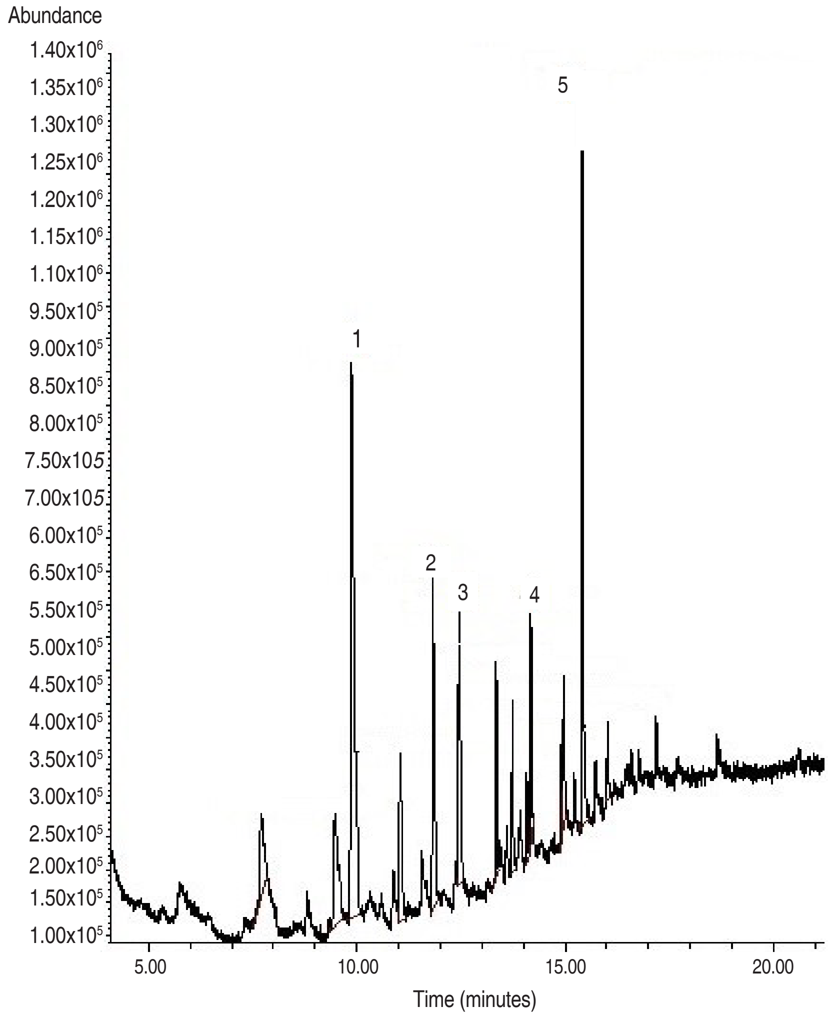

Figure 2. Analytical gas chromatogram of the F3 fraction (60:40 ethyl acetate/methanol), obtained from the biol elaborated at the Guayas province.

Analysis of fraction F3 showed a pasty appearance. Of 5 peaks present in the chromatogram, three esters and two hydrocarbons were identified. The majority of peaks identified corresponded to pentadecanoic acid, 14-methyl-, methyl ester (Figure 2). Another compound with a high percent abundance (14.46%) identified was 1,2-benzenedicarboxylic acid, diisooctyl ester mono (2-ethylhexyl) ester (Table 2).

Table 2. Compounds identified in fraction F3 (ethyl acetate/methanol 60:40), obtained from biol elaborated at the Guayas province.

Peak	Retention time (min)	Compound	Abundance (%)
1	9.87	Pentadecanoic acid, 14-methyl-, methyl ester	28.49
2	11.83	Octadecanoic acid, methyl ester	10.09
3	12.47	Eicosane	7.92
4	14.17	Heptacosane	4.09
5	15.42	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	14.46

1,2-benzenedicarboxylic acid diisooctyl ester identified in F2 was also identified in F3 with the same retention time of 15.42 minutes, but as a different percentage of relative abundance. The compounds reported in this study have also been referenced by other authors. For instance, the 1,2-benzenedicarboxylic acid diisooctyl ester identified in F2, was the main compound observed in the extract of *P. granatum* fruits (Basheera et al. 2021). Some compounds identified in F2 and F3 have pharmacological properties that are more than antimicrobial, such as butyl palmitate (hexadecanoic acid, butyl ester), which is reported to be a potential alternative to combat sickle cell anemia (Tshilanda et al. 2015), was reported as a liquid emollient and solvent/diluent in the cosmetic industry, obtained in a solvent-free system (Khan et al. 2018). Krishnan et al. (2014) reported that the compound 1,2-Benzenedicarboxylic acid, mono (2-Ethylhexyl) ester (F3) exhibited cytotoxic activity against HepG2 and MCF-7 cancer cell lines and low toxicity against normal HaCaT and NIH 3T3 cell lines 30. As shown, F2 fraction presented a greater amount of compounds (11) than F3 (5) from biols from El Oro and Guayas provinces, respectively. This could be caused by typical microorganism and the specific agroclimatic conditions of the location where the biol was prepared, impacting on its production (Compant et al. 2019; Saad et al. 2020).

The analysis and interpretation of the ^{1}H NMR and ^{13}C-NMR (Table 3), compared with the literature, indicates the presence of an alditol, specifically the so-called Mannitol (2*R*, 3*R*, 4*R*, 5*R*-Hexane-1,2,3,4,5,6-hexol) m/z: 182, with the molecular formula $C_6H_{14}O_6$ (Chapelle et al. 1991; SDBS 2019).

In the ^{13}C-NMR spectrum, three peaks corresponding to three carbons were observed, which compared to the mass spectrum (M+ of 182), helped to conclude that the molecule shows symmetry. Of the three carbons observed, two of them appear in the DEPT in the normal phase identified as methins C-2.5 and C-3.4, signals consistent with carbons adjacent to hydroxyl groups (HO-CH), their chemical shifts correspond at δ: 71.32 and δ: 69.38 respectively, whose H-2.5 protons appear as a multiple with a chemical shift of δ: 3.40; H-3.4 appears as a triplet at δ: 3.54 with an 8 and 6.8 Hz coupling constant with their respective adjacent hydrogens. The third signal in the ^{13}C-NMR spectrum was identified in DEPT as methylene (C-1.6), with a chemical shift of δ: 63.87, also unprotected, which is why it is concluded that it is bound to one hydroxyl group. In ^{1}H NMR two groups of complex signals consistent with their enantiotopic character were also identified, corresponding to *d*: 3.38 H-1a, 6a, and *d*: 3.60 H-1b, 6b.

The spectrum of ^{1}H NMR , ^{1}H COSY (Table 3), shows correlations between protons H-1.6↔-OH-1.6 (δH-1a, b,6a, b: 3.38; 3.60*m*; δOH-1.6: 4.31*t*; *J*= 5.6; 6.4), H-2.5↔OH-2.5 (δH-2.5': 3.40*m*; δOH-2.5: 4.40*d* *J*= 5.4), H-3.4↔OH-3.4 (δH-3.4': 3.54 *t*, δOH-3.4: 4.13 *d* *J*=6.8).

Antifungal evaluation

The antifungal assessment carried out against *M. roreri*, revealed significant inhibitory activity on the three compounds evaluated; fractions F2, F3, and the pure compound C1 when compared to the control, 7 days after inoculation (Figure 3 and 4).

Table 3. Chemical shifts (δ) of the pure compound C1 in the ^{1}H NMR (DMSO, 400 MHz) and ^{13}C-NMR (DMSO, 100 MHz), of the (2*R*, 3*R*, 4*R*, 5*R*)-Hexan-1,2,3,4,5,6-hexol.

Type	H	δ^{1}H NMR	m	J(H/H)	J (Hz)	C	δ^{13}C-NMR
-CH_2-O	1.6	3.61 3.38	*m*	-	-	1.6	64.36
>CH-O	2.5	3.45	*m*	-	-	2.5	72.05
>CH-O	3.4	3.54	*t*	H-3/H-4	6.8 8	3.4	70.48
-OH	1	4.31	*t*	0H-1/H-1a, H-1b	6.4 5.6	-	-
-OH	2	4.42	*d*	0H-2/H-2	5.2	-	-
-OH	3	4.15	*t*	0H-3/H-3	6.8	-	-

d = Doublet; δ = Chemical shifts; H= Proton; H/H= Proton distance; *J*= Coupling constant; *m*= Multiplicity; *t* = triplet.

The non-parametric Kruskal-Wallis test confirmed that not all treatments are equal or that there are significant differences between at least one pair of treatments (*P*-val=3.36×10^{-22}). This was confirmed with the post hoc non-parametric comparisons through Dunn's test with Holm correction which revealed all pairwise comparisons between treatments had statistical differences (*P*-Val Holm-Corrected <0.05).

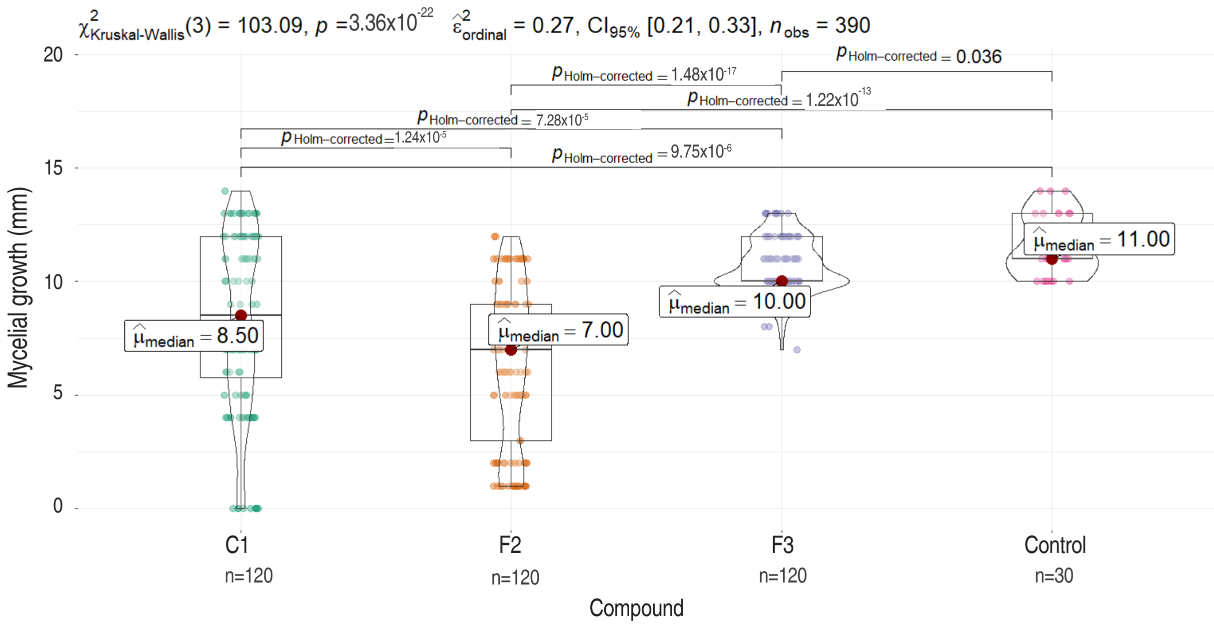

Figure 3. Comparative analysis of *M. roreri* mycelial growth on PDA media containing different compounds (C1, F2, and F3) evaluated 7 days after inoculation. Each plot represents the total measures per compound compared to the control (Dunn test). Significance is shown as corrected *P* values (Holm-correction) for each comparison.

The control treatment exhibited superior stability, with minimal dispersion in the observed data (values ranging from 10 to 15), and the highest median of 11 among all four treatments. Compound F3 demonstrated moderate stability (values ranging from 7 to 13), with a slightly lower median of 10 compared to the control. However, there is an evident skewness in the violin plot, indicating data accumulation above the median and greater dispersion below the median.

Compounds F2 and C1 display higher levels of dispersion and instability in their results, with lower medians of 7.0 and 8.5, respectively (Figure 3). C1 and fraction F2 presented an inversely proportional effect between concentration and growth showing higher inhibition as concentrations of both products were increased. Conversely, fraction F3 showed a directly proportional effect between concentration and growth. Fraction F2 had a higher inhibitory effect of 44.37% compared to C1 and F3 which had values of 28.63% and 8.34%, respectively (Figure 5). The concentration of 100 ppm from fraction F2 showed the most significant control against *M. roreri*, (P<0.05) and was also the treatment with more stable results (less variability) compared to F1 compound evaluated at the same concentration (100 ppm). C1 showed LD_{50} values of 45 ppm and F2 of 32.51 ppm. LD_{50} values for F3 could not be determined. $P<2.2\times10^{-16}$ was obtained by using the Krustal-Wallis method.

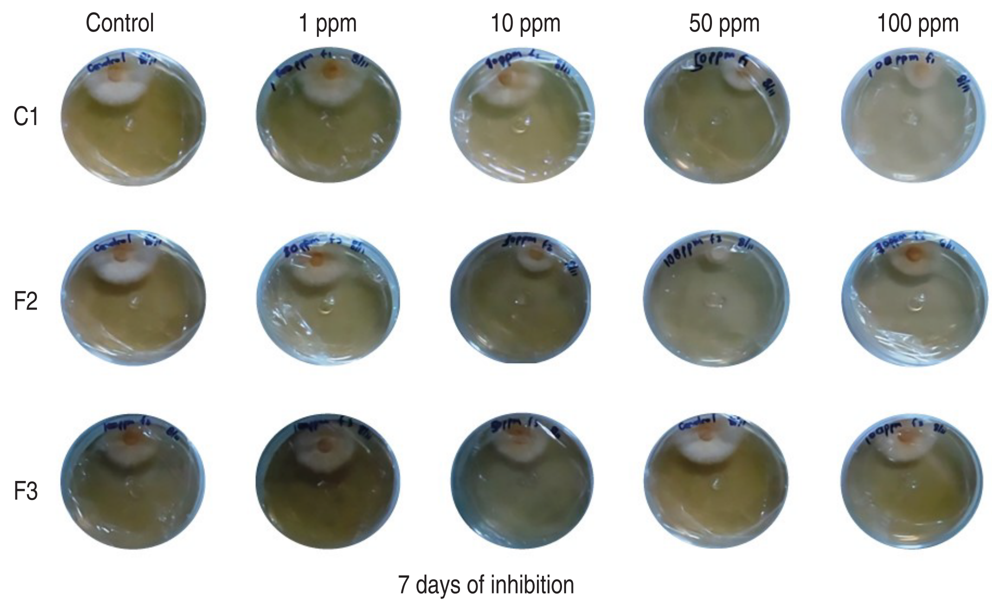

Figure 4. Inhibition of mycelial growth of *M. roreri* in PDA media with different compounds (C1, F2 and F3) evaluated 7 days after inoculation.

It is known that 1,2-benzenedicarboxylic compound reports high antimicrobial activity against gram-positive bacteria such as *Bacillus cereus, Bacillus coagulants, Streptococcus* spp., and *Staphylococcus aureus*; gram-negative bacteria: *Escherichia coli, Proteus mirabilis, Yersinia* spp., and *Pseudomonas aeruginosa*; and two yeasts: *Candida albicans and Candida utilis* (Binti et al. 2019). In F3, the compound 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester with a high percentage of abundance was also present, for which the literature reports having high efficacy in the control of plant diseases with a similar activity like that of a compound identified from a crude extract of ethyl acetate from a strain of *Trichoderma* (Xu et al. 2018) This compound was also present in F2, probably responsible for the main affect against the growth of *M. roreri*. Studies carried out by Ali et al. (2017) report that this compound was the main obtained (58.56%) in a methanolic extract from the roots of *Chenopodium album,* which also showed high antifungal activity against *Sclerotium rolfsii*. There were compounds with antibacterial activity, such as eicosane; 1,2 benzenedicarboxylic acid; and diisocityl ester acid. Eicosane, has been identified in macroalgae (Troncoso et al. 2015) and acid, diisooctyl ester (5.17%) in ethanolic extract of *Phoenix pusilla* fruit (Thangadurai and Velavan, 2018). Other compounds with antifungal activity, such as heptacosane, 1-chloro (7.04% relative abundance), have also been found in ethyl acetate extracts of aerial parts of *Quisqualis indica* (Akriti et al. 2017). The hexadecanoic acid butyl ester was reported in the alcoholic extract of *Chenopoidium* (Ali et al. 2017 and El-Shahir et al. 2022), which differs from what we reported in this study, ethyl acetate/methanol 60:40.

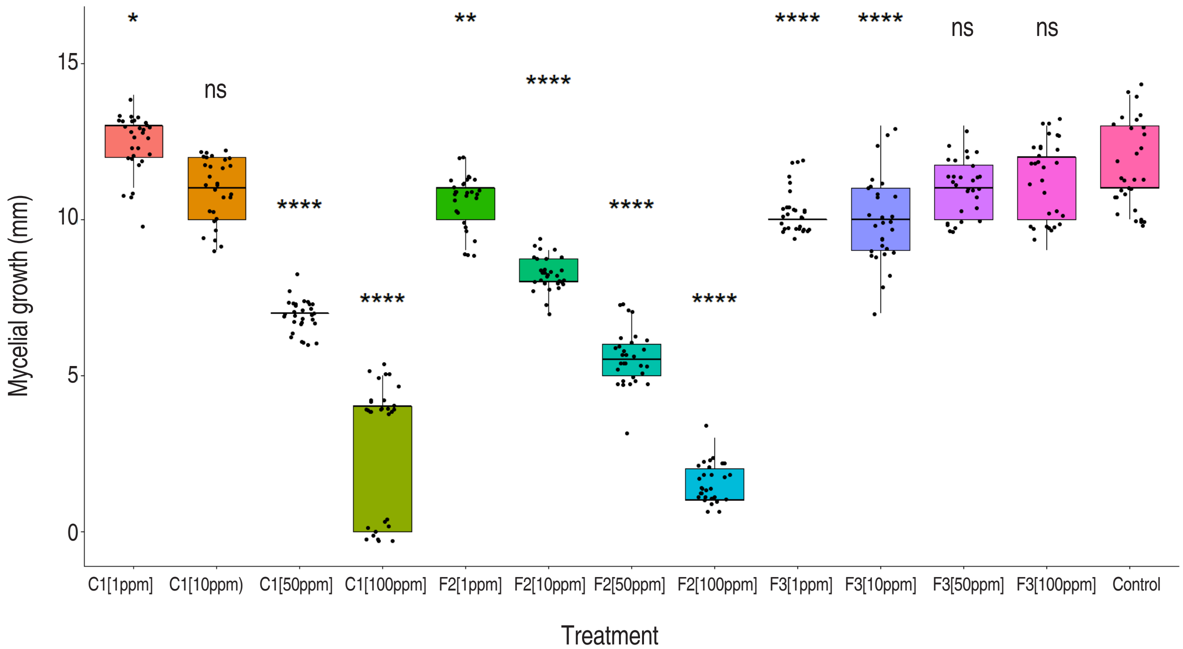

Figure 5. Comparative analysis of *M. roreri* mycelial growth on PDA media containing different concentrations (1, 10, 50 and 100 ppm) of compounds C1, F2, and F3, evaluated 7 days after inoculation. Each plot represents the total measures per treatment compared to the control (Wilcox test). A general test, following the multiple Kruskal-Wallis method, is also shown in the graph. All tests were run with a significance of 95%. Significance shown on the top of each boxplot is read as follows: (0.0001=****), (0.001=***), (0.01=**), (0.05=*), (ns=1).

From the compound isolated from the ethyl acetate fraction of biol (C1) from the province of El Oro. After the isolated we obtained a white solid pellet (60 mg) with a 164 °C melting point, soluble in methanol. The compound C1, identified by NMR in this study as mannitol, has also been isolated from the olive leaves (Lama-Muñoz et al. 2020). The activity of mannitol has been characterized as bacteriostatic (Fernández-Bolaños et al. 2000) against positive bacteria including *Bacillus circulans, Enterococcus avium, Enterococcus avium Proteus vulgaris Bacillus circulans Enterobacter gregoviae, Enterococcus asacchrolyticus, and Bacillus brevis* (Singh, 2014). Other studies show the role of mannitol in increasing the sensitivity of persistent bacteria forming biofilms (*Pseudomonas aeruginosa)* to antibiotics (Barraud et al. 2013), advising it as an attractive raw material for developing next-generation antibiotics (Nguyen et al. 2019). It could be inferred from our results that the biological activity found in the liquid organic amendments of this research, including its effect on reducing the growth of *M. roreri*, could be to the presence of antifungal compounds including 1,2-benzene carboxylic acid, bis (2-methyl propyl) ester, acid 1,2-benzenedicarboxylic acid, mono (2-Ethylhexyl) ester and mannitol. Further research is needed to assess if these compounds have synergistic interactions with other compounds also present in these products.

CONCLUSIONS

The biological activity observed in some liquid organic amendments (Biols), as the ones studied in this work obtained by anaerobic fermentation, can be related to the presence of secondary metabolites and hydrocarbons with antifungal properties. The high abundance (30.88%) of 1,2-Benzenedicarboxylic acid, diisooctyl ester and the rest of the compounds identified in fraction F2 could be related to the higher inhibitory activity against *Moniliophthora roreri*. The use of GC-MS and NMR can be used to identify compounds with high accuracy for further characterization and serve the purpose of molecule discovery for new applications, either in agriculture or related fields. This report presents a novel method of improving bioles to promote multiple crop growth, using a natural fermentation process that produces bioactive compounds capable of inhibiting the growth of *M. roreri*. Notably, the process avoids synthetic chemical products, facilitating organic waste management and promoting green chemistry. Moreover, in this study the biol production process could serve as a potential natural bioreactor for developing other bioactive components, such as antioxidants and antimicrobials, for future applications in the cosmetic, food, and pharmaceutical industries. However, further research is necessary to explore these exciting possibilities fully.

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Weaknesses and potential of green businesses in the Sub-regions of La Mojana and San Jorge, in the department of Sucre, Colombia

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Debilidades y potencialidades de los negocios verdes, en las subregiones La Mojana y San Jorge en el departamento de Sucre, Colombia

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ABSTRACT

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Environmental impact
Natural resources
Subsectors
Sustainable development

This study was aimed to determine the weaknesses and potential of green businesses in La Mojana and San Jorge subregions in the department of Sucre. Businesses in the category of sustainable goods and services from natural resources and subsectors of the Biotrade sector were considered since they were the most representative in the area—the instrument verification sheet version 1.2 F001-2014 established by the Ministry of Environment and Sustainable Development - MADS was used to evaluate them. The fulfillment of sustainability criteria in the evaluated subsectors and between the level of business development in the study municipalities was analyzed using the Chi-square test. As a result, weaknesses in the economic component criteria and strengths in the environmental criteria for most of the businesses were evidenced, as well as significant differences between the municipalities and subsectors. However, restructuring businesses towards sustainability is a path that requires cultural, technological, and management strengthening. This is a change that should gradually happen and must be supported by government authorities.

RESUMEN

Palabras clave:
Cumplimiento de indicadores
Sistemas ecológicos Impacto ambiental
Recursos naturales
Subsectores
Desarrollo sostenible

El propósito de este estudio fue determinar las debilidades y potencialidades de los negocios verdes de las subregiones Mojana y San Jorge en el departamento de Sucre. Se consideraron los negocios pertenecientes a la categoría de bienes y servicios sostenibles provenientes de los recursos naturales y subsectores del sector biocomercio, debido a que tienen mayor representatividad en la zona. Mediante el instrumento ficha de verificación versión 1.2 F001-2014 establecido por el Ministerio de Ambiente y Desarrollo Sostenible- MADS. Además, fue aplicado un análisis sobre el cumplimiento de criterios de sostenibilidad, utilizando la prueba de Chi cuadrado, en los subsectores evaluados y entre el nivel de desarrollo de los negocios en los municipios del estudio. Como resultado se evidenció la existencia de debilidades en los criterios del componente económico y fortalezas en los criterios ambientales en la mayoría de los negocios, así mismo, diferencias significativas en los municipios y subsectores. El proceso de reconversión de los negocios hacia la sostenibilidad es un camino que amerita un fortalecimiento cultural, tecnológico y gerencial, que ocurre en forma paulatina y debe estar acompañado de una asesoría por parte de las autoridades gubernamentales.

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The development of societies is largely due to global economic activities. In many cases, these activities are a permanent source of pollution, with waste dumping affecting all environmental compartments (Taco-taco et al. 2017), which raises concern because of climate variability due to the continued environmental degradation (Severiche et al. 2016). Despite this situation, economic prosperity, quality of life, equity, and social welfare have been placed above the protection of natural resources (Cuartas et al. 2019; Salas and Ortiz 2018), demonstrating a lack of awareness, attitude, apathy, and harmonious relationship with the ecosystems (Ramírez 2015).

This problem, according to the Ministry of Environment and Sustainable Development (MADS by its acronym in Spanish) and the United Nations Development Program (UNDP), is driven by hydroelectric plants, the indiscriminate use of pesticides, water pollution, and depletion, loss, and degradation of elements in native ecosystems (MADS and PNUD 2014). Even though Goal 12 of the Sustainable Development Goals established by the United Nations, titled "responsible consumption and production", indicates that "achieving economic growth and sustainable development requires that we urgently reduce our ecological footprint by changing the way we produce and consume goods and resources" (PNUD 2019). Additionally, the update of the National Green Business Plan 2022–2030 proposes an approximation of the contribution of green businesses and their related actions to achieving the Sustainable Development Goals (SDGs) in each emphasis area. In the environmental dimension, SDGs 6, 13, 14, and 15, in the social dimension, SDGs 1, 2, 3, 4, 5, 7, 11, and 16, and in the economic dimension, they emphasize SDGs 8, 9, 10, and 12 (MADS 2022). Consequently, the goal of creating 12,630 new green businesses by 2030 has been linked to the objective of promoting an increase in productivity and economic competitiveness as part of the country's follow-up goals (CONPES 3934).

Colombia has a green growth approach (CONPES 3934) with the purpose of generating technological and behavioral changes in economic sectors that can contribute to environmental conservation. In this context, the Ministry of Environment and Sustainable Development created, in 2014, the National Plan for Green Businesses (PNNV), which "defines the guidelines and provides tools for planning and decision-making that allow the development and promotion of green and sustainable businesses in the country" (MADS 2014). It includes those economic activities that incorporate good sustainable practices with a life-cycle approach that contribute to the conservation of the natural capital that supports the development of the territory (ONNVS 2014).

Researchs from Universidad de Sucre on the application of sustainable agriculture in this region report a limited potential for agricultural production and an acceptable potential for environmental supply and natural resources (Benítez 2010). In addition, it was reported that the population live off subsistence farming and complementary activities of indiscriminate hunting of wildlife (De la Ossa et al. 2011).

This multi-causal situation in the region may be associated with little knowledge about environmental problems, a lack of rigor in compliance with environmental regulations (Martínez and Sánchez 2019), little research on environmental businesses, and a lack of articulation among key actors that can encourage the establishment of connections (Acevedo et al. 2018).

MATERIALS AND METHODS

The study was conducted in La Mojana and San Jorge subregions. La Mojana is located at the southern end of the department of Sucre. It comprises the municipalities of Sucre, Majagual, and Guaranda and has an area of 2,337 square kilometers (22.6% of the department) (Urquijo and Vargas 2013; IGAC 2018). San Jorge subregion is located in the southwest of the department, consisting of the municipalities of San Marcos, San Benito Abad, La Unión, and Caimito, with a territory of 2,934 square kilometers (28.3% of the total of the department) (Corpomojana 2019; IGAC 2018).

The study is a mixed type of research, transversal cut, not experimental. For data analysis, descriptive statistics was used. The collected information was coded and tabulated, and the study variables data were exported to R Project version 4.0.3 (2021) statistical program. A box analysis was performed based on the results of the evaluation. Likewise, a comparative analysis was made to statistically determine significant differences, using the chi-square test with a significance level of 5%

($P \leq 0.05$), among the level of criteria compliance, the subsectors evaluated, and the location of the companies in the municipalities of La Mojana and San Jorge regions.

The businesses were selected from a diagnosis made for the project "Support for the Implementation of the Sustainable Green Business Window in the Jurisdiction of the Corporation for Sustainable Development of La Mojana and San Jorge - Corpomojana" provided by Corpomojana, based on the category of sustainable goods and services from natural resources, biocommerce sector, and subsectors, (Verification and evaluation guide of Green Business 2016). A total of 40 Companies were evaluated and their selection was based on the following inclusion criteria: having a settlement in the subregions of La Mojana or San Jorge in the department of Sucre, having a chamber of commerce certificate, intending to be a green business, and having signed an informed consent.

The study used the methodology of MADS (2014) and Cerón and Lasso (2020) by applying a survey to business' owners using the tool - Form version 1.2 F001-2016 (verification sheet 2), which evaluated a set of indicators on 12 verification and evaluation criteria for green businesses as framed within the National Plan of Green Businesses (ONNVS 2014; Duarte Ramírez 2019; Martínez et al. 2020), as shown in Table 1.

Table 1. Green business criteria.

1. Economic viability of the business.	7. Efficient and sustainable use of resources to produce the finished product or service offered.
2. Positive environmental impact	8. Social responsibility within the company.
3. Life cycle approach of the good or service.	9. Social responsibility within the value chain of the company.
4. Useful life	10. Social responsibility outside the company.
5. Substitution of hazardous substances or materials.	11. Communication of attributes of the goods and services.
6. Recyclability and/or use of recycled materials.	12. Environmental or social schemes, programs, or recognitions implemented or received.

Verification and evaluation guide of Green Business (2016).

The described criteria were evaluated in the business lines found in the department of Sucre's La Mojana and San Jorge subregions. Indicators for each criterion were graded with values 0, 0.5, 1, or not applicable (N/A), according to the verification and evaluation guide of Green Business Criteria (2016). The estimate of the compliance rate was obtained using an arithmetic average, described in Equation (1):

$$\text{Criterion score i} = \sum_{i}^{n} \frac{x_i}{z} * 100 \qquad (1)$$

Where x = score of sub-criteria of criterion i; z = the number of sub-criteria of criterion i.

Determining the weaknesses and potential of the selected businesses was based on compliance level with the green business criteria. If they are below 50%, they are considered weak criteria; if above 51%, they are considered viable. This tool uses a set of indicators developed with the support of the German Agency for International Cooperation GIZ and validated in the field, in 2015, in different states of Colombia (Lizarazo and Contreras 2021).

RESULTS AND DISCUSSION

Results related to the potential and weaknesses of green businesses

In total, six subsectors of the category of sustainable goods and services from natural resources were identified with their respective economic activities, as shown in Table 2.

The analysis of compliance of the evaluated criteria shows strength in the criteria positive environmental impact; the substitution of hazardous substances or materials; recyclability and/or use of recycled materials; and social responsibility outside the company. In contrast, the analysis shows weaknesses in the criteria the economic viability

Table 2. Subsectors of economic activities prioritized.

Green Business Subsector	Economic activities prioritized - Study region	Participation rate (%)
Non-timber	Craft shops based on arrow cane (caña flecha)	10
Ecological, biological, and organic production systems	Agricultural businesses (aji, plantain, yucca, passion fruit, papaya, ahuyama, bocachico, beans, green beans, and vermicompost)	55
Products derived from wildlife	Beekeeping businesses	10
Ecotourism	Ecotourism - historic	2.5
Sustainable agro-systems	Nursery of ornamental plants	2.5
Food industry	Industrial businesses (popocho, panela, cashew, and cocoa)	20

of the business; social responsibility within the company; social responsibility within the value chain of the company; communication of attributes of goods and services; and schemes, programs, or recognitions implemented or received. On the other hand, the analysis showed that the subsectors of nature tourism, agro-industrial food, non-timber, and biological, ecological production systems have a more significant potential for compliance than those of products derived from wildlife and sustainable Agricultural-systems; nevertheless, it indicates that the most significant participation is in organic products, followed by the food industry. These results are detailed in Figure 1.

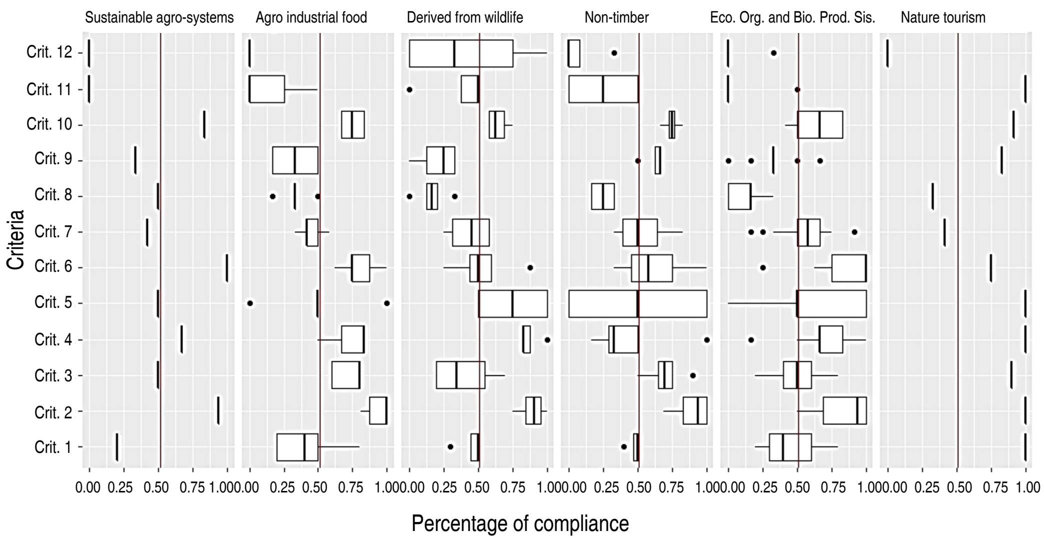

Figure 1. Comparison of the compliance of criteria by subsector.

The comparative analysis of the criteria compliance and location of the companies in the municipalities of La Mojana and San Jorge regions was able to detect that the criteria that exceeded 51% of compliance and have strengths are: positive environmental impact, useful life, substitution of hazardous substances or materials, recyclability and/or use of recycled materials and social responsibility outside the company; and that they were located in the municipalities of San Benito Abad, San Marcos, and Sucre. It detected that the main weaknesses were: efficient and sustainable use of resources for the production of goods or services, social responsibility within the company, social responsibility within the value chain of the company, communication of attributes of goods and services and schemes, programs or recognitions implemented or received; specifically in the municipalities of La Unión and Caimito, as shown in Figure 2.

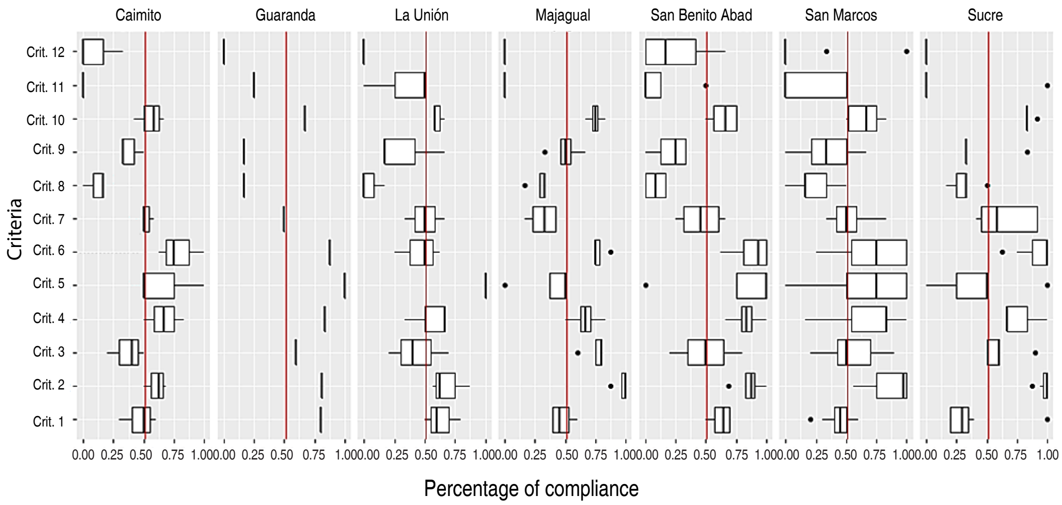

Figure 2. Comparison of the compliance of criteria by the municipality.

Regarding the economic viability of the businesses evaluated, the highest averages were found in the companies of San Benito Abad, and La Unión municipalities, showing significant differences with those located in San Marcos and Sucre municipalities, which have the lowest averages.

The Chi-square test showed that "life cycle approach of the goods or services", "useful life", "recyclability and/or use of recycled materials", "social responsibility within the company", "social responsibility within the value chain of the company", and "communication of attributes of the goods and services" criteria statistically present significant differences ($P<0.05$) with regards to the evaluated sub-sectors. The remaining criteria showed no marked statistical differences, as shown in Table 3.

Table 3. Chi-square test, comparison of the compliance of criteria regarding the subsectors.

Criteria	Chi2	Df	P Chi2
Economic viability of the business.	1.184	3	0.757
Positive environmental impact and contribution to the conservation and preservation of ecosystem resources.	1.429	3	0.699
Life cycle approach of the good or service *	16.520	3	0.001
Useful life *	8.540	3	0.036
Substitution of hazardous substances or materials	0.893	3	0.827
Recyclability and/or use of recycled materials*	9.408	3	0.024
Efficient and sustainable use of resources for the production of goods or services	5.363	3	0.147
Social responsibility within the company*	9.571	3	0.023
Social responsibility within the value chain of the company	11.662	3	0.009
Social responsibility outside the company	3.818	3	0.282
Communication of attributes of goods and services*	9.328	3	0.025
Schemes, programs or recognitions implemented or received.	6.424	3	0.093

*Significant differences**.**

Findings indicated that agro-industrial and non-timber businesses had higher averages than businesses with products derived from wildlife and ecological production systems, regarding essential aspects of sustainability in the life-cycle approach criteria. Businesses in the ecological production systems subsector showed the highest averages on recyclability and use of recycled materials criteria, showing statistically significant differences with non-timber and businesses with products derived from wildlife, which had the lowest scores.

Concerning the social responsibility within the business criteria, the agro-industrial food production businesses have the highest scores. In contrast, the lowest scores were obtained by businesses with products derived from wildlife and ecological production systems. In the communication of attributes of the goods and services criteria, there is a marked difference among businesses with products derived from wildlife, which have the highest scores, and those with ecological production systems, as shown in Table 4.

Table 4. Multiple comparisons according to company subsectors and green business criteria.

Criteria	Subsectors	n	Min	Max	Mean	SD	Groups
Economic viability of the business	Non-timber	4	0.400	0.500	0.475	0.050	A
	Products derived from wildlife	4	0.300	0.500	0.450	0.100	A
	Eco. Org. and Bio. Prod. Sis.	21	0.200	0.800	0.457	0.163	A
	Agro-industrial food	9	0.200	0.800	0.411	0.220	A
Positive environmental impact and contribution to the conservation and preservation of ecosystem resources	Agro-industrial food	9	0.812	1.000	0.938	0.077	A
	Non-timber	4	0.688	1.000	0.891	0.148	A
	Products derived from wildlife	4	0.750	1.000	0.891	0.107	A
	Eco. Org. and Bio. Prod. Sis.	21	0.500	1.000	0.833	0.188	A
Life cycle approach of the goods or services	Agro-industrial food	9	0.600	0.800	0.722	0.097	A
	Non-timber	4	0.500	0.900	0.700	0.163	A
	Eco. Org. and Bio. Prod. Sis	21	0.200	0.800	0.476	0.151	B
	Derivatives of fauna	4	0.200	0.700	0.400	0.245	B
Useful life	Products derived from wildlife	4	0.833	1.000	0.875	0.083	A
	Agro-industrial food	9	0.500	0.833	0.759	0.121	Ab
	Eco. Org. and Bio. Prod. Sis	21	0.167	1.000	0.683	0.166	B
	Non timber	4	0.167	1.000	0.458	0.370	B
Substitution of hazardous substances or materials	Products derived from wildlife	4	0.500	1.000	0.750	0.289	A
	Eco. Org. and Bio. Prod. Sis	21	0.000	1.000	0.595	0.407	A
	Agro-industrial food	9	0.000	1.000	0.556	0.300	A
	Non-timber	4	0.000	1.000	0.500	0.577	A
Recyclability and/or use of recycled materials	Eco. Org. and Bio. Prod. Sis	21	0.250	1.000	0.863	0.205	A
	Agro-industrial food	9	0.625	1.000	0.778	0.121	Ab
	Non-timber	4	0.333	1.000	0.625	0.285	B
	Products derived from wildlife	4	0.250	0.875	0.531	0.258	B

Table 4

Criteria	Subsectors	n	Min	Max	Mean	SD	Groups
Efficient and sustainable use of resources for the production of goods or services	Eco. Org. and Bio. Prod. Sis	21	0.167	0.917	0.587	0.212	A
	Non-timber	4	0.333	0.833	0.542	0.220	Ab
	Products derived from wildlife	4	0.250	0.583	0.438	0.172	Ab
	Agro-industrial food	9	0.333	0.583	0.444	0.072	B
Social responsibility within the company	Agro-industrial food	9	0.167	0.500	0.315	0.100	A
	Non-timber	4	0.167	0.333	0.250	0.096	Ab
	Products derived from wildlife	4	0.000	0.333	0.167	0.136	B
	Eco. Org. and Bio. Prod. Sis.	21	0.000	0.333	0.159	0.123	B
Social responsibility in the company's value chain	Non-timber	4	0.500	0.667	0.625	0.083	A
	Agro-industrial food	9	0.167	0.500	0.333	0.144	B
	Eco. Org. and Bio. Prod. Sis	21	0.000	0.667	0.325	0.153	B
	Products derived from wildlife	4	0.000	0.333	0.208	0.160	B
Social responsibility outside the company	Agro-industrial food	9	0.667	0.833	0.750	0.072	A
	Non-timber	4	0.667	0.833	0.750	0.068	A
	Eco. Org. and Bio. Prod. Sis.	21	0.417	0.833	0.663	0.148	A
	Products derived from wildlife	4	0.583	0.750	0.646	0.080	A
Communication of attributes of the goods and services	Products derived from wildlife	4	0.000	0.500	0.375	0.250	A
	Non-timber	4	0.000	0.500	0.250	0.289	Ab
	Agro-industrial food	9	0.000	0.500	0.139	0.220	Ab
	Eco. Org. and Bio. Prod. Sis.	21	0.000	0.500	0.048	0.150	B
Schemes, programs, or recognitions implemented or received	Products derived from wildlife	4	0.000	1.000	0.417	0.500	A
	Non-timber	4	0.000	0.333	0.083	0.167	Ab
	Eco. Org. and Bio. Prod. Sis.	21	0.000	0.333	0.048	0.120	B
	Agro-industrial food	9	0.000	0.000	0.000	0.000	B

Significant differences were found among the companies in the municipalities of San Benito Abad and La Unión regarding the compliance of economic viability of the business criteria, having the highest averages, compared to the municipalities of San Marcos and Sucre, which had the lowest averages. However, the businesses located in the municipalities of Sucre, Majagual, and San Marcos, although they showed the highest scores in the positive environmental impact criteria, did not register statistically significant differences, as is shown in Table 5.

The findings determined that greater participation in ecological, biological, and organic production systems could suggest that La Mojana and San Jorge sub-regions have greater potential for land use activities. These may be due to the conditions of the regions, which are suitable for agriculture, with a variety of ecosystems represented by swamps, rivers, natural channels, streams, swamp forests (Flooded forests with low trees and scrub), patches of primary and secondary forests, Macrophyte marshes, and a warm and humid tropical climate with constant temperatures close to 28 °C (Benítez 2010).

Table 5. Multiple comparisons according to the compliance of criteria by municipality.

Criteria	Municipality	N	Min	Max	Mean	SD	Groups
Economic viability of the business	San Benito Abad	4	0.500	0.700	0.625	0.096	A
	La Unión	3	0.500	0.800	0.633	0.153	A
	Majagual	4	0.400	0.600	0.475	0.096	Ab
	Caimito	3	0.300	0.600	0.467	0.153	Ab
	San Marcos	14	0.200	0.600	0.429	0.107	B
	Sucre	11	0.200	1.000	0.336	0.234	C
Positive environmental impact and contribution to the conservation and preservation of ecosystem resources	Sucre	11	0.875	1.000	0.972	0.051	A
	Majagual	4	0.875	1.000	0.969	0.062	A
	San Marcos	14	0.562	1.000	0.879	0.156	A
	San Benito Abad	4	0.688	1.000	0.859	0.129	Ab
	La Unión	3	0.562	0.875	0.688	0.165	B
	Caimito	3	0.500	0.688	0.604	0.095	B
Life cycle approach of the goods or services	Majagual	4	0.600	0.800	0.750	0.100	A
	Sucre	11	0.500	0.900	0.591	0.114	Ab
	San Marcos	14	0.200	0.900	0.557	0.217	Ab
	San Benito Abad	4	0.200	0.800	0.500	0.258	Ab
	La Unión	3	0.200	0.700	0.433	0.252	B
	Caimito	3	0.200	0.500	0.367	0.153	B
Useful life	San Benito Abad	4	0.667	1.000	0.833	0.136	A
	Sucre	11	0.667	1.000	0.742	0.115	A
	San Marcos	14	0.167	1.000	0.679	0.281	A
	Caimito	3	0.500	0.833	0.667	0.167	A
	Majagual	4	0.500	0.833	0.667	0.136	A
	La Unión	3	0.333	0.667	0.556	0.192	A
Substitution of hazardous substances or materials	La Unión	3	1000	1.000	1000	0.000	A
	San Benito Abad	4	0.000	1.000	0.750	0.500	Ab
	San Marcos	14	0.000	1.000	0.643	0.413	Ab
	Caimito	3	0.500	1.000	0.667	0.289	Ab
	Sucre	11	0.000	1000	0.409	0.302	B
	Majagual	4	0.000	0.500	0.375	0.250	B
Recyclability and/or use of recycled materials	Sucre	11	0.625	1000	0.909	0.159	A
	San Benito Abad	4	0.625	1.000	0.875	0.177	A
	Caimito	3	0.625	1.000	0.792	0.191	Ab
	Majagual	4	0.750	0.875	0.781	0.062	Ab
	San Marcos	14	0.250	1.000	0.732	0.258	Ab
	La Unión	3	0.250	0.625	0.458	0.191	B
Efficient and sustainable use of resources for the production of goods or services	Sucre	11	0.417	0.917	0.667	0.220	A
	Caimito	3	0.500	0.583	0.528	0.048	Ab
	La Unión	3	0.333	0.667	0.500	0.167	Ab
	San Marcos	14	0.333	0.833	0.506	0.133	Ab
	San Benito Abad	4	0.250	0.667	0.458	0.198	Ab

Table 5

Criteria	Municipality	N	Min	Max	Mean	SD	Groups
Social responsibility within the company	Majagual	4	0.167	0.417	0.312	0.125	b
	Sucre	11	0.167	0.500	0.303	0.101	A
	Majagual	4	0.167	0.333	0.292	0.083	A
	San Marcos	14	0.000	0.500	0.226	0.140	Ab
	Caimito	3	0.000	0.167	0.111	0.096	Bc
	San Benito Abad	4	0.000	0.167	0.083	0.096	C
	La Unión	3	0.000	0.167	0.056	0.096	C
Social responsibility within the value chain of the company	Majagual	4	0.333	0.667	0.500	0.136	A
	Caimito	3	0.333	0.500	0.389	0.096	Ab
	San Marcos	14	0.000	0.667	0.357	0.215	Ab
	Sucre	11	0.333	0.833	0.379	0.151	Ab
	La Unión	3	0.167	0.667	0.333	0.289	Ab
	San Benito Abad	4	0.000	0.333	0.208	0.160	B
Social responsibility outside the company	Sucre	11	0.833	0.917	0.841	0.025	A
	Majagual	4	0.667	0.833	0.750	0.068	B
	San Benito Abad	4	0.500	0.750	0.646	0.125	Bc
	San Marcos	14	0.500	0.833	0.643	0.115	C
	La Unión	3	0.583	0.667	0.611	0.048	C
	Caimito	3	0.417	0.667	0.556	0.127	C
Communication of attributes of the goods and services	La Unión	3	0.000	0.500	0.333	0.289	A
	San Marcos	14	0.000	0.500	0.214	0.257	A
	San Benito Abad	4	0.000	0.500	0.125	0.250	A
	Sucre	11	0.000	1.000	0.091	0.302	A
	Caimito	3	0.000	0.000	0.000	0.000	A
	Majagual	4	0.000	0.000	0.000	0.000	A
Schemes, programs, or recognitions implemented or received	San Benito Abad	4	0.000	0.667	0.250	0.319	A
	Caimito	3	0.000	0.333	0.111	0.192	Ab
	San Marcos	14	0.000	1.000	0.119	0.281	Ab
	La Unión	3	0.000	0.000	0.000	0.000	Ab
	Majagual	4	0.000	0.000	0.000	0.000	B
	Sucre	11	0.000	0.000	0.000	0.000	B

The incipient development in the ecotourism sector is evident. However, among the ecosystem potentials stands out the sighting of birds, especially of exotic species in the lower part of the San Jorge River basin, which are directly related to the ecosystem of the marshes and natural ecosystems (CSB 2019).

This study shows agreement with an analysis of the situation of green business initiatives and biocommerce in the department of Amazonas, which found that no marketing studies, strategic plans, and business plans are implemented, in addition to a lack of knowledge of profitability (Cerón and Lasso 2020), that is to say, that the majority of prioritized economic activities show non-compliance with the management of financial and accounting statements.

The possible causes for these weaknesses are related to low levels of schooling, high levels of poverty, lack of promotion of business culture, and lack of assertiveness by the community to create development alternatives and non-intervention of government agencies, which agrees with those proposed by Otero and Salazar (2017). Therefore, there is a need to strengthen or create business plans, marketing strategies, and budgets and implement monitoring and control plans in the regions (Garay and Contreras 2020) through training processes adapted to meet the needs of the population and its level of education so that it is easy to understand and can help them to build skills in the economic area.

The results show that, although businesses show strengths in compliance with environmental criteria, they should reinforce indicators that are partially met, such as the communication of attributes and description of the product, in each production link, which results in marketing difficulties by not showing its differentiating components in the market. Compliance with environmental criteria is of great importance for the future of the green market in La Mojana and San Jorge sub-regions considering that there is a growth of consumers at a national and international level that wants to contribute to the preservation of the environment and the so-called “responsible consumers” who are interested in supporting natural production processes.

Regarding criteria of the economic viability of the business, companies from the municipalities of San Jorge subregion presented higher averages, indicating a higher organizational level than those from La Mojana subregion. The above demonstrates the need to educate on business training and entrepreneurship subjects.

In the subregions focus of this research, there have been few studies on companies’ sustainability practices projected for the green market, making it necessary to encourage research in this field, promote environmental education, and strengthen infrastructure, skilled labor, and consumer culture in order to achieve sustainable development (Granados 2018). Subregions need to continue strengthening the joint efforts between companies and government agencies to turn their weaknesses into strengths, given that producers benefit from the strategies designed in the Green Business Windows, which help them strengthen their economic activities.

Finally, the challenge for the Corporation for the Sustainable Development of La Mojana and San Jorge - Corpomojana, as the authority in charge of strengthening this type of business, is to continue leveraging and strengthening businesses focusing on sustainable development. To achieve these objectives, it must generate innovative training proposals that meet the new challenges faced by our community to achieve sustainable development by applying interdisciplinary and transdisciplinary approaches (CIN 2010), which would represent a significant strength and opportunity for green businesses in the sub-regions of La Mojana and San Jorge.

CONCLUSIONS

It was determined that La Mojana and San Jorge subregions of the department of Sucre have greater participation in the green business subsector “systems of biological and organic ecological production,” with a high development potential, which could promote a new market line in the area, considering the sustainability criteria established by the Ministry of Environment and Sustainable Development - MADS. On the other hand, the main strengths of the businesses evaluated were found in the criteria related to environmental aspects. In contrast, the main weaknesses were focused on the economic and social sector criteria. These findings suggest that La Mojana and San Jorge sub-regions have more potential for land use activities and nature tourism. However, it is necessary to prioritize these sectors through investment, and technical and financial support, contributing to achieving the country’s goals proposed in CONPES 3934 and the SDGs.

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Optimization of microwave extraction of biocompounds from black pepper using meat quality assessment

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Optimización de la extracción por microondas de biocompuestos de la pimienta negra mediante la evaluación de la calidad de la carne

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ABSTRACT

Black pepper (*Piper nigrum*) is a plant highly valued for its functional aromatic compounds. To increase bioavailability of its phytochemicals, a novel approach to extraction using microwave heating and optimization was investigated. Fixed (microwave power and particle size) and variable (heating time and solvent volume) extraction criteria were applied to obtain aqueous extracts, followed by the optimization of the quality of extracts by subjecting the extracts to meat quality test using the central composite design. Thereafter, quality indices (pH, 2-thiobarbituric acid reactive substance value, refrigeration loss, colour and cooking loss) of meat subjected to storage were determined and the outcomes were analysed using response surface methodology. A total of six optimised solutions were derived but the best extraction criteria (variable) were 87.28 min and 364.26 mL of heating time and solvent volume respectively with a desirability value of 0.624, while microwave power and particle size were kept constant. Afterwards, selected optimised extraction criteria was applied, and black pepper aqueous extract obtained was compositionally analysed using Gas Chromatography Mass Spectrometry. Remarkably, a total of 71 compounds were extracted, comprising element-based compounds such as silicon, nitrogen, sulphur and iodine. Two silicon and nitrogen-based compounds as well as one sulphur and nitrogen-sulphur based compound were obtained while seven iodine-based compounds were discovered alongside other functional compounds. This approach results in green and efficient extraction process with increased bioavailability of functional compounds. However, further modifications of extraction criteria can be applied to upscale the desirability level (score) for increased extraction efficiency.

Keywords:
Aqueous extract
Black pepper
GC-MS
Microwave extraction
Meat quality
Optimization

RESUMEN

La pimienta negra (*Piper nigrum*) es una planta muy apreciada por sus compuestos aromáticos funcionales. Para aumentar la biodisponibilidad de sus fitoquímicos, se investigó un novedoso método de extracción mediante calentamiento por microondas y su optimización. Se aplicaron criterios de extracción fijos (potencia de microondas y tamaño de partícula) y variables (tiempo de calentamiento y volumen de disolvente) para obtener extractos acuosos, seguidos de la optimización de la calidad de los extractos sometiéndolos a una prueba de calidad de la carne mediante un diseño compuesto central. A continuación, se determinaron los índices de calidad (pH, valor de la sustancia reactiva del ácido 2-tiobarbitúrico, pérdida por refrigeración, pérdida de color y pérdida por cocción) de la carne sometida a almacenamiento y se analizaron los resultados mediante la metodología de superficie de respuesta. Se obtuvieron un total de seis soluciones optimizadas, pero los mejores criterios de extracción (variable) fueron 87,28 min y 364,26 mL de tiempo de calentamiento y volumen de disolvente, respectivamente, con un valor de deseabilidad de 0,624, mientras que la potencia de microondas y el tamaño de partícula se mantuvieron constantes. A continuación, se aplicaron los criterios de extracción optimizados seleccionados y se analizó la composición del extracto acuoso de pimienta negra obtenido mediante cromatografía de gases y espectrometría de masas. Se extrajo un total de 71 compuestos, entre los que se encontraban compuestos basados en elementos como el silicio, el nitrógeno, el azufre y el yodo. Se obtuvieron dos compuestos a base de silicio y nitrógeno, así como un compuesto a base de azufre y nitrógeno-azufre, mientras que se descubrieron siete compuestos a base de yodo junto con otros compuestos funcionales. Este enfoque dio lugar a un proceso de extracción ecológico y eficiente con una mayor biodisponibilidad de los compuestos funcionales. Sin embargo, se pueden aplicar otras modificaciones de los criterios de extracción para elevar el nivel de deseabilidad (puntuación) y aumentar la eficacia de la extracción.

Palabras clave:
Extracto acuoso
Pimienta negra
CG-EM
Extracción por microondas
Calidad de la carne
Optimización

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Microwave-assisted extraction is a sought-after procedure applied in industrial and homemade extractions to obtain soluble products via microwave energy. Recently, much attention has been directed to the recovery of bioactive principles from different plant parts considered waste products or of limited competitive significance between man and livestock, such as seeds, leaves, barks, pulp, rind, peels, wood and by-products. Extracts from these substances indicate that the diversity and complexities of phytochemicals constituents and the potency of natural bioactive compounds exist among polyphenols, sterols and alkaloids (secondary metabolites). To obtain sufficient yields of specific phytochemicals for effective protection or regulation of biochemical processes, the need arises for sustainable extraction procedures (Kallel et al. 2014; Aghdase et al. 2017). The reduction in operation time and solvent consumption during microwave heating increases the extraction efficiency as electrical oscillations of microwaves causes repeated rotations of aqueous molecules in the presence of electromagnetic fields that generate heat from the friction of molecules (Rodríguez, 2017; Jimenez et al. 2018). Such updated innovative strategies have resulted in extensive convenient methods of extraction with proven advantages when compared with conventional solid–liquid extraction methods (Olalere et al. 2018a; Zin et al. 2020). Repeated experiments are often conducted to obtain information on constituents of extracts using Gas Chromatography Mass Spectrophotometry (GC-MS), but a wide range of investigations using factorial experiments can be both solvent and time consuming. Limited funds for analysis during such cost-intensive experiments can negatively affect the frequency and integrity of outcomes especially in developing economies. Therefore, a methodology that accommodates these concerns can increase man's application and utilization of beneficial phytochemicals in black pepper. Response surface methodology (RSM) allows for assessing of the impact of multiple factors and their interactions on one or more response variables (Aydar 2018). This presents a methodology for the possibility of overcoming the challenge of excessive solvent consumption and large sample sizes to produce quality extracts.

Black pepper is a crop of nutritional, medicinal and economic significance commonly sought for its desired biological potency and flavour. Black pepper (*Piper nigrum*) is a major plant with varied applications in processing industries as products such as black pepper powder, pepper oil, and pepper aqueous are produced in response to increased quality consciousness and preference for natural flavours and medicinal properties. Agbor et al. (2006) reported that black pepper contains not only aromatic compounds but flavonoids, alkaloids, amides and lignans, and some phytochemicals extracted from high-resolution gas chromatography analysis (GC-MS) include δ-cadinol, δ-guaiene, (Z) (E)-farnesol, (E)-β-ocimene and guaiol (Pino et al. 1990). *Piper nigrum* (family Piperaceae) is a spice widely considered as "The King of spices" for it contains major bioactive phytochemicals and pungent alkaloids like piperine (1-peperoyl piperidine), safrol, pinene, sabinene, limonene, caryophyllene and linalool - compounds that contribute to its overall potency which stimulate beneficial pharmacological actions such as antihypertensive, antioxidant, antitumor, antipyretic, anti-inflammatory, anti-diarrheal, antispasmodic, hepato-protective, antibacterial, antifungal, antiapoptotic and anti-colon toxin (Suvarna and Abdul 2019). *Piper nigrum* leaves and seeds are applied to indigenous systems of medicine in many rural communities, owing to its multidimensional influence on biological systems. In light of the diverse benefits obtainable from phytochemicals in black pepper, it becomes expedient to explore efficient extraction methods to increase the qualitative yield of black pepper extract.

The use of meat quality and RSM as assessment criteria to optimize extraction efficiency is a novel approach. This approach leverages on the rationale that the more the bioactive compounds extracted, the better the extraction process. Optimization of quality indices such as the physico-chemical and oxidative properties of meat incorporated with extracts (Insani et al. 2008; Sanwo et al. 2019) is an untapped potential for the assessment of qualitative yield generated from microwave extraction. The oxidation of lipid in meat can negatively affect the storability of many multifaceted food products as the nutrient becomes depleted and essential polyunsaturated fatty acids turns rancid as a consequence of hydrolytic and/or oxidative reactions during manufacture, processing, storage and distribution of meat leading to the generation of toxic compounds that are detrimental to consumer health (Böttcher et al. 2015; Keller et al. 2015). On this premise, extracts containing more bioactive principles should minimize the extent of nutrient depletion and subsequent deterioration. This novel approach is notably expedient to overcome the demerits associated with other conventional techniques of extraction, such as higher extraction cost and repetitive trials; lab-intensive and time-consuming extraction, isolation and analysis; high solvent consumption and low extraction yield, which are significant drawbacks to the utilization of natural products by man (Olalere et al. 2017; Zhang et al. 2018). This study is therefore designed to explore a novel approach to improve the quality of black pepper extracts produced from microwave extraction using meat quality assessment optimized by RSM.

MATERIALS AND METHODS

Experimental site

Microwave refluxation and major aspects of meat technological quality assessment were carried out at the Animal Product and Processing Laboratory, Department of Animal Production and Health. Extraction of the aqueous component was determined by Gas Chromatography – Mass Spectrometry (GC – MS) analysis in the Chemistry Department Laboratory, Faculty of Science, University of Lagos. Meat colour values were deduced at the Food Science Laboratory, Department of Food Science and Technology, Federal University of Agriculture, Abeokuta, but the fatty acid and lipid oxidation profile were determined at the Laboratory of Veterinary Medicine, University of Ibadan.

Experimental materials

Dried clarified pepper corms of black pepper sourced from a traditional herb–spice market in Ibadan was pulverized into a finely defined powder using an attrition mill. Black pepper powder was temporarily stored in coloured vials. Ground black pepper was sieved using a mesh and the particles were clarified using an 0.40 mm sieve, then stored in airtight container prior to further analysis. Distilled water was provided at the Feed Quality Laboratory of the Department of Animal Production and Health. Daewood (KOR6N9NC) microwave oven was used for heating aqueous black pepper.

Fresh meat from the breast muscles of carcasses of manually deboned broiler chickens (Cobb strain) weighing between 1.9–2.4 kg was sourced. Before the slaughter of broiler chickens, ethical principles outlined by the Ethical Committee Board of the College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, was adhered to.

Experimental design

Central composite design (CCD) as defined by Anderson and Whitcomb (2016) was used for the experiment (process analysis and optimization procedure). 13 experimental runs were generated with 5 repetitions as the central point. Operational factors and ranges were generated from adjustments to the study of Olalere et al. (2018a). Two variable factors – irradiation time (75–85 min) and solvent volume (360–380 mL) were applied while two fixed factors – microwave power and particle size were held constant at 320 W and 0.40 mm. Afterwards, data obtained from a qualitative assessment of extracts via meat quality assessment was optimized using response surface methodology.

Microwave refluxation procedure and extract yield calculation

13 experimental runs were generated using Design Expert software with 5 repetitions generated as central points (Table 1). 8 g of ground black pepper was mixed with 360–380 mL of distilled water per experimental run. Soaking and stirring of dissolved pepper prior to loading was carried out to achieve homogeneity and uniform hydration of the mixture placed in an irradiation-tolerant container before placement in the microwave cavity. Microwave power was fixed at 320 W. Before extraction, pre-heating was performed for 10 min at 160 W – a slight modification to the procedure established by Olalere et al. (2017). Subsequently, the loading and unloading of the mixture and extracts from the cavity were carried out according to the procedure established by previous studies (Olalere et al. 2017; 2018b).

Extracts obtained were cooled and placed in coloured vials prior to the soaking of chicken meat. The extraction yield was calculated by using Equation (1).

$$\text{Extraction Yield} = \frac{\text{Volume of extracts / mL)}}{\text{Volume of mixture (mL)}} * 100 \qquad (1)$$

Experimental birds

20 (4-weeks old) broiler (Cobb strain) chickens reared under an intensive deep litter management system were

Table 1. Central composite design matrix used for optimizing black pepper aqueous extract.

RUN	Irradiation time (min)	Solvent volume (mL)
1	85.00	360.00
2*	80.00	370.00
3	80.00	355.86
4*	80.00	370.00
5	85.00	380.00
6	87.07	370.00
7*	80.00	370.00
8*	80.00	370.00
9*	80.00	370.00
10	80.00	384.14
11	75.00	380.00
12	75.00	360.00
13	72.93	370.00

*Midpoint repeated five times.

sourced, then further reared for four weeks. Commercial diet fed is presented in Table 2. Birds were subjected to uniform management, thereafter, birds were sacrificed and breast muscles extracted, then, weighed before soaking prior to subsequent meat quality analysis.

Table 2. Nutrient composition of commercial finisher diet.

	Composition-value
Energy (kcal (kg ME)$^{-1}$)	2900
Crude protein	20.00 (%)
Fat Oil^{-1}	6.00 (%)
Crude fibre	5.00 (%)
Salt	0.30 (%)
Lysine	0.85 (%)
Methionine	0.35 (%)
Calcium	1.00 (%)
Available Phosphorus	0.40 (%)

DM: dry matter; ME: metabolizable energy.

Determination of microwave extraction temperature

The internal temperature of the microwave was measured for 5 min post-extraction, and temperature ranged between 37±2 °C.

Determination of meat extract uptake

20 g of raw meat from the breast muscles were weighed in triplicates and labelled; then 20 mL of extract was added to each sample and left for 30 min. The samples were subsequently removed and reweighed. The increase in weight of samples indicate the volume of extract absorbed.

Extract quality assessment

Each meat sample soaked in each extract was drained for 5 min prior to qualitative (pH, colour, refrigeration and cooking loss) investigations.

Meat pH and colour evaluation

Meat pH was measured using an Orion 230A pH meter (Thermo Fisher Scientific, Waltham, MA). Chicken meat colour was measured with a CR-400 Chroma Meter instrument (Minolta Co., Osaka, Japan) via CIE L* (lightness), CIE a* (redness), and CIE b* (yellowness) according to specification by Kralik et al. (2017).

Determination of malondialdehyde content in meat

Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS). The preparatory procedure for absorbance: blank was set up following the procedure documented by Adeleye et al. (2021). Lipid oxidation was reported as milligrams of malondialdehyde per kilogram of meat, using Equation (2) (Kim et al. 2020).

$$\text{TBARS}\left(\frac{\text{mg MDA}}{\text{kg of meat}}\right) = (\text{kg of meat absorbance of sample} - \text{absorbance of blank sample}) * 5.88 \quad (2)$$

Meat refrigeration loss

Meat samples soaked in aqueous extracts were refrigerated to determine the refrigeration loss. Each sample was weighed prior to refrigeration, then re-weighed on day 0, 5 and 10. Refrigeration loss and loss percentages were calculated using Equations (3) and (4), respectively:

$$\text{Refrigeration loss (g)} = \text{weight before refrigeration} - \text{weight after refrigeration} \quad (3)$$

$$\text{Refrigeration loss (\%)} = \frac{\text{weight before refrigeration} - \text{weight after refrigeration}}{\text{weight before refrigeration}} * 100 \quad (4)$$

Evaluation of cooking loss

Cook loss percentages were calculated on day 10, post-refrigeration. Meat samples were allowed to drain, then weighed, wrapped in separate air-tight polythene bags and cooked in a water bath at 70 °C for 20 min, applying slight modifications to the procedure stated by Sanwo et al. (2012).

Cooking loss was determined using Equations (5) and (6):

$$\text{Cooking loss (g)} = \text{Weight before cooking} - \text{Weight after cooking} \quad (5)$$

$$\text{Cooking loss (\%)} = \frac{\text{weight before cooking} - \text{weight after cooking}}{\text{wight before cooking}} * 100 \quad (6)$$

Gas Chromatography Mass Spectrometry of aqueous black pepper extract

Spectrometry [Gas Chromatography Mass Spectrometry (GCMS)] analysis of extract generated from the highest and best desirability was carried out. GC (Agilent 7890, USA) and MS (Agilent 5975 model, USA) had 30 mm tubular column diameter, 0.32 mm internal diameter and 0.25 µm thickness. The oven initial temperature hold was operated at 80 °C for 2 min at 12 °C min^{-1} to a temperature of 240 °C held for 6 min. The interface temperature between GC and MS is 250 °C. A small portion of the supernatant solution was filtered using the 0.45 µm micro filter and diluted with analytical grade acetone to 1:10. One microliter of the diluted solution was then injected into the gas chromatography column at 280 °C at a helium gas velocity of 1 mL min^{-1}. The scan ranges between 50 and 500 with a spitless mode of analysis. Filtered extracts (1 µL) were diluted using analytical standard grade acetone extract 1:10 injected into the GC-MS for components identification as described by Olalere et al. (2018a), while the bioactive components present were identified in relation to the peak area fragmentation fingerprints.

Statistical design

Data obtained from responses were analyzed using Design Expert version 13.0.5.0 (Design Expert, 2021). Mean separation at 5% level of significance was carried out according to the regression coefficients examined that were subjected to analysis of variance (ANOVA) to derive response surface and contour plots together with the coefficient of determination (R^2) for each response. Numerical optimization was performed to determine the level of desirability.

RESULTS AND DISCUSSION

Quality characteristics of chicken meat soaked in black pepper aqueous extracts prepared from designed extraction combinations

Optimization of aqueous extract using chicken meat soaked in aqueous black pepper extract subjected to irradiation time and solvent volume is presented in Table 3. Extract volume ranged between 60.42–115.13 mL, while aqueous extract uptake was between 3.15 and 17.09%. Meat pH ranged from 5.82–6.33, 6.05–6.21 and 6.39–7.23 on days 0, 5 and 10 of refrigeration storage, respectively. Meat TBARs value ranged between 0.286–2.088 and 0.11–0.351 on days 5 and 10 of storage, respectively. Loss resulting from refrigeration storage of meat samples soaked in extracts on days 5 and 10 were 9.26–18.92% and 16.4–28.72% while cook loss ranged between 30.6 and 39.73% on day 10. Meat colour parameter (L*, a* and b*) ranged between 47.36–64.62, 6.12–8.77 and 14.42–21.36 respectively on day 5; 49.03–66.35, 6.86–11.45 and 13.68–23.09 respectively on day 10.

Table 3. Mean values of quality attributes of chicken meat soaked in black pepper aqueous extracts.

RUN	Time (min)	Sol Vol (mL)	Ole Ext (mL)	Ole Abs (%)	Meat pH D 0	Meat pH D 5	Meat pH D 10	TBARS D 5	TBARS D 10	Ref Ls D 5 (%)	Ref Ls D 10 (%)	L* D 5	L* D 10	a* D 5	a* D 10	b* D 5	b* D 10	Ck ls D 10 (%)
1	85	360	86.80	4.11	5.89	6.16	7.23	0.40	0.20	13.54	23.88	57.38	57.64	6.77	9.36	18.12	19.33	34.60
2	80	370	71.15	3.47	6.33	6.16	6.58	0.39	0.17	12.92	26.81	64.62	54.55	7.33	11.19	20.48	17.54	34.59
3	80	355.86	81.37	17.09	6.13	6.09	6.78	0.82	0.18	15.70	28.72	57.23	56.24	6.42	9.88	15.45	17.61	30.93
4	80	370	102.49	3.99	6.08	6.13	6.78	0.30	0.18	18.26	25.68	53.92	52.34	8.03	10.17	18.44	16.74	34.42
5	85	380	91.29	7.82	5.93	6.05	6.53	0.37	0.35	9.26	24.12	51.58	54.57	7.62	6.89	16.12	14.21	38.48
6	87.07	370	93.32	7.87	5.97	6.05	6.63	0.66	0.11	10.7	26.95	52.61	55.09	7.92	7.52	18.97	17.36	34.46
7	80	370	115.13	11.33	6.34	6.14	6.61	0.38	0.19	14.56	26.15	53.71	56.87	6.74	7.34	17.13	15.36	37.14
8	80	370	92.38	4.11	6.30	6.08	6.79	0.46	0.31	12.57	31.3	52.28	50.45	6.23	7.49	14.42	15.10	31.51
9	80	370	61.91	3.47	6.16	6.12	7.19	0.29	0.19	15.82	16.4	50.18	61.85	7.92	9.64	18.52	18.27	32.50
10	80	384.14	77.28	3.15	6.21	6.07	7.05	2.09	0.31	13.31	27.82	59.17	65.74	6.12	9.54	15.91	20.79	33.31
11	75	380	108.77	4.17	5.82	6.08	7.20	0.31	0.22	12.38	20.56	63.42	64.77	8.77	11.45	21.36	23.09	31.70
12	75	360	63.26	5.73	6.09	6.21	6.90	0.34	0.24	13.6	25.66	55.51	66.35	8.63	11.14	19.73	21.42	30.60
13	72.93	370	60.42	6.42	6.25	6.05	6.39	0.37	0.27	18.92	27.28	47.36	49.03	6.22	7.44	14.59	13.68	39.73

Values are in triplicates.

Response surface and contour plots of extraction conditions optimized via meat quality assessment

The response surface and contour plots showing the interaction of irradiation time and solvent volume (variable factors) on quality indices of extracts derived is detailed in Figure 1–7. Figure 1 shows the response surface and contour plots in relation to the extract yield and solvent absorbed in meat. Volume of extract obtained was at its peak in at 338.5 mL of distilled water and 90 min of irradiation. Oleoresin uptake (%) increases as irradiation time and solvent volume increased. Extraction method employed increased bioactive compounds harvested. Notably, the temperature of operation impacted extraction efficiency. Microwave power at 320 W which is sandwiched between the 300 and 350 W employed by Olalere et al. (2018a) complemented other extraction factors with more bioactive compounds extracted. Optimal microwave irradiation energy at 320 W likely enhance molecular movement of the electromagnetic field and the substance within the cavity, thereby, positively influencing molecular seeping of solvent into the dissolved matrix and increasing the dissolution and recovery of phytochemical compounds (Zin et al. 2020). Teo et al. (2009) expressed benefits derived from the use of closed vessel system operation. With little evaporation experienced, solvent volume is slightly sustained compared to extraction via open systems where solvent volume increases as extraction duration extends. Furthermore, dielectric conduction and refluxation of microwave cavity at lower temperature further facilitate the extraction of bioactive compounds. Such extraction method identified as green extraction method minimizes solvent consumption and operates an environmentally friendly procedure (Flórez et al. 2015). The solute: solvent ratio likewise affects microwave solvent extraction processes. Tatke and Jaiswal (2011) stressed the limitations solubility or mass transfer impose. Additionally, the quantity of black pepper dissolved in distilled water for this study positively affect the extraction yield. Beyond 8 g, a possibility exists that an optimal value would not be attained nor additional benefits derived, possibly resulting in diluted extracts, with limited solute potency. The capacity for water to absorb energy and refluxation, thereby facilitating the uniform distribution and microwaves exposures likely aided the extraction of functional compounds in this study (Veggi et al. 2013). Similar effects results in expected extraction outcomes as irradiation from specialized microwave allows for easier and proper control of operation with operation at lower cost than pressurized extraction protocols. The effect of ratios of water to raw material can likewise affect the solute extract yield and the number of compounds obtained. In a study conducted by Passos and Coimbra (2013), rescued liquid to solid ratios did not result in higher recovery of polysaccharide but monosaccharides implying solvent volume is critical to extraction yield. Findings confirm this, as revealed by the quantity of oleoresin extracted from white and black pepper that increase as solvent increased (Olalere et al. 2017, 2018a). In this study, however, more solvent volume was used at 360–380 mL than the 120 mL reported by authors previously cited. Significant outcome may be attributed to extraction system (closed system) employed in this study. Extracts can be applied to improve shelf stability of raw products. Also, functional compounds can be isolated for medicinal or therapeutic uses. The quantity of compounds harvested using this methodology exceeds the 55 compounds found in *Piper nigrum* by Mohammed et al. (2016), attesting to the efficiency of extraction method employed.

Figure 2 shows the response surface and contour plots of the pH of meat soaked in extracts on days 0, 5 and 10 of refrigeration storage. Meat pH on day 0 decreased as irradiation time of extracts is extended and the solvent volume is lowered while generating extracts; while on day 5, meat pH reduced among samples soaked in extracts formed from higher irradiation time and 372 mL of distilled water. On day-10, meat pH decreased among samples soaked in aqueous extracts generated from increased irradiation time and solvent volume combination. Extraction process of black pepper reveal solvent volume affects meat pH. Experiment conducted affirms that meat pH is critical to quality of extract obtained. Plant molecules can absorb microwave energy causing cell disruption by internal superheating, leading to desorption of phytochemicals and release of chemical compounds in extracts. An oscillating electric field or refluxation causes vibrations of polar molecules along with molecular friction that extends to volumetric heating, thereby causing water evaporation within cells and subsequent increased pressure that result in cell disruption (Balasubramanian et al. 2011; Tan and Muhamad 2017) and pH of resultant extract.

As seen from Figure 3, the response surface and contour plots of 2-thiobarbituric acid reactive substance count of broiler chicken meat soaked in black pepper extracts, then preserved for 5 and 10 days respectively is presented. The higher the solvent volume used for extraction, the higher the meat 2-thiobarbituric acid reactive substance (TBARs) count, especially at 371 mL in combination with 87 min of irradiation time, but on day-10, stored meat had lowered malondialdehyde value, especially when soaked in extract generated from lower solvent volume. Least meat TBARs value was recorded at 88 min of irradiation time and 367 mL of solvent volume. Extract quality affecting meat TBARs value reveal higher solvent volume increases meat TBARs (malondialdehyde) value. Though Tatke and Jaiswal (2011) explained that higher proportion of solvent volume to solid matrix resulted in higher extraction yields, however, higher moisture content and degree of unsaturation of meat lipid facilitated generation of pro-oxidants. Thus, lower solvent volume corresponds with higher extract concentration as well as generation of phenolic compounds.

Response surface and contour plots of refrigeration and cooking loss percentages obtained after soaking chicken meat in black pepper extract, stored for 5 and 10 days is shown in Figure 4. Meat refrigeration loss was suppressed for extracts generated from lower irradiation time and higher solvent volume, while on day-10, refrigeration loss was minimized at 89 min of irradiation exposure and 367 mL of solvent volume. Cooking loss of meat on day-10 post-refrigeration resulted in higher moisture loss as the irradiation time and solvent volume increased. Meat refrigeration loss was lowered among extracts generated from lower irradiation time and higher solvent volume. On the other hand, loss (%) from cooking meat incorporated with aqueous extracts on day-10 post-refrigeration had more moisture loss as the irradiation time and solvent volume increased. According to USDA (2013), the percentage of naturally occurring water in meat varies with the type of muscle and the pH of the meat. Hence, moisture movement in and out of pore spaces of muscle cells affect the eventual moisture content of meat incorporated with extracts. Aqueous extracts contain acidic compounds that interacts with meat interstitial fat by maintaining a fairly acidic environment (pH) that fairly regulates muscle cell properties and layering, yielding meat with minimal moisture loss. Refrigeration loss of meat was fairly stabilized by aqueous extracts generated at lower irradiation time and solvent volume. Compositional analysis of black pepper by Clery et al. (2006) and Jeleń (2015) reveal black pepper contain terpenes, nitrogen-containing compounds, limonene, α- and β-pinene, methyl propanal, myrcene, 2- and 3-methylbutanal, α-phellandrene, linalool, butyric acid and 3-methylbutyric acid, altering the internal environment within meat, suppressing loose movement of water, water soluble sarcoplasmic proteins, vitamins and enzymes from interstitial spaces and muscle cells (Sanwo et al. 2019). This explains why the moisture loss was higher in meat preserved with extracts generated from higher extraction (irradiation and time) values post-cooking as the quality of bioactive principles in pepper is possibly suppressed.

The response surface and contour plots for meat lightness (L*) of samples soaked in black pepper extract on day 5 and 10 of refrigeration storage is presented in Figure 5. Meat L* value increased as the irradiation time and solvent volume applied to extract decreased after soaking then storing meat for 5 days; however, on day-10, higher L* values were recorded as extraction conditions were sandwiched between 85–87 min and 361–379 mL of irradiation time and solvent volume respectively. The trend of activity of black pepper extracts on meat L* values as storage progresses contradicts the report of Ramamoorthi et al. (2010) that storage decreased meat L* and a* values, possibly pointing to the effectiveness of extraction process in releasing bioactive compounds in pepper, however, that meat lightness increased with decreasing irradiation time and solvent volume indicates minimal dosage of antioxidant application favors extension of meat lightness. Falowo et al. (2014) supports this by stating that higher concentrations of antioxidants may alter expected effects through pro-oxidative action. Notably, from this study, extraction conditions sandwiched between 85–87 min and 361–379 mL of irradiation time and solvent volume respectively in tandem with fixed extraction factors favours the preservation of meat lightness using black pepper extract.

Response surface and contour plots of redness (a*) and yellowness (b*) values after soaking chicken meat in black pepper extract stored in the refrigerator for 5 and 10 days is presented in Figure 6 and 7, respectively. Meat a* value increased as irradiation time and solvent volume increased; but on day-10, as irradiation time and solvent volume lowered, meat a* value reduced. On the other hand, meat yellowness (b*) values were elevated for meat soaked in extracts derived from increased irradiation time and solvent volume on day-5 of storage, but on day-10, meat b* values increased in extracts generated from lower solvent volume and fairly-constant irradiation time (91.50 min). According to Leal (2019), meat redness is perceived by consumers as an indicator of freshness and superior product quality that can further be enhanced by highly oxidizing compounds or additives. Oxidizing principles were extracted from black pepper. The antioxidant components abounded with higher irradiation time and solvent volume, though within defined set-up. The change observed on D 10 may be attributed to the kind of storage employed (refrigeration storage). Extension of the preservative potency of extracts may be achieved if storage at temperatures lower than 4 °C is employed in tandem with other preservation methodologies (active packaging) for better storage. Outcome of this study agrees with Ramamoorthi et al. (2010), whose study reveal all antioxidants increased L* value by two units (lighter), but the irradiation dose had no effect.

Regression coefficient of responses (aqueous extract volume, meat absorption percentage, colour, loss percentage and 2-thiobarbituric acid value) as a function of the independent variables

Model regression coefficient applied for the optimization of white pepper aqueous extracts is shown in Table 4. Linear irradiation time for pH (-2727.52) on day-5; quadratic model for irradiation time and solvent volume for pH on day-5 (-1636.49 and -545.408); as well as cubic model for solvent volume were significant (P<0.05) among models and coefficients assessed.

An overview of the significant models from the regression coefficient table signifies aspects extract

Table 4. Regression coefficient of response parameters of black pepper aqueous extract applied as a function of independent variable.

	Intercept (β^0)	A – Irradiation time	B – Solvent volume	AB	A^2	B^2	A^2B	AB^2	A^3	R^2
Extract vol	85.0438									0.2932
***P*-values**										
Aqueous Uptake	7.38407	0.510116	-2.19552							0.3439
***P* -values**		0.7187	0.1418							
Meat pH Day-0	5.99389	-0.0607484	0.140392	0.0775						0.5323
***P* -values**		0.3713	0.4869	0.4180						
Meat pH Day-5	-1084.96	**-2727.52***	-0.208787	-0.206716	**-1636.49***	**-545.408***	-0.0529289	**-272.693***	**-272.753***	0.4454
***P* -values**		**0.0459***	0.0592	0.0723	**0.0459***	**0.0459***	0.0651	**0.0459***	**0.0459***	
Meat pH Day-10	6.48239	-0.30752	-0.50227	-0.25	-0.0768621	0.125631				0.4582
***P* -values**		0.4833	0.1192	0.1031	0.4722	0.2542				
TBARS Day-5	0.162038	-0.266206	0.216424	-0.00025	-0.0827086	**0.388576***				0.5592
***P* -values**		0.6975	0.6435	0.9991	0.6212	**0.0455***				
TBARs Day 10	0.16793	-0.0279678	0.122606	0.04175	-0.00291569	0.0270869				0.4839
***P* -values**		0.7866	0.1130	0.2321	0.9075	0.2999				
Meat Ref Loss Day-5	44781.3	111930	-4.49502	-2.88503	67160.9	22385.3	-0.530007	11192.7	11193.9	0.5143
***P* -values**		0.2081	0.5120	0.6877	0.2081	0.2080	0.7631	0.2080	0.2081	
Meat Ref Loss Day-10	25.1522	-0.280166	1.9034	1.335	-0.111104	0.466604				0.0082
***P* -values**		0.9706	0.7141	0.5880	0.9521	0.8011				
L* day 5	47.2724	-7.35124	-6.2483	-3.4275	-1.75828	2.34911				0.3980
***P* -values**		0.3835	0.2840	0.2190	0.3915	0.2619				
L* day 10	50.6322	-3.2867	0.353129	-0.3725	-0.49851	3.96596				0.3589
***P* -values**		0.7347	0.9572	0.9044	0.8323	0.1239				
a*-day 5	8.01647	0.842371	0.425717	0.1775	0.229543	-0.170616				0.0830
***P* -values**		0.6572	0.7411	0.7699	0.6201	0.7114				
a* day 10	5.35263	-3.03489	-1.7201	-0.695	-0.564131	0.550779				0.3928
***P* -values**		0.2814	0.3638	0.4326	0.4025	0.4131				
b*-day 5	18.2015	0.485757	-1.77993	-0.9075	0.14196	-0.40834				0.0800
***P* -values**		0.9145	0.5680	0.5377	0.8974	0.7120				
b* day 10	15.1501	-0.730838	-3.2641	-1.6975	-0.00249913	1.83716				0.4092
***P* -values**		0.8744	0.3179	0.2742	0.9982	0.1343				
Cook Loss Day-10	40.2267	5.77859	2.43323	0.695	1.34068	-1.14671				0.3755
***P* -values**		0.2522	0.4656	0.6543	0.2731	0.3429				

- Significant means were separated at P<0.05. TBARs – 2-thiobarbituric acid value; D – Day; Ref – Refrigeration; L -Lightness; a* - Redness; b*- Yellowness.

properties affected meat quality values presented in Table 3. On D 5, as irradiation time increases, solvent volume at 372 mL of distilled water was optimal. Since pH is a fundamental quality index for storage, it can be deduced that decline in potency of extracts corresponds with tendency for meat pH to shift from acidity to basicity. Changes in meat pH and colour result from post mortem glycolysis and glycogen conversion into lactic acid (Honkavaara et al. 2003; Abubakar et al. 2021) and the extent of variation influences the properties of meat. Meat soaked in acidic medium affects its hydrogen ion balance, therefore, significant changes on D 5, implies meat post-mortem changes was affected by extract properties on D 5 than D 0 and 10. *Piper nigrum* contain acidic compounds such as 2-Ethylbenzotriazole and (R)-(-)-14-Methyl-8-hexadecyn-1-ol, but overall, proportions of hydrocarbons in extracts possibly resulted in tissue alterations due to changes in muscle glycolysis with resultant decline in meat pH. Report on spices and herbs reveal these organic substances often contain high concentrations of phenolic components with an extreme H-donating activity by Falowo et al. (2014) further highlight the power of extracts. Hence, pronounced influence from variations among extracts on D 5 signify operational extraction conditions impacted extract quality and acidity with variations in acidic strength and tissue modification on D 5. Further implications and deductions from this study indicate that generally, extraction yield increases proportionally with power, solvent volume, irradiation time and particle size until the increase becomes insignificant or the yield declines (Xiao et al. 2008).

The quadratic model for the influence of solvent volume on 2-thiobarbituric acid (TBARs) value (0.3886) on day-5. Meat TBARs value was influenced ($P<0.05$) at 0.389 on day-5 of refrigeration storage. Linear model (irradiation

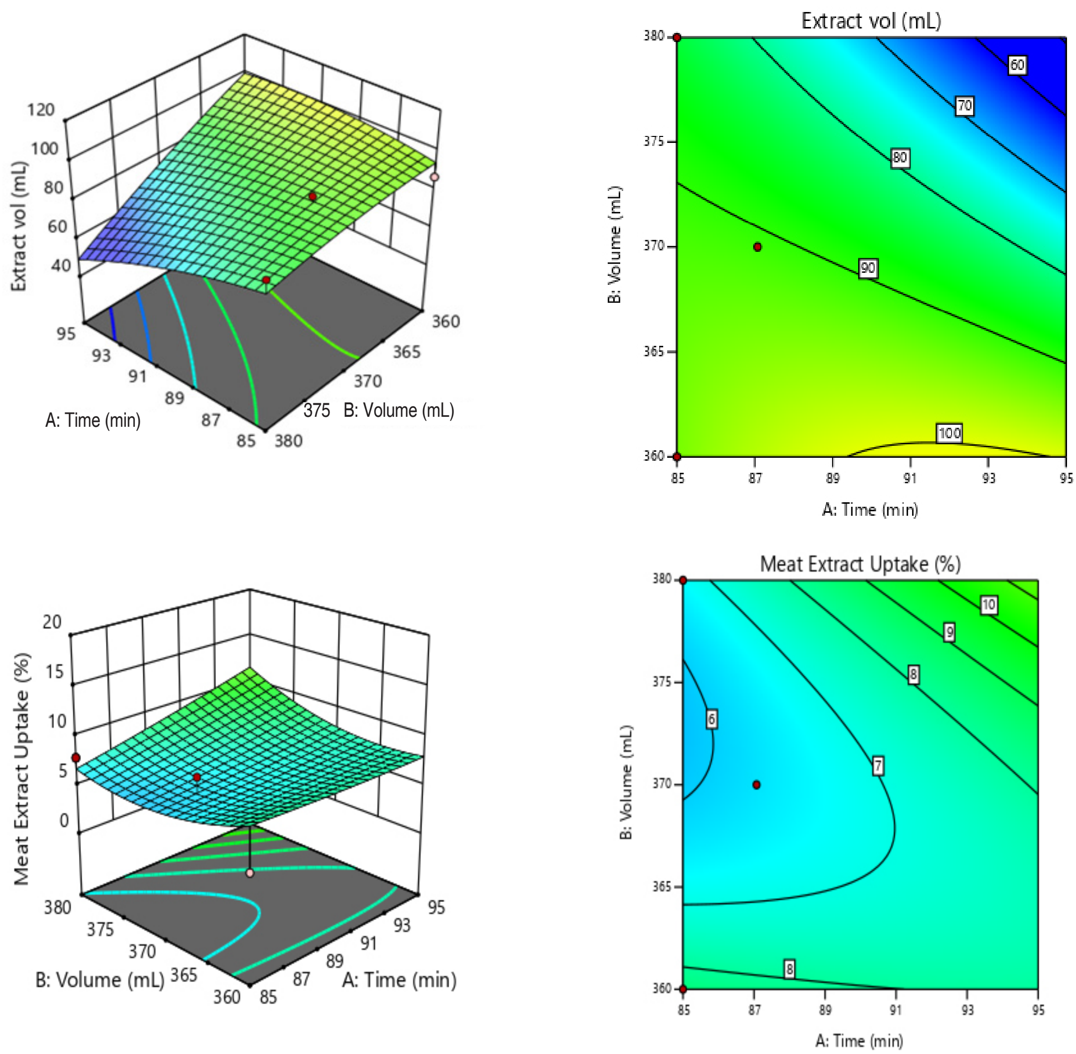

Figure 1. Response surface and contour plots of extract yield and uptake (%) of chicken meat soaked in black pepper extract.

time and solvent volume) values ranged between -7.483 and 5.788; -14.983 and 2.433 for extract volume and cook loss percentage respectively. Similarly, 2FI model and quadratic model were between -10.255 and 0.695 as well as -3.51 to 1.34 for aqueous extract volume and cook loss percentage respectively; while the quadratic model for solvent volume varied from -2.28 to 3.97 for extract volume and meat lightness respectively. Regression coefficient (R^2) ranged between 0.0830 and 0.5592 for meat lightness and malondialdehyde values on day (D) 5 of refrigeration storage. Drosou et al. (2015) explained that the use of water as solvent under microwave extraction significantly increased the yield of polyphenols in red grape, however, this study has demonstrated that increasing solvent volume enhances extraction efficiency up till a limit and beyond this, further release of bioactive principles cannot be achieved. Therefore, increasing extraction criteria values beyond the limit will not positively enhance the extraction of polyphenols in aqueous black pepper extracts, which acts as antioxidants for quenching free radicals progressively generated beyond day 5, as meat is subjected to refrigeration storage.

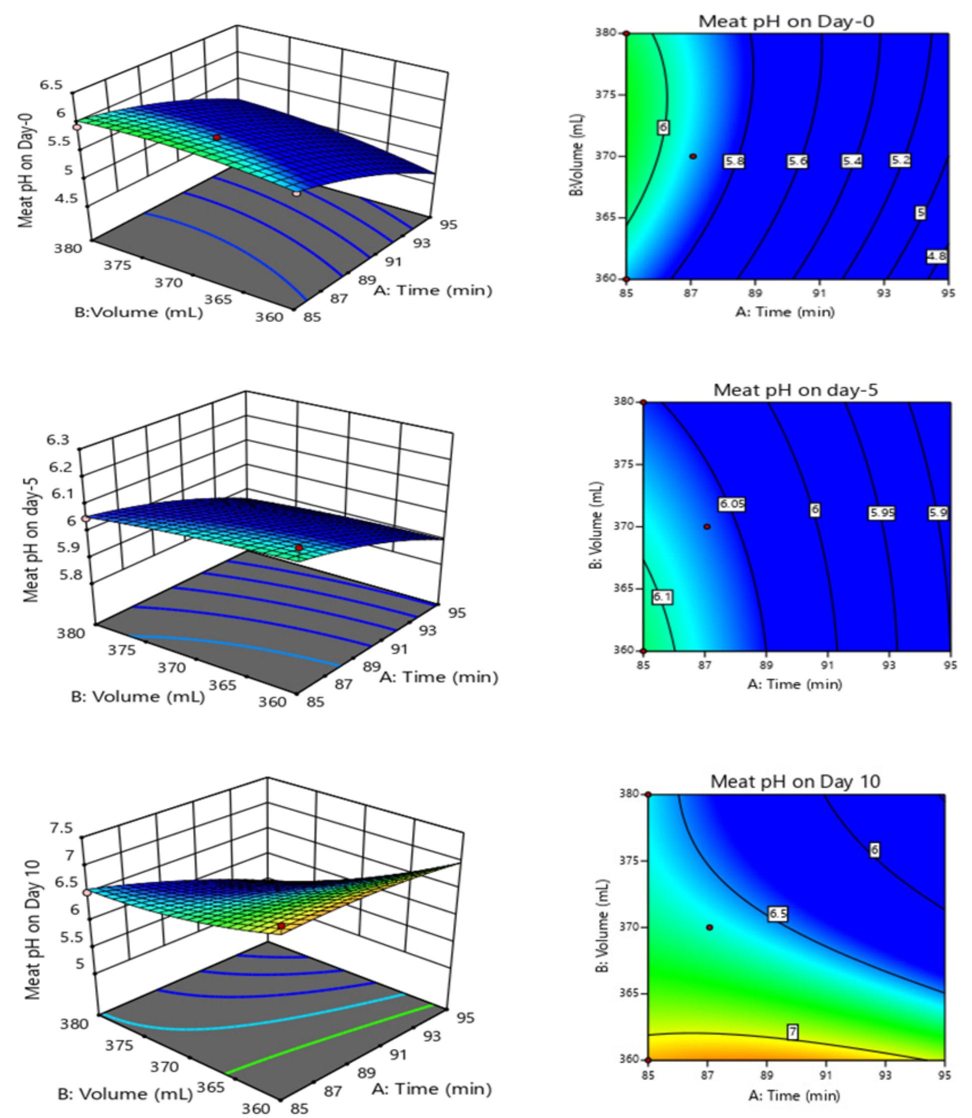

Figure 2. Response surface and contour plots of pH of meat soaked in black pepper aqueous extracts on day-0, 5 and 10 of refrigeration storage.

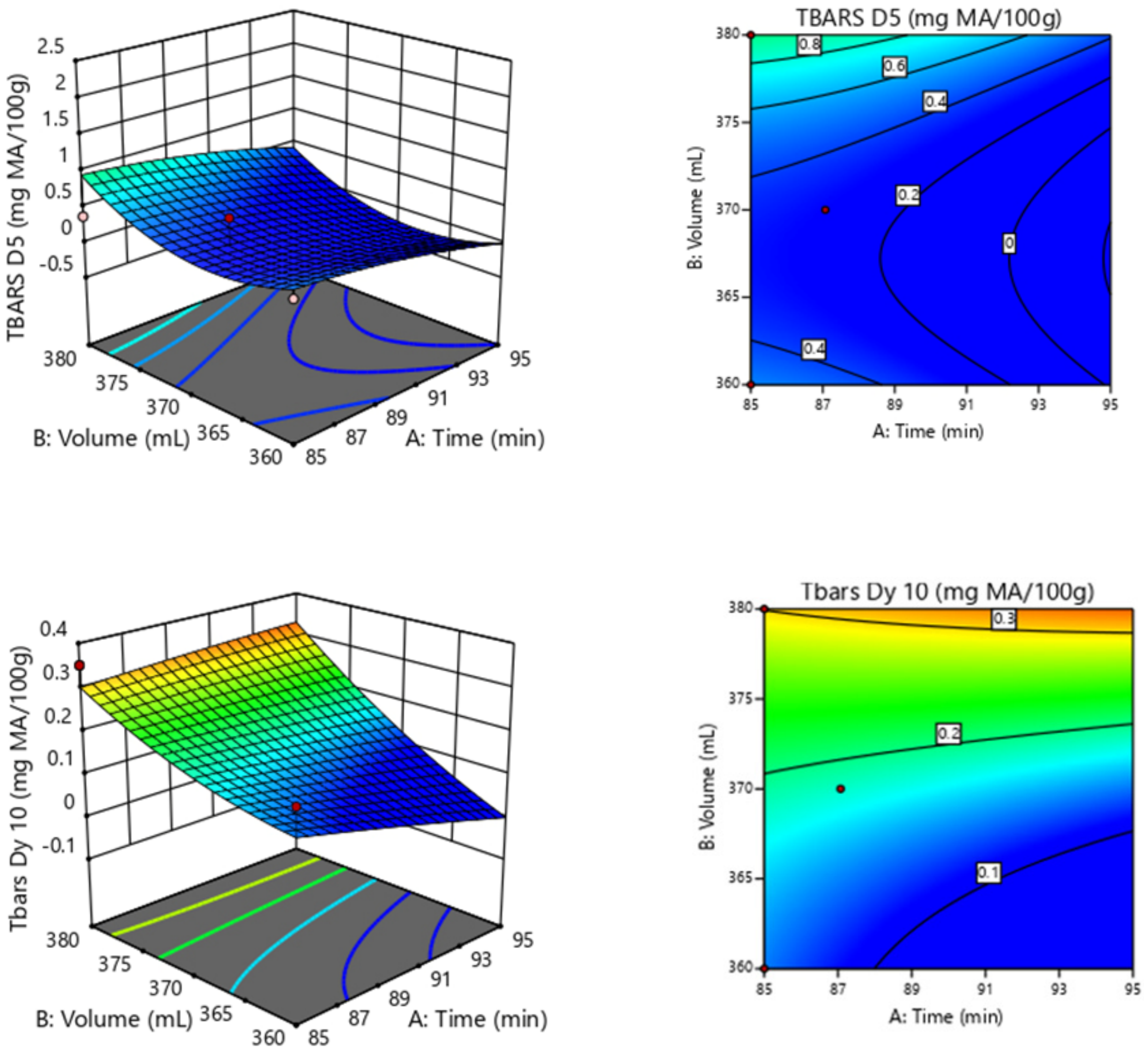

Figure 3. Response surface and contour plots of 2-thiobarbituric acid reactive substance (MDA) in meat of broiler chickens soaked in black pepper aqueous extract stored for 5 and 10 of refrigeration.

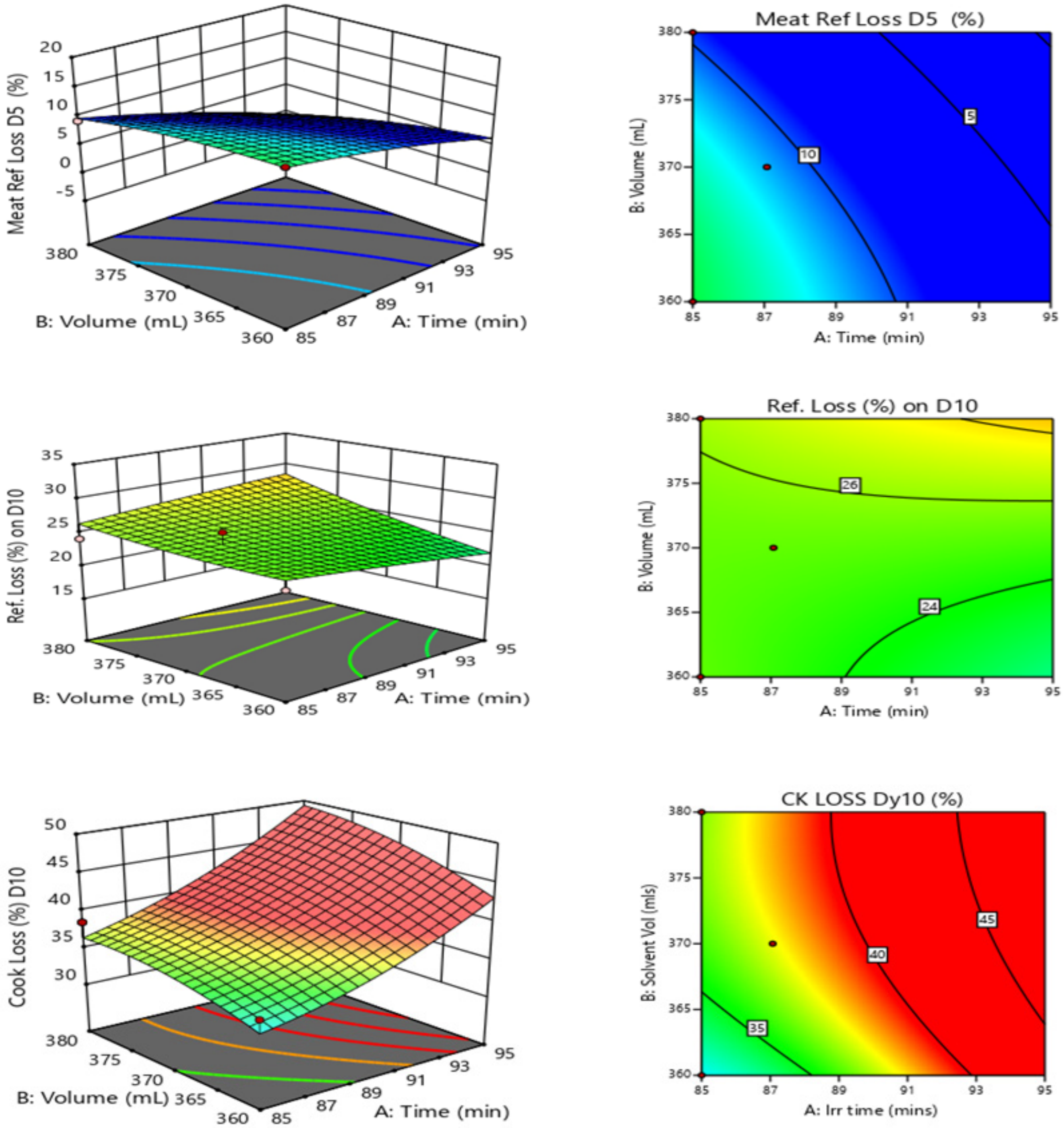

Figure 4. Response surface and contour plots of refrigeration and cook loss percentage of chicken meat soaked in black pepper aqueous extract stored for 5 and 10 days.

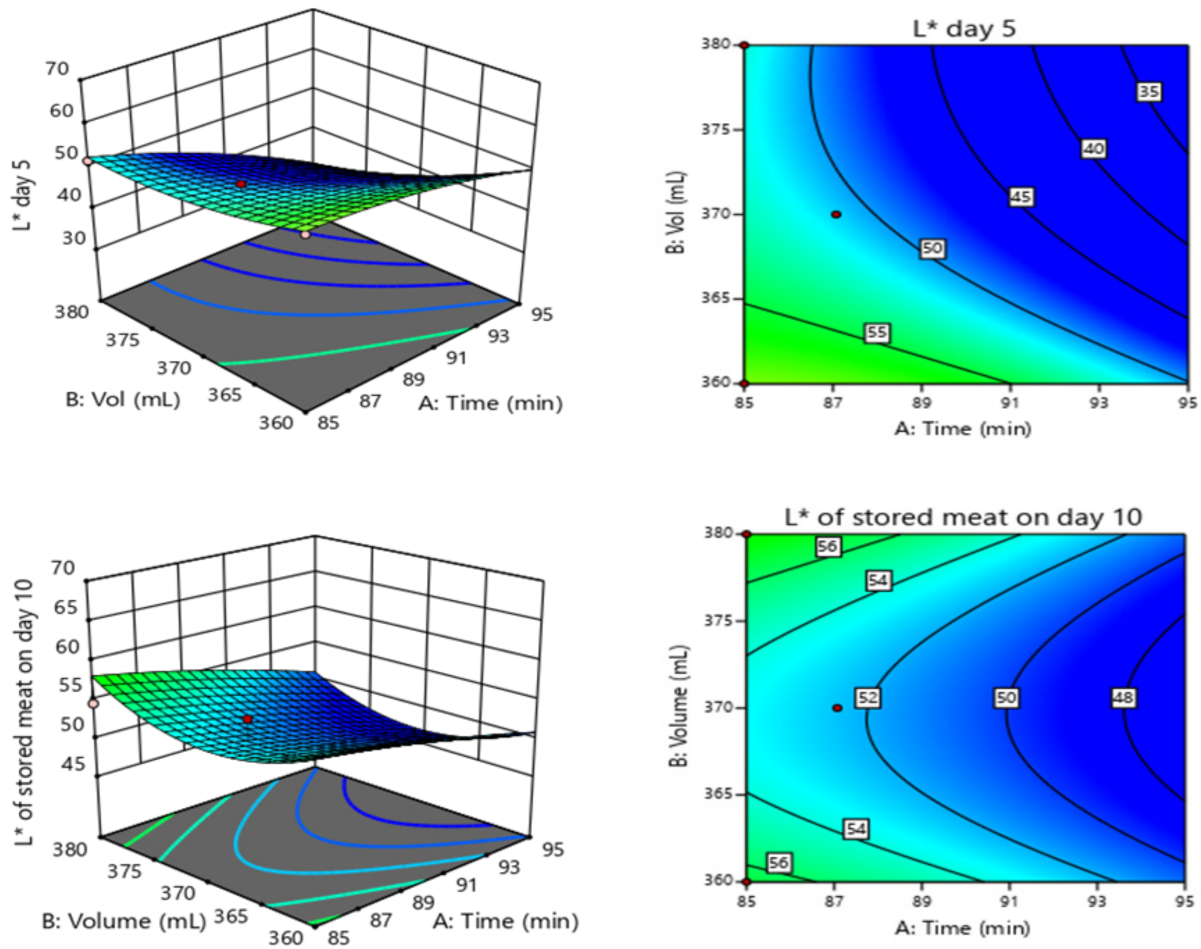

Figure 5. Response surface and contour plots of lightness value of meat soaked in black pepper aqueous extract on days 5 and 10 of refrigeration storage.

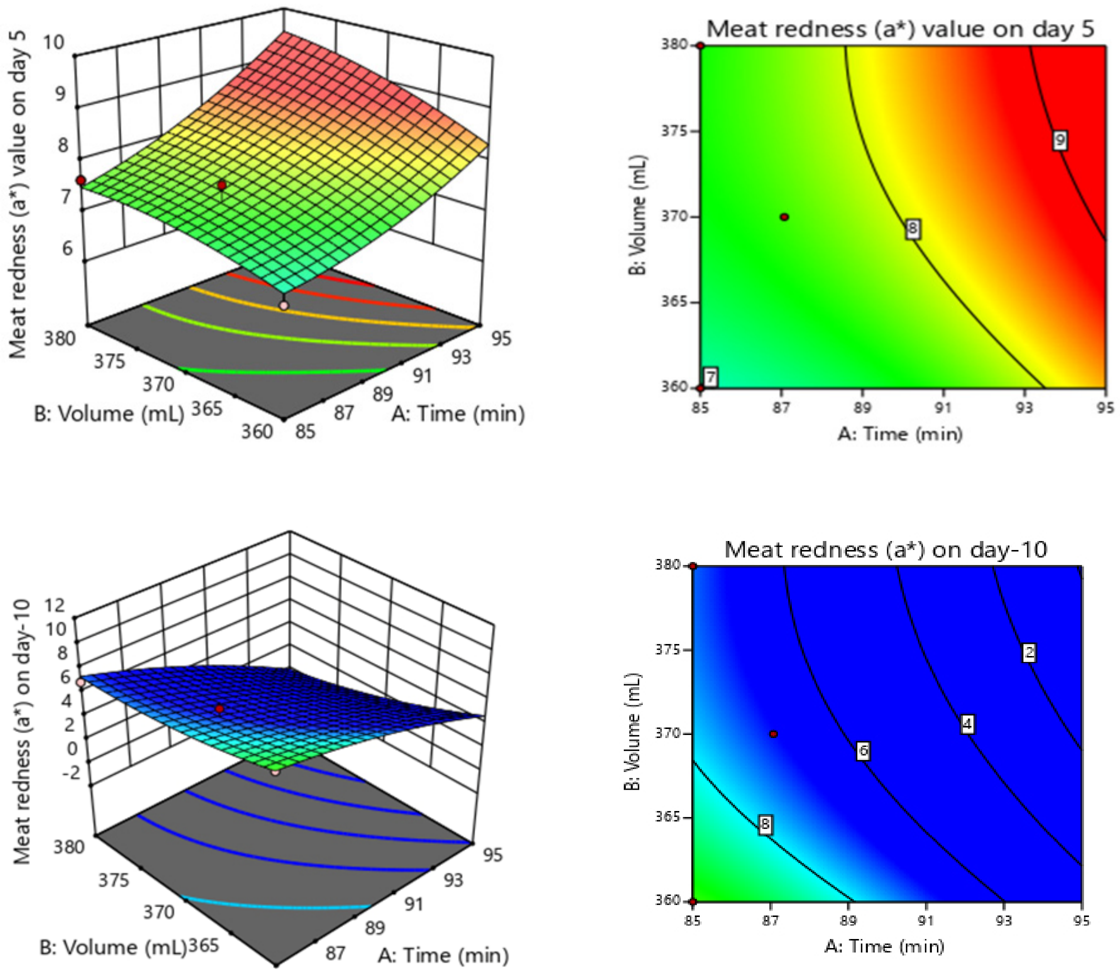

Figure 6. Response surface and contour plots of redness values of chicken meat soaked in black pepper aqueous extract on days 5 and 10 of refrigeration storage.

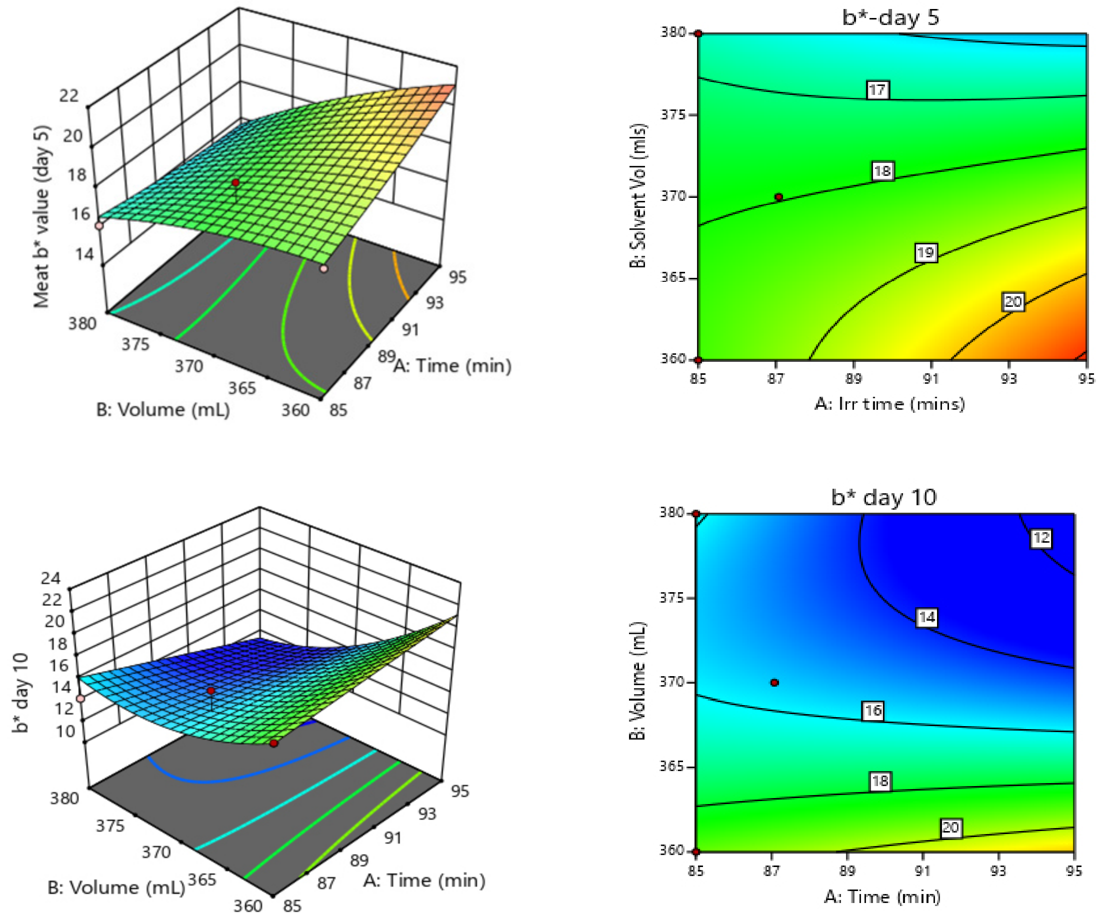

Figure 7. Response and contour plots of yellowness of meat of broiler chickens soaked in black pepper aqueous extract stored for 5 and 10 days in the refrigerator.

Criteria for optimization of production of black pepper aqueous extracts is presented in Table 5. The time for extract exposure to microwave irradiation was fixed between 85–95 min and the solvent volume was likewise fixed between 360 and 380 mL. Volume of extract to be obtained ranged between 60.42 and 115.23 mL, while extract soaked-u by meat was set to be maximized between 3.15 and 17.09. Meat pH of 5.82 was targeted for D 0 but meat pH for D 5 and 10 were minimized, ranging between 6.05-6.21 and 6.39-7.23 respectively. Meat malondialdehyde value range for D 5 was fixed between 0.286-2.088 but targeted at 0.11 for D 10. Meat lightness and redness on D 5 and 10 respectively were varied between 47.36-64.62, 49.03-66.35; 6.12-8.77 and 6.89-11.45, while cook loss of 30.60% was targeted. Refrigeration loss (%) in meat for D 5 and 10 and meat yellowness on D5 were not factored to obtain optimization outcomes.

From the suggested solutions in Table 6, six solutions were proffered. An irradiation time of 87.267 min and 364.285 mL of distilled water would result in best desirability level of 0.624, while holding solvent volume and microwave power constant, however, a similar combination of 87.245 min and 364.189 mL of distilled water can be combined as variable factors to achieve a similar effect. Other suggested solutions have desirability values of 0.623, 0.622, 0.621 and 0.612 respectively. This study has shown that irradiation time and solvent volume are critical factors that can affect the extraction of functional compounds in black pepper. Also, while the change in irradiation time of solutions suggested was close, the solvent volume affected the desirability than the irradiation time. This agrees with the findings of Jeyaratnam et al. (2016) that with increased solvent volume for extraction, a lower heat and by extension the irradiation time reduces for actual extraction as the energy requirement

Table 5. Criteria for optimization of extraction parameters.

Parameter	Goal	Limit
Irradiation time	in range	85-95
Solvent volume	in range	360-380
Extraction volume	in range	60.42-115.23
Oleoresin uptake	maximize	3.15-17.09
Meat pH D0	target	5.82
Meat pH D5	minimize	6.05-6.21
Meat pH D10	minimize	6.39-7.23
TBARs D5	minimize	0.286-2.088
TBARs D10	target	0.11
Ref. Loss D5	none	-
Ref. Loss D10	none	-
L* D5	in range	47.36-64.62
L* D10	in range	49.03-66.35
a* D5	in range	6.12-8.77
a* D10	in range	6.89-11.45
b* D5	none	
b* D10	in range	13.68-23.09
Cook loss	target	30.60

for vaporization increases. Also, from this study, the upper and lower limit for irradiation time and the extraction of white pepper with optimal desirability is between 87.245 and 87.267 min and further increment in irradiation time will not significantly increase extraction yield of bioactive compounds (Mustapa et al. 2015).

Table 6. Solutions found from optimizing aqueous black pepper extracts.

Number	Irr time	Solvent Vol	Extract vol	Oleoresin Uptake	Meat pH Day-0	Meat pH Day-5	Meat pH Day 10	TBARs D5	TBARs Dy 10	Meat Ref Loss D5
1	**87.267**	**364.285**	**95.359**	**7.007**	**5.820**	**6.075**	**6.878**	**0.286**	**0.134**	**11.835**
2	87.245	364.189	95.413	7.027	5.820	6.076	6.883	0.289	0.134	11.862
3	87.202	363.997	95.519	7.069	5.820	6.077	6.893	0.295	0.133	11.914
4	87.403	364.915	94.964	6.886	5.820	6.072	6.843	0.268	0.137	11.658
5	86.984	363.071	95.939	7.291	5.820	6.082	6.944	0.329	0.129	12.161
6	87.747	366.668	93.563	6.637	5.820	6.063	6.749	0.236	0.148	11.140

Number	Meat Ref Loss D10	L* day 5	L* day 10	a*- day 5	a* day 10	b*- day 5	b* day 10	CK Loss Dy10	Desirability
1	**24.754**	**54.033**	**53.257**	**7.381**	**7.789**	**18.579**	**17.484**	**35.920**	**0.624**
2	24.752	54.115	53.306	7.374	7.816	18.583	17.526	35.871	0.624
3	24.748	54.282	53.407	7.360	7.872	18.591	17.610	35.772	0.623
4	24.773	53.496	52.952	7.427	7.610	18.547	17.216	36.237	0.622
5	24.744	55.091	53.937	7.289	8.144	18.618	18.024	35.283	0.621
6	24.874	52.056	52.279	7.549	7.136	18.423	16.516	37.061	0.612

Bioactive compounds gotten from black pepper extract via optimized criteria is presented in Table 7. A total of 71 compounds were identified with a diverse range of element-based compounds such as silicon, nitrogen, sulphur and iodine. Two silicon, and nitrogen-based compounds as well as one sulphur and nitrogen-sulphur based compound were obtained, while seven iodine-based compounds were found. Palmitic acid was the major abundant component (17.05%), followed by Stearic acid (15.84%), 2-Ethylbenzotriazole (10.14%), N-Methyl-1-phenylcyclopropanamine (10.14%) and butylsemithiocarbazide (10.14%). Linoleic acid, petroselenic acid and (R)-(-)-14-Methyl-8-hexadecyn-1-ol were 9.72 % respectively, while methyl linoleate, methyl linolelaidate and 9-cis, 11-trans-octadecadienoate were 8.55% respectively. Slightly lower yield was dibutyl phthalate and butyl isobutyl phthalate. Notably, Bis (2-ethylhexyl) phthalate content of 8.55% was present in aqueous extract. The GC-MS result for optimized black pepper extract contradicts the reports of Zhang and Xu (2015) and Olalere et al. (2018a) that white pepper contains more bioactive compounds than black pepper, thus indicating that the method of extraction employed have a strong impact on the qualitative yield of bioactive components.

Table 7. GC-MS analysis of various components in black pepper aqueous extract.

No	Component	Molecular Formula	Retention time	Area
1	Octamethylcyclotetrasiloxane (D4)	$C_8H_{24}O_4Si_4$	3.187	0.580
2	5-Methylsalicylic acid, 2TMS derivative	$C_{13}H_{22}O_3Si_2$	3.187	0.580
3	4-Methyldecane	$C_{11}H_{24}$	3.409	0.850
4	2,6-Dimethylnonane	$C_{11}H_{24}$	3.409	0.850
5	3,7-Dimethylundecane	$C_{13}H_{28}$	3.865	1.570
6	5,7-Dimethylundecane	$C_{13}H_{28}$	3.865	1.570
7	Tetrahydrolavandulol	$C_{10}H_{22}O$	4.465	0.600
8	3-Methyldecane	$C_{11}H_{24}$	4.465	0.600
9	2-Ethylbenzotriazole	$C_8H_9N_3$	4.598	10.14
10	N-Methyl-1-phenylcyclopropanamine	$C_{10}H_{13}N$	4.598	10.14
11	Butylsemithiocarbazide	$C_5H_{13}N_3S$	4.598	10.14
12	6-Methyldodecane	$C_{13}H_{28}$	6.009	0.650
13	3,6-Dimethylundecane	$C_{13}H_{28}$	6.009	0.650
14	2-Methylnonane	$C_{12}H_{22}$	6.009	0.650
15	4,5-Dimethylnonane	$C_{11}H_{24}$	6.564	0.830
16	2,4-Dimethylheptane	C_9H_{20}	6.686	0.640
17	Sulfurous acid, butyl dodecyl ester	$C_{16}H_{34}O_3S$	6.686	0.640
18	2,6,11-Trimethyldodecane	$C_{15}H_{32}$	6.909	1.360
19	3,7-Dimethyldecane	$C_{12}H_{26}$	6.909	1.360
20	1-Iodohexadecane	$C_{16}H_{33}I$	7.520	0.540
21	Tetracosane	$C_{24}H_{50}$	7.520	0.540
22	1-Iodo-2-methylundecane	$C_{12}H_{25}I$	8.642	0.590
23	9-Methylnonadecane	$C_{20}H_{42}$	9.264	1.22
24	Hexadecane	$C_{16}H_{34}$	9.264	1.22
25	Tetracosane	$C_{24}H_{50}$	9.264	1.22
26	1H-Cyclopropa[a]naphthalene, 1a,2, 3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR- (1a. alpha.,7. alpha.,7a. alpha.,7b. alpha.)] (Caryophyllene)	$C_{15}H_{24}$	9.464	1.53

Table 7

No	Component	Molecular Formula	Retention time	Area
27	1s,4R,7R,11R-1,3,4,7-Tetramethyltricyclo [5.3.1.0(4,11)] undec-2-en-8-one	$C_{15}H_{22}O$	9.464	1.53
28	Heptacosane	$C_{27}H_{56}$	9.597	0.93
29	1-Iodooctadecane	$C_{18}H_{37}I$	9.597	0.93
30	Tridecane, 1-iodo-	$C_{13}H_{27}I$	9.675	1.56
31	3,8-Dimethyldecane	$C_{12}H_{26}$	9.675	1.56
32	2,4-di-tert-butylphenol	$C_{14}H_{22}O$	9.897	1.91
33	2,5-Di-tert-butylphenol	$C_{14}H_{22}O$	9.897	1.91
34	6,6-Diethylhoctadecane	$C_{22}H_{46}$	11.308	0.64
35	Undecane	$C_{11}H_{24}$	11.486	0.62
36	Heptadecane	$C_{17}H_{36}$	12.041	0.67
37	2,6,10-Trimethyltetradecane	$C_{17}H_{36}$	12.041	0.67
38	Hentriacontane	$C_{31}H_{64}$	12.041	1.260
39	9-Octylheptadecane	$C_{25}H_{52}$	12.041	1.260
40	Hentriacontane	$C_{31}H_{64}$	12.608	0.55
41	Octadecane	$C_{18}H_{38}$	13.108	0.80
42	2-Methyltetracosane	$C_{25}H_{52}$	13.108	0.80
43	Octacosane	$C_{28}H_{58}$	13.586	0.67
44	Triacontane	$C_{30}H_{62}$	13.586	0.67
45	Dotriacontane, 1-iodo-	$C_{32}H_{65}I$	14.030	0.98
46	3-Ethyl-5-(2-ethylbutyl) octadecane	$C_{26}H_{54}$	14.030	0.98
47	Methyl palmitate	$C_{17}H_{34}O_2$	14.408	2.80
48	Methyl 14-methylpentadecanoate	$C_{17}H_{34}O_2$	14.408	2.80
49	Dibutyl phthalate	$C_{16}H_{22}O_4$	14.741	6.20
50	Butyl isobutyl phthalate	$C_{16}H_{22}O_4$	14.741	6.20
51	Palmitic acid	$C_{16}H_{32}O_2$	14.885	17.050
52	Methyl linoleate	$C_{18}H_{32}O_2$	15.996	8.55
53	Methyl (9E,12Z)-9,12-octadecadienoate (Methyl linolelaidate)	$C_{19}H_{34}O_2$	15.996	8.55
54	9-cis,11-trans-Octadecadienoate	$C_{18}H_{31}O_2$	15.996	8.55
55	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	16.052	4.29
56	9-Octadecenoic acid, methyl ester, (E) (methyl eladiate)	$C_{19}H_{36}O_2$	16.052	4.29
57	cis-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	16.052	4.29
58	methyl stearate	$C_{19}H_{38}O_2$	16.296	0.72
59	Ethyl heptadecanoate	$C_{19}H_{38}O_2$	16.296	0.72
60	Petroselinic acid (6-Octadecenoic acid)	$C_{16}H_{34}O_2$	16.452	9.72
61	(9E,12Z)-linoleic acid	$C_{18}H_{32}O_2$	16.452	9.72
62	R) - (-) - 14-Methyl-8-hexadecyn-1-ol	$C_{17}H_{32}O$	16.452	9.72
63	Stearic acid	$C_{18}H_{36}O_2$	16.719	15.84
64	Carbonic acid, decyl undecyl ester	$C_{22}H_{44}O_3$	18.163	0.73

Table 7

No	Component	Molecular Formula	Retention time	Area
65	Tetracosane, 1-iodo-	$C_{24}H_{49}I$	18.163	0.73
66	Dotriacontane, 1-iodo-	$C_{32}H_{65}I$	18.163	0.73
67	(Z)-14-Methylhexadec-8-enal	$C_{17}H_{32}O$	19.507	0.56
68	Eicosane	$C_{20}H_{42}$	19.507	0.56
69	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	19.774	1.87
70	Phthalic acid, dodecyl 2-ethylhexyl ester	$C_{28}H_{46}O_4$	19.774	1.87
71	Phthalic acid, hexadecyl 4-methylhept-3-yl ester	$C_{32}H_{54}O_4$	19.774	1.87

CONCLUSIONS

Meat quality is a major criterion for purchase that drives consumer preference and decision. This study has demonstrated that the number of compounds extracted correspond with extraction efficiency, evidenced by the influence of extracts on meat pH and MDA count on D 5; meat lightness and eventual number of compounds extracted. Extraction conditions sandwiched between 85–87 min and 361–379 mL of irradiation time and solvent volume respectively, in tandem with other fixed extraction operational factors favors the preservation of lightness in meat, while the least meat TBARs value was recorded at 88 min of irradiation time and 367 mL of solvent volume. After optimization was performed, a total of six solutions were suggested for the extraction of aqueous black pepper, and the recommended extraction criteria had desirability level of 0.624 was for two solutions. With irradiation power and particle size fixed at 320 W and 8 g respectively, alongside 0.40 mm particle size; 87.268 min of irradiation time and 364.285 mL of solvent volume was suggested as the recommended variable extraction values, while an irradiation time of 87.245 min and 364.189 mL can be applied as effective alternative. Applying the suggested extraction conditions in a closed system, a total of 71 compounds were found which is a remarkable improvement in the extraction yield and efficiency.

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Effect of particle size on sensory and bioactive properties of chocolates with *Physalis peruviana* L and *Vaccinium* spp

UNIVERSIDAD NACIONAL DE COLOMBIA

Efecto del tamaño de partícula sobre las propiedades sensoriales y bioactivas de chocolates con *Physalis peruviana* L y *Vaccinium* spp

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ABSTRACT

Keywords:
Anthocyanins
Cocoa
Particle size
Total phenols

Chocolates are considered functional foods and are consumed worldwide. The objective was to evaluate the effect of particle size and the inclusion of *Physalis peruviana* L (Goldenberry) and *Vaccinium* spp (Andean blueberry) on sensory attributes, color, total phenols, and anthocyanins in dark and milk chocolate. The attributes of gloss, surface, breakage, melting, grittiness, odor, fruit scents, aftertaste, sweetness, mouthfeel, bitterness, and astringency were evaluated for sensory evaluation. To measure color, the CIELAB system was used. Phenols were evaluated using spectrophotometry and anthocyanins using the pH differential method. According to principal components analysis (PCA), dark chocolates with the inclusion of goldenberries had the best sensory scores ($Ø_p$=10-20 µm); the results for the correlations were 0.97 for surface and melting, 0.98 for surface and aftertaste, 1.0 for aftertaste and astringency, and -0.92 melting and odor. Milk chocolate with the inclusion of goldenberries ($Ø_p$=10-20 µm) was superior in attributes such as fruit scents, sweetness, grittiness, gloss, odor, aftertaste, and mouthfeel. At the same time, there was a correlation between melting and aftertaste of -0.92 and between melting and mouthfeel of -0.94, odor with fruit scents and sweetness were 0.96 and 0.98, and fruit scents with aftertaste and sweetness were 0.94 and 0.99. Dark and milk chocolates with the inclusion of goldenberries ($Ø_p$=10-15 µm) were slightly superior in the phenol content, while chocolates with the inclusion of Andean blueberries ($Ø_p$=10-20 µm) had greater anthocyanin contents.

RESUMEN

Palabras clave:
Antocianinas
Cacao
Tamaño de partícula
Fenoles totales

Los chocolates son productos considerados como alimentos funcionales y son consumidos en todo el mundo. El objetivo de esta investigación fue evaluar el efecto del tamaño de partícula y la inclusión de *Physalis peruviana* L (Aguaymanto) y *Vaccinium* spp (Gongapa) sobre los atributos sensoriales, color, fenoles totales y antocianinas en chocolate oscuro y con leche. Para la evaluación sensorial se consideró los atributos de brillo, superficie, rotura, fusión, arenosidad, olor, aroma a fruta, retrogusto, dulzor, sensación en la boca, amargura y astringencia. Para medir el color se utilizó el sistema CIELAB. Los fenoles fueron evaluados por espectrofotometría y las antocianinas mediante el método de pH-diferencial. Según el análisis de componentes principales (ACP), los chocolates oscuros ($Ø_p$=10-20 µm) con inclusión de aguaymanto fueron mejores sensorialmente; los resultados de sus correlaciones entre superficie y fusión (0,97), superficie y retrogusto (0,98), retrogusto y astringencia (1,0), fusión y olor (-0,92). En el chocolate con leche ($Ø_p$=10-20 µm) con inclusión de aguaymanto fue superior en los atributos de aroma a frutas, dulzor, arenosidad, brillo, olor, retrogusto y sensación en la boca. Asimismo, este presentó correlación entre fusión con retrogusto (-0,92) y fusión con sensación en la boca (-0,95); olor con aromas a frutas y dulzor (0,96 y 0,98), y aromas a frutas con retrogusto y dulzor (0,94 y 0,99). Los chocolates oscuros y con leche ($Ø_p$=10-15 µm) e inclusión de aguaymanto fueron ligeramente superiores en el contenido fenólico; mientras que los chocolates con inclusión de gongapa ($Ø_p$=10-20 µm) fueron mayores en el contenido de antocianinas.

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Chocolate is a very popular product worldwide. Its market value has risen to 500 million dollars since 2017, with an annual growth of 5.6%, and is expected to reach an average of 730 million dollars in the world market by 2025 (Kim and Jeon 2019). Chocolate is categorized as a functional, nutraceutical food, and is consumed around the world; at the same time, it is valued for its polyphenol content because it has been demonstrated to protect the cardiovascular system due to its antioxidant activity (Deus et al. 2020). It is also considered good for increasing longevity, libido, and fertility, as well as preventing diseases such as cancer, diabetes, and others related to oxidative stress (Halib et al. 2020).

Chocolates are made with cocoa beans, which contain three groups of polyphenols: catechins or flavan-3-ols (37%), anthocyanins (4%) and proanthocyanidins (58%). Furthermore, the total phenols content in dark and milk chocolates varies depending on the proportion of cocoa solids used in formulation (Afoakwa 2016). For example, dark chocolate contains cocoa butter, a low sugar content, and high cocoa solids content. The latter affects the sensory profile, highlighting bitterness and astringency, which are related to phenol compounds that potentially influence consumers acceptance (Ostrowska-Ligęza et al. 2018).

To make chocolate, cocoa must go through a process of fermentation, drying, roasting, grinding, refining, conching, and tempering. During these operations, there is a noticeable decrease in the content of bioactive compounds. Phenolic compounds can be reduced to up to one-tenth of their original condition and some flavonoids can disappear as a result of non-enzymatic browning (Mattia et al. 2017). Anthocyanins and procyanidins are degraded by hydrolysis during the fermentation process and about 50% of epicatechin is lost in drying (Zugravu and Otelea 2019). To balance these enormous losses of phytochemicals, fortification with fruits, spices, or probiotics, is one of the most effective alternatives. This fortification can also improve the physical, rheological, and organoleptic properties of chocolates (Sharmistha et al. 2022).

Refining and conching are operations that determine the particle size distribution, consistency, and viscosity of chocolate to produce specific textural and the sensory qualities (Afoakwa 2016). The structure and size of non-fat particles in chocolates are two factors that influence sensory quality, because inhomogeneous particles give a pasty consistency and are more difficult to handle in the mouth (Tan and Balasubramanian 2017). The sensory attributes of melting, flavor, smell, and texture of chocolate are associated with roasting temperature and particle size (Hinneh et al. 2019).

Perú is very diverse in promising fruit, that develops perennially on the coast, mountain region and jungle, due to the various microclimates, peaks, and soils within the country. The fruit of the *Vaccinium* spp*. and Physalis peruviana* L. species are native to the high Andean regions and have achieved great importance due to their bioactive, functional, and mineral compounds (Bazalar et al. 2020). The goldenberry (*Physalis peruviana* L.) originated in the Peruvian and Ecuatorian Andes and is known as uchuva, uvilla or aguaymanto, and as goldenberry in English-speaking countries. This fruit is consumed fresh and processed. At the same time, it is valued for its organoleptic properties, nutritional value, and health benefits due to compounds such as vitamin C, carotenoids, flavonoids, and antioxidant activity (El-Beltagi et al. 2019). The Andean blueberry (*Vaccinium* spp.) grows wild in the Huánuco region but has no commercial value and is only consumed by those who live in the area. The Ericaceae family and the *Vaccinium* spp genre are widespread and represent around 40 species. The berries are dark blue in color and have delicious sensory properties as well as antioxidant and dye properties. This fruit also contains phenolic compounds (anthocyanins), sugars, fiber, lipids, and vitamins and has nutraceutical properties that act as protectors against cancer, age-related neurodegenerative diseases, and inflammatory responses (Quintero-Quiroz et al. 2019). The objective was to evaluate the effect of particle size and the inclusion of *Physalis peruviana* L (Goldenberry) and *Vaccinium* spp (Andean blueberry) on sensory attributes, color, total phenols, and anthocyanins in dark and milk chocolate.

MATERIALS AND METHODS

The different varieties of cocoa liquor from the Amazon region were obtained from the company "Agro Industrias MAKAO Perú S.A.C." The Andean blueberry fruit

(*Vaccinium* spp.) was harvested from the Rondos district (3566 masl; 10°0'52.733" S and 76°42'53.046" W), Lauricocha province, and the goldenberries (*Physalis peruviana* L.) were harvested from Nauyan Rondos province of Huánuco, (2748 masl; 9°57'5.004" S; 76°14'49.885" W).

Experimental methodology

Elaboration

For the 70% dark chocolate, 65% cocoa paste, 5% cocoa butter, 29.5% sugar, and 0.5% lecithin were used. For milk chocolate, 14.5% cocoa paste, 19.5% cocoa butter, 20% milk powder, 45.5% sugar, 0.45% lecithin, and 0.05% vanilla were used. The final particle sizes (Øp) were 10-15 µm and 16-20 µm (refiner/shelling machine QYJ-20. China). Concerning the inclusions, they were 12% goldenberries (DCP10 and DCP16) and 12% Andean blueberries (DCV10 and DCV16).

Measure of color

A colorimeter (Lovibond LC-100/SV-100-United Kingdom) was used. The system used for measurements was CIELAB, gloss (L*), and chromaticity (a* and b*), with four repetitions for each sample (Chire-Fajardo et al. 2019).

The sensory evaluation

It was done following the Belščak-Cvitanović et al. (2012) method, with some modifications. The judges that were used were six with experience in tasting cocoa liquor and chocolates. For the dark chocolate, an evaluation sheet with 11 attributes was used: gloss, surface, breakage, melting, grittiness, odor, fruit scents, aftertaste, sweetness, mouthfeel, bitterness, and astringency; and for the milk chocolate, 10 attributes were used, similar to the dark chocolate, except for bitterness and astringency. The hedonic scale was five points, with one being "tasteless" and five being "extremely desirable."

Total phenols

The method proposed by Delgado et al. (2018) was followed, with some modifications. A mix of 100 µL of diluted extract (2.5 mg mL^{-1}) with 500 µL of Folin-Ciocalteu's reagent (0.2 N) was made; later, 400 µL of sodium carbonate 7.5% (w v^{-1}) was added. The mix was stored for two hours at room temperature and in darkness before measuring the absorbance at 740 nm in a UV-visible spectrophotometer (Genesys 150, USA). A standard curve for gallic acid was elaborated (1 to 10 µg mL^{-1}) in order to express the absorbency of the sample as a concentration of phenols, equivalent in gallic acid (g EAG 100 g^{-1}).

Anthocyanins: The pH differential method was followed (Zapata et al. 2014). Two distinct buffer solutions were prepared, one from KCl (0.025 M, pH=1) and sodium acetate (0.4 M, pH=4.5). For each sample, the appropriate dilution factor was determined, taking into account that the absorbance should not pass 1.2 without exceeding a 20% dilution factor, since that would surpass the saturation indices of the buffers. The maximum wavelength depends on the pattern chosen (cyanidin-3-glucoside, 510 nm). For the calculations, the following Equation (1) was used:

$$\text{cyanidin–3–glucoside}\left(\frac{mg}{L}\right)=\frac{[(A_{510}-A_{710})_{pH1}-(A_{510}-A_{710})_{pH4.5}]*MW*DF*1000}{\varepsilon*1} \quad (1)$$

The molecular mass of cyanidin-3-glucoside was (MW) 449.2 g mol^{-1}, the molar extinction coefficient was (ε) 26900 L mol^{-1} cm^{-1}, and the l, the length of the optical path (1 cm).

Statistical analysis

The results were analyzed through the use of the unifactorial ANOVA, and for the treatments, where there was a statistical difference, the Tukey test (P<0.05) was applied. On the other hand, the description of the principal variation of the sensory data, obtaining the descriptive profiles, and the graph of the response in a cluster on a dendrogram were done through the quantitative descriptive analysis (QDA) and the multivariate principal component's analysis (PCA). Finally, Pearson's linear correlation was calculated using the program InfoStat 2019P, Universidad Nacional de Córdoba, Argentina.

RESULTS AND DISCUSSION

Color of the chocolates

The highest gloss (L) of the dark chocolate (Table 1) was for the $Ø_p$=10-15 µm particle size with goldenberries and Andean blueberries; the opposite case occurred for the $Ø_p$=16-20 µm size. According to the results, it can be observed that particle size affects gloss; this concurs with Hinneh et al. (2019), who mentions that the highest gloss values were for the dark chocolate with small particles, $Ø_p$=25.25±0.09 µm, and the lowest was for the large particles, $Ø_p$=27.98±0.02 µm. At the same time, Chire-Fajardo et al. (2019) obtained L=27.28±0.30 to 26.19±0.34 for dark chocolates, indicating that the gloss is affected by the factors such as formula, tempering, bean color, temperature, roasting time, and shelling. On the other hand, the highest gloss for the milk chocolate corresponded to the $Ø_p$=10-15 µm particle size with goldenberries and the lowest was for the $Ø_p$=16-20 µm particle size with Andean blueberries; the values that were reported agree with the results from Konar et al. (2018), L=31–28 for milk chocolates. It is worth mentioning that a shiny, glossy, and smooth chocolate will have greater acceptance on behalf of the consumer (Sözeri Atik et al. 2020).

The results of the a* chroma for the dark chocolate presented differences; the highest value was for the chocolates with a $Ø_p$=16-20 µm size particles with goldenberries and Andean blueberries. This result was very similar to that reported by Hinneh et al. (2019) for chocolates refined with two types of mills, which obtained values of a*=12.7±0.2 to 10.06±0.3. Meanwhile, the milk chocolates with goldenberries and Andean blueberries had a range from a* 12.30 to 12.70; these values were superior to the dark chocolate, which could be due to the fact that the solid cocoa content was lower. The a* chroma is seen to be influenced by the formula (sugar, milk, fruit, etc.), because it generates an effect of diluting the intensity of the chocolate's color. A decrease in the cocoa liquor percentage in the chocolate provokes a decrease of the dark brown color, and thus, the value of the a* chroma increases (De Jesus Silva et al. 2019).

Table 1. Color Parameters (L, a*, b*) of dark and milk chocolates.

Treatment	L	a*	b*
DCP10	27.63±0.10[a]	10.53±0.03[ab]	5.85±0.03[b]
DCP16	26.60±0.21[b]	10.65±0.06[a]	6.50±0.12[a]
DCV10	27.58±0.16[a]	10.25±0.13[b]	5.60±0.21[bc]
DCV16	25.95±0.23[b]	10.65±0,06[a]	5.18±0.06[c]
MCP10	31.72±0.34[a]	12.70±0.14[a]	9.30±0.15[a]
MCP16	30.37±0.09[b]	12.43±0.09[a]	9.95±0.33[a]
MCV10	31.50±0.12[a]	12.30±0.15[a]	9.43±0.18[a]
MCV16	30.20±0.12[b]	12.35±0.12[a]	9.83±0.05[a]

The values represent the average±SEM; repetitions (n=4); values from the same column with different superindices are significant ($P \leq 0.05$). DCP10=Dark chocolate, Øp=10-15 µm, *Physalis peruviana* L; DCP16=Dark chocolate, Øp=16-20 µm, *Physalis peruviana* L; DCV10=Dark chocolate, Øp=10-15 µm, *Vaccinium* spp; DCV16=Dark chocolate, Øp=16-20 µm, *Vaccinium* spp; MCP10=Milk chocolate, Øp=10-15 µm, *Physalis peruviana* L; MCP16=Milk chocolate, Øp=16-20 µm, *Physalis peruviana* L; MCV10= Milk chocolate, Øp=10-15 µm, *Vaccinium* spp; MCV16=Milk chocolate, Øp=16-20 µm, *Vaccinium* spp.

On the other hand, the highest b* chroma for the dark chocolate was for the $Ø_p$=16-20 µm particle size with goldenberries; the positive sign indicates the presence of the color yellow; to which Etzbach et al. (2018) indicated that the goldenberry is a yellow–orange color fruit, rich in α, β carotenes and lutein. The lowest b* was presented by the chocolate with $Ø_p$=16-20 µm particle size and Andean blueberries, the fruit of which is a dark blue color, very similar to the blueberry (Quintero-Quiroz et al. 2019).

Sensory evaluation for the dark chocolate

According to the sensory profile from the descriptive quantitative analysis DQA (Figure 1), it can be observed that particle size did not affect the gloss attribute. The gloss and brown color positively influenced the acceptance of the consumer (Kiumarsi et al. 2020). The breakage was not affected by the particle size; a good breakage, gloss, texture, and melting is achieved with a good tempering, because during this operation the polymorphic changes of the cocoa butter occur (Halim et al. 2019). The melting was classified as "desirable" for the chocolates ($Ø_p$=10-15 µm) with the inclusion of goldenberries and Andean blueberries; this attribute is perceived through the level of melting when it is in contact with the hand for 30 seconds (Toker et al. 2019). The grittiness was classified as "desirable"; particle sizes superior to 30 µm tend to cause the perception of a gritty sensation in the mouth. The chocolate with the inclusion of Andean blueberries presented a better classification for the fruit scents attribute. The best mouthfeel was presented by the ($Ø_p$=10-15 and 16-20 µm) chocolates with goldenberries; this attribute is related to the texture of the chocolate and is evaluated using the terms stickiness, grittiness, dryness, creaminess, and coating (De Pelsmaeker et al. 2019). The bitterness was classified as "adequate", and the astringency as "desirable"; these classifications were due to the presence of the polyphenols in the cocoa beans. It is worth highlighting that the chocolate with a $Ø_p$=16-20 µm particle size with the inclusion of Andean blueberries was classified as "tolerable" with respect to the astringency. Lin et al. (2020) indicated that blueberries present astringency, due to the presence of proline with tannins and phenolic compounds.

According to the correlation matrix between the tasting attributes of the dark chocolates (Table 2), the "gloss" attribute presented a high correlation between the surface, melting, aftertaste, and astringency. The surface was correlated with the melting, aftertaste, and astringency; with values of 0.97, 0.98 and 1.0, respectively. To this effect, De Pelsmaeker et al. (2019) indicated that the melting depends on the textural properties (cohesion, consistency, hardness, and surface). The melting vs. the odor had a correlation of -0.92; this behavior was similar to that reported by Muhammad et al. (2018), the chocolate with cinnamon particles significantly affected the melting profile.

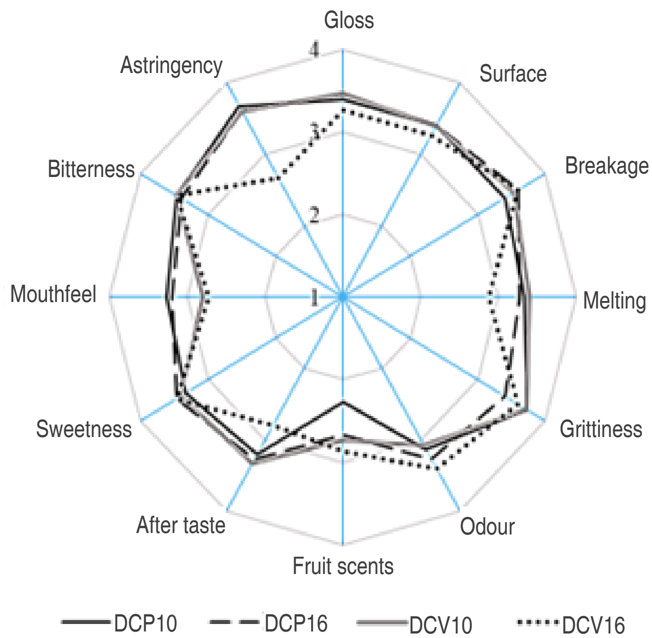

Figure 1. Sensory profile of dark chocolate. DCP10=Dark chocolate, Øp=10-15 µm, *Physalis peruviana* L; DCP16=Dark chocolate, Øp=16-20 µm, *Physalis peruviana* L; DCV10=Dark chocolate, Øp=10-15 µm, *Vaccinium* spp; DCV16=Dark chocolate, Øp=16-20 µm, *Vaccinium* spp.

The behavior of the principal components analysis (PCA) biplot (Figure 2) for the dark chocolate was divided into two dimensions and explains the general variance at 87.9% of the total variability for the data between the CP1 and CP2. For the CP1 it can be observed that the grittiness attribute stood out for the chocolates with a particle size of $Ø_p$=10-15 µm, with goldenberries and Andean blueberries. For the CP2, it can be observed that the bitterness attribute stood out and was associated with the chocolate with Andean blueberries with a particle size of $Ø_p$=16-20 µm. The bitterness possibly stood out because the Andean blueberry is an edible berry with an astringent and acidic flavor, which presents a high antioxidant capacity and varieties of bioactive phenolic compounds (Garzón et al. 2020).

Table 2. Pearson correlation coefficients of the sensory attributes of dark chocolate.

	Gl	Su	Br	Me	Gr	Od	Fs	At	Sw	Mo	Be	As
Gl	1.00											
Su	0.94	1.00										
Br	-0.16	-0.48	1.00									
Me	0.92	0.97	-0.52	1.00								
Gr	-0.11	0.06	-0.70	0.25	1.00							
Od	-0.73	-0.82	0.67	-0.92	-0.59	1.00						
Fs	-0.29	-0.61	0.91	-0.56	-0.35	0.55	1.00					
At	0.98	0.98	-0.33	0.98	0.05	-0.83	-0.43	1.00				
Sw	-0.23	-0.52	0.99	-0.60	-0.77	0.76	0.86	-0.41	1.00			
Mo	0.45	0.66	-0.52	0.50	-0.24	-0.27	-0.82	0.49	-0.43	1.00		
Be	-0.48	-0.33	-0.48	-0.15	0.92	-0.24	-0.09	-0.33	-0.52	-0.49	1.00	
As	0.91	1.00	-0.53	0.97	0.10	-0.83	-0.66	0.96	-0.57	0.69	-0.30	1.00

Gl – Gloss; Su - Surface; Br - Breakage; Me – Melting; Gr – Grittiness; Od – Odor; Fs – Fruit scents; At– Aftertaste; Sw– Sweetness; Mo – Mouthfeel; Be – Bitterness; As – Astringency.

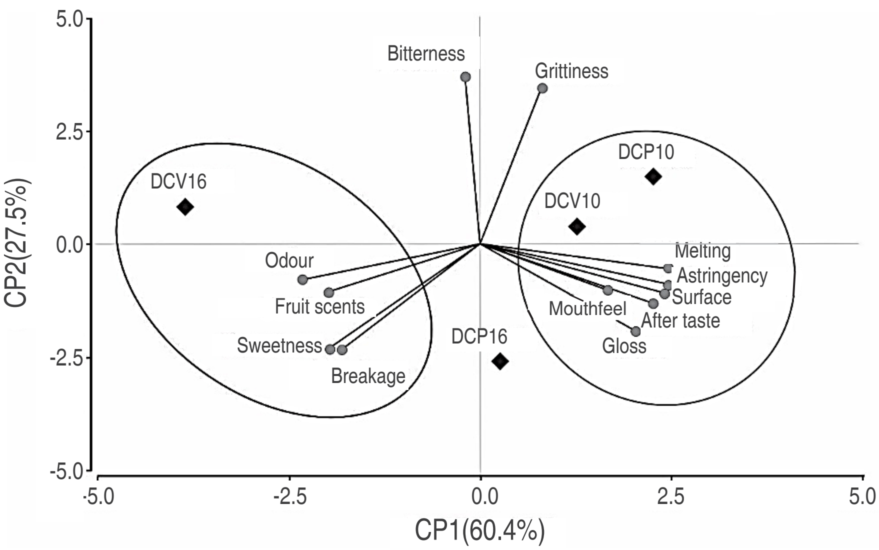

Figure 2. Behavior of the biplot of the sensory evaluation for dark chocolate. DCP10=Dark chocolate, Øp=10-15 μm, *Physalis peruviana* L; DCP16=Dark chocolate, Øp=16-20 μm, *Physalis peruviana* L; DCV10=Dark chocolate, Øp=10-15 μm, *Vaccinium* spp; DCV16=Dark chocolate, Øp=16-20 μm, *Vaccinium* spp.

According to the analysis of the dendrogram (Figure 3), two groups can be observed. The first considers just the chocolate with Andean blueberries and a particle size of $Ø_p$=16-20 μm, which had only five attributes that stood out and one of them was astringency. To this effect, Mirković et al. (2018) indicated that the volatile acids and polyphenols are responsible for the astringency in the chocolates. The second was made up of chocolates between $Ø_p$=10-15 μm with goldenberries and Andean blueberries and $Ø_p$=16-20 μm with goldenberries. This group had better classifications in the attributes of gloss, mouthfeel, aftertaste, surface, astringency, and melting.

Sensory evaluation for the milk chocolate

The chocolates had a classification of “desirable” for the attributes of gloss, surface, and breakage (Figure 4).

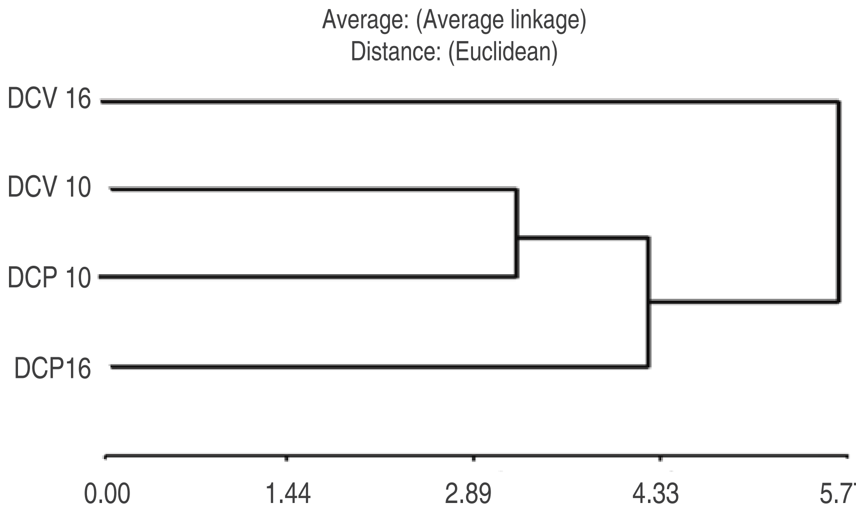

Figure 3. Representation of the analysis of conglomerates for dark chocolate. DCP10=Dark chocolate, Øp=10-15 µm, *Physalis peruviana* L; DCP16=Dark chocolate, Øp=16-20 µm, *Physalis peruviana* L; DCV10=Dark chocolate, Øp=10-15 µm, *Vaccinium* spp; DCV16=Dark chocolate, Øp=16-20 µm, *Vaccinium* spp.

The shelling process promotes the coating of the solid cocoa particles with the butter, developing good textural properties (Toker et al. 2019). The melting of the chocolate with Andean blueberries, for both particle sizes, presented the greatest score against the chocolate with goldenberries; given that the melting depends on the nature of the fatty acids present in the chocolate. To this effect, Handiati et al. (2019) indicated that a soft texture and good melting in the mouth are provided by the stearic (34%), palmitic (27%) and oleic (34%) fatty acids. All the chocolates were classified as "acceptable" with respect to the grittiness. The greatest score for the odor was for the chocolates with goldenberries; very fine particles allow for the

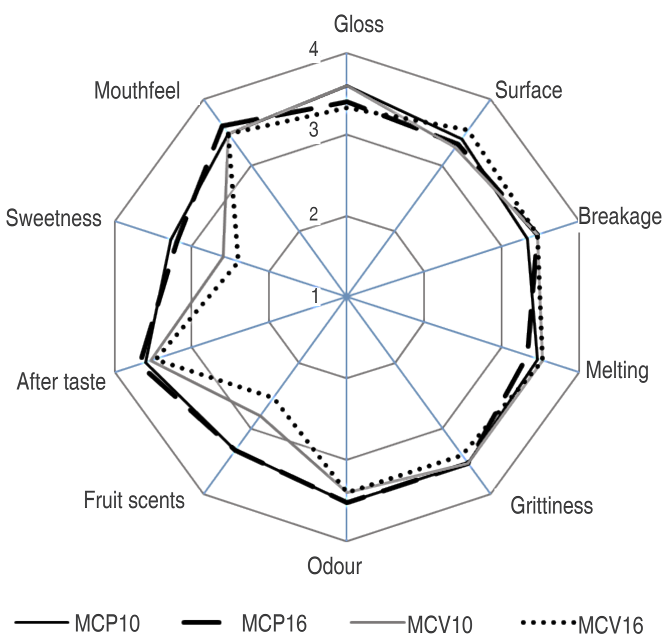

Figure 4. Sensory profile of milk chocolate. MCP10=Milk chocolate, Øp=10-15 µm, *Physalis peruviana* L; MCP16=Milk chocolate, Øp=16-20 µm, *Physalis peruviana* L; MCV10=Milk chocolate, Øp=10-15 µm, *Vaccinium* spp; MCV16=Milk chocolate, Øp=16-20 µm, *Vaccinium* spp.

volatile components to provide a strong impression of the chocolate odor (Nurhayati et al. 2019). The fruit scents attributed in the chocolates with goldenberries were superior to those with Andean blueberries due to the fact that the goldenberry has a bittersweet flavor with a pleasant aroma. For the sweetness, differences were presented with respect to the fruit that was added, but not with respect to the particle size, thus, the chocolates with goldenberries were better than those with Andean blueberries. This could be due to the fact that the goldenberry is a fruit with good acidity and easy to mask with the addition of sugar (Bazalar et al. 2020). All the chocolates were classified as “acceptable” for the mouthfeel attribute. To this respect, Hinneh et al. (2020) mentioned that the mouthfeel is perceptible when the chocolates surpass a particle size of 35 μm.

According to the correlation matrix (Table 3) between the tasting attributes for the chocolates, the melting vs. aftertaste (-0.92) and the mouthfeel (-0.95) concurs with the studies done by Rosales et al. (2018), where it was found that the mouthfeel and the melting velocity are negatively correlated -0.87. This indicates that the addition of products to the chocolate mix will have an effect on the behavior of the melting of the fats and the final product. The odor was positively correlated to the fruit scents and the sweetness. To this affect, Augusto et al. (2019) indicated that the cocoa beans, when roasted, develop aromatic compounds.

Table 3. Analysis of the correlation matrix for the sensory attributes of milk chocolate.

	Gl	Su	Br	Me	Gr	Od	Fs	At	Sw	Mo
Gl	1.00									
Su	-0.62	1.00								
Br	-0.56	-0.10	1.00							
Me	0.24	0.30	-0.04	1.00						
Gr	0.73	-0.88	-0.33	-0.46	1.00					
Od	0.15	-0.18	-0.58	-0.80	0.58	1.00				
Fs	0.36	-0.44	-0.55	-0.76	0.78	0.96	1.00			
At	0.16	-0.52	-0.25	-0.92	0.75	0.90	0.94	1.00		
Sw	0.33	-0.34	-0.62	-0.74	0.72	0.98	0.99	0.91	1.00	
Mo	-0.44	-0.31	0.33	-0.95	0.33	0.58	0.55	0.76	0.51	1.00

The primary component (CP1) for the biplot of the milk chocolate tasting (Figure 5) were the attributes of fruit scents, sweetness, grittiness, and gloss. These were positively associated with the goldenberry and the $Ø_p$=10-15 μm size, which represents 60.6% of the total variability. The sweetness attribute is highly related to the inclusion of goldenberries and negatively related to that of the Andean blueberries. According to Delgado et al. (2018), the polyphenols are highly related to the astringency and bitterness of the chocolates. At the same time, the melting, surface, and breakage attributes of the chocolates represent 23.9% of the variability of the second component (CP2), and in general both components represent 84.5% of the total variability. The results for the CP2 show that the chocolate with Andean blueberries and a particle size of 16-20 μm was associated with the surface and breakage attributes, but not with the attributes of gloss, grittiness, sweetness, and fruit scents.

Total phenols in the dark and milk chocolates

The phenols for the dark chocolates (Table 4) varied between 1.049±0.03 and 1.231±0.03 g EAG 100 g^{-1}. These chocolates presented significantly high phenolic compounds, which are attributed to the phenolic fraction of the cocoa beans and depend on the percentage of cocoa liquor employed in the elaboration. At the same time, the results obtained were similar to those reported by Martini et al. (2018) in dark chocolates with 70% liquor. The total phenols were greater for the $Ø_p$=10-15 μm particle size when compared to the $Ø_p$=16-20 μm particle size, for both fruits. This behavior was similar to the studies done by Nurhayati et al. (2019) with dark chocolate coverings. On the other hand, the total

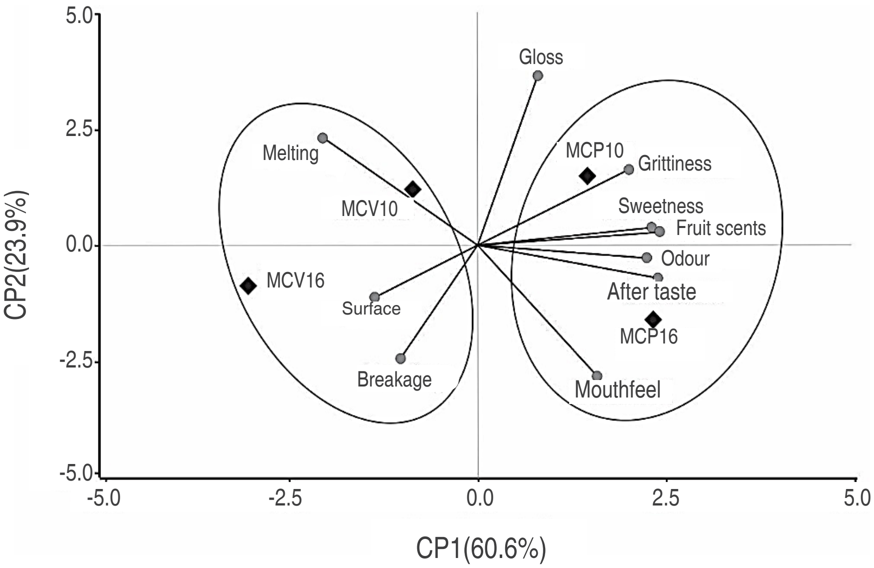

Figure 5. Behavior of the biplot for the sensory evaluation of milk chocolate. MCP10=Milk chocolate, Øp=10-15 µm, *Physalis peruviana* L; MCP16=Milk chocolate, Øp=16-20 µm, *Physalis peruviana* L; MCV10=Milk chocolate, Øp=10-15 µm, *Vaccinium* spp; MCV16=Milk chocolate, Øp=16-20 µm, *Vaccinium* spp.

phenols for the milk chocolate were less in comparison to the dark; this is possibly due to the fact they have a lower content of cocoa solids.

The anthocyanin concentrations of the dark chocolates (Table 4) were Andean blueberries>goldenberries>control without fruit. The results show that the goldenberries and the Andean blueberries increased the concentration of anthocyanins in comparison to the control. In the studies done by Calva-Estrada et al. (2020), it was reported that the anthocyanin content was 0.04–0.08 mg Cy3GE g^{-1} in a sample of five commercial brands of dark chocolate

Table 4. Quantification of the total phenols and anthocyanins in dark and milk chocolate.

Chocolate	Treatment	Total phenols (g EAG 100 g^{-1})	Anthocyanins (mg Cy3GE g^{-1})
Dark	DCP10	1.231±0.03^{a}	0.105±0.002^{c}
	DCP16	1.085±0.02^{bc}	0.102±0.001^{cd}
	DCV10	1.200±0.03^{ab}	0.184±0.005^{b}
	DCV16	1.049±0.03^{c}	0.229±0.002^{a}
	Control	1.136±0.01^{abc}	0.090±0.004^{d}
Milk	MCP10	0.651±0.02^{a}	0.039±0.001^{b}
	MCP16	0.576±0.01^{bc}	0.024±0.001^{c}
	MCV10	0.579±0.01^{bc}	0.037±0.001^{b}
	MCV16	0.532±0.02^{c}	0.099±0.001^{a}
	Control	0.621±0.01^{ab}	0.040±0.002^{b}

The values represent the average±SEM; repetitions (n=3); values from the same column with different superindices are significant ($P \leq 0.05$). DCP10=Dark chocolate, Øp=10-15 µm, *Physalis peruviana* L; DCP16=Dark chocolate, Øp=16-20 µm, *Physalis peruviana* L; DCV10=Dark chocolate, Øp=10-15 µm, *Vaccinium* spp; DCV16=Dark chocolate, Øp=16-20 µm, *Vaccinium* spp; MCP10=Milk chocolate, particle size, Øp=10-15 µm, *Physalis peruviana* L; MCP16=Milk chocolate, Øp=16-20 µm, *Physalis peruviana*; MCV10 =Milk chocolate, Øp=10-15 µm, *Vaccinium* spp; MCV16=Milk chocolate, Øp=16-20 µm, *Vaccinium* spp.

produced in different geographic zones, with different varieties of cocoa, and different percentages of cocoa solids. The concentration of anthocyanins in the milk chocolates was between 0.024 and 0.099 mg Cy3GE g^{-1} for the sample; the greatest concentration was for the $Ø_p$=16-20 size with Andean blueberries; this could be due to the purple color of the fruit and to the high phenol content associated with the antioxidant activity.

CONCLUSIONS

The sensory attributes in dark and milk chocolates improved at particle sizes of 10-15 µm, being brightness, the favorable attribute for both cases. The incorporation of *Physalis peruviana* L, improved sensory acceptance in the attributes of fruit aroma, sweetness, grittiness, smell, aftertaste, and mouthfeel compared to *Vaccinium* spp. In the bioactive compounds evaluated, the addition of *Vaccinium* spp increased the anthocyanin content, however, it produced a decrease in sensory acceptance due to its relationship with increased astringency. A particle size of 10-15 µm improved luminosity L* in color evaluation, however berry addition influenced chromatic coordinates a* with *Physalis peruviana* L and b* with *vaccinium* spp, possibly due to the contribution of orange and purple pigments respectively. It is necessary to study new formulations and drying conditions of berries that can minimize losses of their bioactive compounds.

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Application of antioxidants and hot water treatments to improve shelf life of fresh-cut eggplants (*Solanum melongena* L.) during storage

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La aplicación de antioxidantes y tratamientos con agua caliente para mejorar la vida útil de berenjenas (*Solanum melongena* L.) recién cortadas durante el almacenamiento

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ABSTRACT

The objective of this study was to evaluate the effect of antioxidant treatments on the conservation of cut eggplants (*Solanum melongena* L.), which was carried out in two stages. Initially, the effect of citric acid (CA), ascorbic acid (AA) and cysteine (Cys) solution at 0.5 and 1% on sensory attributes (general appearance and browning), and color parameters during storage were evaluated. Immersion in 1% AA was considered the best antioxidant since it maintained visual quality for 6 days. Subsequently, hot water dipping (HWD) treatments followed by the 1% AA solution were evaluated and optimized through the Response Surface Methodology (RSM). Sensory attributes, color parameters, respiration rate (RR), phenolic compounds (PhC), antioxidant capacity, as well as the activity of polyphenol oxidase (PPO) and peroxidase (POD) were assessed during cold storage. The results showed that HWD at 50 °C, 60 s and 1% AA was the optimal combination to control enzymatic browning and extend its fresh quality for up to 8 days. Furthermore, that combination of treatments reduced the PPO and POD activities and increased the PhC compared to the control (untreated), not finding significant differences between them in antioxidant capacity and RR. Therefore, the application of this combination would be the most appropriate to preserve the quality of the fresh-cut eggplants for 8 days of storage at 4 °C.

Keywords:
Anti-browning treatments
Minimally processed eggplants
Quality
Shelf life

RESUMEN

Este estudio tuvo como objetivo evaluar el efecto de tratamientos antioxidantes en la conservación de berenjenas cortadas (*Solanum melongena* L.), el cual se realizó en dos etapas. Inicialmente, se evaluó el efecto de las soluciones de ácido cítrico (CA), ácido ascórbico (AA) y cisteína (Cys) al 0,5 y 1% sobre los atributos sensoriales (aspecto general y pardeamiento) y parámetros de color durante el almacenamiento. La inmersión en AA al 1% se consideró el mejor antioxidante ya que mantuvo la calidad visual durante 6 días. Posteriormente, se evaluaron y optimizaron los tratamientos de inmersión en agua caliente (HWD) seguidos de la solución de AA al 1%, mediante la aplicación de la metodología de superficie de respuesta (RSM). Durante el almacenamiento refrigerado, se evaluaron los atributos sensoriales, parámetros de color, tasa de respiración (RR), compuestos fenólicos (PhC), capacidad antioxidante, así como la actividad del polifenol oxidasa (PPO) y la peroxidasa (POD). Los resultados mostraron que HWD a 50 °C, 60 s y AA al 1% fue la combinación óptima para controlar el pardeamiento enzimático y prolongar su calidad de fresco hasta los 8 días. Además, esa combinación de tratamientos redujo las actividades de la PPO y la POD y aumentó los PhC respecto al control (sin tratar), sin diferencias significativas entre ellos en la capacidad antioxidante y la RR. Por lo tanto, la aplicación de esta combinación sería la más adecuada para conservar la calidad de las berenjenas recién cortadas durante 8 días de almacenamiento a 4 °C.

Palabras clave:
Tratamientos antipardeantes
Berenjenas mínimamente procesadas
Calidad
Vida útil

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The eggplant (*Solanum melongena* L.) is a non-climacteric vegetable belonging to the Solanaceae family containing mostly water and high phenolic content. Nowadays, this vegetable is marketed also as fresh-cut vegetable for its easy preparation and nutritional value (Barbagallo et al. 2012). However, postharvest processing, such as cutting or slicing, accelerates senescence and induces an increase in loss of commercial quality, reducing the shelf life of fresh-cut vegetables (Ansorena et al. 2014). Browning is the main cause of quality loss of fresh-cut eggplants, due to the action of enzymes such as polyphenol oxidase (PPO) and peroxidase (POD) mainly (Zhou et al. 2018), which causes the oxidation of phenolic compounds to o-quinones and subsequent brown pigments (Ghidelli et al. 2013). Therefore, reducing the PPO and POD activities is an important way to prevent browning.

Several studies have shown that low pH organic acids solutions (oxalic acid, ascorbic acid, citric acid, cysteine, kojic acid, oxaloacetic acid, erythorbic acid, 4-hexylresorcinol, calcium chloride) are used as antioxidants to inhibition/reduction of enzymatic browning, since they have a high capacity to inhibit PPO in fresh-cut products (Chiabrando and Giacalone 2012). Ascorbic acid (AA) is potentially effective in reducing enzymatic browning as it can convert soluble quinones to diphenols, and thus significantly reduce browning in treated products (Ali et al. 2021). AA was found to have been used as an anti-browning agent in mangoes (Lo'ay and Ameer 2019), lotus root slices (Ali et al. 2020a) and longan fruit (Khan et al. 2019). Oxalic acid (OA) is another organic acid that has anti-browning properties and has been applied in different horticultural crops, such as bamboo shoots (Zheng et al. 2019), longan (Khan et al. 2019), and slices of lotus root (Ali et al. 2020b). In this same way, it was found that citric acid retarded browning on litchi fruit pericarp (Ducamp-Collin et al. 2008), fresh-cut artichokes (Amodio et al. 2011), and apple slices (Chiabrando and Giacalone 2012). However, most of these chemical compounds have limited success because their inhibitory action is only temporary (Rojas-Grau et al. 2006).

On other hand, it has been recorded that heat shock treatments at 45-55 °C can be a good alternative to prevent browning since they can reduce the polymerization step of browning reactions (Aguayo et al. 2015). The hot water dipping (HWD) treatment is a technique that characterizes by being waste-free and easy to use (Aguayo et al. 2015). Thus, it was found that the use of HWD preserved the quality of fresh-cut apples (Bai et al. 2004) and onions (Siddiq et al. 2013). However, the negative effects of HWD can occur when using high temperatures and longer exposure times. Therefore, it is necessary to study the optimal processing conditions that improve conservation.

Besides, it has been reported that with the use of combined treatments an even greater extension of the shelf life can be achieved (Goyeneche et al. 2014), for example, Aguayo et al. (2015) found that the combination of heat treatments followed by immersion in calcium ascorbate helped maintain the quality of the fresh-cut apple. Similarly, the application of Ca solutions at 60 °C maintained the quality and texture of the fresh-cut melon (Silveira et al. 2011), eggplant cubes (Barbagallo et al. 2012), and the sliced carrot (50 °C, 1 min.) (Rico et al. 2007) compared to the hot water (control). However, different combinations of temperature and immersion time were not evaluated in these studies. Therefore, the objective of this paper was to evaluate different anti-browning solutions, and a combination of mild heat shock (HWD) with antioxidant dip to increase the shelf-life of fresh-cut eggplants.

MATERIALS AND METHODS

Experimental design

This work was divided into two parts: in the first evaluated the effect of dipping treatments with different antioxidants solutions upon eggplant cut into slices, while in the second the combined effect of HWD with the best antioxidant solution selected in the previous stage was study and optimized.

Response modeling

In the second part of this work the effect of two technological barriers (hot water and the antioxidant solution) were studied and modeled in function of the color and the organoleptic characteristics of fresh-cut eggplant slices through the Response Surface Methodology (RSM) using the Central Composite Design (CCD). The RSM is a group of statistical and mathematic techniques that can be used to define the relationships between independent variables and the response (Kumari and Farid 2020). The CCD selected is a full factorial with points to the center plus added star points that are used to model the curvature related to each factor and to construct a second-order response surface model (Van de Velde et al. 2016). The total number of experiments needed (N) of the CCD was determined using Equation (1) as follows:

$$N = 2^k + 2k + N_0 \quad (1)$$

Where k corresponds to the number of variables studied, in this case temperature and time (k=2). While 2^k, 2k and N_0 are the cubic, axial and center point runs, respectively. Therefore, N was calculated using Equation (1) and equals 11, corresponding to four factorial design points + four axial points + three replicates in the central point. Each variable was examined at five different levels ($-\alpha$, -1, 0, 1, and $+\alpha$) as follows: T=35.9, 40, 50, 60 and 64.1 °C whereas t=17.7, 30, 60, 90, and 102.3 s. The selection of the extreme levels was carried out in previous studies.

It was assumed that a mathematic function existed between the responses studied according to both variables related to HWD processing given in Equation (2).

$$Y = f(T,t) \quad (2)$$

A second order equation was used to model Equation (2) as shown in Equation (3) for each response:

$$Y= \beta_0 + \beta_T x T + \beta_t x\ t + \beta_{TT}\ x\ T^2 + \beta_{tt}\ x\ t^2 + \beta_{Tt}\ x\ T\ x\ t \quad (3)$$

Where: β_0, β_T, β_t, β_{TT}, β_{tt} and β_{Tt} are the regression coefficients while T and t are the variables under study. The responses studied (Y) on each eggplant slice were general appearance, browning and browning index.

Eggplants preparation

The eggplants (*Solanum melongena* cv. Black Nite) were obtained from a local producer (Santiago del Estero, Argentina). After washing them with chlorinated water (150 ppm, 3 min), they were drained and sliced 1 cm thick with a sharp stainless-steel knife. They were soaked in running water for 5 min to remove phenolic compounds and enzymes from the surface; eventually, the pieces were air-dried on a clean surface. The process was carried out in a room at a controlled temperature of 15±2 °C under highly cared hygienic sanitary conditions.

Preparation of samples and application of antioxidant solutions

The cut eggplants, with optimal visual characteristics, without tissue damage and without microbial growth, were randomly divided into seven batches to apply the antioxidant solutions. For this, they were immersed for 3 min at 4 °C in different antioxidant solutions (Sigma Chemical Co) at 0.5 and 1% w v^{-1}, of citric acid (CA), ascorbic acid (AA), cysteine (Cys) and water (control). They were then drained and packaged in a passive modified atmosphere as described below. In this stage the samples were analyzed at 0, 2, 4, 6 and 8 days of storage.

Preparation of samples and application of hot water treatments and antioxidant solution

To determine the optimal combination of HWD with the previously selected antioxidant, a Central Composite Design (CCD) was applied to define the different combinations of temperature and immersion time of the distilled water. Thus, a total of 11 tests were obtained with temperature variations between 35.9 and 64.1 °C and immersion times of 17.7 to 102.3 s. After HWD, the samples were immersed into a pH=4.5 buffer solution of 1% AA at 4 °C for 3 min and finally drained. Eggplant slices immersed in distilled water at 4 °C for 3 min were used as control.

Packaging and storage conditions

After treatments, three eggplants slices were manually packed in 17.4×13.8×4.8 cm polypropylene (PP) trays (Cellpack S.A., Santa Fe, Argentina) and heat-sealed with 35 µm thick bi-oriented PP film featuring an O_2-transmission rate of 5,000 mL m^{-2} 24 h^{-1} atm^{-1} and 18,000 mL CO_2 m^{-2} 24 h^{-1} atm^{-1} and a water vapor transmission rate of 110 g m^{-2} 24 h^{-1} atm^{-1} (data provided by INTI, Argentina) and then were stored at 4 °C.

Each fresh-cut eggplant package with three slices was considered as one experimental unit. Samples were immediately evaluated in the case of the determinations of sensory evaluation, color measurement and respiration rate, or immediately frozen at –80 °C (Ultrafrezzer Righi, Argentina) and stored until chemical analysis were performed. On each evaluation day, at least three packages per treatment were collected for analysis and the results were expressed as the mean values with SE.

Sensory evaluation

During storage, 10 trained judges evaluated visually the eggplant slices. The general appearance (such as shape, color, and texture) and browning were scored on a 1-9 scale, being one for poor and nine for excellent. Scores below five were considered the cut-off point for quality attributes (Ghidelli et al. 2013).

Color measurement

The surface color values of eggplants samples using a CR-300 Minolta Chroma Meter (Osaka, Japan) and the CIELAB color parameters, namely, L* (lightness), a* (red-green chromaticity), and b* (yellow-blue chromaticity) were determined. For that, a total of eight readings were taken from both sides of each eggplant slices from each experimental unit. The Browning Index (BI) was calculated as follows Equation (4) (Bal et al. 2011):

$$BI = \frac{(x - 0.31) * 100}{0.17} \qquad (4)$$

Where:

$$x = \frac{(a + 1.75 * L)}{(5.645 * L + a - 3.012 * b}$$

Respiration rate

The respiration rate (RR) of the control and optimized treatment samples was measured on days 0 and 8 of storage using a closed system. On each sampling day, three slices of eggplant (approximately 120 g) were placed within 1,000 mL glass jars and stored at 4 °C. The increase in CO_2 as monitored 0.5 h after closing the jars using a gas chromatograph (SRI 8610C, EUA), equipped with a thermal conductivity detector (150 °C), oven (80 °C), injector (150 °C), and Poropack-Q 80/100 column. He (20 mL min^{-1}) was used as the carrier gas. All measurements were conducted in triplicate and the CO_2 production was expressed in mL CO_2 kg^{-1} h^{-1}.

Phenolic compounds and antioxidant capacity

The phenolic compounds were determined as described by Concellón et al. (2004) with slight modifications. For this, a methanolic extract was prepared by crushing 1 g of frozen tissue with 10 mL of methanol, which was subsequently centrifuged and the methanolic supernatant extracted. Then, 350 µL of the methanolic extract was mixed with 50 µL of Foling-Ciocalteu and 500 µL of distilled water, allowing the mixture to react for 30 min and measuring the absorbance at 760 nm, using commercial chlorogenic acid as standard. The results were expressed in g kg^{-1} of fresh tissue.

To determine the electron donating capacity of the samples, the radical DPPH• was used, following the procedure described by Brand-Williams et al. (1995). Thus, 50 µL of the methanolic extract prepared as described above, was mixed with 500 µL of DPPH• in a methanolic solution. The absorbance was determined at 515 nm using Trolox as a standard, and the results were expressed in g kg^{-1} of fresh tissue. All determinations were made in triplicate using a JASCO V-630 spectrophotometer.

Determination of polyphenol oxidase (PPO) and peroxidase (POD) activity

The PPO activity was determined according to Concellón et al. (2004), grinding 5 g of frozen sample with 20 mL of phosphate buffer (0.05 M, pH=6.5, 4 °C), 10 g L^{-1} of polyvinylpolypyrrolidone (PVPP); and 0.1% v v^{-1} Triton X-100. The mixture was centrifuged at 15,000xg for 20 min at 4 °C and the supernatants were collected using as enzyme extracts. The reaction mixture was prepared from 50 µL of the extract with 1 mL of pyrocatechol (20 mM) in phosphate buffer (0.10 M, pH=6.5) and incubating at 25 °C. The absorbance was then measured spectrophotometrically at 410 nm.

The POD activity was analyzed according to the method described by Li et al. (2014) with slight modifications, mixing 4 g of sample with 25 mL of phosphate buffer (0.05 M, pH=6.5) for extraction. The homogenate was centrifuged at 15,000xg for 20 min at 4 °C and the supernatant (1 mL) was mixed with phosphate buffer (0.05 M, pH=6.5), 300 µL of 0.5% H_2O_2, and 0.1 mL of guaiacol 2%. The reaction mixture was incubated at 25 °C and the enzymatic activity was determined spectrophotometrically at 430 nm.

One unit of enzyme activity (UEA) was defined as the amount of extract that produces an absorbance change of 0.001 units per min under assay conditions. For both enzymes, the results were expressed as UEA per mg protein. Three extracts were prepared for each treatment and storage time.

Protein determination

The protein content was determined by Bradford's method (1976) in the different extracts using bovine serum albumin as the standard protein.

Statistical analysis

All experiments and analysis were carried out in triplicate. The statistical analysis was performed using the Infostat software in its 2011 release (National University of Cordoba, Argentina). The means specific differences were determined by the least significant difference (LSD) method applied following the analysis of variance (ANOVA); the significance of differences was defined at $P<0.05$.

RESULTS AND DISCUSSION

Selection of antioxidant treatment

Sensory evaluation

Figure 1 shows the general appearance and browning of fresh-cut eggplant slices treated with different antioxidants during storage at 4 °C. All the sensory parameters decreased as time of storage due to the natural oxidative processes which are particularly high in fresh-cut products (Amodio et al. 2011).

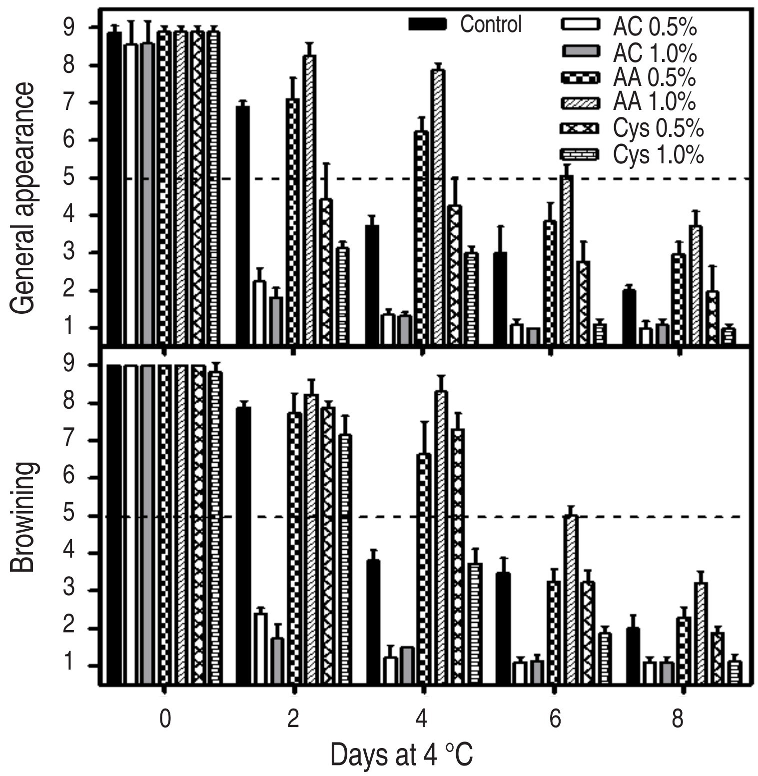

Figure 1. Effect of antioxidant type and concentrations on general appearance and browning of fresh-cut eggplants stored for 8 days at 4 °C. Data shown are mean ± standard error. LSD ($P<0.05$). General appearance=0.44. LSD ($P<0.05$) Browing=0.50.

Control samples reached the cut-off point before 4 days of storage, due to excessive browning, affecting the general appearance. Eggplant slices treated with AA at both concentrations presented very good scores on the general appearance and browning on day 4 of storage. However, on day 6, only the concentration of 1% allowed to preserve acceptable characteristics for commercialization, with values close to 5 (limit of acceptability). Therefore, the antioxidant effect of AA solutions was dependent on the concentration used. This agrees with what was reported by Ghidelli et al. (2014), who reported that the use of AA in persimmon at 1.12% and 2.25% allowed reaching a marketability limit of up to 7 and 5 days, respectively. Similarly, the same trend was observed in eggplant cv. Telma cut into cubes, since the 0.35 and 0.88% AA concentrations showed better results in color maintenance than the control (untreated), while 1.75% AA increased the browning of the fruits (Ghidelli et al. 2013).

The samples treated with 0.5 and 1% Cys presented inappropriate visual characteristics from day 2, with a decrease in their general appearance and a significantly higher browning than those treated with 1% AA. This effect is attributed to the action of Cys, which affected both the pulp, increasing browning, and the skin, since it modified the characteristic purple color of the fruit towards a reddish-pink, spreading towards part of the pulp that is in contact with the skin. These characteristics were similar to those recorded in pear slices treated with a 0.5% Cys solution as it caused an undesirable appearance due to the formation of pinkish-red pigments (Gorny et al. 2002; Siddiqui et al. 2020). Furthermore, this antioxidant in both concentrations was excluded as they also produced an unpleasant odor inside the package (data not shown).

On the other hand, CA at both concentrations (0.5 and 1%) was the antioxidant that caused the highest degree of browning and decrease in the general appearance in eggplants slices during storage resulting in a commercial shelf-life shorter than 2 days. However, in other products, CA treatment may be adequate to prevent browning such as persimmon, in which the application of a 0.21% CA solution allowed to preserve its quality for 7 days at 5 °C (Ghidelli et al. 2013). According to these sensory quality attributes, the AA 1% dipping treatment was considered as the best antioxidant solution since maintained the highest visual quality and least browning of fresh-cut eggplants up to 6 days of storage at 4 °C.

Color parameters

Different authors have used the values of lightness (L*) and browning index (BI) for describing browning in vegetables, the latter being a good indicator of the darkening of the samples due to increased pigments concentrations (Arias et al. 2008). The surface color parameters L* and BI for the eggplant slices treated with different antioxidant solutions were evaluated during storage (Figure 2). Browning was accompanied by a decrease in L* and an increase in BI.

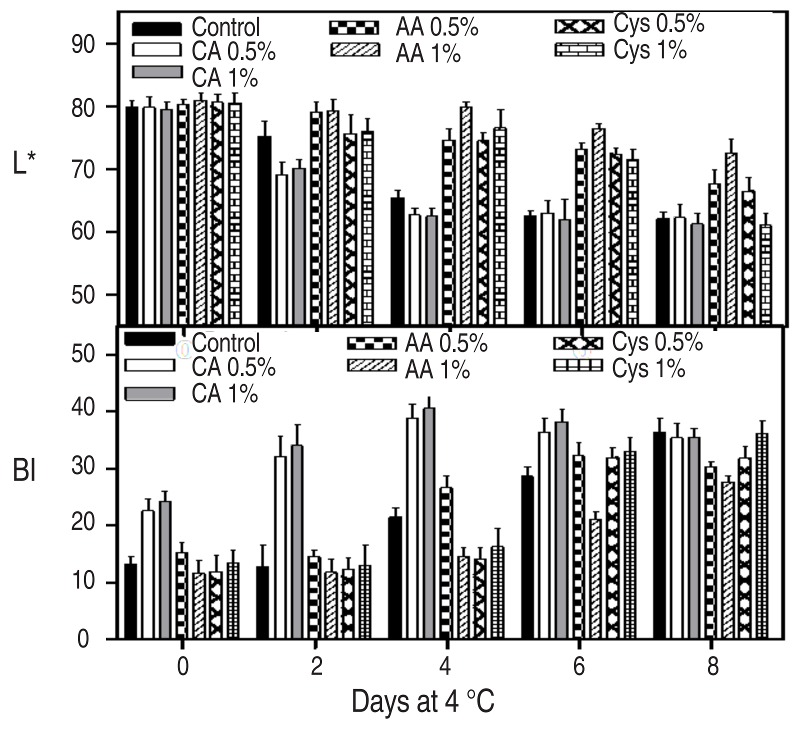

Figure 2. Effect of antioxidant type and concentrations on colorimetric parameters of lightness (L*) and browning index (BI) of fresh-cut eggplant stored for 8 days at 4 °C. Values are mean ± SD of three experiments and 24 readings at each time, n=72.

The control showed a decrease and a significant increase in L* and BI, respectively, during the first 6 days, remaining unchanged afterwards, the eggplants presenting a very high superficial darkening at the end of the storage. The treatment with 1% AA allowed to control enzymatic browning during first 4 days at 4 °C, since that maintained stable values of L* and BI parameters. The 0.5% AA treatment was not adequate to reduce browning, as it presented values of L* and BI significantly lower and higher respectively, after 2 days with respect to the concentration of 1%. Using AA as inhibitor of enzymatic browning in vegetables is widely used since reduces the change of quinones into non-colored phenolic compounds (Ghidelli et al. 2014; Chiabrando and Giacalone 2012). In this regard, very good results on browning inhibition and shelf-life prolongation were reported in litchi fruit (Ali et al. 2021), diced eggplants (Ghidelli et al. 2014) and fresh-cut apples (Chiabrando and Giacalone 2012) treated with AA compared to untreated samples.

Cys was also effective in controlling browning in the eggplant slices, showing practically stable BI values during the 4 days of storage. However, the samples presented lower L* and higher BI values than those immersed in 1% AA from 6 days of storage. This may be due to discoloration in the skin fresh-cut eggplants that appeared with an intense reddish-pink color which spread to the pulp. These results do not agree with what was reported by Ghidelli et al. (2014) since 1% Cys was considered the most effective antioxidant to reduce browning of cubed eggplant. However, in that study an organoleptic evaluation was not carried out and no changes in odor or reddish coloration were reported due to the absence of skin in the samples. On the other hand, CA at concentrations of 0.5 and 1% was the antioxidant that caused the highest degree of browning in fresh-cut eggplants even higher than the control samples, presenting the lowest L* and the highest BI values during 4 and 6 days at 4 °C respectively, and remained stable until the end of the storage. This could be due to the fact that in certain cases the CA in the presence of ascorbic acid typical of the vegetable, would participate in the formation of brown pigments, either acting as a catalyst for the oxidation of ascorbic acid, a catalyst in the accumulation of brown pigments, influence in the process of decomposition of ascorbic acid to a variety of carbonyls, or else behave similarly to amino acids and react with some products of ascorbic acid oxidation (Clegg 1966; Zeki 2016). These results obtained agree with Ghidelli et al. (2014) who worked with fresh-cut eggplants (cv. Telma) and found that the CA solutions increased browning and reduced L* values with respect the control.

These results of color parameters were in agreement with the sensory attributes of general appearance and browning. Therefore, the data collected demonstrated that the 1% AA solution was effective in reducing browning in fresh-cut eggplants for 6 days of storage at 4 °C.

Optimization of HWD treatment combined with antioxidant solution

In accordance with the previous results, the 1% AA solution was effective in controlling browning in fresh-cut eggplant up to 6 days of storage at 4 °C. From this, a second experiment to evaluate the use of combined HWD treatments followed by immersion in 1% AA as a double barrier to reduce the enzymatic browning of fresh-cut eggplant and preserve for a longer time. To optimize the process, the RSM was used and through the application of a CCD, different combinations of temperatures and immersion times of the HWD were established, evaluating the general appearance, browning and BI at day 8 of storage, whose results are presented in Table 1. On the basis of these results, three-dimensional response surface plots were generated to demonstrate the effects of exposure times and temperatures on the general appearance, browning, and BI of fresh-cut eggplants.

Sensory evaluation

Figure 3A shows the effects of HWD immersion times and temperatures followed by 1% AA immersion on the overall appearance of fresh-cut eggplants at 8 days storage at 4 °C. Both temperatures and times significantly ($P<0.05$) affected this attribute. As can be seen, treatments with temperatures above 60 °C and below 40 °C caused a significant decrease in the overall appearance scores of the treated samples. Similarly, immersion times greater than 80 s and less than 40 s also produced significant reductions in scores for this attribute. This may be because prolonged temperatures and times could damage the tissue and thereby affect the visual quality of the eggplant during storage. For example, Aguayo et al. (2015) discovered that high temperatures induce damage to the surface of fresh-cut apples by decreasing the L* parameter and color (hue angle and chroma). On the contrary, low temperatures and times did not generate a positive effect on the conservation of the product, which may be associated with the fact that temperatures below 40 °C do not inhibit the PPO enzyme in this product, according to what was reported by Concellón et al. (2004). When analyzing the browning, it is concluded that its behavior was similar to that of the general appearance (Figure 3B), since temperatures below 40 °C and above 60 °C and immersion times below 40 s and above 80 s caused a significant decrease in the scores of this parameter.

Table 1. Analysis of variance and regression coefficients of reduced models of temperature and times of immersion.

Model parameters	Browning	General Appearance	BI
R^2	0.99	0.95	0.98
P<	0.05	0.05	0.05
Coefficients[a]			
β_0	0.434[a]	-2.982[a]	91.17[a]
β_T	0.182[a]	0.312[a]	-2.584[a]
β_t	0.027[a]	0.046[a]	-0.345[a]
β_{TT}	-0.002[a]	-0.0031[a]	0.026[a]
β_{tt}	-0.0002[a]	-0.0004[a]	0.003[a]
B_{Tt}	0.00002	0.00015	0.00004

[a]Coefficients: β_0=Independent, T=temperature (°C), t=immersion time (s).

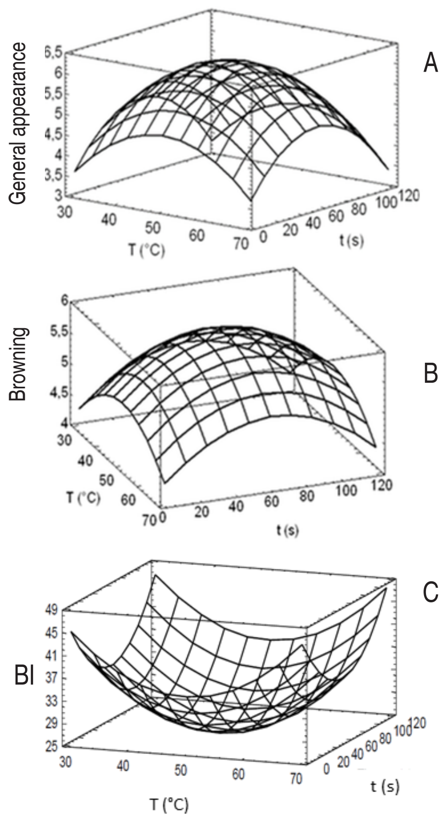

Figure 3. Effects of hot water dipping treatment temperatures (°C) and immersion times (s) on the A. general appearance. B. browning and C. BI of fresh-cut eggplants at 8 days of storage at 4 °C.

Based on this experiment, heat treatment with an intermediate temperature and time ratio of 50 °C and 60 s respectively was selected as the best combination to maintain visual quality and reduce the browning of fresh-cut eggplants for 8 days. This agrees with what was reported by Constant et al. (2021), who reported that heat treatment at 65 °C for 30 min considerably reduced browning by inhibiting PPO activity, as well as the use of the antioxidant ascorbic acid, reducing this enzyme by approximately 80% in whole eggplants. In this way, Loaiza-Velarde et al. (2003) also discovered that applying heat shock at 50 °C for 90 s delayed the browning potential increase in fresh-cut celery petioles by three weeks.

The results show that the combination of HWD (50 °C, 60 s) followed by immersion in 1% AA solutions, delayed the browning of the tissue and maintained the visual quality of the fresh cut eggplants for up to 8 days at 4 °C, while than individual 1% AA treatment for only 6 days.

Color parameters

Thermal treatments had a relevant impact on the BI of fresh-cut eggplants (Figure 3C). As can be seen, treatments with temperatures above 60 °C and below 40 °C caused an increased significant BI values. In the same way, immersion times below 40 s and above 80 s caused the same result. Therefore, a thermal treatment with an intermediate ratio temperature and time of 50 °C and 60 s to maintain the color of fresh-cut eggplants during storage was select. In addition, these results were consistent with those obtained in the sensory evaluations performed by the trained panel.

In that same sense Goyeneche et al. (2014) reported similar results in sliced radish treated by dipping with hot water at 50 °C for 1 min followed of 0.3% CA. On the other hand, Aguayo et al. (2015) reported that treatments with hot water (48 °C, 2 min) followed by 6% of Ca ascorbate showed a greater reduction of browning than when treated with hot water without Ca ascorbate in fresh-cut apples. Based on the analysis of these parameters, the combination of HWD (50 °C, 60 s) followed by immersion in solutions of 1% AA, were determined as the optimal conditions for maintaining the color of fresh-cut eggplants during 8 days of storage at 4 °C.

Respiration rate

Figure 4 shows the respiration rate (RR) of fresh-cut eggplants with the optimized antioxidant treatment and control (untreated) during storage at 4 °C. This parameter tended to increase during storage no observing significant differences observed among them, indicating that the applied treatment did not induce additional tissue damage and that the plant tissue is still alive. However, different authors reported that the application of thermal treatments reduced the RR of various fruits and vegetables (Ansorena et al. 2014).

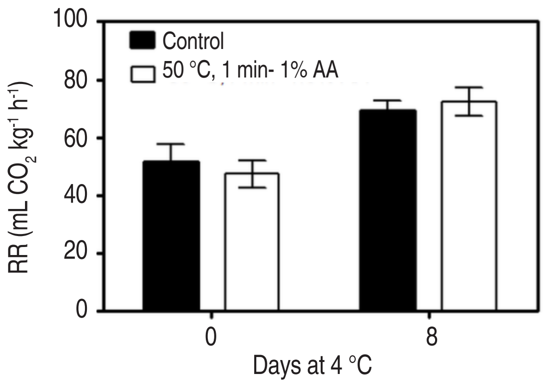

Figure 4. Effect of optimized treatment HWD+AA (50 °C, 1 min+1% AA) on respiration rate (RR) of fresh-cut eggplants stored at 4 °C for 8 days. LSD (*P*<0.05)=5.7.

Phenolic compounds and antioxidant capacity

Phenolic compounds (PhC) are a group of secondary metabolites belonging to the plant kingdom, of great functional value due to their antioxidant action (Goyeneche et al. 2015). As a result of the application of optimized treatment, PhC values were initially significantly higher than those of the control by approximately 20% (Table 2). At the end of storage, the values decreased in the control, while in the treated samples remained practically unchanged. Likewise, in studies carried out Barbagallo et al. (2012) with "Birgah" eggplants cut into cubes and treated with a 60 °C solution of citrate and calcium ascorbate, an increase in the PhC content was also reported.

Regarding antioxidant capacity measured by DPPH• (Table 2) no significant differences were observed (*P*>0.05) between the treatment and the control during the time evaluated. This probably is associated with its high antioxidant capacity because the high content of phenols in eggplants, and also that possibly other substances of a different nature than polyphenols contribute to the antioxidant capacity, such as anthocyanins, beta-carotenes, etc. However, these results differ from what was observed in radish slices treated with hot water (50 °C, 1.5 min) followed by immersion in a 2% AA solution, in which it was determined that the antioxidant capacity was always significantly higher than the untreated samples (Goyeneche et al. 2015).

Table 2. Phenolic compounds (PhC), antioxidant capacity (DPPH•), and enzyme activity PPO and POD for the control and treated samples during cold storage.

Storage time (days)	Treatments	
	Control	50 °C-1min, 1% AA
PhC (EAC g kg^{-1} FW)		
0	14.50±0.71a^{B}	17.30±0.60a^{A}
8	11.02±0.39b^{B}	16.00±0.80a^{A}
DPPH (g TROLOX kg^{-1} FW)		
0	0.76±0.01a^{A}	0.76±0.01a^{A}
8	0.66±0.01b^{A}	0.69±0.04b^{A}
PPO (UEA mg prot^{-1})		
0	6.90±0.77b^{A}	3.60±0.35a^{B}
8	10.80±0.80a^{A}	3.20±0.61a^{B}
POD (UEA mg prot^{-1})		
0	3.10±0.08b^{A}	2.20±0.05a^{B}
8	4.21±0.05a^{A}	2.51±0.04a^{B}

Mean values in the same row with different superscript letters or in the same column for same type of sample with different lowercase letters were significantly different according to LSD test at *P*<0.05.

Polyphenol oxidase and peroxidase activity

During the minimal processing of fruits and vegetables, a series of deterioration reactions are generated that involve enzymes that act on the nutrients and deteriorate their quality. Among these enzymes, we can mention the PPO and POD. PPO acts mainly in the oxidative processes of phenolic compounds, while POD participates in various deterioration reactions (Siddiqui et al. 2020). Therefore, researchers have reported that increases in POD activity may be associated with increased browning (Ciou et al. 2011).

Table 2 shows the effect of the optimized antioxidant treatment on the PPO enzyme activity of the fresh-cut eggplants. Initially, PPO activity was significantly reduced by 50% in treated samples compared to untreated samples (control). Subsequently, it increased significantly for the control, however the PPO activity of the treated samples remained without significant variations. Regarding the POD activity, the Table 2 shows that it had a similar trend to that registered for PPO. According to ANOVA, the treated samples showed a significant reduction (*P*<0.05) in their activity compared to the control, which was maintained for 8 days, relating these values to that of PhC since they registered higher levels than the control. These results are in agreement with Goyeneche et al. (2015), who reported that immersion in hot water at 60 °C for 1.5 min followed by immersion on 2% AA were effective to reducing and maintained the PPO activity during 7 days of storage in fresh-cut radish. Similarly, the beneficial effect of heat treatment on browning was related to a reduction in PPO activity in apple cut into slices (Bai et al. 2004). Furthermore, AA has good potential to reduce the incidence of browning by suppressing PPO and POD activities (Ali et al. 2021).

Therefore, the combined treatment optimized through this work was effective in reducing the browning of the fresh cut eggplant slices, since it reduced the activity of PPO and POD allowing to maintain visual quality, and thus extending the shelf life up to 8 days at 4 °C.

CONCLUSIONS

Dipping treatments in a solution containing 1% AA was considered as the best antioxidant solution, since can effectively reduce browning of fresh-cut eggplants up to 6 days of storage at 4 °C. However, the combined application of HWD at 50 °C for 60 s followed by dipping in solution of 1% AA, allowed control for longer time the browning of fresh-cut eggplants without significantly affecting the RR, extending their shelf life up 8 days at 4 °C. The positive effect of HWD+1% AA on browning control and color retention of eggplant slices was related to low levels of enzyme activity of PPO and POD. Furthermore, the phenols content was significantly increased by applying this optimized treatment, but without effect on its antioxidant capacity. These results suggest that hot water dipping treatment (50 °C, 60 s) combined with 1% AA can be considered a good alternative to maintain the overall appearance and browning control of fresh-cut eggplants.

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Molecular prevalence of Bovine Leukemia Virus in specialized dairies in the department of Antioquia, Colombia

UNIVERSIDAD NACIONAL DE COLOMBIA

Prevalencia molecular del Virus de Leucosis Bovina en lecherías especializadas del departamento de Antioquia, Colombia

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ABSTRACT

Dairy production systems are a sensitive sector of the primary economy frequently affected by pathogens that negatively impact production parameters, the bovine leukemia virus (BLV) one of these. In this study, the molecular prevalence of BLV was determined in the specialized dairy sector of Antioquia using the viral marker of the envelope gene *(env)*. Blood samples were taken from 575 bovines from specialized dairies in Antioquia distributed in 53 herds and located in the three specialized milk production areas of Antioquia (north, east, and Valle de Aburrá). DNA extraction was performed by salting out, and a nested PCR was performed to detect the *env* gene. The products were visualized on a 2% agarose gel with GelRed as an intercalator. A molecular prevalence of BLV of 17.0% in animals and 71.7% in herds were found, being Valle de Aburrá the area where the highest rate of positive animals was obtained (21.1%), unlike the northern area with the lowest rate (15.6%). The molecular prevalence of BLV in this study is lower than that of previous studies in the department, which ranged between 47 and 73%, and this may be associated with factors of breed resistance, the age of the animals, or management practices in the herds. These results can contribute to creating BLV control strategies and optimizing milk production in the department of Antioquia, being relevant to paying attention to the behavior of this pathogen under different production system conditions.

Keywords:
DNA
Enzootic bovine leukemia
Lymphocytes
Prevalence
Polymerase chain reaction
Retrovirus

RESUMEN

Los sistemas de producción de leche son un sector sensible de la economía primaria, viéndose frecuentemente afectados por patógenos que impactan negativamente parámetros productivos, siendo el virus de la leucosis bovina (BLV) uno de estos. En este estudio, se determinó la prevalencia molecular a BLV en la lechería especializada de Antioquia mediante el marcador viral del gen de la envoltura *(env)*. Se tomó muestra de sangre de 575 bovinos de lecherías especializadas de Antioquia distribuidos en 53 hatos, ubicados en las tres zonas de producción de leche especializada de Antioquia (norte, oriente y Valle de Aburrá). Se realizó extracción de ADN por Salting out y se realizó una PCR anidada para detección del gen *env*. Los productos se visualizaron en un gel de agarosa al 2% con GelRed como intercalante. Se encontró una prevalencia molecular a BLV del 17,0% en animales y 71,7% en hatos, siendo el Valle de Aburrá la zona donde se obtuvo la mayor tasa de animales positivos (21,1%), a diferencia del norte que tuvo la más baja (15,6%). Se halló que la prevalencia molecular a BLV de este estudio es menor a la de investigaciones previas en el departamento, la cual se encontraba entre el 47 y 73%, y esto puede estar asociado a factores de resistencia raciales, edad de los animales o las prácticas de manejo en los hatos. Estos resultados pueden contribuir a crear estrategias de control del BLV y optimizar la producción lechera en el departamento de Antioquia, siendo relevante poner atención al comportamiento de este patógeno bajo las diferentes condiciones que tienen los sistemas de producción.

Palabras clave:
ADN
Leucosis Bovina Enzoótica
Linfocitos
Prevalencia
Reacción en cadena de la polimerasa
Retrovirus

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The specialized milk production systems with bovines constantly face the presence of pathogens that affect the sanitary conditions and the economy of herds. One of these pathogens is bovine leukemia virus (BLV), whose target cells are B lymphocytes, causing enzootic bovine leukemia (EBL), which affects the immune system of animals causing immunosuppression and allowing entry to other pathogens (Bartlett et al. 2020; Marawan et al. 2021). Most animals are asymptomatic to the disease; only 30% of infected animals develop persistent lymphocytosis and only 1-5% develop lymphomas (Barez et al. 2015). In addition to affecting the health of animals, it generates a detriment in the quantity of milk produced and the compositional and sanitary quality of the milk, having adverse effects on the economy of dairy herds (Marawan et al. 2021; Úsuga-Monroy et al. 2018a).

The BLV belongs to the Retroviridae family; these viruses are characterized by transcribing their genome from RNA to DNA through the reverse transcriptase enzyme and inserting themselves into the host cell genome (Polat et al. 2017). When this occurs, the virus is renamed provirus; infections of this type are permanent. BLV transmission occurs through contact with fluids that have the presence of infected cells (blood, colostrum, and milk) from an infected animal to another healthy one, for which good management practices in production systems are vital to prevent virus transmission (Bartlett et al. 2014; Ruiz et al. 2018). It has also been found that it can be transmitted in the womb and at the time of delivery (Kuczewski et al. 2021).

The viral genome consists of four primordial genes that code for structural proteins and enzymes (*env, gag, pol,* and *pro*). In addition, it has two regulatory genes (*tax* and *rex*) and the R3 and R4 genes that code for accessory proteins, and a part that encodes microRNAs. The envelope gene *(env)* encodes proteins *gp30* and *gp51,* which play a fundamental role in the infection process, are the recognition center of B lymphocytes and modulate the immune response, so changes in their structure can affect the infective capacity of the virus (Canova et al. 2021; de Brogniez et al. 2016). Therefore, it is a highly conserved genome region and is the most used worldwide for virus detection and genetic research (Blazhko et al. 2020).

In 2016, the molecular prevalence of BLV in animals of the Holstein breed of the department of Antioquia was evaluated, finding 47.6% of positivity (Úsuga-Monroy et al. 2018c). Two years later, in a study carried out in six regions of the country, 73% of positivity was obtained in Antioquia, but only one municipality was evaluated (Corredor-Figueroa et al. 2020). Thus, this is the first extensive study that covers different breeds and geographical regions of specialized dairy in Antioquia. Although various studies have shown the implications of BLV in milk production systems (Bartlett et al. 2014; Úsuga-Monroy et al. 2018a), the virus is not included among the mandatory control diseases in the country by the Colombian Agricultural Institute (Instituto Colombiano Agropecuario - ICA), although, since 2015 and according to Resolution No. 003714 of 2015 (Instituto Colombiano Agropecuario 2015), it is mandatory to declare if its presence is found.

The lack of control mechanisms and knowledge regarding this pathogen by producers have contributed to the increase in the number of positive animals, facilitating the circulation of BLV within dairy herds. Milk production is a sensitive sector of the national primary economy, and the department of Antioquia is one of the leading milk producers in the country, with three regions (north, east, and Valle de Aburrá) where this activity prevails for the peasant economy. With this context in mind, the aim of this study is to evaluate the prevalence of BLV in the main dairy breeds and their crosses in the specialized dairy herds of this department.

MATERIALS AND METHODS

Experimental design

The study population consisted of 224,714 mechanical and traditional specialized dairy milking cows from Valle de Aburrá and the north, and east regions of Antioquia, Colombia, the largest milk-producing regions in the department (Secretaría de Agricultura y Desarrollo Rural, 2019). The sample size was calculated from the study population, with an expected molecular prevalence of 47% (Úsuga-Monroy et al. 2018c), a relative error of 10% (i.e., an absolute error of 4.4%), and a design effect of 1.2, obtaining a sample size of 575 bovines that were selected in 16 municipalities of the three regions mentioned above. The number of bovines sampled per herd was proportional to the sample size and the total number of bovines in the herd; these were selected randomly. Each herd was given a list of bovines that were selected.

Ethical aspects: Ethics Committee

This research is part of the macroproject "Bovine leukemia virus in dairies of Antioquia: Evaluation of the zoonotic potential and the effect on reproductive performance", endorsed by the Institutional Committee for the Care and Use of Animals (CICUA) of Universidad Nacional de Colombia "020-2020". For the development of the project, no permits or licenses were required. However, in the execution of the project, the legislation and other current regulations pertaining to it in terms of ethics, environmental regulations, or access to genetic resources were complied with. The permits for taking blood samples from the animals and obtaining the required information from each herd were processed with the owners of the herds included in the research, who signed a consent.

Blood sampling

Blood samples were taken from the middle coccygeal vein with an 18G needle with a vacutainer vacuum system (VACUETTE®) and tubes with EDTA as an anticoagulant to obtain the DNA of the nucleated peripheral blood cells. All samples were homogenized by inversion and transferred to the Animal Biotechnology Laboratory of Universidad Nacional de Colombia, Medellin, under refrigeration conditions (4 °C).

DNA extraction

Blood samples with anticoagulant were centrifuged to obtain the layer of white cells from which the DNA was extracted using the salting out technique (Miller et al. 1988). The DNA obtained was resuspended in TE 1X pH=8.0 buffer (1 M Tris HCl and 0.5 M EDTA) and stored at 4 °C until analysis. The sample was run on a 1% agarose gel stained with SYBR-safe to verify the integrity of the extracted DNA. Extracted DNA was quantified in a spectrophotometer (NanoDrop2000®, Massachusetts, United States) at 260 and 280 nm optical densities to verify its concentration as well as the quality through the 260:280 ratio. In addition, a PCR for a bovine constitutive gene (Toll-like receptor 6) was performed with random samples to verify that there were no inhibitors in the samples.

Molecular analysis for the detection of BLV in cattle

Controls

As a positive control for the PCR tests, a mixture of DNA from bovines previously diagnosed positive serologically by the ELISA technique and by PCR for the *env* gene by the BIOGEM group was used. Negative controls were performed with a reaction in the absence of DNA and with DNA from a bovine previously diagnosed by serology and molecularly as negative for BLV infection.

Nested PCR of the *env* gene

The PCR to detect the BLV *env* gene was performed in a final volume of 25 µL with 150 ng of DNA, 0.2 mM of dNTPs, 1 IU of Taq polymerase, 1X of buffer, and 1.5 µL of 10 mM of each envFw and envRv oligonucleotide (Fechner et al. 1996) for the first reaction, and envFW2 and envRV2 (Fechner et al. 1996) for the second reaction (Table 1). The reaction conditions for the two PCR cycles were 5 min at 94 °C, 40 cycles of 30 s at 94 °C, 30 s at 60 °C, 1 min at 72 °C, and 5 min at 72 °C. At the end of the second reaction, a 444 bp fragment of the BLV provirus *env* gene was obtained in the positive animals. Agarose gel electrophoresis was performed to observe the PCR results in which the positive animals for the BLV exhibited a band at the height of 444 pb.

Table 1. Primers used for diagnostic PCR to BLV.

Name	Nucleotide sequences	Expected band size (pb)
envFw	TCTGTGCCAAGTCTCCCAGATA	598
envRv	AACAACAACCTCTGGGGAGGGT	
envFw2	CCCACAAGGGCGGCGCCGGTTT	444
envRv2	GCGAGGCCGGGTCCAGAGCTGG	

Statistical analysis

The results of the PCR were integrated into an Excel database with the information of the sampled animals (breed, age, parity, region and herd), and descriptive statistics were used to analyze the results. The qualitative traits were summarized in absolute and relative frequencies, estimating 95% confidence intervals. Quantitative variables were summarized using the mean and standard deviation or median with quartiles, depending on data normality. The Chi-square test (X^2) was performed to determine the possible association between breed and the presence or absence of the virus. To establish the association between breed and the presence of BLV, breeds and breed types were grouped into: Holstein x Crossbreed: where crossbred can be Viking Red, Ayrshire, BON, Brown Swiss, Normande, Angus; Other purebred: where it can be: Jersey, Ayrshire, BON, Brown Swiss, Viking Red and Other crossbreeds: where there were more than three crossbreds. And they were analyzed through an Odds ratio (OR) by means of a 2x2 contingency table. It was determined that if the OR is more than one, the breed or crossbreed has a low risk of being positive for BLV and if the OR is less than one, the breed or crossbreed has a high risk of being positive for BLV. The significance of the data was accepted as $P<0.05$. OR analysis was performed using Epi Info™ v7.2 CDC software.

RESULTS AND DISCUSSION

The fragment amplified in the PCR corresponds to the region that codes for the surface protein *gp51*, vital in the infectivity cycle of the virus since it is responsible for the recognition of the receptor and the binding of the virus to the host cell. Therefore, it is decisive to know the range of virus hosts (Gao et al. 2020). The primers selected to carry out the nested PCR of the *env* gene have been used in various diagnostic and genotyping studies of the virus since 1996 due to the low genetic variability of this region concerning other genome regions (Fechner et al. 1996; Polat et al. 2017).

The results obtained in this study for the 575 bovines belonging to the specialized dairy in Antioquia show that the prevalence of bovine leukemia virus in the department is 17.04% (Table 2), i.e., lower than the results obtained in previous studies (Corredor-Figueroa et al. 2020; Ortiz et al. 2016; Úsuga-Monroy et al. 2018c). This result may be due to the fact that this is the first extensive study carried out in the region where cattle of different racial components (Holstein, Jersey, Viking Red, Ayrshire, and their Jerhol crosses and trihybrids, among others), age groups (calves, heifers, and cows in production) and geographical regions of the department were sampled. Furthermore, this research contrasts with previous studies where only the population of one breed (Úsuga-Monroy et al. 2018c), a municipality, or an age group were sampled (Corredor-Figueroa et al. 2020; Ortiz et al. 2016).

Table 2. Molecular prevalence to BLV of the *env* gene.

Result	Number of animals	Percentage (%)
Negative	477	82.96
Positive	98	17.04

The low prevalence may also be the result of improved sanitary measures in specialized dairy herds in the department, the implementation of good livestock practices, and biosecurity protocols in the herds that prevent the transmission of the virus, such as the use of one needle per animal, effective as a control strategy for the BLV (Kuczewski et al. 2019). In Colombia, since 2012, the payment of raw milk to producers dependent on compositional and hygienic factors has been implemented, finding that this measure has been effective in stimulating progress in production systems (Carulla and Ortega 2016) and is directly related to the improvement of the sanitary scheme of the production systems and the general management of the animals in it. Nevertheless, the prevalence in herds was relatively high (71.7%) in this study, indicating that in more than half of the productive systems, there is at least one infected animal, existing the risk of an increase in the number of infected animals by contagion. Therefore, it is necessary to evaluate what factors can increase or decrease the risks of spreading the disease in the dairy systems of the department. It is well known that practices such as using shared needles for the application of medicines are one of the main risk factors for the infection of bovines in herds (Kuczewski et al. 2021; Ortiz et al. 2016). Moreover, it is essential to transmit information to producers, creating control strategies to contribute to the progress of this productive sector.

In the department of Antioquia, the main breeds found in specialized dairies are Holstein and Jersey. Crosses between them and with other reference breeds are also widely used when analyzing prevalence data according to racial components (Table 3). It can be inferred that this factor may be influencing the results obtained since the categories Holstein x (Cross) and other crossbreeds are associated with an increased risk of being infected with BLV. There is evidence that genetic factors can make animals susceptible or resistant to diseases. An example is the Colombian Blanco Orejinegro (BON) breed, where some studies have determined the resistance/susceptibility of these individuals to some viral infections such as foot-and-mouth disease and vesicular stomatitis (Lopez-Herrera et al. 2000, 2002).

In the case of EBL, it has been found that the molecular prevalence of the virus is lower in BON compared to data from pure breeds (Herrera et al. 2011; Úsuga-Monroy et al. 2018b). The heritability of this susceptibility factor to the bovine leukemia virus infection among Holstein and Jersey cattle populations has also been estimated at 8%, indicating that genetics plays an important role in the incidence of BLV in different cattle breeds (Marawan et al. 2021). In order to analyze the breed factor, several categories were combined to determine whether or not the racial factor has an influence on BLV infection. These results can be a precedent for later studies where this hypothesis was evaluated in depth. It should be noted that the results obtained on the prevalence of BLV in the specialized dairy in Antioquia for the different racial components are not sufficient to make comparative statements between susceptibility/resistance to BLV infection due to the number of individuals for some breeds. However, to establish the association between the breed and the presence of BLV, the following groupings were made: Holstein x Cross, others purebreds and other crossbreeds and compared with the Holstein breed (Table 3). It was identified that the racial component (Holstein x Cross and other crossbreeds) does influence the probability of not having BLV infection (P<0.05) compared to the Holstein breed. Therefore, these results show a trend that generates the hypothesis that there may be breeds with greater resistance to infection by the virus in the Antioquia dairy, however, our data do not allow us to establish individually which breed or breeds are more resistant to BLV infection.

Table 3. Odds Ratio (OR) for other breeds and crosses with respect to the Holstein breed.

Category	OR[1]	95% Confidence interval		*Pr*>Chisq
Holstein x (Cross[2])	2.4528	1.2739	4.7229	0.006*
Others purebreeds[3]	1.766	0.8145	3.8292	0.146
Other crossbreeds[4]	2.4528	1.4608	4.1187	0.0005*

[1]OR>1, P<0.05: probability of resistance to BLV; OR>1, P<0.05: probability of susceptibility to BLV. [2]Viking Red, Ayrshire, BON, Brown Swiss, Normande, Angus. [3]Jersey, Ayrshire, BON, Brown Swiss, Viking Red. [4]More than three crossbreds.

It has been found that in the bovine major histocompatibility complex (BoLA), with a fundamental role in the presentation of antigens and the immune response of the organism against infection, there is a genetic region (*BoLA-DRB3*) that influences the development of BLV infection (Lo et al. 2020; Notsu et al. 2021). Most cattle with specific mutations in the allele (*DRB3*009:02*) maintain undetectable proviral loads and are positively related to the development of lymphomas (Lo et al. 2020). Identifying these genetic variations associated with resistance to BLV infection helps develop control programs based on the genetic selection of animals with these genes (Andoh et al. 2021). Studies recommend the evaluation of this factor in BLV positive and negative bovines from dairies in Antioquia to determine if it is the determining factor for the low molecular prevalence in the population studied.

It has been found that the association between BoLA-DRB3 gene polymorphisms and BLV infection varies between breeds (Hernandez et al. 2018; Lo et al. 2021a), supporting the hypothesis that some breeds are more susceptible than others to infection with BLV. Moreover, environmental factors also relate to the susceptibility/resistance that cattle may have (Andoh et al. 2021). Therefore, more in-depth studies are needed to test this hypothesis as, so far, only animals heterozygous for BoLA-DRB3 have been shown to have advantages against BLV infection regardless of their breed (Hernandez et al. 2018; Lo et al. 2021b).

Another factor that can be decisive in the prevalence results of BLV in this study is the age of the sampled animals. Other studies have found that the number of infected animals increases as age increases (Murakami et al. 2011). Calves are less likely to be infected, as the main transmission route of the virus is iatrogenic in production systems (Ruiz et al. 2018). It is until after the first calving that prevalence rates increase due to increased exposure to the virus. Nevertheless, the presence of the virus has been found in calves because there is a risk of transmission during pregnancy and birth and later with milk consumption (Marawan et al. 2021; Ruiz et al. 2018). It should also be considered that the onset of the persistent lymphocytosis stage in infected cattle generally occurs before 5 years of age, and after that, between 5 and 8 years of age, it passes to the lymphoma stage (Zyrianova and Kovalchuk 2019). Therefore, many adult cattle individuals may be positive for viral infection and show positive serological tests but are negative for the molecular detection test by PCR since BLV-positive cells may be sequestered in lymphomas and not be circulating in the blood.

When detailing the seropositivity of the study samples by the number of calvings (Figure 1), it is observed that as the number of calvings increases, the prevalence rate also increases, reaching the highest percentage in the group of cows from four to six calvings (20.7%). Further, the positivity rate decreases in the interval of more than seven calvings, possibly due to the sequestration of BLV-positive cells in internal tissues. On the other hand, it is known that most of the cattle in specialized dairies throughout the country are discarded before reaching seven calvings due to the reduction in productive traits. It is then possible that the lower prevalence in this range of calvings is because the animals that continue in the system after seven calvings have good health conditions, and their productivity has been efficient throughout their lives. Knowing the effects of BLV infection on productive and reproductive characteristics, the possibility that these animals were not infected with BLV during their productive life and, therefore, have not been discarded should be considered.

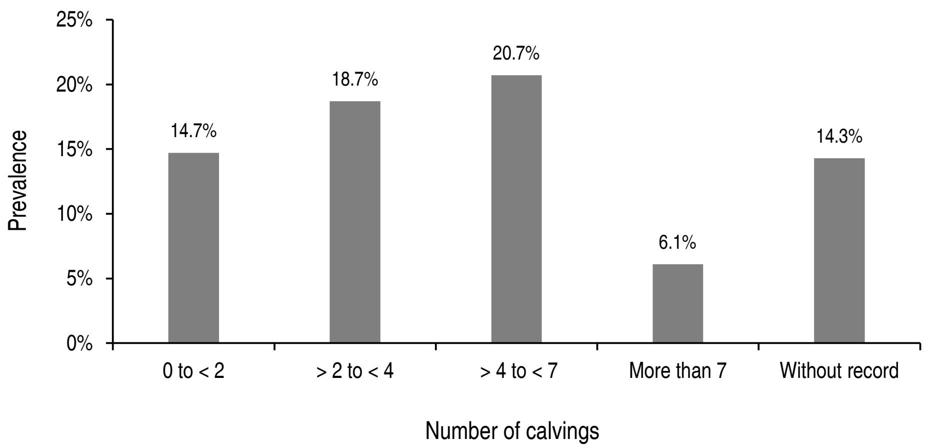

Figure 1. Results of molecular prevalence to BLV for bovines with a different number of calvings in the specialized dairy in the department of Antioquia. Group 0<2 calvings includes animals from three months of age until the first calving.

Detailing the analysis of seropositivity in the three geographical areas where the study was carried out (Figure 2), the area with the highest prevalence was Valle de Aburrá, with 21.05% positivity to BLV. This area has a lower percentage of livestock production systems dedicated to dairy than the north region of Antioquia, in which the lowest molecular prevalence was found (15.65%).

The analysis of the results obtained in the 16 municipalities evaluated in three regions (Figure 3) shows that there is variability in these, finding values from 7.1% (Carmen de Viboral from the eastern region) to 61.5% (Girardota from the Valle de Aburrá region). Nonetheless, it should be noted that these values cannot be compared because the experimental design was based on the population of each region and not on the one at the municipality

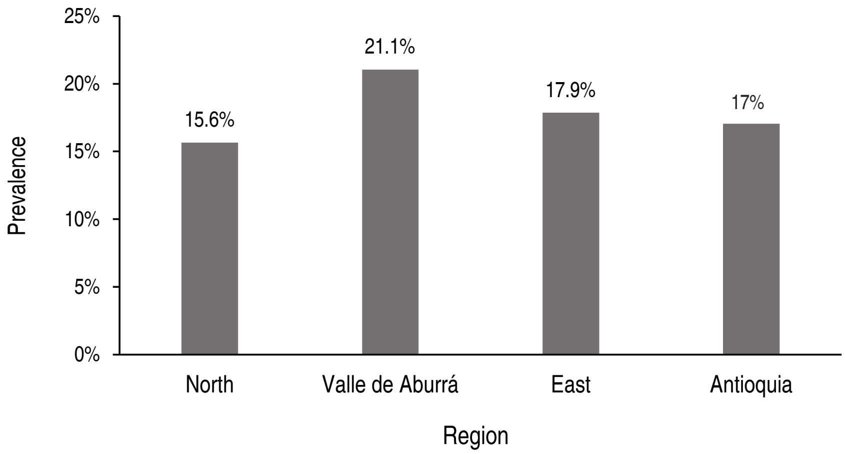

Figure 2. Results of molecular prevalence to BLV for the north (15.6%), east (17.9%), and Valle de Aburrá (21.1%) regions in the department of Antioquia, Colombia

level. However, it is a result that must be considered when proposing future studies. In Valle de Aburrá, the municipality with the highest prevalence was Girardota (Figure 3A). Of the three municipalities evaluated in the region, Medellín has the lowest prevalence despite being the one with the largest bovine population included in the study for this region. The second is the east region (Figure 3B), with the municipality of Santuario exhibiting the highest prevalence (50%) with respect to the other four evaluated. Conversely, the municipality with the lowest BLV seropositivity, Carmen de Viboral (7.1%), is also found in this municipality. The north region (Figure 3C) was the one where a higher number of municipalities were evaluated (eight) since it is the one with the most specialized dairy,

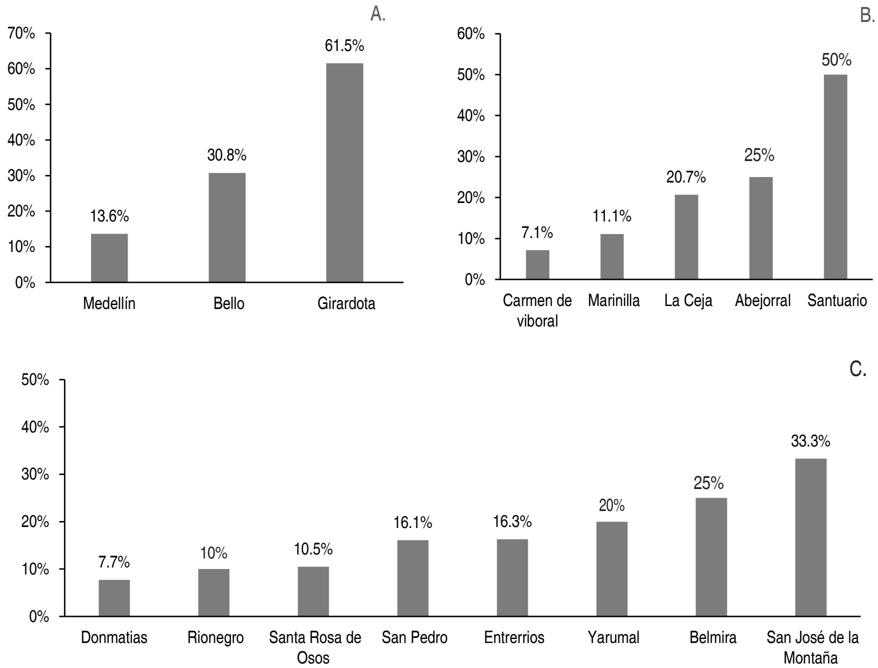

Figure 3. Molecular prevalence of bovine leukemia virus in municipalities of the department of Antioquia A. Molecular prevalence in the municipalities of Valle de Aburrá, B. Molecular prevalence in the municipalities of the east region, and C. Molecular prevalence in the municipalities of the north region.

having most a low prevalence rate (between 7.7 and 33.3%) compared to the municipalities of other regions. On average, seropositivity for this region is 17%, which is the same value that the molecular prevalence of BLV had for the entire department. These results show a variation between the different geographical areas of the department, and future studies would be necessary to evaluate this and the possible reasons for these differences.

CONCLUSIONS

The bovine leukemia virus is a pathogen found in most of the specialized dairy systems of the department of Antioquia, generating economic losses to producers. Thus, it is necessary to implement measures to control and prevent this disease in the territory. The results of this study contribute to the creation of BLV control strategies in the specialized dairy of Antioquia since differences in molecular positivity to BLV were detected by the number of calvings (age of the cows), racial component, herd, region, and municipality. It is necessary to identify factors contributing to reducing the presence of the virus in the territory to optimize this agricultural sector of the economy in the department. Thus, future studies are necessary to identify in greater detail the behavior of this pathogen under the different conditions that exist in the dairy production systems of Antioquia, as well as the influence that the different racial components may have.

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Fine root biomass and its relationship with the soil in degraded areas by mining in biogeographic Chocó

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Biomasa de raíces finas y su relación con el suelo en áreas degradadas por minería en el Chocó biogeográfico

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ABSTRACT

Fine root biomass (FRB) is essential for the functioning of tropical forests. However, due to the degradation generated by mining, it is necessary to evaluate the influence of the soil and the successional time on the FRB, to contribute to its restoration. Forests of biogeographic Chocó have been affected by mining; for this reason, the objective of evaluating the influence of the soil and the successional time on the FRB in abandoned mines was proposed. For this, plots were established in three successional stages: two abandoned mines (12-15 and 30-35 years) and a primary forest. Subsequently, the physicochemical conditions of the soil and the FRB (<2 mm) at 20 cm depth were measured. The registered FRB was 5.73 t ha^{-1} in stages of 12-15 years, 2.56 t ha^{-1} in stages of 30-35 years, and 5.91 t ha^{-1} forests; that is, it recovered quickly, but did not increase with successional time. In general, the FRB was determined by successional age, fertility, OM, and clay. In stages of 12-15 years, it was positively associated with OM and slime; and negatively with clay and sand. While, in stages of 30-35 years, it was positively related to Al, N, Ca and clay; but, with K, MO and Mg it was negative. In summary, it was determined that the soils have a different influence on the FRB in each successional stag. In addition, the limitation of belowground biomass storage due to multiple nutrients, which affect the mitigation of global climate change, was noted.

Keywords:
Ecological succession
Global climate change
Mining
Nutrient limitation
Organic matter
Restoration

RESUMEN

La biomasa de raíces finas (BRF) es fundamental para el funcionamiento de bosques tropicales. Sin embargo, debido a la degradación generada por la minería, se requiere evaluar la influencia del suelo y el tiempo sucesional sobre la BRF, para contribuir a su restauración. Los bosques del Chocó biogeográfico han sido afectados por la minería, por tal razón, se planteó el objetivo de evaluar la influencia del suelo y el tiempo sucesional sobre la BRF en minas abandonadas. Para ello, se establecieron parcelas en tres estadios sucesionales: dos minas abandonadas (12-15 y 30-35 años) y un bosque primario. Posteriormente, se midieron las condiciones fisicoquímicas del suelo y la BRF (<2 mm) a 20 cm de profundidad. La BRF registrada fue de 5,73 t ha^{-1} en estadios de 12-15 años, de 2,56 t ha^{-1} en etapas de 30-35 años, y de 5,91 t ha^{-1} bosques; es decir, se recuperó rápidamente, pero no aumentó con el tiempo sucesional. En general, la BRF estuvo determinada por edad sucesión, fertilidad, MO y arcilla. En etapas de 12-15 años, se asoció positivamente con MO y limo; y negativamente con arcilla y arena. Mientras que, en etapas de 30-35 años, se relacionó positivamente con Al, N, Ca y arcilla; pero, con K, MO y Mg fue negativa. En síntesis, se determinó que los suelos influyen de forma distinta sobre la BRF en cada etapa sucesional; además, se denotó la limitación del almacenamiento de biomasa subterránea por múltiples nutrientes, que afectan la mitigación del cambio climático global.

Palabras clave:
Sucesión ecológica
Cambio climático global
Minería
Limitación de nutrientes
Materia orgánica
Restauración

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Tropical forests store about 55% of the total carbon (C) present in terrestrial ecosystems (Pan et al. 2011); thus, they significantly contribute to the dynamics of the C cycle and to the mitigation of global climate change (IPCC 2014; Morrison-Vila et al. 2021). This C is stored mainly in the biomass (above and below ground) of trees and in the soil (Pan et al. 2011). For this reason, interest has increased in determining forest C stocks (Pugh et al. 2019) and revealing the influence of environmental and anthropogenic factors that determine its dynamics (Poorter et al. 2016), in the context of the problem of global warming.

Belowground biomass (fine and coarse roots) represents more than 25% of the total biomass of tropical rain forests (Cairns et al. 1997; Saugier et al. 2001), so they are fundamental in the functioning of the ecosystem (Chapin III et al. 2002). In particular, fine roots (<2.0 mm in diameter) are important for the acquisition, transport, and accumulation of water and nutrients from the soil (Hendricks et al. 1993; Brearley 2011); furthermore, they can store between 3 and 120 t ha^{-1} in tropical forests (Vogt et al. 1986). Therefore, they can be considered as a significant sink and reservoir of atmospheric C. However, it has been shown that the biomass of fine roots (FRB) is determined by factors such as the type of ecosystem (Cairns et al. 1997), the basal area (Finér et al. 2011), climatic conditions (Roy and Singh 1995), soil fertility (Powers et al. 2004; Powers and Peréz-Aviles 2013), the dynamics of disturbances and forest clearings (Ostertag 1998), the type of predominant tree species (Valverde-Barrantes et al. 2007), the availability of water and nutrients (Cavelier et al. 1999; Espeleta and Clark 2007; Brearley 2011), rates of mineralization, nitrification, ammonification, and total availability of nitrogen (N) (Singha et al. 2020) and the time (age) of succession (Hertel et al. 2006; Quinto et al. 2013; Singha et al. 2020), which indicates that the variation in these factors affects the C reserves of the ecosystem, and its function in the face of climate change.

Controversially, some studies evaluating the influence of soil fertility and successional age on FRB show contrasting trends. Indeed, in some cases a negative correlation has been shown between soil nutrients (especially N) and FRB (Brearley 2011; Powers and Peréz-Aviles 2013; Singha et al. 2020), while other studies do not show such correlations (Quinto et al. 2016a), and cases have even been reported in which fine roots proliferate within nutrient-rich patches (Roy and Singh 1995). On one hand, in the studies that evaluate successional processes, two contrasting tendencies have been evidenced. On the other hand, Deans et al. (1996); Hertel et al. (2006); Quinto et al. (2013) observed significant increases in FRB with increasing successional age, while the analyzes of Power and Peréz-Aviles (2013) and Brearley (2011) show that the FRB is poorly determined by age or time of succession in tropical forests, apparently because FRB recovers early in succession or very quickly after disturbances (Brearley 2011; Power and Peréz-Aviles 2013; Quinto et al. 2013). Recently Singha et al. (2020) observed that the FRB was positively associated with the time (age) of succession; but, with the total N content of the soil, the relationship was negative, which also increased with the age of the ecosystem. Thus, the lack of consensus and current debate on the influence of nutrients on FRB in tropical successional ecosystems is evident.

Assessing these patterns is fundamental because, although FRB represents a considerable portion of total C of the forest (Jackson et al. 1997), it plays a crucial role in plant nutrition, absorption, and recycling of minerals, soil enrichment with organic matter, and ooverall ecosystem productivity (Singha et al. 2020). Particularly tropical forests that have suffered in recent years from strong effects due to human activities such as deforestation, degradation, Selective logging, forest fires, open-pit mining, among others (FAO and PNUMA 2020), it is essential to understand the changes of the FRB in the different successional stages; because with the passage of time, soil conditions change (Guariguata and Ostertag 2001); and with this, it is likely that the influence of edaphic nutrient content on the stocks and recovery of belowground biomass will also vary; therefore, it is necessary to evaluate the relationship that soil physicochemical parameters have on FRB in different successional stages, with the aim of contributing to the ecological and functional restoration of degraded tropical ecosystems.

In particular, open-pit mining of metals such as gold has become one of the main drivers of deforestation and degradation of tropical forests (Primack and Vidal 2019; Kalamandeen et al. 2020). It is estimated that between 2001 to 2013, around 1,680 km^2 of tropical forests in South America were lost (Primack and Vidal 2019); of which 41% corresponds to mining carried out in Guyana, 28% in the Southwest of the Amazon, 11% in the Tapajos-Xingú forests in Brazil, and 9% in the Magdalena basin in Colombia (Álvarez-Berrios and Aide 2015). Between 2010 to 2017, it is estimated that between 57,000 and 60,000 ha of natural forests were lost to gold mining in Guyana and Perú, respectively (Kalamandeen et al. 2020). A particular case has been recorded in Colombia, in the state of Chocó (Colombian Pacific), where in 2015 licenses were granted for the mining exploitation of 302,381 ha of natural forest, representing 6.49% of the territory (Tierra Digna 2016); with which, not only the biodiversity of the region is threatened, but it also affects the carbon content stored and the capacity of ecosystems to mitigate global climate change (Quinto et al. 2013).

Chocó biogeography is one of the rainiest regions in the world, with places that have rainfall levels higher than 10,000 mm per year (Poveda et al. 2004). In this region, open pit gold mining generates the deforestation and degradation of more than 360 ha of forest annually (IIAP 2001; Ramírez and Ledezma 2007). Due to this, these ecosystems offer us an opportunity to evaluate the aforementioned hypotheses about the influence of soil fertility and successional age on the FRB in areas degraded by open pit mining. In this sense, in this investigation the following questions were raised: How does the FRB vary as a function of the time of succession, in areas degraded by mining in these high rainfall tropical ecosystems? How much do the edaphic conditions, especially the contents of nutrients, determine the FRB of areas degraded by mining with different successional stages? In areas degraded by mining, which factor has a greater influence on the variation of the FRB, soil fertility or successional age? Even more so if one considers that, according to Austin and Vitousek (1998), with high precipitation the nutrient content tends to decrease due to runoff and leaching. For this reason, the objective of evaluating the biomass of fine roots and its relationship with the soil in areas degraded by mining in the biogeographical Chocó (Colombia) was established.

MATERIALS AND METHODS

Study area

This study was carried out in forested areas previously degraded by open-pit gold mining, in the locality of Jigualito (5°06'01" N - 76°32'44" W), municipality of Condoto, in the Colombian Pacific (Figure 1).

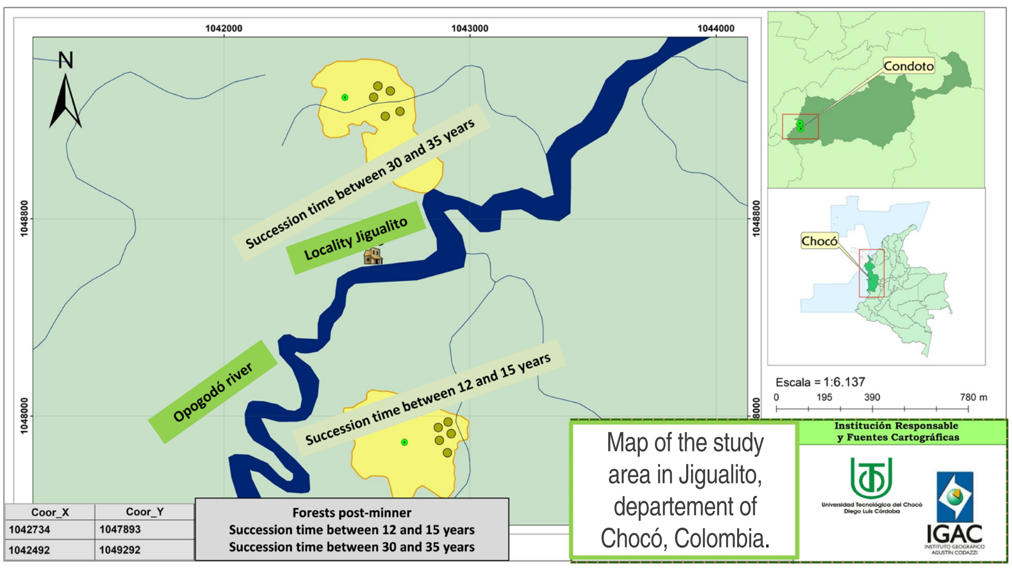

Figure 1. Map of the study area in the town of Jigualito (municipality of Condoto), department of Chocó, Colombia. Modified from Quinto et al. (2022).

This has an average rainfall of 8,000 mm per year, an altitude of 70 masl and flat topography. This locality is part of the north central Chocó biogeographical subregion, which includes the upper basins of the Atrato and San Juan rivers, in Piedemonte and Colinas low landscape units with humid terraced soils and with a type of transitional sedimentary rock (Poveda et al. 2004). The forests are mostly secondary, and with different ages of recovery due to the fact that mining has been carried out in the area at different times (Figure 2).

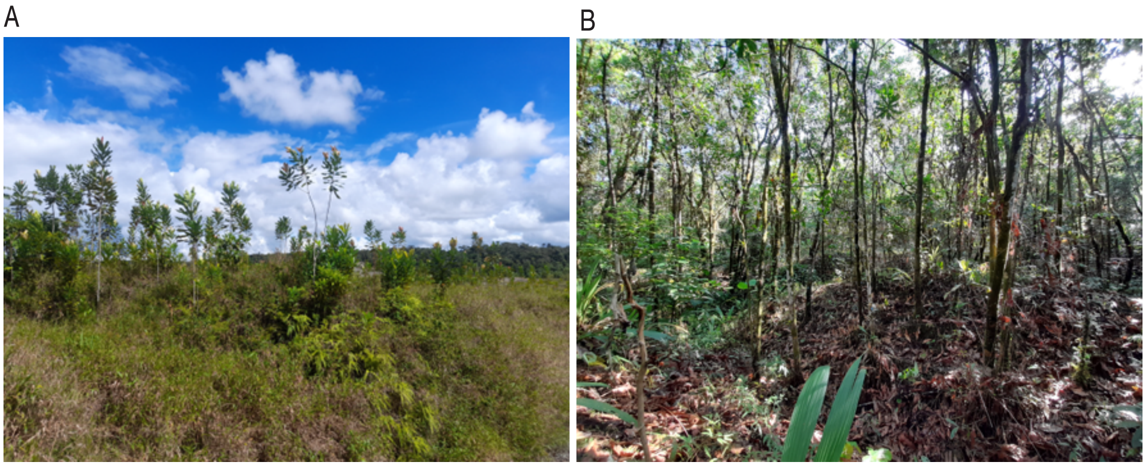

Figure 2. Forested areas previously degraded by open-pit gold mining, in the locality of Jigualito, municipality of Condoto, in the Colombian Pacific. Where A. are areas with a succession time of between 12 to 15 years. B. Are areas with a succession time of between 30 to 35 years.

The soils are ultisols, but due to mining they were characterized by a lot of rocky material and sand. In addition, they are acidic and have high contents of OM, total N, available P, Al and clay; while the concentrations of Ca, K, Mg, CICE and silt are very low in areas of recent mining activity, but their content is higher in areas with more recovery time (Ramírez et al. 2019). On the other hand, the soils of the forested areas surrounding the mines present extreme acidity, clayey with high contents of Al, OM and total N, and low amounts of P, Mg and Ca, likewise, the K contents are intermediate and the CICE is low (Quinto and Moreno 2016).

In the mature forest, the most abundant tree species were *Wettinia quinaria, Mabea occidentalis, Eschweilera sclerophylla, Vismia* sp., *Inga* sp., *Pourouma chocoana, Vismia macrophylla, Matisia* sp., *Protium apiculatum, Couepia platycalyx, Miconia* sp., *Sloanea grandifora, Sloanea fragrans, Anaxagorea crassipetala, Humiriastrum melanocarpum, Faramea jefensis, Cespedesia spathulata, Chrysochlamys clusiifolia,* and *Brosimum utile* (Ramirez et al. 2019). While, in post-mining forests, the most dominant tree species were *Cecropia peltata, Vismia baccifera, Cosmibuena macrocarpa, Ochroma pyramidalis, Welfia regia, Pityrogramma calomelanos, Cespedesia spathulata, Perebea xanthochyma, Inga chocoensis, Pourouma bicolor* and *Ocotea cernua* (Ramírez and Rangel 2019).

Experimental design

A design stratified by age of succession was used, with three strata for sampling. Stratum 1, initial age (EI) included areas recently degraded by mining, with a succession time between 12 to 15 years. This stratum presented small shrubby and woody vegetation, with a smaller average diameter and richness of tree species. This ecosystem is dominated by plant species such as *Cespedesia spathulata* (Ruiz & Pav.) Planch, *Cyathea* sp., *Ficus* sp., *Vismia* sp., *Cosmibuena macrocarpa, Hampea romeroi* Cuatrec., *Xylopia macrantha* Planch. & Triana, *Piptocoma discolor* (Kunth) Pruski, *Abarema barbouriana* (Standl.) Barneby & J.W.Grimes, *Isertia pittieri* Standl, *Lunania parviflora* Spruce ex Benth, *Bellucia pentamera* Naudin, *Cecropia peltata* L., *Croton* sp., *Faramea multiflora* A. Rich., *Tococa guianensis* Aubl., and *Inga acrocephala* Steud. The second stratum, stratum 2, called recovery age (ER) corresponded to areas that had a recovery time between 30 to 35 years; in which, an arboreal vegetation with greater diameter and specific richness was found.

This ecosystem is dominated by plant species such as *Cespedesia spathulata* (Ruiz & Pav.) Planch., *Cosmibuena macrocarpa*, *Clidemia septuplinervia* Cogn., *Cecropia peltata* L., *Inga lopadadenia* Harms., *Croton* sp., *Vismia* sp., *Socratea exorrhiza* (Mart.) H.Wendl., *Ficus* sp., and *Licania mambranaceae* Sagot ex Laness. The time elapsed since the mining activity ended was determined with interviews with the miners in the area, with the reports of the mining permits issued with the Mayor's Office, and with the information provided by the community council "Consejo comunitario" of the locality. Finally, the last stratum 3, corresponded to primary forests present in the region, and was taken as a reference scenario.

Establishment of plots

In stratum 1 (EI), mines with a recovery time between 12 to 15 years were selected. Five plots of 25x25 m were established in these mines. Similarly, in stratum 2 (ER), which corresponded to another forest area previously affected by mining and with a regeneration time between 30 to 35 years, five permanent plots of 10x50 m were installed. In stratum 3, three plots of 1 ha (100x100 m) located in forests of the localities of Opogodó, in the Colombian Pacific, were used, which were divided into 25 quadrants of 20x20 m. The latter were used as reference units. The sampling units were installed randomly within the mines, avoiding the edge effect. The plots were installed with different sizes in the succession ages, due to differential characteristics in the structure of the vegetation of the post-mining areas; however, this situation did not influence the analyzes and results obtained, because the samples in which the fine roots were collected were the same in all the sites.

Estimation of the biomass of fine roots (FRB)

For the measurement of the FRB, ingrowth cores growth cylinders were used (Moreno-Hurtado 2004; Hendricks et al. 2006). In this sense, by means of a soil hole (8 cm in diameter and 20 cm deep), pits (samples) of a known volume of soil from each of the quadrants of the plots were extracted from the forest, and the fine roots present were removed, first in the field with the help of different caliber sieves and then in the laboratory. The soil extracted and free of roots was placed in the holes left by the hole, without any kind of packing, in such a way that the new soil is completely in contact with the surrounding environment and thus eliminate the obstacles that could limit the growth of the plants. To line the filling space and facilitate the subsequent collection of the sample, thick metal wires were used on the walls of the hole at three different points.

The fine root samples were taken in the center of the quadrants of the plots at a depth of 20 cm, which were separated according to their diameter and those with diameters $\leq$2 mm were determined as fine roots. Sand and clay were removed from the fine roots sampled every six months using sieves with different sieves (0.5 and 1 mm wide) and subjecting the samples to different water pressures. The collected fine roots were dried at 70 °C for 48 h in the Acequilab Ltda® drying oven and weighed dry with a precision analytical balance (0.0001 g). These procedures were carried out in the Laboratory of Botany and Ecology of Universidad Tecnológica del Chocó "D.L.C". The dry weight values in grams of the fine roots were expressed in terms of t ha^{-1}.

Measurement of edaphic characteristics

In each of the sampling units, five composite soil samples were taken at a depth of 20 cm, in the corners and the center of each of the quadrants of 5x5 m, 10x10 m and 20x20m. In total, 300 composite soil samples were taken, 125 in the EI and ER successional strata of the areas degraded by mining and 175 in primary natural forests. The soil samples collected were sent to the Biogeochemistry Laboratory of Universidad Nacional de Colombia – Medellin Headquarters. In said laboratory, texture (percentage of sand, silt, and clay), pH, OM content, Al, effective cation exchange capacity (ECEC), and nutrient content (N, P, K, Ca and Mg) were analyzed, using the techniques referenced below: the texture with the Bouyoucos technique, the pH with the potentiometer of soils: water 1:2 ratio, the OM with the Walkley and Black technique and with volumetrics, the P available with L-ascorbic acid, UV–VIS spectrophotometer, total N with the Micro-Kjeldahl analytical technique, Al with 1 M KCl/volumetry, NTC 5 263, nutrients (Ca, Mg, K) with the 1 N ammonium acetate method, neutral, atomic absorption (Osorio 2014). The results of the physicochemical characteristics (nutrients, acidity, and texture) evaluated in the two post-mining forests and in the reference primary forest are described in greater detail in a recent study carried out in the study area (Quinto et al. 2022).

Statistical analysis

The Kruscal-Wallis (KW) non-parametric test was used to evaluate the variation of the FRB as a function of the recovery time, given that the assumptions of normality and homogeneity of variances of the data and their residuals were evaluated with the Bartlett, Hartley and Kurtosis statistics (between +2.0 and -2.0). Then, to determine the linear correlations and associations between edaphic variables (pH, MO, Al, total N, P, K, Ca, Mg, CICE, sand, silt, and clay) of each of the successional stages (strata 1 and 2) and the FRB, multiple regressions were used with the method of selection of significant variables *backward*; for which, the FRB and soil data were transformed with the natural logarithm, due to their lack of statistical normality. In addition, a principal component analysis (PCA) was used to evaluate the co-linearity and relationship between the edaphic variables, with the PCA data, the principal component with the highest significance (eigenvalue>1.0) was selected as an integrated soil fertility variable, similar to what was done by Powers and Peréz-Aviles (2013). The values of the principal component (Component 1) were related to those of the FRB using simple exponential regression models. Finally, General Linear Models (GLM) were used to evaluate the influence of successional age, the main component (Component 1) as an integrated measure of edaphic fertility, and the physicochemical variables of the soil on the FRB together. The analyzes were performed in the R programming environment (R Development Core Team 2012).

RESULTS AND DISCUSSION

The FRB presented statistically significant differences between the different successional stages in the areas degraded by open pit mining (KW=151.8; P<0.00001). However, these differences did not show a trend of increasing or decreasing FRB over time (Figure 3). Specifically, in successional stages between 12 and 15 years, with sandy textured soils, the FRB was on average (±standard error) 5.73±0.87 t ha^{-1}; while, in intermediate stages of recovery between 30 to 35 years, with sandy textured soils, the FRB was lower, with average values of 2.56±0.51 t ha^{-1}. Finally, in reference forests, with clay-textured soils, the FRB was 5.91±0.85 t ha^{-1} (Figure 3).

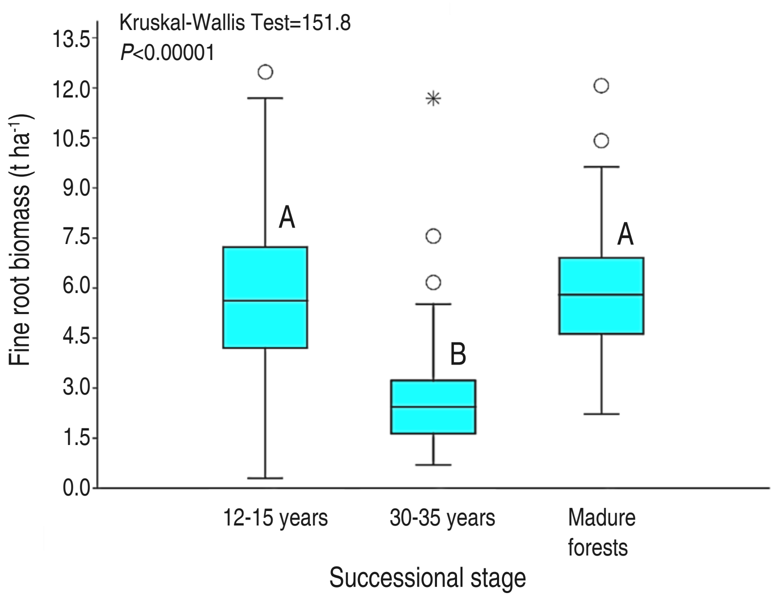

Figure 3. Fine root biomass as a function of successional stage in abandoned mines in Jigualito, Colombian Pacific. The letters "A" and "B" indicate significant differences.

The FRB recorded in areas degraded by mining in the Chocó biogeography presented values between 2.56 and 5.73 t ha^{-1}; which is a value that is within the range between 3 and 120 t ha^{-1}, reported for tropical forests (Vogt et al. 1986). Likewise, these values are similar to the belowground biomass recorded in areas degraded by open-pit mining in the biogeographical Chocó, with a recovery period between 1.5 to 6 years, with averages of 4.88 t ha^{-1}, and a range between 0.45 and 12.81 t ha^{-1} (Quinto et al. 2013). These quantifications show that, in areas affected by open pit mining, the FRB recovers quickly; and in less than 12 years, FRB values were similar to those recorded in primary reference forests of 5.91 t ha^{-1} reported in this study, and also to those quantified in rain forests of Opogodó (5.91 t ha^{-1}) and Pacurita (6.28 t ha^{-1}), in the biogeographic Chocó (Quinto et al. 2016a). This similarity in the FRB of mature forests and areas degraded by mining denotes that with little time for plant succession, high amounts of belowground biomass can be reached, since 10 years of ecosystem recovery was sufficient time for the recovery of the FRB in soils.

The fact of not observing increases in FRB with the passage of succession time in areas degraded by mining is similar to that recorded by Brearley (2011) and Powers and Peréz-Aviles (2013), who also noted little relationship or increases in the FRB with successional age. Among the possible reasons that explain why there was no evidence of increases in FRB with succession time, the following can be mentioned: first, the recovery of FRB is very fast in disturbed and/or deforested areas (Brearley 2011; Powers and Peréz-Aviles 2013), even in areas affected by mining where the soil structure is affected (Quinto et al. 2013); surely due to the need to colonize new edaphic spaces left by the disturbance, and due to the need to quickly acquire the nutrients that are being released in this recently affected ecosystem. Second, due to the changes that occur in the distribution of aboveground and belowground biomass with the passage of time of succession (Feldpausch et al. 2004; Quinto et al. 2013); since, in the initial stages, the acquisition of nutrients from the soil is required for the development of the plants, which generates a greater FRB; subsequently, when a high proportion of nutrients (N, P, K, Ca, and Mg) has already been stored in the aboveground biomass as the time of succession progresses (Feldpausch et al. 2004), it becomes necessary to carry out more photosynthesis and growth in diameter and height, for which greater aerial biomass is produced, while the belowground biomass remains constant (Quinto et al. 2013), which is related to what was stated by Bloom et al. (1985), who propose that plants respond homeostatically to the imbalance of resources in the environment, for example, the deficit of nutrients and/or sunlight, through the allocation of the resources for the acquisition of the elements that are more limiting to growth (Bloom et al. 1985). Therefore, it is denoted that in the initial stages, more fine roots are produced due to the limitation by soil nutrients, and in the later successional stages, more aboveground biomass and litter production are generated due to the competition for solar radiation for photosynthesis, as evidenced in areas altered by mining (Quinto et al. 2013) and in this investigation.

The third reason is related to changes in the composition of plant species that occur with succession in areas affected by mining (Valois-Cuesta and Martínez-Ruiz 2017; Ramírez and Rangel 2019); since, in the initial stages, a greater number of herbaceous and small woody species that have a greater number of fine roots are recorded; while, in late successional stages, there is a greater presence of trees and shrubs, which tend to have a higher biomass of thick roots. Therefore, these changes in species composition would be partially explaining the variation in FRB in these areas affected by mining. This assertion is related to what was reported by Valverde-Barrantes et al. (2007), who observed variations in the FRB content according to the type of dominant tree species in the ecosystem, which is partially similar to what was reported in this study; since, with the changes in the floristic composition, there were also changes in the FRB. In summary, it is concluded that the little increase in FRB with succession is due to factors such as the rapid colonization of fine roots in degraded areas due to the need to acquire nutrients and occupy previously affected soil spaces, variations in the distribution aboveground and belowground biomass with succession, and changes in floristic composition over time. Likewise, the little increase of the FRB with the succession denotes the need to restore the forest ecosystem; therefore, there is evidence of a retrogression with the time of succession that needs to be corrected.

Spearman's correlation analysis showed a negative relationship between fine root biomass and O.M., total N, P, Ca, Mg, and CICE contents, in post-mining areas in general (S2-Table 2, S3-Figure 1, Figure 4B). Using PCA, it was denoted that the first two principal components (component 1 eigenvalue=4.41 (36.7% variance); component 2 eigenvalue=3.03 (25.28% variance); explained the 61.98% of the total variation of the physicochemical variables of the soil of the mines together (Figure 3A). Likewise, an association was observed between edaphic variables such as OM, total N, Ca, CICE, Mg, Al, and K (Figure 4A). For its part, the ACP including the FRB denoted that biomass was positively associated with edaphic variables such as clay, silt and pH; while, with OM, total N, Ca, CICE, Mg, Al, and K denoted an inverse relationship (Figure 4B). Through the GLM, it was determined that, in the areas degraded by mining, in general, the FRB was significantly determined by the successional age, the Principal Component 1, OM, and soil clay (Table 1). However, in degraded areas between 12 and 15 years of recovery, FRB was positively associated with OM and silt; and negatively with clay and sand (Table 2). While, in the degraded areas between 30 and 35 years old, the FRB was positively related to Al, N, Ca and the percentage of clay; but, with K, OM and Mg it was negative (Table 3).

Table 1. General Linear Models of fine root biomass as a function of edaphic variables in abandoned mines in Jigualito, Colombian Pacific. Where R^2=32.09%, R^2 (adjusted)=24.7%.

Analysis of Variance for fine root biomass					
Source	**Sum of Squares**	**Df**	**Mean Square**	**F-Ratio**	***P*-Value**
Model	3913.37	11	355.76	4.34	0.0000
Residual	8279.01	101	81.97	-	-
Total (Corr.)	12192.4	112	-	-	-
Type III Sums of Squares					
Source	**Sum of Squares**	**Df**	**Mean Square**	**F-Ratio**	***P*-Value**
Successional age	1828.05	1	1828.05	22.30	0.0000
Component 1	291.8	1	291.8	3.56	0.0621
pH	47.70	1	47.7	0.58	0.4473
Al	1.47	1	1.47	0.02	0.8937
MO	418.98	1	418.9	5.11	0.0259
N	46.58	1	46.5	0.57	0.4527
P	0.543	1	0.54	0.01	0.9353
Ca	2.77	1	2.77	0.03	0.8544
Mg	17.14	1	17.14	0.21	0.6484
CICE	6.48	1	6.48	0.08	0.7791
Clay	543.03	1	543.03	6.62	0.0115
Residual	8279.01	101	81.97	-	-
Total (corrected)	12192.4	112	-	-	-

Table 2. Multiple regression of fine root biomass as a function of edaphic variables in abandoned mines in Jigualito, Colombian Pacific. Where R^2=19.81%, R^2 (adjusted)=9.78%. *Backward* variable selection method. (12-15 year).

Parameter	Estimate	Standard Error	T Statistic	*P*-Value
CONSTANT	-6.97	34.56	-0.201	0.84
pH	5.069	5.004	1.013	0.31
Al	-1.435	2.869	-0.501	0.61
LOG(MO)	7.527	3.092	2.433	0.017
LOG(N)	-5.081	3.839	-1.323	0.18
P	-0.138	0.077	-1.796	0.075

Table 2

Parameter	Estimate	Standard Error	T Statistic	*P*-Value
LOG(K)	-3.681	3.054	-1.205	0.23
LOG(Ca)	-0.425	3.83	-0.111	0.91
LOG(Mg)	-2.637	3.85	-0.683	0.49
LOG(CICE)	11.351	11.73	0.967	0.33
Clay	-0.837	0.327	-2.55	0.012
Sand	-0.254	0.119	-2.13	0.035
Silt	0.298	0.097	3.04	0.003

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	*P*-Value
Model	1863.41	11	169.401	1.98	0.0402
Residual	7542.45	88	85.7096		
Total (Corr.)	9405.86	99			

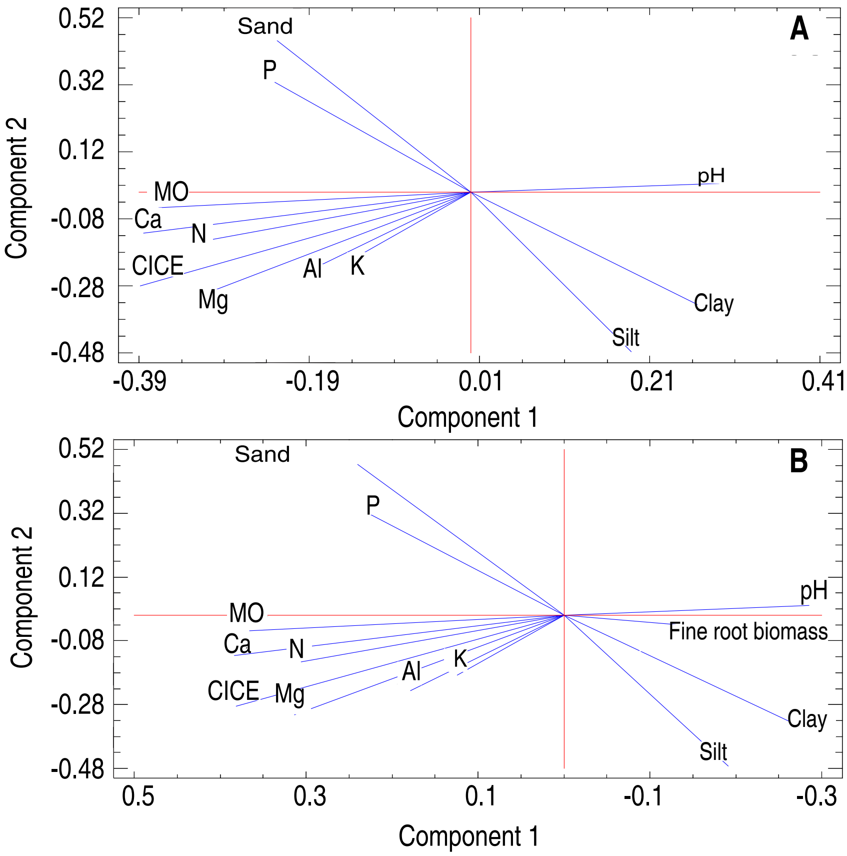

Figure 4. Principal component analysis (PCA) for fine root biomass variables and soil parameters in abandoned mines in Jigualito, Colombian Pacific. Where the component 1 eigenvalue=4.41 (36.7% variance); component 2 eigenvalue=3.03 (25.28% variance) of the total variation.

Table 3. Multiple regression of fine root biomass as a function of edaphic variables in abandoned mines in Jigualito, Colombian Pacific. Where R^2=95.64%, R^2 (adjusted)=89.53%. *Backward* variable selection method. (30-35 year).

Parameter	Estimate	Standard Error	T Statistic	*P*-Value
CONSTANT	-13.38	5.767	-2.320	0.068
LOG(K)	-8.582	1.928	-4.450	0.006
Al	1.584	0.373	4.239	0.008
LOG(Clay)	2.46	1.203	2.047	0.095
MO	-1.737	0.243	-7.145	0.0008
Mg	-2.99	0.666	-4.497	0.006
N	49.357	5.761	8.566	0.0004
LOG(Ca)	3.2856	0.952	3.450	0.018

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	*P*-Value
Model	130.912	7	18.7018	15.67	0.004
Residual	5.96788	5	1.19358		
Total (Corr.)	136.88	12			

Relationship of the FRB with the physicochemical variables of the soil in areas affected by mining

FRB in areas degraded by mining was associated with edaphic variables such as texture (percentage of clay and silt), pH, and soil fertility (nutrient content, OM, and Principal Component 1). Specifically, a negative relationship between FRB and variables such as MO, total N, Ca, Mg, and K was denoted; which is similar to that previously reported in various studies carried out in tropical forests, in which it is evident that the mass (biomass and/or necromass) of fine roots is negatively related to the edaphic content of N (Maycock and Congdon 2000; Powers et al. 2004; Brearley 2011, Powers and Peréz-Aviles 2013; Singha et al. 2020), P available (Gower 1987; Powers et al. 2005; Powers and Peréz-Aviles 2013; Quinto et al. 2016a), Mg (Powers and Peréz-Aviles 2013), and Ca (Gower 1987; Powers and Peréz-Aviles 2013). These negative correlations between FRB and soil nutrients denote nutritional limitations for the growth of trees in the ecosystem (Brearley 2011), which is evidenced in the present study, since the edaphic content of Ca, K, Mg and CICE was very low. In addition, the contents of OM, total N, and P available, although very high, their absorption may be limited due to the high contents of Al that are recorded in the area (Ramírez et al. 2019). This is due to the fact that the high availability of Al produces a decrease in root elongation, reduces stem growth, alters metabolic and physiological processes, and decreases the productivity of the ecosystem (Shetty et al. 2021). Likewise, soil Al reduces nutrient absorption, produces nutritional stress, chlorosis and necrosis, decreases leaf size, and affects photosynthesis (Chandra and Keshavkant 2021), with which, the acquisition of nutrients is affected, and nutritional limitation is generated.

With open pit mining, the texture of the soil is generally altered, which presents considerable relevance, since the FRB was positively related to the percentage of silt and clay. This positive association is explained by the fact that in soils with high clay and silt contents some cations are retained (P, Ca, Mg, K) (Osorio 2014), against which, the FRB presents increases to be able to absorb them. This increase in FRB is commonly referred to as proliferation; and in the present study, it was observed in small patches of clay, silt, and OM, in soils that are predominantly sandy, as a strategy to acquire nutrients fixed there, similar to what was observed by Roy and Singh (1995), who recorded higher FRB in the soils when there was greater availability of N and C, as well as, they are related to the results of Metcalfe et al. (2008); Jiménez et al. (2009); Kochsiek et al. (2013) and Quinto et al. (2016b), who observed higher rates of productivity and turnover of fine roots in small patches of sandy and/or nutrient-rich soils, as a strategy to acquire nutrients in tropical oligotrophic soils. This greater biomass and dynamics of fine roots in sandy soils is possibly due to the fact that the high sand content favors aeration (oxygen content) and macroporosity (Osorio 2014), and with this the growth and biomass of fine roots the soils is promoted.

The relationship between the soils and the FRB was different in the successional stage; in early stages (12-15 years), where soils are sandy, acidic and poor in nutrients (Ca, Mg, K), with high contents of available P and OM, FRB was positively associated with OM and silt content, similar to what occurs in poor and infertile soils, where fine roots proliferate in small areas and soil patches with higher nutrient content (Kochsiek et al. 2013; Quinto et al. 2016b). This is similar to that reported by Roy and Singh (1995), who observed increases in FRB related to seasonal increases in edaphic N and C content in acid and sandy soils present in tropical dry forests in India. This evidence a strategy of rapid production of FRB in environments that are very poor in nutrients, as is surely happening in areas degraded by open-pit mining, but in the initial stages of plant succession.

While, in areas affected by mining with a longer time (30-35 years) of succession, the FRB was negatively related to the content of K, MO, and Mg; This is similar to the pattern observed in low altitude tropical forests, where the mass of fine roots is negatively related to edaphic fertility in response to the nutritional limitation of the ecosystem (Gower 1987; Maycock and Congdon 2000; Powers et al. 2004; Brearley 2011; Powers and Peréz-Aviles 2013; Quinto et al. 2016a; Singha et al. 2020). However, in these sites, a positive relationship between FRB and soil Al content was also denoted, which may possibly be related to the presence of tree species tolerant to high Al contents recorded in these successional stages (Ramírez et al. 2019). Since, with the toxicity generated by the availability of Al, some tree species may be benefiting more than others (Watanabe and Osaki 2002); and probably, they would be responsible for the positive relations between the FRB and the edaphic Al. In particular, trees from botanical families such as Melastomataceae, Rubiaceae, Euforbiaceae, and Lauraceae, recorded in these areas affected by mining (Valois-Cuesta and Martínez-Ruiz 2017; Ramírez and Rangel 2019) have been previously described as Al tolerant and bioaccumulative (Jansen et al. 2002). Therefore, they would surely be responsible for the relationships denoted between the FRB and the edaphic Al. This phenomenon is similar to that recorded with the species *Acacia mangium*, considered to be tolerant to Al toxicity (Watanabe and Osaki 2002), and which has been successfully used in restoration processes of areas degraded by mining in some regions of Colombia (León and Osorio 2014), including the Colombian Pacific.

CONCLUSIONS

In the areas degraded by mining in Colombian Pacific, the relationship between the FRB and the physicochemical conditions of the soil changes as succession progresses. In addition, the FRB trends show nutrient limitations of the ecosystem. These limitations are determined by the lack or scarcity of nutrients generated by mining, sandy, and rocky textures with little mineral retention, the influence of high edaphic Al contents, and changes in the composition of tree species, especially, the N fixers and those tolerant to Al toxicity, which would be the main responsible for the recovery of nutrients and the FRB of the ecosystem. In synthesis, after the degradation of the ecosystem, as occurs with mining, the adaptations that the forest develops are denoted, such as the development of abundant biomass of fine roots, to acquire mineral nutrients from the soil, which evidences the strong affectation that is caused by mining. In addition, it is shown that it is necessary the restoration and that with a recovery of soil fertility in areas affected by anthropic activities, the underground carbon storage of the ecosystem is improved in the long term, and with it, the mitigation of global climate change.

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