Periodicidad: Cuatrimestral
Vol. 76 No. 1- 2023

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Teléfono: (*4) 430 90 06; Fax: (* 4) 230 04 20
Diagramación: Miryam Ospina Ocampo
Marcación: LandSoft S.A.
Diseño e Impresión: Centro de Publicaciones UN, Medellín.
Primera edición: Año 1939
ISSN: 0304-2847
ISSN formato web: 2248-7026
doi: 10.15446/rfnam

Licencia Ministerio de Gobierno: 275/64

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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EDITORIAL

The importance of the plants, fungi and bacteria network in maintaining sustainable plant production

The global demographic explosion triggers an alarming situation of food demand. This implies sustainable plant production through the rational and healthy use of long-term soils. This can only happen by reducing the use of chemical fertilizers and pesticides since they have toxic effects on the health of the soil and the ecosystem in general. However, to maintain plant production, it is essential to seek other more effective and sustainable alternatives. Soil harbors a panoply of interactions between the major players in the rhizosphere, mycorrhizal fungi and plant growth-promoting bacteria, which improve plant growth, health and development. These beneficial interactions lead to additive and/or synergistic effects which translate positively into the sustainable production of agrosystems and stop the use of products with toxic effects on the ecosystem.

In return to nature, for better resistance to biotic and abiotic stress, and efficient absorption of water and nutrients; the majority of terrestrial plants are forced to associate with mycorrhizal fungi. As a result, mycorrhizae have attracted more attention, but unfortunately, apart from bacteria which represent the third component of mycorrhizal associations. The rare studies on this subject show the close association between mycorrhizal fungi and the associated bacterial flora. These latest advances should change our way of seeing mycorrhizal symbioses and redefine mycorrhizae as tripartite associations. Therefore, it is necessary to expand research on the understanding of plant-fungus-bacteria interactions and the use of this tripartite association as bioinoculants to improve plant production; in order to meet the increasing demand for nutrients.

The importance of the third bacterial partner comes from the fact that these prokaryotic microorganisms are associated with symbiotic fungi during the different stages of their life cycle. They colonize mycorrhizal roots, extraradical hyphae, sporocarps and also live in the fungal cytoplasm as endobacteria. However, those identified as endobacteria should be given more importance, despite the difficulty that they are not culturable outside of their hosts. Because these endobacteria are widespread in mycorrhizal fungi, 10 out of 11 Gigasporaceae isolates contain endobacteria. Also, are themselves obligatory symbionts of plants, thus proving the direct link between the fungus and the plant. In general, the rhizospheric bacterial flora are responsible for multiple auxiliary effects on the development of mycorrhizal symbiosis. These beneficial effects can take place on the host plant or the associated mycorrhizal fungus. These effects can be summarized in the following points:

- Facilitating the acquisition of nutrient resources through the solubilization and mineralization of different nutritional resources and the fixation of atmospheric N_2.
- Improving the resistance of plants to pathogens by competition for space at the root level, the triggering of systemic resistance induced in plants, or also by a direct effect on the pathogen; through the production of antimicrobial compounds that restrict the functioning of pathogens and the production of enzymes that lyse the cell walls of oomycetes and pathogenic fungi.
- The production of phytohormones, including indoleacetic acid, cytokinins, gibberellin, aseismic acid, salicylic acid, brassinosteroids and jasmonate, which regulate the development of the root system to become more receptive to mycorrhiza.

- Stimulation of fungal spore germination and enhancement of presymbiotic growth of the mycorrhizal fungus; through increased elongation and branching of hyphae and maintenance of the saprophytic life of fungi until the development of plant roots to form mycorrhiza.

On the other hand, several bacterial strains with different beneficial mechanisms can act in synergy and complement each other to improve the growth and production of host plants as well as create a sustainable balance in the entire ecosystem. Therefore, there is considerable interest in deciphering the mechanisms of tripartite interactions between mycorrhizae, bacteria and plants in order to build an excellent strategy for sustainable agricultural production. In summary, rhizospheric interactions between mycorrhizae and bacteria are vital to improve plant production and fight against various biotic and abiotic stresses. Therefore, the final objectives will be to understand the complex interactions established by the trinomial mycorrhizal fungi - plant cells - bacteria, in order to be able to develop highly productive models in sustainable agriculture. To achieve these objectives, it is essential to rely on new high-throughput sequencing methods and invites the scientific committee to develop the following aspects:

- Develop metagenomic, ecological and functional analyzes in the mycorrhizosphere.
- Decipher the complete scenario of the development of tripartite mycorrhiza.
- Identify bacterial strains associated with fungi and mycorrhizal roots and their functional relationships.
- Identify the effects of specific bacterial species on fungi, plants or both at the same time.
- Identify the effects and mechanisms of influence on the presymbiotic development of symbiotic fungi.
- Identify the effects of fungal species and plants on the activity of the bacterial microflora of the mycorrhizosphere.
- Decipher nutritional strategies in the mycorrhizal fungus-plant-bacteria network.

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Research article

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Revista Facultad Nacional de Agronomía

Effect of water deficit on water status and growth of five tropical species used in urban forestry

Efecto del déficit hídrico en el estado hídrico y el crecimiento de cinco especies tropicales usadas en silvicultura urbana

https://doi.org/10.15446/rfnam.v76n1.101470

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ABSTRACT

Due to the urban environment urban trees must deal with drier and hotter than in rural areas. Knowing the water consumption of each species and the indicators of water deficit is useful to decide the frequency and volume of irrigation and to select species according to the microclimate of the location. To determine approximately the irrigation frequency and to identify physiological variables that indicate water stress, it was carried out an experiment in which five tropical species (*Citharexylum montanum* M., *Citharexylum sulcatum* M., *Caesalpinia spinosa* K., *Inga edulis* M. and *Retrophyllum rospigliosii* P.) were subjected to water deficit. After a month of planting, eight trees per species were subjected to four treatments: control treatment (volumetric water content higher than 45% (TC)), the volumetric water content of 20% (VM20), fifteen and thirty days after the soil had reached VM20 (T15 and T30, respectively). In trees with similar height, it was found that the descending order of water consumption was *I. edulis*, *C. montanum*, *C. spinosa*, *C. sulcatum*, *R. rospigliosii* and that the best indicator of water deficit was the stem water potential. In general, volumetric moisture of soil of 20% was a suitable threshold to decide when irrigating regardless of the species. Deeming the effect of the treatments on the growth of the assessed species, T30 diminished severely the growth by 50% in comparison to the control, except for *C. sulcatum* in which there were no significant differences.

Keywords:
Drought
Stomatal conductance
Evapotranspiration
Stress
Transpiration
Vapor pressure

RESUMEN

Debido al ambiente urbano los árboles urbanos deben enfrentar condiciones más secas y calientes que en áreas rurales. Conocer el consumo de agua de las especies e indicadores de déficit hídrico es útil para decidir el volumen y la frecuencia de riego y para seleccionar especies de acuerdo al microclima de cada lugar. Para determinar aproximadamente la frecuencia de irrigación e identificar variables fisiológicas indicadoras de déficit hídrico, se llevó a cabo un experimento en el cual cinco especies tropicales (*Citharexylum montanum* M., *Citharexylum sulcatum* M., *Caesalpinia spinosa* K., *Inga edulis* M. y *Retrophyllum rospigliosii* P.) fueron sometidas a déficit hídrico. Después de un mes de plantadas, ocho arboles por especie fueron sometidos a cuatro tratamientos: tratamiento control (contenido volumétrico de agua superior al 45% (TC)), contenido volumétrico de agua del 20% (VM20), quince y treinta días después de que el suelo había alcanzado un contenido volumétrico de agua del 20% VM20 (T15 y T30, respectivamente). En árboles con altura similar se encontró que el consumo de agua en orden descendente fue *I. edulis*, *C. montanum*, *C. spinosa*, *C. sulcatum*, *R. rospigliosii* y que el mejor indicador de déficit hídrico fue el potencial hídrico del tallo. En general, una humedad volumétrica del 20% fue un umbral adecuado para decidir el momento de riego, sin importar la especie. Considerando el efecto de los tratamientos sobre las especies evaluadas, T30 disminuyó severamente el crecimiento en un 50%, en comparación con el tratamiento control, excepto para *C. sulcatum* en la cual no hubo diferencias significativas.

Palabras clave:
Sequía
Conductancia estomática
Evapotranspiración
Estrés
Transpiración
Presión de vapor

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Received: October 12, 2022; Accepted: November 04, 2022
Rev. Fac. Nac. Agron. Medellín 76(1): 10125-10134. 2023

ISSN 0304-2847 / e-ISSN 2248-7026

Water deficit in plants is more common in urban than in rural areas. This is due to the impervious surface and the high vapour pressure deficit of urban space, which is caused by high temperature and low relative humidity (Czaja *et al.,* 2020). For instance, in the late afternoon, there is a difference of 2 °C between the city centre of Adelaide (Australia) and its suburbs. Meanwhile, there is a difference of 5.9 °C at midnight between the city and a rural area located 56 Km far (Soltani and Sharifi, 2017).

Water deficit can be caused by either water shortage in the substrate where the plants are growing and/or by the evaporative demand of the atmosphere (Grossiord *et al*., 2020). The evaporative demand can be represented by the potential evapotranspiration, which is the water loss of a reference crop through evaporation and transpiration under certain environmental conditions (Paredes *et al*., 2017). Taking evapotranspiration as a measurement of the integrated evaporative demand over time instead of a measure of time like days makes easier the comparison among studies of water deficit.

Water deficit is more frequent in young trees because the root confinement of urban space prevents water absorption (Czaja *et al*., 2020), while in adult trees, roots can reach deeper water sources. The consequence of water deficit is the reduction in physiological processes like photosynthesis (Flexas *et al.,* 2012), transpiration, water absorption, hydraulic conductance, cell expansion and nutrient availability (Rouphael *et al*., 2012). In the end, the reduction of such variables reduces the tree growth and produces symptoms like wilt, yellow and brown leaves and in extreme cases tree death (Flexas *et al*., 2012).

Considering that climate change has been affecting water availability and that water has been used in activities different from irrigating urban trees, water has to be used efficiently to maintain urban health (Joy *et al*., 2020). To achieve this efficiency, there are strategies like efficient irrigation, understanding the site hydrology, determining tree water demand and planting species tolerant to drought conditions (Joy *et al*., 2020). Among these factors, determining the water tree requirements of each species and establishing their tolerance degree to drought in early stages are extremely useful for choosing species and planning irrigation in the city.

Water deficit affects several plant processes like growth, water status, photosynthesis and fertilization (Czaja *et al*., 2020), some of which can be used to identify trees undergoing water deficit (stress markers). These markers have been useful to decide when to irrigate trees and to identify tolerant species to water deficit. For example, Cole and Pagay (2015) found that stem water potential is useful for detecting drought in grapevine and that this potential is more stable under environmental conditions than stomatal conductance and leaf water potential. Although stomatal conductance and water potential can be used to assess the negative effects of impermeable surfaces (Savi *et al*., 2014), there is little information about which stress markers are useful to diagnose water deficit in most urban species, especially in native ones.

Additionally, long-term effects of water deficit can be diagnosed using the chlorophyll content or the growth rate (this last is even more important since the bigger is an urban tree, the more ecosystem services provides). Tree-ring and therefore stem growth analysis can be used to identify tolerant genotypes to drought and to assess their historical growth under different drought seasons (Britta and Rigling, 2012). Besides, when the stress is so severe that the photochemical molecules are damaged, chlorophyll is also affected by water deficit after stomatal conductance and water status (Flexas *et al*., 2012). Consequently, chlorophyll content and stem growth might also be used as markers of long-term water deficit.

In descending order, the variables considered to choose urban tree species have been: climate adaptation, pest and disease tolerance and ecosystem services provided (Sjöman and Busse Nielsen, 2010). In Bogotá (Colombia), urban trees have been chosen considering their tolerance to the urban environment, their aesthetic traits and their popularity, which are the reasons why most of the species planted are non-native. However, there has been deemed neither the role of native diversity on the forest adaptation to future climate conditions, the role in conserving rare tree species (Ordóñez and Duinker, 2014) nor that some native species probably thrive in urban conditions. For this reason, it is important to evaluate the suitability of native species for water deficit, a common condition in the urban environment.

All the species assessed in this research are native and have been used in urban forestry due to either their apparent

tolerance to urban conditions or their ecosystem services. The species *Citharexylum montanum* M., *Citharexylum sulcatum* M. and *Caesalpinia spinosa* K. have been used as ornamental trees in Bogotá (Colombia) because of their tolerance to urban conditions like drought and high temperatures. Meanwhile, *Inga edulis* M. and *Retrophyllum Rospigliosii P.* have been mainly used because of their ornamental beauty and their particle deposition capacity (Vasquez and Maya, 2019).

Accordingly, the aims of this research were to i) make an approximation of water requirements for *Citharexylum montanum*, *Citharexylum sulcatum*, *Caesalpinia spinosa*, *Inga edulis* and *Retrophyllum rospigliosii*, ii) identify the most sensitive and the most tolerant species to water deficit, iii) evaluate how many days without irrigation can tolerate each species and iv) find variables that can be used to characterise water deficit in these species.

MATERIALS AND METHODS

The experiment was carried out at "Universidad Militar Nueva Granada" in Cajica, Colombia (4°56'33.5502"N, 74°0'36.417"W) where the mean temperature and relative humidity were 14.2 °C and 40%, respectively. The evaluated trees had a basal diameter of 2.2-3.5 cm and a height of 1.7-2.0 m. Eight trees per species (*C. montanum*, *C. sulcatum*, *C. spinosa*, *I. edulis* and *R. Rospigliosii)* were planted in plastic bags filled with 60 litters of a mix of soil (pH of 6.1, organic carbon of 3.13%, wet density of 0.84 g cm^{-3}, Cation Exchange Capacity (CEC) of 35.2 $cmol^{+}$ kg^{-1}, porosity of 65% and Field capacity of 40% volumetric water content) and compost (pH: 6.9, organic carbon 9.94%, electrical conductivity 2.0 dS m^{-1}, CEC: 35.2 $cmol^{+}$ kg^{-1}, bulk density: 0.84 g cm^{-3}, C/N rate: 10.54) in a rate of 7:1 v v^{-1}.

Treatments

Treatments started one month after planting. To reach the moisture level of each treatment, the soil was covered with plastic to prevent the soil moistening and water runoff. In each treatment, the irrigation was stopped at 12/10/2017 until each treatment reached the moisture level desired (when at least the soil of four trees reached the desirable volumetric moisture). The treatments were: control treatment (volumetric water content higher than 45% (TC)), volumetric water content of 20% (VM20), fifteen days after the soil had reached VM20 (T15) and thirty days after the soil had reached VM20 (T30). Each treatment represented a moment after irrigation had been stopped, e.g, For the VM20 treatment, the physiological traits were measured after the soil reached 20% of volumetric water content, for the T15 treatment, fifteen days after, and for T30 thirty days after. After having done the physiological measurements in each treatment, irrigation was restored and approximately one month later, growth variables were measured. In each measurement, eight trees for each treatment and each species were measured. Additionally, in four of eight bags where the trees were growing, ten grams of hydro-absorbent (polyacrylamide with a real density of 0.83 g cm^{-2} and a water retention capacity of 300 g H_2O g^{-1}) were mixed with the soil, and in the other four not.

Potential Evapotranspiration

To employ an energy variable instead of a chronological scale, the number of days was transformed into cumulative potential evapotranspiration units (mm) using the Hargreaves and Samani (1982) equation 1:

$$PE = 0.0023(T_{mean} + 17.8)(T_{max} + T_{min})^{0.5} R_a \qquad (1)$$

Where:

PE Potential evapotranspiration (mm day^{-1})
T_{mean} Average daily temperature (°C)
T_{max} Average daily maximum temperature (°C)
T_{min} Average daily minimum temperature (°C)
R_a Extra-terrestrial radiation (MJ m^{-2} day^{-1})

Soil moisture

To know the water consumption, every two days, the volumetric water was measured using a moisture sensor ML3 (Delta T Devices, ± 1% accuracy). The sensor was inserted in four points of the plastic bags until 15 cm deep and the volumetric water content was registered.

Stomatal Conductance and stem water potential

Stomatal conductance was measured with a steady-state porometer SC-1 (Decagon Devices) in two areas from a completely expanded leaf from the middle part of the tree´s canopy. Stem water potential was measured in one of the leaves used for measuring the stomatal conductance. Leaves were covered, for thirty minutes, with a plastic aluminized bag before the stem water potential measurements. After that, each leaf was cut

down from the tree and the water potential was measured using a Schollander pressure chamber. Both stomatal conductance and stem water potential measurements were measured between 9:00 and 13:00 hours. Due to the small petiole of *R. rospligiosii*, a stem structure of approximately 8 cm long was taken for measuring its water potential.

Relative Chlorophyll content

These measurements were determined between 9:00 and 13:00 hours. Chlorophyll content was measured in four places of a leaf in three leaves completely developed of each tree using a chlorophyll meter MC-100 (Apogee Instruments, United Kindom).

Stem Height and Diameter Growth

Considering that the moisture of each treatment was reached on different dates (Table 1), growth variables were also measured at different times. For instance, growth measurements of V20 in *I. edulis* were done on 14/12/2017, but *R. rospigliosii* was measured on 09/02/2018 for the same treatment. Stem height and diameter were assessed using a meter and a digital calliper, respectively. The height was measured from the base to the apex of the stem, where there were two main stems, the longest was measured. The diameter was measured where the stem and the root joint. Tree growth was determined from the increase in height and diameter.

Fresh and dry leaf mass

One month after irrigation was restored in each species; fresh mass from one leaf of each tree was measured. After that, each leaf was dried in an oven at 80 °C for 48 hours. All growth variables, except fresh and dry leaf mass, were calculated by subtracting the initial measurement on 12/10/2017.

Data Analysis

To assess differences in volumetric soil moisture among species, a nonparametric profile analysis was conducted in which species, hydroabsorbent and potential evapotranspiration were the factors (Feys, 2016). For this a nonparametric rank-based analysis was employed for longitudinal data using the F2-LD-F1 design in nparLD package of R software (Noguchi *et al.,* 2012). After identifying the differences between factors, the same profile analysis was used to evaluate differences between species.

To identify species potentially tolerant to water deficit and to determine stress markers, each treatment (VM20, T15 and T30) was compared with its respective control treatment (TC). The T-student or Wilcoxon Test was used depending on data normality (Shapiro test) and homoscedasticity (Levene test). All Statistical analyses were performed using R software version 3.5.3 (R. Core Team *et al.,* 2017).

RESULTS AND DISCUSSION

Water consumption by species

I. edulis spend less time in reaching the volumetric water content of 20% (32 days), meanwhile, *C. sulcatum* spent 73 days more and *R. rospigliosii* almost 90 more. Consequently, while *I. edulis* needed potential evapotranspiration (PE) of 154.3 mm to decrease the volumetric water content of soil from 45% to 20%, *R. rospigliosii* needed almost four times more energy (Table 1). Considering PE is a representation of the evaporation capacity of the atmosphere, *I. edulis* needed less energy (PE) to transpire the same water amount as the other species. This might have happened because of its bigger crown, which has more leaf area thus more surface for transpiration.

Table 1. Cumulative potential evapotranspiration and volumetric moisture for VM20, T15 and T30.

Species	Date when VM20 was reached	Days to reach VM20	PE to reach VM20	VM at VM20 (%)	VM at T15 (%)	VM at T30 (%)
I. edulis	13/11/2017	32	154.3	15.80±7.3	16.13±7.3	13.88±1.2
C. montanum	14/12/2017	63	304.5	17.5±4.4	14.05±1.5	18.5±10.3
C. spinosa	29/12/2017	78	379.3	22.43±17.4	14.32±2.4	11.48±2.3
C. sulcatum	25/01/2018	105	508.2	5.1±16.65	13.8±2.1	13.6±1.06
R. rospigliosii	09/02/2018	120	587.3	23.06±10.7	16.3±4.13	16.08±10.6

In all species, irrigation was suspended in 12/10/2017. VM20: Volumetric moisture of 20%. T15 and T30: fifteen and thirty days after having reached VM20, respectively. PE: Potential evapotranspiration (mm). VM: volumetric moisture of soil (%). Values of volumetric moisture represent medians±interquartile range.

Considering that bags, where trees were growing, were impervious, moisture depletion was directly related to transpiration (Joy *et al.,* 2020). The higher number of days necessary to reach VM20 of *I. edulis* in comparison to *R. rospigliosii* might be related to their relative growth, with *I. edulis* with the highest growth rate and *R. rospigliosii* with the lowest. Mitchell *et al.*, (2012) mention that rapid growth species like *Eucalyptus globulus* and *Eucalyptus smithii* use more water than *Pinus radiata*, which has a lower growth rate.

Considering the water depletion order, the irrigation frequency should be: 1) *I. edulis*, 2) *C. montanum*, 3) *C. spinosa*, 4) *C. sulcatum* and 5) *R. rospigliosii*. Although, height and diameter are important traits for choosing and managing urban trees, it is necessary to consider the crown size and water consumption for choosing the frequency and volume of irrigation.

There were significant differences among species, among potential transpiration rates (time) and among the interaction Species-Evapotranspiration (Figure 1A), but not for hydroabsorbent. *I. edulis* followed by *C. montanum* had a higher water-consumption rate than the other ones. *C. sulcatum* and *R. rospigliossi* were the species with the slower water consumption (Figure 1B). In general, *I. edulis* showed a sharp decrease in volumetric water content while the rest of the species had a progressive decrease during the first thirty-two days when the cumulative transpiration was of 154 mm (Figure 1B). The water rate consumption of *I. edulis* (0.16% VM mm^{-1} EP) was two times higher than *C. montanum* (0.08% VM mm^{-1} EP) and three times higher than *R. rospigliosii* with 0.04 VM mm^{-1} EP.

Possible reasons why the hydroabsorbent did not affect soil moisture are: i) In experiments in which the hydroabsorbent raised soil water retention or tree growth (Subhadip *et al.,* 2018), there were used sand or sandy soils, which held less water (Saha *et al.*, 2020) than the soil used in this study, ii) Organic matter content of the soil used might have provided similar water retention (Paradelo *et al.,* 2019) and nutritional functions of the hydroabsorbent and iii) the amount of hydroabsorbent polymer used here was five or until thirty times (Subhadip *et al.*, 2018) less than the used in other experiments. In the Colombian context, because of hydroabsorbent price and soil type, applying more than 160 mg L^{-1} of this amend instead of compost will be profitless; consequently, as long as the soil had a high-water retention capacity, there is unnecessary to apply hydroabsorbent.

A

Factor	ATS	df	*P*-value
Sp	40.14	3.36	<0.0001
Hyd	0.21	1	0.65
PE	168.01	5.40	<0.0001
Sp:Hyd	1.47	3.36	0.22
Sp:PE	5.07	17.38	<0.0001
Hyd:PE	1.36	5.40	0.23
Sp:Hyd:PE	0.88	17.38	0.60

B

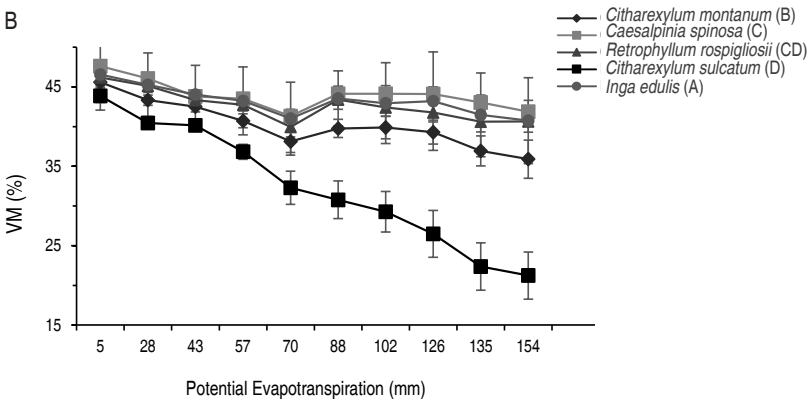

Figure 1. A) Effect of Species, Hydroabsorbent, potential evapotranspiration and their interaction on volumetric water content (ANOVA-Type Statistic profile analysis) and B) Graph profile analysis plot for volumetric water content. sp: Species, Hyd: Hydroabsorbent, PE: Potential Evapotranspiration, ATS: ANOVA Type statistic, df: Degree of Freedom VM: Volumetric moisture. In B; species following by the same letters (C and D) are not significantly different according to non-parametric profile analysis (P<0.01).

Physiological Response to Water deficit

At a volumetric water content of 20%, neither the chlorophyll nor the water status variables nor were affected, except the stem water potential of *C. montanum* (Table 1). After 15 days of having reached VM20 (T15), stomatal conductance and stem water potential

decreased in *I. edulis*, *C. sulcatum* and *R. rospigliosii* and there was only a decrease in stem water potential for *C. montanum* and *C. spinosa*. After T30, several physiological variables felt: all in *I. edulis*, stomatal conductance and stem water potential in *Caesalpinia spinosa*, stomatal conductance in *C. montanum*, stem water potential in *C. sulcatum* and none of them in *R. rospigliosii* (Table 1). A soil moisture content of 20% VM may have been enough to supply the water requirements of all the species, except for *C. montanum*. In the soil used for this experiment 20% VM equals a tension of ~70cb which is not enough to induce water deficit in plants (Intrigliolo and Castel, 2004).

Each species showed a different response to water deficit: *I. edulis* showed first (T15) a reduction in stem water potential (-32% to Control) and stomatal conductance (-27%). Then, under severe stress (T30) *I. edulis* presented a reduction of 46% and 77% in both variables, respectively, and in chlorophyll content (-17%). *C. sulcatum* showed a reduction in gS (48%) and W.P (94%) at T15 and only a reduction of W.P (162%) at T30. On the other hand, *R. rospigliosii* only presented a decrease in gS (18%) and W.P (43%) at T15 but there was not effect on the treatments VM20 and T30. *C. spinosa* presented a similar pattern to *I. edulis* with a decrease of W.P and gS at T15 and T30, respectively (Table 2).

Table 2. Stomatal conductance (gS), stem water potential (W.P) and relative chlorophyll content (Chl) of *Inga edulis*, *Citharexylum montanum*, *Citharexylum sulcatum, Caesalpinia spinosa* and *Retrophyllum rospigliosii* submitted to three water deficit periods.

Inga edulis									
	VM20	**Control VM20**	**Sig**	**T15 days**	**Control 15**	**Sig**	**T30 days**	**Control 30**	**Sig**
gS	212.19±83.8	311.57±51.01	T^{-}	215.53±37.54	295.76±50.95	**T***	24.37±39.26	231.47±32	**T****
W.P	-2.1±0.16	-1.9±0.23	T^{-}	-1.97±0.24	-1.49±0.28	**T***	-2.59±0.2	-1.46±0.46	**T****
Chl	68.88±11.82	65.03±12.05	T^{-}	60.62±12.83	64.89±9.08	T^{-}	54.87±6.23	66.52±5.26	**T***
Citharexylum montanum									
gS	213.62±49.65	272.98±40.32	T^{-}	284.96±22.78	322.25±41.68	T-	318.7±9.83	359.01±21.99	**T****
W.P	-1.95±0.55	-0.74±0.12	**T****	-1.55±0.35	-0.71±0.11	**T****	-0.79±0.23	-0.64±0.10	T^{-}
Chl	29.48±7.42	23.26±4.31	T^{-}	21.89±3.9	27.55±7.4	T^{-}	26.13±5.89	25.57±4.36	T^{-}
Citharexylum sulcatum									
gS	236.51±64.33	294.41±64.14	T^{-}	202.17±73.5	390.54±47.26	**T****	285.89±64.49	354.26±37.72	W^{-}
W.P	-1.58±0.63	-0.97±0.24	T^{-}	-1.63±0.45	-0.84±0.13	**T***	-2.36±1.54	-0.90±0.29	**W****
Chl	31.94±2.59	34.71±7.86	T^{-}	32.69±1.38	35.74±11.49	T^{-}	39.33±7.98	38.19±7.04	W^{-}
Caesalpinia spinosa									
gS	306.8±48.46	304.83±20.69	T^{-}	245.6±50.51	305.51±36.98	T^{-}	121.75±26	331.93±63.74	**W*****
W.P	-1.76±0.44	-1.26±0.15	T^{-}	-2.87±0.25	-1.77±0.30	**T*****	-3.83±0.35	-1.84±0.14	**W*****
Chl	74.85±12.92	59.06±13.42	T^{-}	69.61±11.16	63.49±9.3	T^{-}	56.17±8.49	50.83±5.49	T^{-}
Retrophyllum rospigliosii									
gS	104.44±20.93	134.96±18.91	T^{-}	122.57±11.7	151.56±11.51	**T****	154.49±29.36	148.07±20.9	T^{-}
W.P	-0.29±0.12	-0.45±0.13	T^{-}	-0.99±0.08	-0.69±0.15	**W***	-0.89±0.15	-0.78±0.10	T^{-}
Chl	NA	NA	NA	NA	NA	NA	NA	NA	NA

Means and medians ± confidence interval (95%) or ½ interquartile range, respectively. T: t-student test. W: Wilcoxon test. $^{-}$ No significant. *, **, ***, significant differences at $P<0.05$, $P<0.01$ and $P<0.001$, respectively. gS: Stomatal conductance (mmol H_2O m^{-2} s^{-1}). W.P: Stem Water Potential (MPa). Chl: Relative Chlorophyll Content (CCI units), NA: Non-available.

To reduce water loss, one of the first plant strategies to avoid water deficit is the stomatal closure (Osmolovskaya *et al*., 2018), meanwhile enzyme activity and Chl content decrease sharply when the water deficit changes from mild to severe (Flexas *et al.,* 2012). In this study, there was a similar situation in which there was a reduction in stomatal conductance and stem water potential under T15, while, chlorophyll content diminished only in *I. edulis* after 30 days of having reached VM20 (Table 2). The chlorophyll content is less susceptible to mild than to severe water stress and there is a metabolic impairment consisting in inhibition of photosynthetic enzymes and a decrease in chlorophyll content (Flexas *et al.,* 2012).

The quick decrease of water potential has been proved in the same species or species of the same genus. For example, Cordero (2016) proved that leaf water potential and stomatal conductance decreased faster than leaflet movement in *C. spinosa*. However, Stomatal conductance was more negative in that assay with stomatal conductance less than 150 mmol H_2O m^{-2} s^{-1} in control plants and less than 25 mmol H_2O m^{-2} s^{-1} in stressed plants.

In all the species, stem water potential was the first and in *C. montanum* and *C. spinosa* the first and only indicator of water deficit. This variable was the most susceptible to water deficit since there were 9 of 15 cases in which this potential decreased significantly while stomatal conductance (the second more susceptible) did it in six cases. Cole and Pagay (2015) found something similar: stem water potential was the best water deficit marker, being less sensitive to environmental conditions than stomatal conductance and leaf water potential. Deeming that the water potential of stem decreased faster than the stomatal conductance species from *Citharexylum genus* and *C. spinosa* might be anisohydric species.

Besides, the volumetric water content of soil correlated positively (spearman method, rho>0.65 and *P*<0.001) with stem water potential and negatively with stomatal conductance (rho<-0.5 and *P*<0.001) for all species, except for *R. rospigliosii*. A positive correlation between leaf water potential and the volumetric water content of the soil was also discovered by Cordero (2016) in *C. spinosa*. Its frequency to detect water deficit and its correlation with volumetric water content endorses the usefulness of stem water potential as a water deficit marker, and its use as a predictor of soil moisture.

Growth Response to Water deficit

As time passed, the effects of water deficit on tree growth were more severe (Table 3). The longer was the water deficit, the higher were the differences between the control and water deficit treatments. For example, after 15 days of having reached VM20, a few growth variables were affected by water deficit decreasing until 40%. However, after 30 days all the variables from all species decreased more than 50% except FLM and DLM. After the soil had reached a volumetric water content of 20%, none of the growth variables was affected except ΔBST in *C. montanum*. After 15 days, growth variables diminished in *I. edulis*, *C. montanum* and *C. spinosa* but not in *C. sulcatum* and *R. rospigliosii*. Meanwhile, after 30 days, at least one growth variable, except in *C. Sulcatum*, decreases in all species. A permanent growth reduction after 30 days of water deficit was also reported by Cordero (2016) in the same species *C. spinosa* after reducing water supply for more than 20 days.

Making an analogy with physiological variables, the descending order of growth variables would be stem diameter, height and dry leaf mass. In *R. rospigliossi* there was a faster physiological response to water deficit, in comparison to growth. While in the rest of the species, there were both a growth reduction and a physiological response. However, *C. sulcatum* showed an opposite response: there was a physiological change but without a growth decrease.

Considering the reduction of growth as well as of stomatal conductance, growth reduction might have been caused by one of these reasons: i) cell growth reduction, which is one of the most sensitive processes to water deficit (Czaja *et al.,* 2020) or ii) a fall in photosynthesis, transpiration and mesophyll conductance due to stomatal close (Flexas *et al.,* 2012), which was also proved by Cordero (2016) in one of the evaluated species (*C. spinosa*). Since some species did not show a relationship between instantaneous physiological variables and growth parameters, it is important to use growth variables to evaluate water deficit. However, it should be also considered physiological variables that indicate some degree of water deficit adaptation or

Table 3. Increases of stem diameter (ΔBSD), diameter at breast height (ΔDBH), height (ΔHeight), fresh (FLM) and dry leaves mass (DLM) of *Inga edulis*, *Citharexylum montanum*, *Citharexylum sulcatum*, *Caesalpinia spinosa* and *Retrophyllum rospigliosii* submitted to three water deficit periods.

				Inga edulis					
	TVM20	**Control VM20**	**Sig**	**T15 days**	**Control 15**	**Sig**	**T30 days**	**Control 30**	**Sig**
ΔBSD	0.17±0.04	0.21±0.03	T^{-}	0.19±0.09	0.34±0.12	**W***	0.21±0.1	0.47±0.06	**T*****
ΔDBH	0.13±0.13	0.14±0.04	W^{-}	0.18±0.09	0.2±0.06	T^{-}	0.12±0.05	0.23±0.03	**W****
ΔHeight	0.16±0.08	0.12±0.06	W^{-}	0.16±0.09	0.13±0.04	T	0.06±0.04	0.15±0.05	**T****
FLM	5.41±0.83	6.88±1.79	T^{-}	9.11±1.51	7.83±1.11	T^{-}	6.39±1.67	8.58±1.38	T^{-}
DLM	2.56±0.4	3.02±0.25	T^{-}	3.91±0.62	3.1±0.55	T^{-}	2.51±0.74	3.74±0.8	T*
				Citharexylum montanum					
ΔBSD	0.23±0.06	0.29±0.07	T	0.14±0.06	0.35±0.07	**T***	0.16±0.03	0.4±0.08	**T*****
ΔDBH	0.16±0.1	0.18±0.07	W^{-}	0.17±0.06	0.22±0.21	W-	0.14±0.06	0.21±0.08	T^{-}
ΔHeight	0.13±0.1	0.16±0.12	W^{-}	0.07±0.03	0.19±0.08	**W***	0.15±0.07	0.2±0.11	T^{-}
FLM	2.65±0.26	2.37±0.38	T^{-}	2.14±0.48	2.02±0.21	T^{-}	2.94±0.57	3.3±1.26	T^{-}
DLM	1.1±0.14	0.79±0.26	T^{-}	0.93±0.21	0.85±0.11	T^{-}	1.17±0.32	1.18±0.33	T^{-}
				Citharexylum sulcatum					
ΔBSD	0.32±0.12	0.55±0.44	W^{-}	0.26±0.37	0.4±0.21	W^{-}	0.33±0.08	0.47±0.2	W^{-}
ΔDBH	0.17±0.02	0.09±0.09	W^{-}	0.8±0.06	0.12±0.05	T^{-}	0.14±0.08	0.11±0.06	T^{-}
ΔHeight	0.14±0.05	0.17±0.08	W^{-}	0.19±0.09	0.19±0.09	T^{-}	0.14±0.07	0.2±0.09	T^{-}
FLM	1.31±0.2	1.35±0.1	T^{-}	1.01±0.17	1.32±0.3	T^{-}	1.25±0.34	1.22±0.33	W^{-}
DLM	0.57±0.12	0.63±0.09	T^{-}	0.46±0.11	0.66±0.16	T^{-}	0.56±0.07	0.59±0.2	W^{-}
				Caesalpinia spinosa					
ΔBSD	0.18±0.06	0.14±0.05	T^{-}	0.25±0.13	0.21±0.06	T^{-}	0.06±0.02	0.14±0.06	**W***
ΔDBH	0.11±0.05	0.09±0.04	T^{-}	0.09±0.06	0.1±0.03	T^{-}	0.08±0.07	0.09±0.04	T^{-}
ΔHeight	0.18±0.16	0.19±0.05	W^{-}	0.13±0.07	0.23±0.07	**T****	0.06±0.06	0.19±0.13	**W****
FLM	1.08±0.34	1.01±0.11	T^{-}	0.72±0.09	1.01±0.15	**T****	1.14±0.19	1.01±0.11	T^{-}
DLM	0.58±0.17	0.49±0.08	T^{-}	0.41±0.08	0.52±0.07	T^{-}	0.58±0.22	0.49±0.16	T^{-}
				Retrophyllum rospigliosii					
ΔBSD	0.18±0.06	0.17±0.06	T^{-}	0.13±0.03	0.19±0.05	T^{-}	0.11±0.02	0.3±0.15	**T****
ΔDBH	0.19±0.07	0.27±0.09	T^{-}	0.15±0.1	0.28±0.11	T^{-}	0.1±0.03	0.35±0.22	**W****
ΔHeight	0.16±0.14	0.2±0.1	W^{-}	0.13±0.12	0.21±0.12	W^{-}	0.2±0.1	0.24±0.09	W^{-}
FLM	1.21±0.21	1.4±0.23	T^{-}	1.36±0.37	1.34±0.32	T^{-}	1.32±0.47	1.76±0.32	**T***
DLM	0.46±0.08	0.5±0.09	T^{-}	0.51±0.32	0.51±0.06	T^{-}	0.46±0.15	0.62±0.21	**T***

Means and medians±confidence interval (95%) or ½ interquartile range. T: t-student test. W: Wilcoxon test^{-} No significant. *, **, ***, significant differences at $P<0.05$, $P<0.01$ and $P<0.001$, respectively. ΔBSD basal stem diameter increment (cm). ΔDBH diameter increment at breast height (cm). ΔHeight: Height increment (m). FLM: Fresh leaves mass (g). DLM: Dry leaves mass (g).

response, even if those variables do not translate into growth reductions.

C. sulcatum and *R. rospligiosii* showed certain tolerance to water deficit possibly because of their conservative strategy (low growth rate and transpiration): both species had a slower rate of water consumption (Figure 1), low growth and in the case of *R. rospigliosii* the lowest gS rate (Table 2). Mitchell *et al.* (2012) propose that a conservative strategy reduces the risk of hydraulic failure, and decreases the rate of carbon depletion, which in turn extend the tree's lifespan under drought conditions, which would explain the tolerance of *C. sulcatum* to all treatments and of *R. rospigliosii* to T15.

The tolerance of *R. rospigliosii* to water deficit might be also due to its wood anatomy. Having narrow tracheids (25.2-54.6 µm) and a high frequency of these (740-128 tracheids mm^{-2}) (Vásquez Correa *et al*., 2010), this species has less vulnerability to high vapour pressure deficit, embolism and drought (Aroca, 2012). By contrast, some species of Citharexylum genus like *Citharexylum myrianthum* (Marcati *et al*., 2014) and Inga genus (María Martín-Seijo *et al.,* 2021) have ring-porous wood, whose vessels are wider and less frequent. Additionally, *I. edulis* with rapid growth and high transpiration usually is more vulnerable to drought (Mitchell *et al.,* 2012).

Trees that keep their growth rate and show certain tolerance to water deficit are desirable for urban trees since they will reach their final size faster than those that will not, which means to rapidly provide services like shade, temperature reduction, rainwater interception and pollution removal (Eisenman *et al*., 2021). Additionally, tolerance to water deficit is an advantage for trees growing under an urban environment, since temperature and water deficit are more frequent in urban than in rural areas (Czaja *et al*., 2020).

CONCLUSIONS

i) The water consumption, in descending order, was *I. edulis*, *C. montanum*, *C. spinosa*, *C sulcatum* and *R rospigliosii*, ii) A volumetric water content of 20% is enough to guarantee all species growth, iii) Stem water potential is the best variable for using as a water deficit marker for these species. Because this study was carried out on young trees, there would be important to assess the water requirements of each species in several phenological stages under the urban landscape. Neither basal stem diameter nor height alone should be considered to plan irrigation but any variable related to potential transpiration like the leaf area of trees.

ACKNOWLEDGMENTS

This project was supported by Jardín Botánico José Celestino Mutis and Universidad Militar Nueva Granada. Thanks to Gustavo Ardila, María Mercedes Pérez, Sandra Pulido, Omar Cantor, Ariel López Esmit Galeano, Daniel Franco and Ivonne Quiroga for the logistical support provided during the development of this research.

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https://doi.org/10.15446/rfnam.v76n1.101474

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ABSTRACT

This study evaluates the impact of agricultural credit on productivity of Musaceae (banana and plantain) in farmers from Valle del Cauca (Colombia) using data from National Agricultural Census of 2014. Additionally, the effect of credit on two productivity indices (PI_1 y PI_2) was evaluated, PI_1 measured in tons of production per hectare and PI_2 in ton of production per employee. To evaluate this impact, the counterfactual without treatment was estimated using the information of those farmers who obtained a credit and similar farmers who did not. Therefore, to control the selection bias, derived from the fact that the credits are not awarded randomly, this study uses the Propensity Score Matching (PSM) methodology applying the 4-nearest neighbor matching algorithm. In general, for banana producers, the results suggest that access to agricultural credit has positive and significant effects with an increase in productivity per hectare (PI_1) of 8.4%; on the other hand, for PI_2 the result was not statistically significant, however, it may be an indicator that the farmer is not using human resources efficiently to achieve the increase obtained in PI_1 . Finally, this study suggests that access to agricultural credit may not be decisive in increasing the productivity of the plantain crop, given that the effect on the two indices evaluated was indeterminate.

Keywords:
Banana
Plantain
Production
Propensity score matching
Yield

RESUMEN

Este estudio evalúa el impacto del crédito agrícola sobre la productividad de Musáceas (plátano y banano) en productores del Valle del Cauca (Colombia) utilizando los datos del Censo Nacional Agropecuario del 2014. Adicionalmente, se evaluó el efecto del crédito en dos índices de productividad (PI_1 y PI_2), PI_1 medido en toneladas de producción por hectárea y PI_2 en toneladas de producción por empleado. Para evaluar dicho impacto se estimó el contrafactual sin tratamiento utilizando la información de aquellos agricultores que recibieron el crédito y agricultores similares que no. Por lo tanto, para controlar el sesgo de selección, derivado de que los créditos no se otorgan aleatoriamente, este trabajo utilizó la metodología del Propensity Score Matching (PSM) aplicando el algoritmo de emparejamiento 4-nearest neighbor. En general, para los productores de banano, los resultados sugieren que acceder a un crédito agrícola tiene efectos positivos y significativos con un aumento de la productividad por hectárea (PI_1) del 8,4%; por otra parte, para PI_2 el resultado fue estadísticamente no significativo, sin embargo, puede ser indicador de que el agricultor no usó eficientemente el recurso humano para lograr el incremento obtenido en PI_1. Finalmente, este estudio sugiere que el acceso al crédito agrícola puede no ser decisivo para aumentar la productividad del cultivo de plátano, dado el efecto indeterminado en los dos índices evaluados.

Palabras clave:
Banano
Plátano
Producción
Propensity score matching
Rendimiento

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Received: October 12, 2022; Accepted: November 06, 2022
Rev. Fac. Nac. Agron. Medellín 76(1): 10135-10148. 2023
ISSN 0304-2847 / e-ISSN 2248-7026

Musaceae (banana and plantain) are part of the main food products in the world, these crops play an important role in the socioeconomic growth of developing countries located in tropical and subtropical regions. According to FAO in 2019 the world production of bananas was 116 million tons and of plantain 41 million tons.

In Colombia, banana production in 2020 exceeded 2.4 million tons with a harvested area of more than 103,000 hectares, and with 86% of the national production destined for export. The average yield per hectare of bananas was 24.4 t ha^{-1} in 2020. The department of Valle del Cauca had a total banana production in 2019 of around 68,300 tons, being the third department with the highest production nationwide and an average yield of 14 t ha^{-1}, at the same time, it should be noted that in terms of production for the national consumption, it occupies the first place with a 25% stake (MADR, 2021a).

According to the MADR (2021b) (Ministry of Agriculture and Rural Development from Colombia by its acronym in Spanish) in 2019, plantain production in Colombia exceeded 4.3 million tons with a planted area of more than 450,000 hectares, in this way, the plantain became the most planted crop in the country, and the most important in food security. In Colombia, the average yield per hectare of plantain has been 8.3 t ha^{-1} for the year 2019, a higher yield than the world average, which for 2018 was calculated at 7 t ha^{-1}. For its part, the department of Valle del Cauca for 2019 had a total production of about 320,000 tons, being the fourth department with the highest production nationwide and an average yield of 11 t ha^{-1}.

This research has the interest of studying the department of Valle del Cauca since according to the results of the last National Agricultural Census of 2014, the food production of it was 3.2 million tons, while the national production was around 33.2 million tons, placing Valle del Cauca in the first place of production with a stake of 9.6% (DANE, 2015).

In this way, to promote and increase the productivity of Musaceae crops in the department of Valle del Cauca, economic and technical resources are necessary that lead to the modernization of the production chain. According to Yang and Zhu (2013) agricultural modernization implies increasing the efficiency in the use of natural resources, monitoring and subsequent improvement of the organization of the production process and the active implementation of innovative technologies, therefore large investments and financial and productive resources are necessary.

Agricultural production is related to the period that elapses from the initial investments, the purchase of the inputs required for the establishment, and subsequent maintenance of the crop until the time of harvest and/or marketing of the products, said period includes stages of risk and uncertainty for the production process (Seven and Tumen, 2020). Therefore, access to agricultural credit programs can play a crucial role in the possible management of these risks, thus sustainably achieving growth in agricultural productivity and supporting decisions during the production process (Eswaran and Kotwal, 1986). Likewise, the main objective of granting credit is not only to improve the production and commercialization of the agricultural sector, is also to promote technological change (Fernández Moreno *et al*., 2011).

For the reasons mentioned above, many authors have used different methods and models to evaluate the impact that accesses to an agricultural credit program generates for a certain group of people on productivity, quality of life, and others.

International authors as Ciaian *et al*. (2012) estimated how access to agricultural credit affected input requirements and agricultural efficiency in CEE transition countries (Central and Eastern Europe). To do so, they turned to a farm-level single-panel dataset with 37,409 observations and used a matching estimator. Within their results, they found that access to agricultural credit increases total factor productivity by up to 1.9% for every 1,000 euros of additional credit, this in turn is based on a negative effect of access to credit on labor, suggesting that these two are substitutes.

Chandio *et al*. (2019) examined the impact of agricultural credit and farm size on the technical efficiency of rice productivity in Sindh, Pakistan. For that, they collected

data from 180 rice farmers using a cross-sectional random sampling technique and did analysis through Maximum Likelihood Estimation (MLE). Among their results they found that credit, farm size, fertilizers, and labor have positive and significantly influenced rice productivity.

It is also important to mention that credit restrictions are of special interest when evaluating the access that a farmer may have, for example, Seck (2021) applied an endogenous commutation regression model to examine heterogeneous credit constraints and their effect on the productivity of small farmers in Senegal, obtaining results that indicate that credit restrictions hinder the productive performance of farmers.

A study conducted for Elahi *et al*. (2018) in 48 villages in the Sargodha district of Punjab, Pakistan, analyzed farmers access to agricultural advisory and financial services, and their impact on wheat productivity using Propensity Score Matching methodology (PSM). They found results showing that access to farm advisory services improves wheat productivity, as well as significant differences between farmers who had simultaneous access to farm advisory and financial services compared to those who had access to one or neither, were also found.

Owusu, (2017) applied also PSM methodology to assess the effect of access to credit on agricultural productivity of cassava in Ghana. The results showed that access to credit was determined by different factors such as: age, gender, level of education, size of the household and of the farm, agricultural experience, and extension service, as well as the hired labor and the distance between the farmer and the lender. Finally, the author found that credit has a positive and significant effect on cassava productivity.

Contrary to what was previously presented, other authors such as Nakano and Magezi (2020) found results in which financing is not necessarily related to increased productivity. They analyzed the impact of microcredit on technology adoption and rice crop productivity in Tanzania by conducting a randomized control trial (RCT) to estimate the intention-to-treat (ITT) effect as well as local average treatment effect (LATE) of microcredit, using treatment status as instrumental variable (IV). They obtained results in which it is evident that the financing programs granted do not result in an increase in rice yields, profits from rice cultivation or family income.

In the case of Colombia, Echavarría, Villamizar-Villegas, and Mcallister (2017) evaluated the impact of credit in the coffee sector, using a panel data model with fixed effects and instruments, together with common support given by estimated propensity scores, their results suggest that credit has a beneficial and significant effect on outcome variables. Echavarría, Villamizar-Villegas, Restrepo-Tamayo *et al.* (2017) through a Propensity Score Matching (PSM) analysis, studied the effect on some variables such as farm yield, and the Multidimensional Poverty Index (MPI) for long and short cycle crops. In general, the results suggested that the various types of credit have a positive and significant effect on yield (between 3% and 28%).

The main purpose of this research is to investigate the question of how access to agricultural credit affects agricultural productivity in Musaceae crops (plantain and bananas) in the department of Valle del Cauca (Colombia). To answer this question, data from the 2014 national agricultural census was used, and econometric methods were applied to analyze the relationship between access to agricultural credit and two productivity indices considered as production per hectare and production per employee. Following the existing literature, the main contributions of this study be listed below: it is the first study that has been developed that assesses the impact of agricultural credit at the departmental level, analyzing the crops of banana and plantain in specific way. Second, in this study two important factors or productive resources are considered to evaluate and analyze the efficiency and effectiveness in agricultural productivity, which are the surface of the land in which the crop is established and the labor. Third, this study considers the possible endogenous problems caused by the "selection bias" of the sample, so the non-parametric Propensity Score Matching (PSM) method was applied to estimate the impact of access to different credit programs in agricultural productivity in Musaceae crops.

MATERIALS AND METHODS

Data

This research will be based on survey data published by the National Administrative Department of Statistics

(known by its acronym in Spanish DANE) in 2017, obtained from the National Agricultural Census (CNA for its acronym in Spanish) carried out in 2014 in Colombia; said census is identified at the level of the Agricultural Production Unit (UPA for its acronym in Spanish respectively)[1]. Therefore, UPA was the analysis unit for this research. Moreover, the results of CNA questionnaire, were structured in several modules where each one of them provided unique information. For this reason, files related to the characteristics of UPAs, people, crops, machinery, and infrastructure were of interest.

As CNA was carried out in 2014, all the information about agricultural production and earned credit correspond to 2013. Therefore, the results found express the changes in productivity of that year based on access to credit in that year.

The CNA covered a total of 2,370,099 UPAs nationwide. Valle del Cauca, which is the zone of interest for this study located in the southwest of the country has 3.2% of the UPAs registered (76,874). To meet the objectives proposed in this research, the data base was filtered, and it was reduced to banana producers (1,501), plantain producers (4,232) and farmers with both crops (880).

Treatment variable

Initially, the agricultural credit as "treatment variable" responds to the CNA question: "Was the requested credit or financing approved?", it is a dichotomous variable in which if the credit was approved the value it takes is 1 and 0 if it was not obtained. As the credit acceptability rate is quite high, since in average 87% of the Musaceae producers obtained the credit (DANE, 2017), this becomes a limitation since the application of the PSM methodology requires a big population to form the control group, therefore it is proposed to include those farmers who did not request financing or credit in the control group and manage the possibly systematic differences produced with the implementation of said methodology. The credits granted come from any entity such as banks, cooperatives, individuals or moneylenders, government programs, or warehouses of agricultural and agro-industrial inputs.

Dependent variable (Productivity Index 1 and 2)

The variable of interest or result was agricultural productivity measured in two indices proposed in this study. The first productivity index PI_1 was calculated as the natural logarithm of the division of production (in tons) by the harvested area (in hectares), the second index PI_2 was calculated as the natural logarithm of the division of production (in tons) by the total number of employees in the farm (including permanent and daily employees belonging or not to the family, that is, all the labor available for the agricultural activity).

In general, in this research, the natural logarithm was applied to some variables, as can be seen in Tables 1 and 2 (including productivity indices), since for the econometric analysis, applying the natural logarithm, the effect of the units of the variables on the coefficients is eliminated, and given the properties of the logarithms, some complex mathematical operations are facilitated.

Method

Because credits are not awarded randomly among farmers, there is a selection bias problem, which will cause ordinary least squares (OLS) estimate to produce a biased effect of the impact of credit on agricultural productivity. Consequently, this research applies the Propensity Score Matching PSM methodology to obtain the causal effect of the granting of agricultural credit, however, its application assumes of conditional independence, which establishes that the selection of the treatment is given exclusively by observable variables.

According to Vinha (2006) the general idea of this methodology is to evaluate the impact of estimating the counterfactual without treatment using the information of those individuals who have received the treatment and similar individuals who did not receive. In the same way, Heinrich *et al.* (2010) point out that the PSM solves the question of what would have happened to the participating individuals in the absence of treatment using information from the group of those individuals who did not participate.

[1] According to DANE (2014), UPA is all land that is fully or partially dedicated to agricultural production and that is worked, directed, or administered as a technical and economic unit, directly by a person or with the help of other persons without regard to the tenure system, legal status, size, or location.

For this study, the objective is to form a control group with farmers who have a propensity scores (PSCORE) or probability $b(x)$ similar with those who received agricultural credit. By comparing how results differ between participating and nonparticipating individuals who have equivalent observable characteristics (control variables), the intervention effect is estimated by averaging the differences between the participants and their matched comparison cases. In this way, the PSM methodology allows to calculate the Average Treatment Effect on the Treated (ATT). Therefore, the impact of the agricultural credit on productivity indices studied can be given by:

$$ATT_1 = E\{PI_1^1 | b(x), WAC = 1\} - E\{PI_1^0 | b(x), WAC = 0\} = E\{PI_1^1 - PI_1^0 | b(x)\}$$

$$ATT_2 = E\{PI_2^1 | b(x), WAC = 1\} - E\{PI_2^0 | b(x), WAC = 0\} = E\{PI_2^1 - PI_2^1 | b(x)\}$$

Where, treatment condition (With Agricultural Credit) is denoted by WAC =1 and WAC = 0 otherwise, and the impact variable (PI_1 and PI_2) of interest is denoted by PI_1^1, PI_2^1 if the credit was received, and PI_1^0, PI_2^0 otherwise.

It is worth to mentioning the weaknesses of this method as Hoz Aguilar, (2019) did, who summarizes the following limitations: PSM technique requires large databases, one of its conditions to be used is that the region of common support between the treated and untreated must be met and also it is not possible to establish or demonstrate that there are no differences in unobserved variables, since these can affect both the probability of participation and the results.

Control variables

The control variables included two main categories. The first category included factors that can affect the agricultural productivity as systems or technology used in the crops, for this research it was taken into account if the farmer implements a pressure irrigation system (drip, sprinkler, pumping) or a surface system by gravity or manual, another indicator of the modernization of agricultural work can be the existence or not of agricultural machinery, use of fertilizer organic, chemical or other method to improve the soil. Other determinants that can affect productivity were the area (in hectares) allocated to agricultural infrastructure such as warehouses, ponds, silos, wells, etc., the land area (in hectares) occupied by the UPA and if the farmer received technical assistance.

And the second category covers the personal characteristics of the farmers including level of education calculated as percentage of people without education living in the farm, as well as their race, understanding by majority those who do not belong to an indigenous, gypsy, raizal or black groups, and if farmers use of permanent and/or temporary employees including family members.

Thus, these variables become control variables, since they can systematically generate different groups. This will be corroborated in the following analysis and results section (Descriptive Statistics), where it will be possible to observe if there are significant differences between the group made up of farmers who accessed agricultural credit and those who did not, according to each of the control variables.

RESULTS AND DISCUSSION

Descriptive statistics

This section presents the descriptive statistics corresponding to the sample used to estimate the impact of agricultural credit. According to the results of the descriptive statistics alone (Table 1 and Table 2), when the agricultural credit is approved, the average value of the PI_1 for plantain crops and PI_2 for plantain and banana crops are higher and significantly different, this suggests a possible positive correlation between access to credit and the productivity indexes studied. However, in the case of PI_1 for banana crop, although it presents higher average values for the treated group, the difference is not statistically significant, suggesting that there is no positive effect of agricultural financing on this.

Another interesting result is about technical assistance, which does not have significant differences between the average values of the two groups but has a higher

value in the treatment group than in the control group. However, technical assistance was considered within the group of control variables, since according to the census questionnaire, advice on credit and financing was part of the technical assistance received by some of the farmers.

Table 1. Definition and descriptive statistics of dependent variables with and without agricultural credit.

The dependent variable	Definition	Untreated (Without credit)		Treated (With credit)		Mean difference
		Mean	SD	Mean	SD	
ln_banana_prod_1	Banana PI_1	2.188	0.252	2.195	0.184	-0.007
ln_banana_prod_2	Banana PI_2	-0.540	1.896	1.034	1.905	-1.573***
ln_plantain_prod_1	Plantain PI_1	1.940	0.181	1.957	0.192	-0.018**
ln_plantain_prod_2	Plantain PI_2	0.320	1.939	0.827	1.806	-0.507***

*P<0.10, **P<0.05, ***P<0.01.

Table 2. Definition of control variables obtained from questionnaire of CNA and descriptive statistics with and without agricultural credit.

Control variables	Definition	Untreated (Without credit)		Treated (With credit)		Mean difference
		Mean	SD	Mean	SD	
irr_pres	A dichotomous variable was created for the following categories: • irr_pres = Pressure irrigation systems (Aspersion, Drip, Pumping), • irr_sup = Surface irrigation system (Gravity and Manual) For example: irr_pres: it will take a value of one (1) if any pressure irrigation system were used and zero (0) in any other case.	0.111	0.314	0.128	0.334	-0.017
irr_sup		0.141	0.348	0.211	0.408	-0.070***
agr_mach	Existence of agricultural machinery. It takes a value of one (1) if the answer was "yes" and zero (0) for the others.	0.335	0.472	0.603	0.490	-0.268***
soil_1	A dichotomous variable was created for the following categories: • soil_1 =Organic Fertilizer • soil_2 =Chemical Fertilizer • soil_other =Other (Corrector of soil acidity, burns, prayers, rites, payments) • soil_8 =Did not apply For example: soil_1: it will take a value of one (1) if organic fertilizer was used and zero (0) in any other case.	0.331	0.471	0.462	0.499	-0.131***
soil_2		0.315	0.465	0.626	0.484	-0.310***
soil_other		0.030	0.172	0.043	0.202	-0.012*
soil_8		0.465	0.499	0.145	0.352	0.321***
ln_agr_infr	Measure the total area in constructions or agricultural infrastructure of the UPA (ln)	3.728	1.494	3.989	1.498	-0.261***
ln_area_apu_ha	Measure the total area of the UPA (ln)	0.704	1.752	1.140	1.422	-0.436***

Table 2

The dependent variable	Definition	Untreated (Without credit)		Treated (With credit)		Mean difference
		Mean	SD	Mean	SD	
Technical_ assistance	Agricultural assistance or advice. It takes a value of one (1) if the answer was "Yes" option and zero (0) for the "No" option.	0.318	0.466	0.340	0.474	-0.022
without_education_ pct	• Percentage of people with basic education (Preschool, Basic primary, Basic secondary, Medium) • Percentage of people with high education (Technician, Technological, University, Postgraduate) • Percentage of people with without education (None)	15.301	28.055	8.312	20.030	6.989***
majority_pct	Percentage majority, for which option "f" is considered for majorities and for minorities the other cases. a) Indigenous b) Gypsy c) Raizal d) Black e) Palenquero f) None of the above	49.466	49.503	86.315	33.877	-36.849***
ln_employees	Number of permanent employees (ln)	0.603	0.650	0.720	0.676	-0.117***
ln_dayly_ employees	Number of daily employees (ln)	1.337	1.127	1.599	1.265	-0.262***

*P<0.10, **P<0.05, ***P<0.01.

There are significant differences between the control and treatment groups if the farmer implements some type of innovation on the farm, such as the use of agricultural machinery, application of some method to improve soil fertility, or if an irrigation system is installed. On average, who accessed an agricultural credit has the highest percentage of the population with owns agricultural machinery (61%), implements chemical fertilization (63%) over organic fertilization (46.2%), and uses irrigation systems (on average, the surface irrigation system is more implemented than pressurized).

The group that did not receive any type of credit have an average of 15.3% of its population without education (basic or high) while those who received the credit, only 8.3%. On the other hand, on average 86% of people who received some types of agricultural credit do not belong to some ethnic minority group, while those who did not receive it, have an average of 50% minorities.

In general, as can be seen in Table 1 and Table 2, the groups have different means in the group that had access to agricultural credit and in those that did not receive it, which leads to the conclusion that they are not randomly distributed. Therefore, these differences confirm the need to create a control group that is comparable to the group that accessed agricultural credit and to apply the methodology proposed in this research.

Common support

The common support shows the probabilities of participation for both UPAs with credit and those without. As can be seen in Figure 1, exist a common support, since each UPA with credit and with a defined probability can be associated or "matched" to a unit without credit with a similar probability.

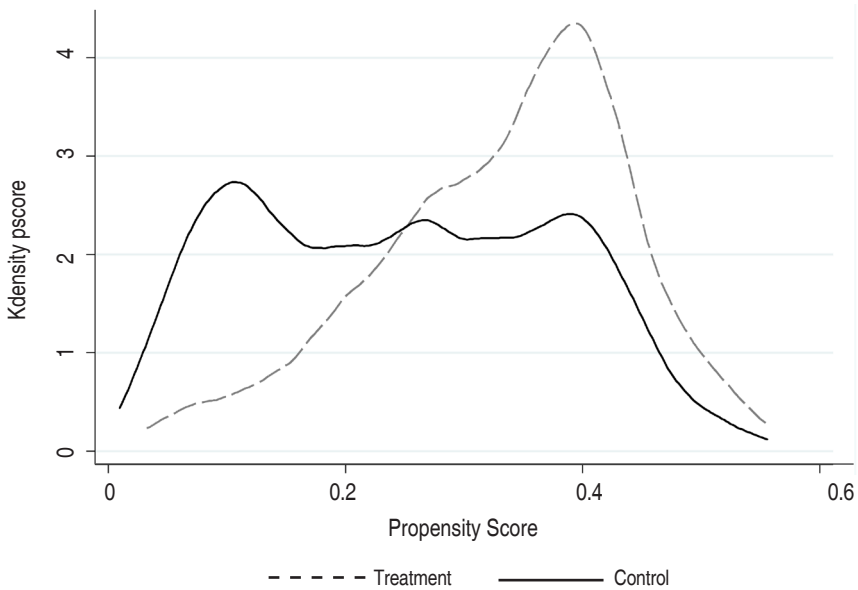

Figure 1. Propensity Score histogram for treatment and control groups.

Balance test

Before using PSM, the samples must pass the balance test, which ensures that there is no systematic difference between the treatment group and the control group after pairing. In other words, it is necessary to verify that the post-pairing rocking has corrected the problem of selection in observables, which was evidenced by the tests of differences of means carried out in the item "Descriptive statistics". As can see below, Table 3 and Table 4 show balance test results for each crop.

Table 3. Balance test results for 4-nearest neighbor matching (Banana crop).

Variable	Unmatched	Mean		%reduct		
	Matched	Treated	Control	%bias	|bias|	t
Implementation of an agricultural irrigation system (Pressure)	U	0.17143	0.03065	47.7		4.51***
	M	0.14706	0.09191	18.7	60.8	0.99
Implementation of an agricultural irrigation system (Superficial)	U	0.28571	0.06897	58.8		5.24***
	M	0.26471	0.27206	-2	96.6	-0.1
Existence of agricultural machinery.	U	0.82857	0.50958	71.8		4.96***
	M	0.82353	0.84926	-5.8	91.9	-0.4
Use of Organic fertilizers.	U	0.54286	0.44061	20.5		1.52
	M	0.54412	0.54412	0	100	0
Use of Chemical fertilizers	U	0.61429	0.25287	77.9		6***
	M	0.60294	0.64338	-8.7	88.8	-0.48

Table 3

Variable	Unmatched	Mean		%reduct		
	Matched	Treated	Control	%bias	|bias|	t
Other types of methods to improve the soil (burning, prayers, rituals, etc.)	U	0.08571	0.05747	10.9		0.86
	M	0.07353	0.07721	-1.4	87	-0.08
No application of any methods to improve the soil.	U	0.12857	0.42912	-70.9		-4.78***
	M	0.13235	0.12132	2.6	96.3	0.19
Area with agricultural infrastructure.	U	4.0958	3.4896	40		3***
	M	4.1079	4.1455	-2.5	93.8	-0.13
APU (Agricultural Production Unit) Area.	U	1.6202	1.2309	29.9		2
	M	1.5914	1.514	5.9	80.1	0.36
Agricultural assistance or advice.	U	0.38571	0.29119	20		1.52
	M	0.38235	0.34191	8.5	57.2	0.49
Level of education (% Without education).	U	8.0602	11.176	-14		-1.04
	M	8.2973	7.9915	1.4	90.2	0.09
Ethnic group (% Majorities).	U	76.19	26.693	115.6		8.56***
	M	75.49	77.934	-5.7	95.1	-0.34
Number Permanent Employees.	U	0.9197	1.0192	-12.9		-0.98
	M	0.9306	0.95572	-3.3	74.7	-0.19
Daily employees on the farm.	U	1.6894	1.4701	18		1.47
	M	1.7025	1.5513	12.4	31	0.63

*P<0.10, **P<0.05, ***P<0.01.

Table 4. Balance test results for 4-nearest neighbor matching (Plantain crop).

Variable	Unmatched	Mean			%reduct	
	Matched	Treated	Control	%bias	|bias|	t
Implementation of an agricultural irrigation system (Pressure)	U	0.15897	0.12343	10.2		1.26
	M	0.15979	0.14562	4.1	60.1	0.39
Implementation of an agricultural irrigation system (Superficial)	U	0.25641	0.16279	23.1		2.9***
	M	0.25258	0.21263	9.9	57.3	0.93
Existence of agricultural machinery.	U	0.77436	0.60644	36.9		4.28***
	M	0.7732	0.78995	-3.7	90	-0.4
Use of Organic fertilizers.	U	0.54872	0.49195	11.4		1.37
	M	0.55155	0.52062	6.2	45.5	0.61

Table 4

Variable	Unmatched	Mean			%reduct	
	Matched	Treated	Control	%bias	|bias|	t
Use of Chemical fertilizers	U	0.68718	0.50805	37.1		4.38***
	M	0.68557	0.70103	-3.2	91.4	-0.33
Other types of methods to improve the soil (burning, prayers, rituals, etc.)	U	0.08205	0.05546	10.5		1.32
	M	0.08247	0.08119	0.5	95.2	0.05
No application of any methods to improve the soil.	U	0.08718	0.23614	-41.3		-4.55***
	M	0.08763	0.10052	-3.6	91.3	-0.43
Area with agricultural infrastructure.	U	4.0337	3.8601	12.1		1.39
	M	4.0301	3.9007	9.1	25.5	0.94
APU (Agricultural Production Unit) Area.	U	1.4955	1.2544	17.6		2.02**
	M	1.4923	1.3803	8.2	53.6	0.84
Agricultural assistance or advice.	U	0.33846	0.3542	-3.3		-0.4
	M	0.34021	0.34021	0	100	0
Level of education (% Without education).	U	6.12	9.6041	-18		-2.08**
	M	6.1516	6.1318	0.1	99.4	0.01
Ethnic group (% Majorities).	U	89.42	63.89	64.1		7.02***
	M	89.366	90.108	-1.9	97.1	-0.24
Number Permanent Employees.	U	0.85719	0.78704	9.7		1.19
	M	0.85446	0.8328	3	69.1	0.29
Daily employees on the farm.	U	1.7464	1.4652	22.2		2.69***
	M	1.7296	1.733	-0.3	98.8	-0.03

* $P<0.10$, ** $P<0.05$, *** $P<0.01$

According to balance test (Table 3, Table 4), there is evidence of a reduction in the selection bias in observables. Therefore, it is argued that the consistency of the results through the balancing test is an indication of the robustness and reliability of the results found.

Banana productivity

As can be seen in Table 5, the impact of agricultural credit on PI_1 is positive and significant (8.4%). Therefore, it can be affirmed that banana producers who have access to agricultural credit, they achieve an average 8.4% increase in tons produced per hectare, contrary what was initially suggested in basic analysis of descriptive statistics, where a possible non-relationship between access to agricultural credit and the PI_1 was intuited. To get an idea of the magnitude of the effect calculated with PSM methodology in terms of the unit of measurement of the result variable, an average an increasing the yield from 8.8 to 9.5 t ha^{-1} is expected, in other words an increase of almost 1 t ha^{-1}.

As mentioned in the introduction, the production of bananas in Valle del Cauca is destined for national consumption, however, this crop at the national level

Table 5. Results of propensity score matching for banana crops applying 4-nearest neighbor matching and its standard error.

4-Nearest neighbor matching	ATT	
	Difference	S. E
PI_1	0.08430**	0.04255
PI_2	0.50902	0.35741
Sample number of Control group	261	
Sample number of Treatment group	68	

*P<0.10, **P<0.05, ***P<0.01.

mainly has an export market. According of this, FINAGRO (2018) (Financial entity for the Colombian agricultural that provides resources through banks, cooperatives, and microfinance intermediaries) affirm that for export-type bananas, systems production of high technological level is required. However, according to the results of the CNA, only 2% of the resources obtained by agricultural credit were invested in post-harvest processing, and other 16% were destined to the purchase of machinery.

Regarding PI_2, this had a positive but statistically non-significant effect with high SE value (35%) obtained. Therefore, for this index, accurate results are not obtained that allow affirming the causal effect of agricultural credit in the labor productivity. According to CNA, approximately 20% of the resources obtained from the granting of agricultural credit is used to pay the labor, the third highest items in which it is invested (32% is invested in inputs and 20% in installation of the crop). To improve PI_2 with the existing labor, the employee should receive training and use tools that allow optimize work such as specialized machinery or the application of best agricultural practices.

Relating the results obtained between the two productivity indices, it is found that if there is no significant increasing in PI_2 with the increase in PI_1 when a credit is obtained, it is probably because the employees continue to have the same performance generating a low variation in the PI_2. For this reason, especially in this case, it can be said that PI_2 is an indicator that the farmer would be working inefficiently, that is, the human resources are not being optimized.

At this point it should be noted that the Colombian rural labor market presents great challenges related to increasing the quality of jobs, increasing formality, and female participation (Parra-Peña, Puyana, and Yepes Chica, 2021). All these factors mentioned, play an essential role in improving agricultural productivity. According to Otero-Cortés (2019) during the 2010-2019 period in rural areas, the labor informality has rates significantly higher than urban ones; female labor participation continues very low in rural areas compared to that of men and the unemployment rate for them is higher than in the capitals; on the other hand, child labor continues to present high levels.

Dulal and Kattel (2020) mention another important point of view that should be noted within this research, they in their study carried out in Nepal affirm that there were no more opportunities to increase banana production by investing in land preparation, labor, and fertilizers, instead, suggest that an insurance scheme helps improve banana production and income, as they make farmers take risks, market more and seek more business opportunities. For the present study, this information was not accessed, but it is exhorted that the entities that oversee granting credits or financing, offer a suitable insurance scheme, as well as to raise awareness in the producer of the benefits of this.

Plantain productivity

As can be seen in Table 6, for PI_1 a negative effect that suggest a decrease in productivity equivalent on 0.08% was found, and for PI_2 a positive coefficient interpretated as a productivity increase of 19.13% was obtained; however, both were non-significant results. Therefore, there is not enough statistic evidence to demonstrate any effect of access to agricultural credit in the indexes evaluated for this crop.

Table 6. Results of propensity score matching for plantain crops applying 4-nearest neighbor matching and its standard error.

4-Nearest neighbor matching	ATT	
	Difference	S. E
PI_1	-0.00080	0.02887
PI_2	0.19129	0.20154
Sample number of Control group	559	
Sample number of Treatment group	194	

*P<0.10, **P<0.05, ***P<0.01.

As stated in the introduction, plantain crops currently work as a crop destined for national consumption with important growth projections, however, the results obtained have shown that the financing is not generating positive results in the productive improvement of plantain in the farmers from Valle del Cauca. For his part, Jesús Alberto Rodríguez Paz, (2020) points out in his work on the impact of agricultural credit on productivity at the national level, that some negative results may be since farmers in Colombia allocate the resources obtained to maintain the crop and not to improve the productivity.

According to CNA data, the plantain producers use the resources mainly to pay for agricultural inputs, labor, and installation of crops. However, no investment has been made in agricultural infrastructure or post-harvest processes, variables that in practice have been considered essential to increase productivity. There are some factors that influence decision-making on the advisability of investing in the variables mentioned above, such as specialized advice, knowledge, the farmer's openness to new technologies, among others.

The negative and indifferent effect of credit on productivity may also be due to the time the survey was carried out, since the crop could be in sowing, and only until the time of harvest are the results of the investment expected to be obtained, as is explained by Echavarría *et al,* (2017) who in their study at the national level, found positive and significant effects of credit in transitory or short-cycle crops and a negative effect in permanent and annual crops.

On the other hand, Dépigny (2019) also highlight that the high cost of crop plantain is particularly due to the necessary inputs, this is considered by farmers as one of the main reasons for the low success of the crop. In the case of plantain producers in Valle del Cauca, around 35% of the resources obtained through agricultural financing are used for agricultural inputs (seeds, fertilizers, insecticides, pesticides).

Finally, it should be noted that in Valle del Cauca only 13 % of Musaceae producers apply for credit, even though 87% are approved by some financial entity, which indicates that the main problem is not access to credit specifically. The difficulty may rather lie in the conflicts over land in Colombia, which can be summarized as: armed conflict, land with an agricultural vocation dedicated to other activities, and social inequality (Deininger, Castagnini, and González, 2013).

CONCLUSION

The impact of agricultural credit on the productivity of *Musaceae* crops in Valle de Cauca Colombia was analyzed. For banana crops, a positive and significant effect of credit on the tons produced per hectare was obtained (increase of 8.4%), however, significant results regarding the effect of credit on tons produced per employee were not found. Relating the results found from PI_1 and PI_2, it can be concluded that the farmer has not optimized and managed the available human resource. On the other hand, there was no statistical evidence of an increase or decrease in plantain productivity as consequence of the credit access.

Considering the results of the agricultural census, the investment of agricultural credit in both crops differs mainly in two items:

1. The low percentage of investment in post-harvest processes in plantain (0.6%) while in banana it was 2%.
2. There is 4% more investment in inputs in plantain crops than in banana crops.

As mentioned in the analysis of results, when agricultural credit has a maintenance approach and not productivity improvements, it has a direct impact on the results obtained; however, considering the Valle del Cauca banana market and the differences in investment of the credit in each crop, can be the reasons for the difference in the results obtained for both crops, however, is invited to develop researches that consider the effects of the credit programs on agricultural productivity when is investing in specific items.

As the impact evaluations are valuable tools for the design of good public policies, helping to reveal their quality and effect. The most important contribution of this research was to reveal the impact of accessing agricultural credit in *Musaceae* producers from Valle del Cauca, considering two transcendental productive factors: land and labor. In addition, it was shown what items were being invested in once accessed it and the openness that the farmer had to agricultural credit. In other words, through this research was understanded the investment priorities of the farmer and the rate of participation in said credit programs, demonstrating that despite the high rate of credit granting compared to the low participation, it opens the door to the discussion of the weaknesses of financing policies to reach all farmers. In addition, with the results obtained once again, the high costs of inputs that the farmer faces and that prevent investing in other items that allow them to increase productivity efficiently are demonstrated.

Additionally, it is invited to compare the impact of credit programs on agricultural productivity of other crops of departmental and/or national interest with different methods and approaches. Moreover, it is considered important to take into account how credit programs are designed and its target population.

ACKNOWLEDGMENTS

The authors thank the professor Mingwang Cheng for his support, constructive comments during the structuring of the project and subsequent suggestions in the analysis and data processing.

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Research article

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Revista Facultad Nacional de Agronomía

Effect of different concentrations of bioslurry on the germination and production of tomato seedlings (*Solanum lycopersicum* L.)

Efecto de diferentes concentraciones de biol en la germinación y producción de plantines de tomate (*Solanum lycopersicum* L.)

https://doi.org/10.15446/rfnam.v76n1.99647

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ABSTRACT

Tomato is one of the main horticultural products in Argentina. Its cultivation is intensive in the use of fertilizers and pesticides, which negatively impact the environment. The chemical fertilizers commonly used are, to some extent, gradually being replaced by liquid biofertilizers. A liquid biofertilizer (bioslurry) made from goat manure, fresh plant residues, and some mineral inputs was physicochemically characterized. To evaluate its effect on tomato (*Solanum lycopersicum* L.) performance, two trials were conducted between October and November 2020: a seed germination test with increasing bioslurry dilutions (0 to 15%); and another trial in a greenhouse located in Luján de Cuyo, Mendoza, to evaluate the effect of different doses of bioslurry (5, 10 and 15%), compared to a commercial fertilization plan for seedlings in plastic trays. The experimental design used was completely randomized plots in both cases. Bioslurry at concentrations above 5% negatively affected tomato seed germination. The biofertilizer achieved a nutritional effect on seedlings compared to the unfertilized control. However, this effect was inferior to the treatment with commercial fertilizers. It is advisable to initiate applications of bioslurry after seedlings have emerged. Further studies are needed on biofertilizer use concentrations, doses, application frequencies, and suitability for different crops. Also, to achieve the effect of a commercial fertilization program, it will be necessary to combine enriched bioslurry with other bio inputs that complement plant nutrition.

Keywords:
Digestate
Inhibition
Nutrition
Plant growth
Seedling

RESUMEN

El tomate es uno de los principales productos hortícolas en la Argentina. Su cultivo es intensivo en el uso de fertilizantes y pesticidas, que impactan negativamente al ambiente. Los fertilizantes químicos de uso habitual son, en cierta medida, gradualmente reemplazados por biofertilizantes líquidos. Un biofertilizante líquido (biol) elaborado en base a estiércol de cabra, restos vegetales frescos y algunos insumos minerales, fue caracterizado fisicoquímicamente. Para evaluar su efecto en el desempeño del tomate (*Solanum lycopersicum* L.), se realizaron dos ensayos entre octubre y noviembre de 2020: una prueba de germinación de semillas con diluciones crecientes biol (0 a 15%); y otro ensayo en un invernadero ubicado en Luján de Cuyo, Mendoza, para evaluar el efecto de las diferentes dosis de biol (5, 10 y 15%), comparado con un plan de fertilización comercial de plántulas en bandejas plásticas. En ambos casos se utilizó un diseño de parcelas completamente aleatorizadas. El biol en concentraciones superiores al 5% afectó negativamente la germinación de las semillas de tomate. El biofertilizante logró un efecto nutricional en las plántulas, comparado con el testigo sin fertilizar. Sin embargo, este efecto fue inferior al tratamiento con fertilizantes comerciales. Es recomendable iniciar las aplicaciones de biol luego de que las plántulas hayan emergido. Se necesitan mayores estudios respecto de concentraciones de uso, dosis y frecuencias de aplicación de los biofertilizantes y su adecuación a diferentes cultivos. Asimismo, para lograr el efecto de un programa de fertilización comercial, será necesario combinar bioles enriquecidos, con otros bioinsumos que complementen la nutrición vegetal.

Palabras clave:
Digestato
Inhibición
Nutrición
Crecimiento vegetal
Plántulas

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Received: June 21, 2022; Accepted: November 04, 2022
Rev. Fac. Nac. Agron. Medellín 76(1): 10149-10156. 2023
ISSN 0304-2847 / e-ISSN 2248-7026

Tomatoes are one of the main horticultural crops in Argentina, with a cultivated area of 17,000 ha and an average annual production of around 1,100,000 t. In the provinces of Mendoza and San Juan, located in Cuyo region, tomato production is one of the leading economic activity, being one of the most important areas of the country with that end (Ministerio de Agricultura, Ganadería y Pesca, 2020).

In recent years, different studies have shown that the use of synthetic fertilizers and pesticides causes several environmental problems, mainly affecting soil and water. The main negative effects are related to the affection of non-target organisms, like adjacent crops, benefic insects, arachnids and microorganisms, and aquatic life in general (Krasilnikov *et al*., 2022; Bowmer, 2018). In this sense, it is observed that agricultural productions begin to adopt the use of bio-inputs more frequently (Kumar 2018; Liriano González *et al*., 2021). Biological products are commonly made from organic waste, with lower costs than traditional pesticides and less environmental impact. The efficiency in the use of these bio-inputs lies mainly in the presence of beneficial microorganisms for plant growth, and the nutritional content is dependent on the raw material used (Bonten *et al*., 2014).

Although there are a wide variety of bio-products, from aerobic or anaerobic digestions, relatively few experiences studied the effects, characteristics, and mechanisms by which they benefit plant growth. One of them is the bioslurry or digestate, which consists in the liquid fraction of anaerobic digestion, containing a high microbial and nutritional load (Bonten *et al*., 2014). While most studies focus on bioslurry resulting from biogas production (Groot and Bogdanski, 2013), there are others developed specifically for plant nutrition, where the methanogenic generation is dismissed (FAO, 2013). In this case, it is expected a superior performance as a promoter of plant growth, due to the addition of mineral salts and organic material that favor benefic microbial growth and nutrient content in the final product.

In general, bioslurry can play an important role as a source of nutrients for crop production, because they are readily available, allowing short-term effects of fertilization (Möller and Müller, 2012). Fang-Bo *et al.* (2010) demonstrated in tomato crops, that bioslurry significantly improves the macronutrient contents available in the soil, compared to the control without fertilizer and the conventional fertilization methods. Likewise, its use significantly increases the quality of the fruits (content of amino acids, proteins, ß-carotene, soluble solids, and vitamin C in tomatoes), but not the yield or weight of the fruits.

However, there does not appear to be a consensus on the forms of implementation and the concentrations to be used. For their part, Bonillo *et al.* (2015) evaluated frequent foliar applications of organic fertilizers with different concentrations, including supermagro (enriched bioslurry), obtaining positive effects in lettuce seedlings (*Lactuca sativa* L.). The effect of this last and other biofertilizers was also confirmed in the yields of tomatoes growing in a greenhouse (Parodi *et al*., 2020). On the other hand, Silva *et al.* (2011) suggest possible phytotoxicity of the same at concentrations higher than 10%, which was corroborated in periodic applications in the neck of bean plants. In addition, Díaz Montoya (2017) showed that the percentage of germinated lettuce seeds decreased when the dose increased from 2 to 4%.

Due to the scarcity of scientific studies, regarding the use of bioslurries as liquid fertilizers in the production of tomato seedlings, the present work evaluated the effect of different doses of bioslurry, on seed germination and the production of biomass of tomato seedlings for fresh consumption.

MATERIALS AND METHODS

The study was conducted in a greenhouse located at INTA's Mendoza Agricultural Experimental Station, Luján de Cuyo, Mendoza (33° 00 '20"S, 68° 51' 54" W, 929 masl) during October and November 2020. The cultivar used to evaluate the liquid biofertilizer (bioslurry) was the "cocktail type" tomato, selected by the INTA La Consulta and extracted from tomatoes grown during season 2019-2020, at INTA Mendoza.

Preparation and characteristics of liquid biofertilizer (bioslurry)

The bioslurry was elaborated according to the methodology for “enriched liquid biofertilizer” preparation, proposed by the FAO (2013). To do this, a 220-liter plastic drum was used, where the components, listed in Table 1, were

Table 1. Components incorporated into the bioslurry production process.

Component	Quantity	Unit
Fresh chopped alfalfa	10	kg
Goat manure	60	L
Bentonite	4	kg
Ground eggshell	0.5	kg
Wood ash	3	kg
Bone ash	3	kg
Cow's milk	5	L
Water	160	L

placed. Anaerobic digestion was performed for four months. The mixture was stirred weekly to homogenize the materials. At the end of the elaboration process, the mixture was filtered by a canvas fabric, and the liquid fraction was stored in a plastic drum with hermetic closure, protected from direct radiation. The final composition of the bioslurries in solution is presented in Table 2.

Table 2. Physio-chemical characteristics of the dilutions used in the germination evaluation.

Parameter	Dilution of bioslurry in distilled water				
	15%	10%	5%	3%	1%
EC (dS m^{-1})	1.857	1.302	0.702	0.418	0.148
N-NO_3 (mg L^{-1})	11.790	7.860	3.930	2.358	0.786
N-NH_4 (mg L^{-1})	61.155	40.770	20.385	12.231	4.077
P (mg L^{-1})	4.425	2.950	1.475	0.885	0.295
K (mg L^{-1})	215.175	143.450	71.725	43.035	14.345
Ca (mg L^{-1})	169.500	113.000	56.500	33.900	11.300
Mg (mg L^{-1})	45.000	30.000	15.000	9.000	3.000
Na (mg L^{-1})	154.755	103.170	51.585	30.951	10.317
Fe (mg L^{-1})	0.090	0.060	0.030	0.018	0.006
Cu (mg L^{-1})	0.060	0.040	0.020	0.012	0.004
Zn (mg L^{-1})	0.045	0.030	0.015	0.009	0.003
Mn (mg L^{-1})	0.015	0.010	0.005	0.003	0.001

EC: electrical conductivity; N-NO_3: nitrate nitrogen; N-NH_4: ammonia nitrogen.

Germination test

To evaluate the effect of bioslurry on germination, 25 tomato seeds were placed on filter paper in 90 mm Petri dishes, based on Sobrero and Ronco (2004). The experimental design used was completely randomized, with six treatments, consisting in 4 cm^3 per plate of bioslurry at 15% (Biol 15), 10% (Biol 10), 5% (Biol 5), 3% (Biol 3), 1% (Biol 1) and distilled water as control treatment (CW). Each treatment was repeated thrice. Once the seeds were placed on the plates, they were brought to the stove at 22 °C for 120 h in darkness.

The germination proportion (GP = number of seeds germinated in each dilution/number of seeds germinated in the control), length of hypocotyl, and radicle were determined. The effect of each dilution on the germination

of tomato seeds was analyzed statistically using a non-parametric analysis of Kruskal Wallis (P<0.05), due to the lack of normality of data.

Seedling production test

One tomato seed per cell (17.15 cm^3) was sown in plastic trays with 40 alveoli. The substrate used was Cocomix (Línea profesional, Carluccio, Bs. As., Argentina), composed of sphagnum brown peat, perlite, and coconut fiber. Irrigation and nutrition treatments were provided through immersion of the culture trays in plastic polypropylene containers, once a week. Periodically and according to demand by evapotranspiration, all containers were irrigated and remained in conditions close to field capacity during the test period. For irrigation and dilutions, free chlorine water, extracted from a subterranean well was used.

The treatments were: fertilization with a 5% solution of bioslurry (Biol 5); 10% solution of bioslurry (Biol 10); 15% solution of bioslurry (Biol 15); control with well-water (CW) and a fertilized control (CF), supplemented with nutrient complexes (Rootex 8-46-5.5, Cosmoflor S. A., Mexico, and Plant-Prod Iniciador, 10-52-10), Cosmoflor S. A., Canada, both dissolved at 2 g L^{-1} in irrigation water. Prior to the incorporation, electrical conductivity expressed as dS m^{-1} was measured in each treatment at 23 °C, obtaining CW: 1.070; Biol 5: 1.772; Biol 10: 2.372; Biol 15: 2.927 and CF: 2.280. The experimental design used was completely randomized with 15 experimental plots, where three replications were arranged per treatment. The effect of treatments on the emergence and development of seedlings was evaluated by analysis of variance and comparison of means (Fisher's LSD test; P<0.05), using the Infostat statistical software.

At 7, 10, 12, and 14 days after sowing (DAS), the number of emerged plants was recorded. With the data obtained, the percentage of emergence was calculated, as a relationship between the number of germinated seeds and the number of seeds sown (Prado-Urbina *et al.*, 2015). The evaluation of total fresh biomass (aerial and root) was carried out at 28 DAS, when seedlings of the CF treatment had three true leaves, and an approximate height of 10 cm from the base to the vegetative apex. From the central lines of each replica of the tray, ten seedlings were extracted, roots washed, and weighed.

RESULTS AND DISCUSION

Germination proportion

This variable was significantly (P<0.05) reduced by Biol 15 (0.20±0.12). Meanwhile, Biol 5 and Biol 10 (0.91±0.10 and 0.56±0.11) did not differ significantly from the Biol 15 and CW, although showing a tendency to affect germination. The values obtained with dilutions of Biol 1 and Biol 3 (0.98±0.03 and 1.00±0.00) presented no differences regarding to CW (1.00±0.00, Figure 1).

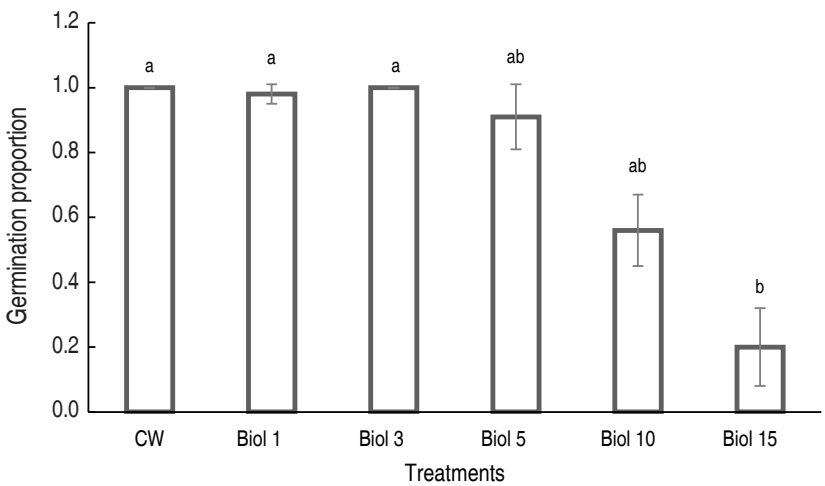

Figure 1. Germination proportion (number of seeds germinated in each dilution/number of seeds germinated in the control) of tomato seeds for each solution of bioslurry tested (Kruskal Wallis, P<0.05). Bars correspond to the standard deviation. CW: Water control; Biol 1: 1% bioslurry dilution; Biol 3: 3% dilution; Biol 5: 5% dilution; Biol 10: 10% dilution; and Biol 15: 15% dilution.

Hypocotyl length

In this case, none of the dilutions differed significantly from the CW. However, at concentrations 1, 3 and 5% (4.17±0.54; 4.12±0.79 and 4.09±0.41 cm), hypocotyls were significantly longer than 15% (0.99±0.94 cm), showing values higher than CW (3.14±0.64 cm, Figure 2).

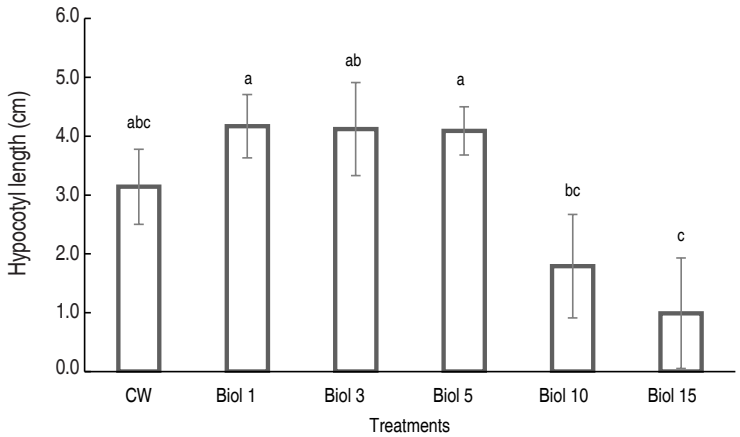

Figure 2. Length of tomato seedling hypocotyl for each bioslurry dilution. Averages with a common letter are not significantly different (Kruskal Wallis, $P<0.05$). Bars correspond to the standard deviation. CW: Water control; Biol 1: 1% bioslurry dilution; Biol 3: 3% dilution; Biol 5: 5% dilution; Biol 10: 10% dilution; and Biol 15: 15% dilution.

Radicle length

Dilutions 1, 3, and 5% (4.01±0.34; 4.84±0.92 and 3.90±1.22 cm) did not differ from CW (5.42±1.51 cm), while 10 and 15% (3.00±0.47; 0.69±0.35 cm) were significantly lower than CW. In this sense, the responses generated by dilutions lesser than 5% indicated an absence of negative effects on tomato seedlings (Figure 3).

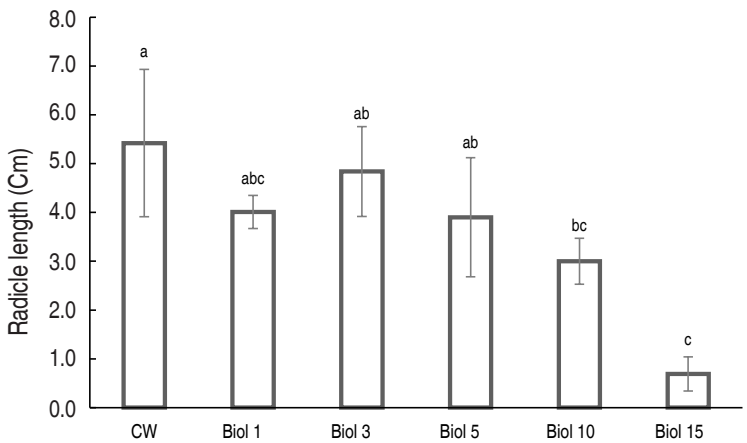

Figure 3. Tomato seedling radicle length for each bioslurry solution tested. Mean values with a common letter are not significantly different ($P<0.05$, Kruskal Wallis). Bars correspond to the standard deviation. CW: Water control; Biol 1: 1% bioslurry dilution; Biol 3: 3% dilution; Biol 5: 5% dilution; Biol 10: 10% dilution; and Biol 15: 15% dilution.

In general, it was observed that after germination, tomato hypocotyl and radicle length decreased with increasing bioslurry concentration. According to Medina *et al.* (2015), very high concentrations of bioslurries produced from sheep manure, inhibit the germination of lettuce seeds and limit the growth of the radicle, possibly by increasing

the electrical conductivity of the solution. Likewise, Goykovic *et al.* (2014) verified in tomato seeds, the osmotic and non-ionic detrimental effects by saline solutions during the germination process.

Díaz (2017) confirmed the presence of precursors of hormonal action, such as gibberellins, auxins, and cytokinins. In this sense, bioslurry concentrations between 2 and 5% presented a stimulating effect on lettuce, cotton, and alfalfa germination. Also, Medina Vargas (1990) points out that there are several hormonal precursors in the composition of bioslurry, but also certain repressors such as methionine.

It is important to consider that bioslurry is a complex solution of macro and micronutrients, microorganisms and growth precursors. Therefore, it became difficult to conclude and demonstrate that the seed's response is related to the presence of a particular chemical element, or a specific growth precursor. In this sense, the plants would react to the whole; that is, the interaction between chemical elements, growth precursors, and microbial population present in the bioslurry.

Emergence and biomass of seedlings

At 7 days, all treatments obtained significantly lower values than the control (53.5±7.37%). At 10 days, only the Biol 15 differed from the rest of the treatments with the lowest values. After 10 days, no statistical differences were observed among the treatments and the CW, although the trend observed at the beginning of the emergency assessment was maintained (Figure 4).

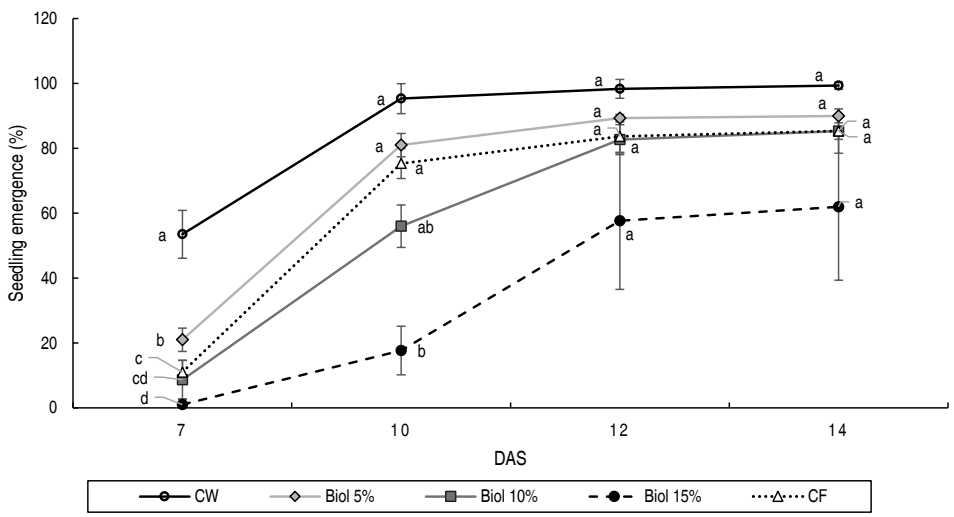

Figure 4. Emergence of tomato seedlings with solutions at different concentrations of bioslurry. Averages with a common letter are not significantly different (P<0.05, LSD Fisher). Bars correspond to the standard deviation. CW: Water control; CF: fertilized control; Biol 5: 5% bioslurry dilution; Biol 10: 10% dilution; and Biol 15: 15% dilution.

The fresh biomass obtained in the seedlings after 28 days, showed a significantly higher response for fertilized control (CF), while CW presented lower values than the rest of the treatments. On the other hand, the solutions of Biol 5 and 10% (340.66±49.69; 357.75±67.26 mg) obtained a fresh weight lower than CF (707.79±108.34 mg) but significantly higher than Biol 15% (227.21±4.03 mg, Figure 5).

Periodic applications of 15% bioslurry were less effective than 5 and 10% dilutions, both of which behaved similarly. These values correspond to the recommendations or the use of these types of biofertilizers (FAO, 2013). Higher concentrations tend to limit the growth of seedlings.

Finally, fertilization plans to achieve complete seedling nutrition should be complemented by the design of a substrate containing organic compounds, as it was shown that both the development of the aerial part of the seedlings and the root system is favored (Moraes *et al.*, 2021).

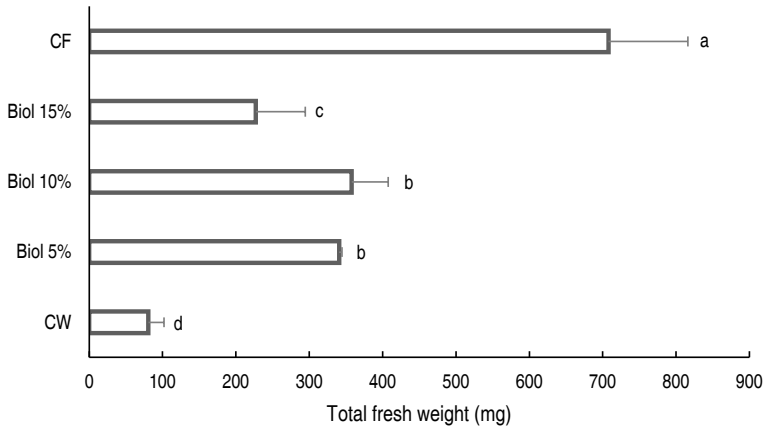

Figure 5. Fresh total biomass of tomato seedlings with solutions at different concentrations of bioslurry. Mean values with a common letter are not significantly different (P<0.05; LSD Fisher). Bars correspond to the standard deviation. CW: Water control; CF: Fertilized control; Biol 5: 5% bioslurry dilution; Biol 10: 10% dilution; and Biol 15: 15% dilution.

CONCLUSIONS

Bioslurry at concentrations above 5% negatively affected tomato seed germination, and applications of the biofertilizer initiated from sowing in seedling trays, reduced the emergence of tomato seeds. Nevertheless, bioslurry achieved a nutritional effect on tomato seedlings compared to the unfertilized control. However, this effect was inferior to the treatment with commercial fertilizers. Results suggested that in weekly applications, will be advisable bioslurry concentration of 5 to 10% for positive tomato seedling growth, higher concentration will be detrimental. It is advisable to start biofertilizer applications after the seedlings have emerged.

The simple and low-cost elaboration of this biofertilizer allows its adoption as a nutrient solution for the production of tomato seedlings. However, it is necessary to deepen studies on concentrations of use, doses, and frequencies of biofertilizer application and their adaptation to different crops. It will also be appropriate to test, in future trials, alternatives of enriched liquid biofertilizers, such as "supermagro" or the combination of bio inputs (e.g., compost or other organic compounds as substrate's components, compost tea, etc.), to verify whether it is possible to achieve the yield of commercial seedling nutrition.

ACKNOWLEDGMENT

To the memory of Hernán Vila, former Director of INTA Mendoza, who gave all his support to be able to carry out the experimentations, in an extremely adverse context determined by the pandemic. To Federico DeBiazi for his invaluable assistance.

The trial was funded by the projects "Intensificación sostenible de los sistemas bajo cubierta" and "Desarrollo de sistemas productivos para áreas de amortiguamiento" of the Instituto Nacional de Tecnología Agropecuaria (INTA), and by the Fondo para la Investigación Científica y Tecnológica (FONCYT, PICT 2015-1727 to Ernesto Martín Uliarte).

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Effect of Bt (Cry1Ac and Cry2Ab) and non-Bt cotton on the temporal variation of *A. grandis* and representatives of the *Spodoptera* complex in Tolima, Colombia

UNIVERSIDAD NACIONAL DE COLOMBIA

Efecto del algodón Bt (Cry1Ac y Cry2Ab) y no Bt en la variación temporal de *A. grandis* y representantes del complejo *Spodoptera* en Tolima, Colombia

https://doi.org/10.15446/rfnam.v76n1.100904

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ABSTRACT

Transgenic cotton plants (*Bacillus thuringiensis* Berliner (Bt)) has significant influenced the integrated pest management around the world. In Colombia, *Anthonomus grandis* Boheman and *Spodoptera* complex are currently considered the main pests in cotton crops. Therefore, this study evaluated the effect of Bt (Cry1Ac and Cry2Ab) and non-Bt cotton on the population fluctuation during two years in Tolima region. A Pearson correlation matrix was carried out between the pest variables and yield, while climatic variables and insect populations were correlated in four phenological stages with Spearman rank correlations. Additionally, a factor analysis for mixed data was performed in order to compare the effect of genotypes on the population fluctuation of the insects. For *A. grandis*, no differences in their populations were presented. However, in yield non-Bt cotton plants showed a higher inverse correlation with the perforated bolls compared to Bt cotton. In relation to the *Spodoptera* complex, the Bt genotype had 67.4% fewer larvae compared to non-Bt cotton. Statistically significant differences were presented. However, there was not a total absence of the pest during the entire crop cycle. These results suggest that if refuge zones and pest management practices are not determined in the study area, *Spodoptera* complex could generate resistance to genetically modified plants.

Keywords:
Pest resistance
Population density
Transgenics plants

RESUMEN

Las plantas de algodón transgénicas (*Bacillus thuringiensis* Berliner (Bt)) han influido significativamente en el manejo integrado de plagas en todo el mundo. En Colombia, *Anthonomus grandis* Boheman y el complejo *Spodoptera* son considerados actualmente las principales plagas en los cultivos de algodón. Por lo tanto, este estudio evaluó el efecto del algodón Bt (Cry1Ac y Cry2Ab) y no Bt en la fluctuación poblacional durante dos años en la región del Tolima. Se realizó una matriz de correlación de Pearson entre las variables plaga y rendimiento, mientras que las variables climáticas y las poblaciones de insectos fueron correlacionadas en cuatro estados fenológicos con la correlación de rangos de Spearman. Adicionalmente, se realizó un análisis factorial para datos mixtos con el fin de comparar el efecto de los genotipos sobre la fluctuación poblacional de los insectos. Para *A. grandis*, no se presentaron diferencias en sus poblaciones. Sin embargo, en rendimiento, las plantas de algodón no Bt mostraron una mayor correlación inversa con las cápsulas perforadas en comparación con el algodón Bt. Con relación al complejo *Spodoptera*, el genotipo Bt tuvo un 67,4% menos de larvas en comparación con el algodón no Bt. Se presentaron diferencias estadísticamente significativas. Sin embargo, no hubo ausencia total de la plaga durante todo el ciclo del cultivo. Estos resultados sugieren que, si en el área de estudio no se fijan zonas de refugio y prácticas de manejo de plagas, el complejo *Spodoptera* podría generar resistencia a plantas genéticamente modificadas.

Palabras clave:
Resistencia a plagas
Densidad de población
Plantas transgénicas

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Received: October 01, 2022; Accepted: December 02, 2022
Rev. Fac. Nac. Agron. Medellín 76(1): 10157-10166. 2023
ISSN 0304-2847 / e-ISSN 2248-7026

The cotton crop (*Gossypium hirsutum* L.) is highly related to the economic, social, and agro-industrial development of many communities. In Colombia, for the year 2019, 18,327 hectares were planted, with total yields of 922 kg of fiber per ha^{-1} (Conalgodon, 2020). However, this crop is attacked by a wide variety of arthropod phytophagous with the potential to cause serious damage to the plant (Ribeiro *et al.,* 2015). This crop is mainly affected by sucking and chewing insect species. It is estimated that 50-60% of losses are due to chewing insect infestations, since they affect fiber quality, decrease crop yield and increase production costs (Shad *et al.,* 2022).

Among the most important pests, the *Spodoptera* complex (*Spodoptera frugiperda*, *Spodoptera ornithogalli*, and *Spodoptera sunia*) (Lepidoptera: Noctuidae) and *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) are highlighted (Santos *et al.,* 2009; de Oliveira *et al.,* 2016). Insecticide control of *S. frugiperda* is difficult because it prefers to oviposit on the lower surface of leaves at almost all phenological stages of the crop (Barros *et al.,* 2010). While for larval stages, different preferences between species have been reported; for *S. frugiperda* its predominance in flowers and bolls have been indicated, while *S. ornithogalli*, in leaves and flowers and *S. sunia*, in flowers (Santos *et al.,* 2009).

On the other hand, *A. grandis* is an economically important pest in crops in the New World (Salvador *et al.,* 2014). Damage of this insect to cotton crops is caused by the larvae and the adult, when the female determines that the site is suitable for oviposition, she pierces the flower bud or boll with her face and inserts an egg into the tissue, inside of the which develops the larval stages (da Silva *et al.,* 2008; Salvador *et al.,* 2021). The bracts of infested floral buds usually turned yellow and flared, and the floral buds dropped from the plant. Infested bolls may or may not have dropped (Sorenson and Stevens, 2019).

Bollgard II® transgenic cotton (which expresses two Bt toxin genes, Cry1Ac and Cry2Ab) is effective against many lepidopteran pests (Yang *et al.,* 2015; Bahar *et al.,* 2019). By 2015 approximately 84% of cotton crops planted in the United States had the *Bacillus thuringiensis* (Bt) protein (James, 2015), leading to a decline in pest populations and the use of chemical synthesis insecticides The sustainable management of Bt crops is threatened by the increase in pest resistance to this type of technology; therefore, the implementation of strategies that minimize insect resistance is critical to ensure the long-term success of this technology (Yang *et al*., 2017; Khakwani *et al*., 2022). To reduce pest resistance, in recent years many countries have implemented the use of pyramid crops that produce more than two Bt toxins different in their mode of action but targeting the same pest, thus achieving a decrease in crop damage (Carrière *et al*., 2021). Although none of the commercial transgenic varieties contain resistance genes against *A. grandis* or the *Spodoptera* complex, several Cry proteins show biological activity against these pests and at least one of them, the Cry1Ia12 and Cry10Aa proteins, has been inserted into cotton plants, conferring partial resistance to these pests (De Oliveira *et al.,* 2016; Ribeiro *et al.,* 2017).

To prevent resistance by pests, Bollgard II®, has been used since 2004, giving it the ability to bind at different sites in the midgut of larvae, thus increasing plant efficiency by decreasing the probability of cross-resistance in pest species (Knight *et al.,* 2016; Meissle and Romeis, 2018). Studies developed in Valle del Sinú (Colombia) by Osorio-Almanza *et al.* (2018) indicate that the combination Bollgard II® together with Cry1Ia12 protein, reduced *A. grandis* and *S. frugiperda* management costs by more than 40% and limited the emergence of combined resistance.

Currently, there are no data on population fluctuation or damage caused by these pests in genotypes with Bt (Cry1Ac+Cry2Ab) and without this technology in Colombia. Therefore, this research aimed to determine the effect of Bt (Cry1Ac and Cry2Ab) and non-Bt cotton on the population dynamics of *A. grandis* and representatives of the *Spodoptera* complex in El Espinal, Tolima, Colombia.

MATERIALS AND METHODS

Experimental area

The present study was performed in the Colombian agricultural research corporation (Agrosavia) C.I. Nataima, situated in El Espinal, Tolima, Colombia at 04° 11'32.49" LN and 74°57'35.10" LW for two years 2015

and 2016. The climatic conditions during the time of the experiments were mean temperature 26.62±0.15 and 27.64±0.12 °C, mean relative humidity 80.04±0.62 and 75.53±0.68% and accumulated precipitation of 73.82 and 124.60 mm during 2015 and 2016 respectively. The plantings were carried out in March for both years, with a non-Bt cotton variety (Deltapine® 90) and a genetically modified variety with *B. thuringiensis* genes Cry1Ac and Cry2Ab (Fibermax1740B2F- FM1740B2F). The experimental design was paired plots (blocks) with three repetitions (800 m^2 each). This was executed for two treatments (Bt and non-Bt cotton) with a total area of 2500 m^2 in each. The established cotton crops in this study are part of the rice, corn-cotton rotational cropping system in the region.

For the management of the crop, insecticide applications were not made for *A. grandis* or *Spodoptera* spp., the fertilization plan was carried out based on the results of the soil analysis, dividing the nutrition into three applications: vegetative stage, beginning of flowering and formation of bolls. The physiological management, with application of Thidiazuron (defoliant) when 70% of bolls opening were presented and the control of weeds were carried out with applications of glyphosate directly to the Bt-cotton variety and directed to the streets in the non Bt-cotton, complemented with manual weeding with a hoe.

Evaluation of temporal variation

To define the temporal variation of pest insects, assessments were started 4 days after emergence (DAE) for *Spodoptera* spp. and at 34 DAE for *A. grandis*, on April 7, 2015, April 8, 2016, at the beginning of the crop May 11, 2015 and May 12, 2016, at which time the first flower buds appeared. The cotton plant was used as a sampling unit, in each repetition 10 randomly selected plants were taken, the samplings were carried out every four days, ending at 118 DAE covering the flowering and fruiting period of the two cotton varieties (Grigolli *et al.,* 2013). The response variables evaluated in each plant were: for *A. grandis* the number of total flower buds (*TFB*) and total bolls (*TB*), measured by counting each structure from the lower third to the upper third of the plant; number of perforated flower buds (*PFB*) and bolls (*PB*) by oviposition, detecting the gummy substance and the visible obstruction left by the female after oviposition; and number of adult insects (*NBW*) present in the plant structures (Grigolli *et al.,* 2013; Silva *et al.,* 2015). For *Spodoptera* spp. were the number of larvae per plant (NLarvae) and the presence of damage (Larv_dam) (Abd El-Salam *et al.,* 2011; Zakir *et al.,* 2017).

During the crop cycles, the variables temperature (°C) (average, maximum and minimum), relative humidity (%), solar radiation (w m^{-2}) and precipitation (mm) were obtained of a weather station watchdog series 2000. At harvest, to evaluate the damage (%) in production, 99 plants were randomly selected per variety (33 per repetition), which were packed in cotton canvas and individually labeled, where the percentage of damage was evaluated from the number of open bolls per plant and number of open bolls with damage of *A. grandis* in one or more locules, total weight of the plant to estimate the yield and average weight per open boll.

Statistical analyses

For all variables an exploratory and descriptive analysis were carried out. Subsequently, in each variety, a Pearson correlation matrix was carried out between the insect variables and yield. Additionally, a factor analysis for mixed data was performed. Then, for each variable, its accumulation curves throughout the cycle were calculated using the technique of "accumulated insect days or damage" based on the area under the curve progression stairs (ABEP) (Jaramillo-Barrios *et al.,* 2019). The variables were compared by an analysis of variance for the randomized complete block design with a combinatorial arrangement of fixed effects (variety, year, and variety * year) and randomized (blocks). Normality was assessed using the Shapiro-wilks test and homoscedasticity through a scatter plot of residuals on the *y* axis and fitted values on the *x* axis. If there were statistical differences, these were compared with Fisher's LSD test at 5%.

The relationship of the climatic factors on the insect damage variables and the productive variables were compared by Pearson correlation coefficient. Also, Spearman rank correlations were calculated between the insect populations and the climatic variables in four phenological stages: vegetative, juvenile, reproductive and maturation. Statistical software R v. 3.3.2 was used in the analyzes (R Core Team, 2016).

RESULTS AND DISCUSSION

Temporal variation of *A. grandis* in non-Bt and Bt cotton

The temporal variation of *PFB* by *A. grandis* started with a progressive increase from 60 DAE in 2015 and 50 DAE in 2016 (Figure 1A-B). The highest population peaks of *PB* in 2015 were recorded at 90 DAE with 4.4±0.56 and 3.5±0.41 in non-Bt and Bt cotton, respectively. In 2016, they were presented in DP90® at 78 DAE with 10.6±1.3 and in FM1740B2F at 74 DAE with 7.1±0.43. The fluctuation of *PB* took place with a gradual increase to 82 DAE in 2015 and to 74 DAE in 2016 (Figure 1C-D). The largest population peaks of *PB* in 2015 were presented in DP90® at 94 DAE with 5.4±0.47 and in FM1740B2F at 90 DAE with 3.5±0.40. In 2016, the 94 DAE were presented with 14.8±0.75 and 13.4±0.97 in non-Bt and Bt cotton in their order. The number of individuals increased their populations after 70 DAE, the highest peaks occurred at 104 DAE and 100 DAE in 2015 and 2016 (Figure 1E-F).

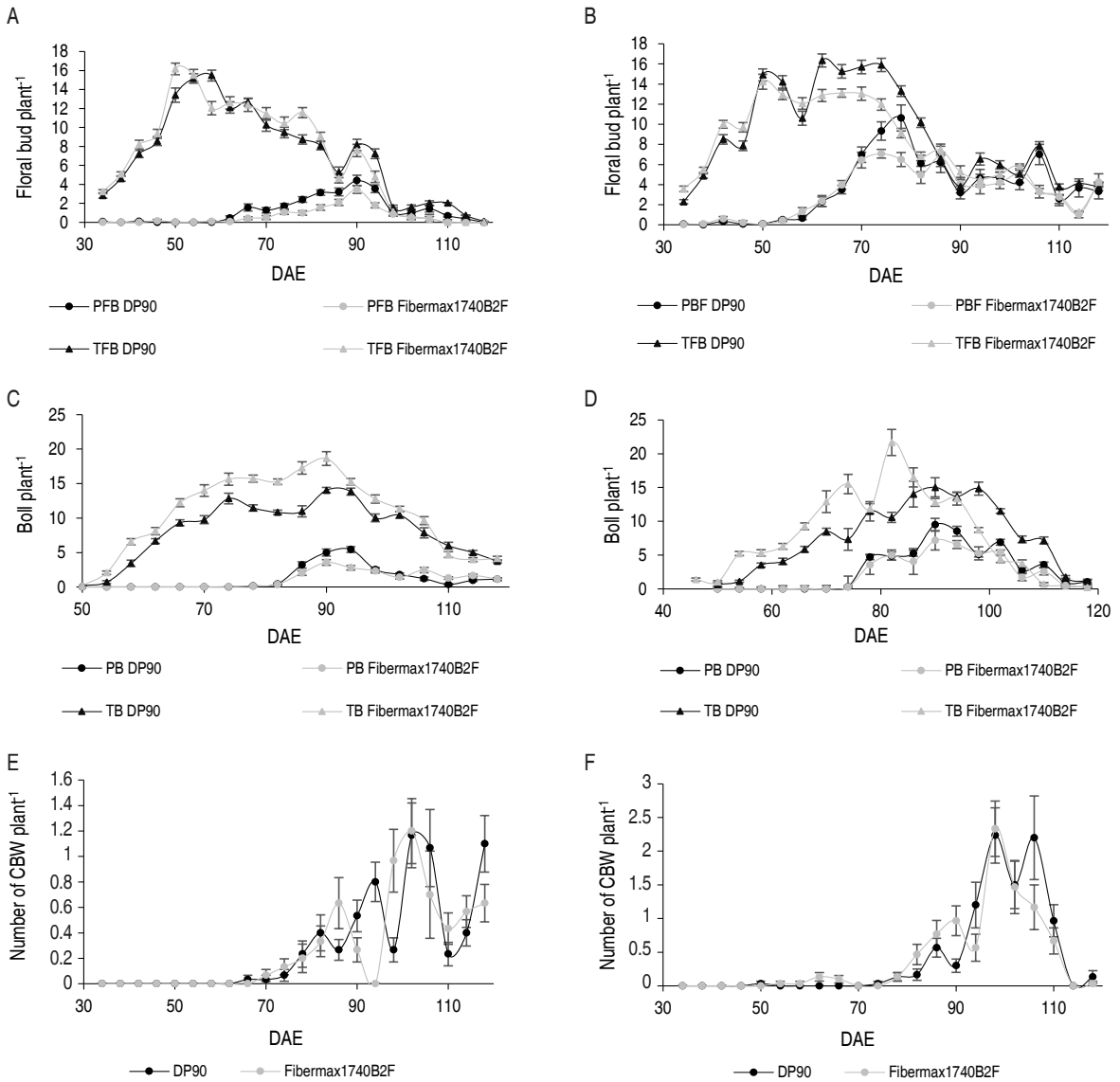

Figure 1. Temporal variation of *A. grandis*. A. Number of total and perforated average flower buds 2015; B. Number of total and perforated average flower buds 2016; C. Average number of total and perforated bolls 2015; D. Number of total and perforated average bolls 2016; E. Number of average cotton boll weevils (CBW) per plant 2015; F. Number of average cotton boll weevil adults per plant 2016. The black color represents the DP90® variety, while the light gray represents the FM1740B2F variety.

In relation to the percentage of damage caused in *PFB*, in 2015, the highest damage was reached at 86 DAE with 61.31±4.81% and 46.45±5.96% in non-Bt and Bt, respectively. In 2016, this level of damage reached its maximum potential at 86 DAE with 74.97±7.77% and 80.74±6.96% in their order. These results are similar to the reported by Oliveira *et al.* (2022), who evaluated the spatio-temporal distribution of *Anthonomus grandis* and reported higher percentages of infected floral buds during the phenological stages of late flowering and open bolls in the wet and dry season in cotton crops in Brazil. Also, an increase in the infested reproductive structures is expected until 'cut-off' (end of floral bud production). After that, floral buds decline quickly, and the boll weevil populations generally plateau (Showler *et al.,* 2005).

Population fluctuation of complex *Spodoptera* in non-Bt and Bt cotton

In Figure 2 A-B, shows the populational variation of the *Spodoptera* complex in cotton crops. These herbivore-insects can affect in different phenological stages, as in the vegetative phase, consuming leaves (Pascua and Pascua, 2002), and in the reproductive phase, feeding on flower buds, flowers, and bolls (Gomes *et al.,* 2017). Regarding the number of larvae, the highest population levels were observed in 2015 (Figure 2A). At 38 DAE, the highest peak was evidenced with 0.7±0.19 larvae per plant in DP90®. From the 90 DAE a constant decrease of the populations is observed. In 2016, a different behavior is presented, where from 2 to 46 DAE larvae were constantly present. At 6 DAE in variety DP90®, the population peak was recorded with 0.6±0.17. After 46 DAE the levels dropped considerably. A lower average number of larvae was found in Bt cotton (0.045±0.01) compared to the conventional (0.138±0.02), which explains the joint efficacy of the Cry1Ac and Cry2Ab proteins on *Spodoptera* spp. Specifically, for *Spodoptera frugiperda*, Valencia-Cataño *et al.* (2014) concluded that the larvae that fed on the variety that contained Cry1Ac + Cry2Ab proteins had a lower survival rate compared to the variety with only Cry1Ac and conventional. However, the levels of *Spodoptera* spp. in transgenic cotton were not constantly reduced throughout the cotton crop cycle. This has been explained because the mortality of second instar *S. frugiperda* larvae evaluated in Cry1Ac / Cry2Ab cotton leaf discs are in the range between 69 and 93%, depending on the age of the plant (Sivasupramaniam *et al.,* 2008). Efficiency in the use of pyramided Bt crop technologies is reduced by cross-resistance, antagonism between Bt toxins generated by poor field pest management strategies, similar Bt proteins used between neighboring crops and cross-crop target pests, causes insects to possess resistance to more than one Bt protein present (Liu *et al.,* 2017; Yang *et al.,* 2020; Huang, 2021; Shad *et al.,* 2022). The widespread use of Bt corn has led to the emergence of resistance to the Cry1F protein in *Spodoptera frugiperda*, which has caused problems in the management of this pest and sustainable use of this technology in countries such as the United States, Brazil and Puerto Rico (Bernardi *et al.,* 2017; Huang, 2021). Research indicates that Cry1Ac endotoxin has mortality levels between 6% and 20% in *S. exigua* and *S. frugiperda* species, indicating low toxicity, while Cry2Ab protein has low efficiency in the control of different *Spodoptera* species (Sivasupramaniam *et al.,* 2008; Britz *et al.,* 2020).

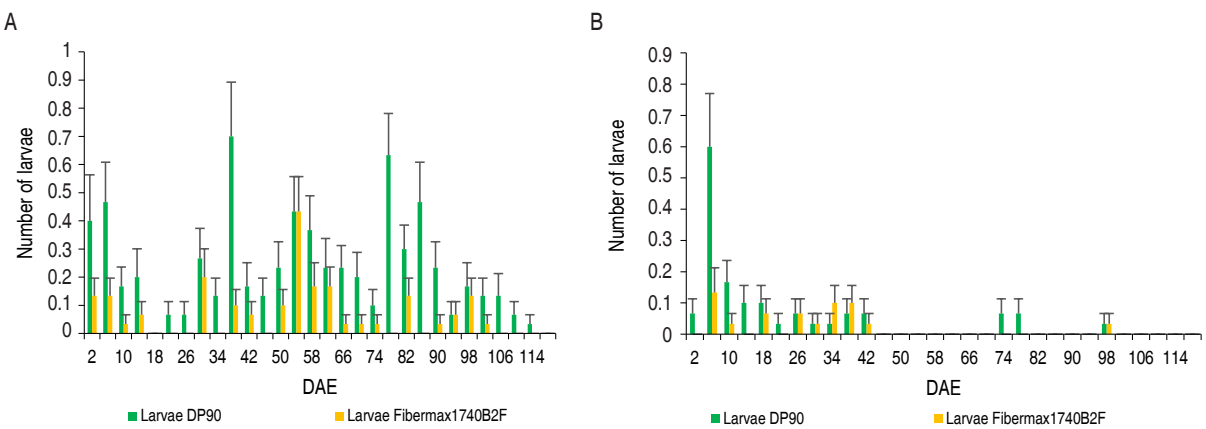

Figure 2. Populations of *Spodoptera* spp. complex in cotton genotypes. A. 2015. B. 2016.

Relationship between yield and cotton pest in non-Bt and Bt cotton

Figure 3 shows the relationship between insect variables and yield. In Figure 3A the correlation matrix for variety DP90® is observed. In this, a direct relationship between *TB* with yield was indicated (R=0.88), while *PB* with yield presented a negative correlation with R=-0.71. In floral buds, both *PFB* and *TFB*, showed an inverse relationship with -0.49 and -0.66 respectively. Between pest, an opposite relationship between the number of larvae and damage with the number of *PFB* and *PB* is registered with a value of -0.71. In Figure 3B, the correlations for FM1740B2F are shown. Yield was inversely related to the number of *PB* (R=-0.49) and directly correlated with *TB* (R=0.60). Pest were negatively correlated, the *PFB* with the number of larvae (R=-0.98), and with the damage (R=-0.75) is highlighted. On the other hand, the number of larvae was inversely correlated with the number of weevils (R=-0.55) and with *PB* by *A. grandis* (R=-0.81). The effect of *A. grandis* on the yield in Bt and non-Bt cotton is explained due to boll weevil is considered the main problem-pest cotton in Colombia. Its management actual includes the remaining 4–6 insecticide applications and when improper management is carried out, losses in yield of between 50 and 250 kg of cotton seed per hectare can be caused (Ñañez, 2012; Brookes, 2020).

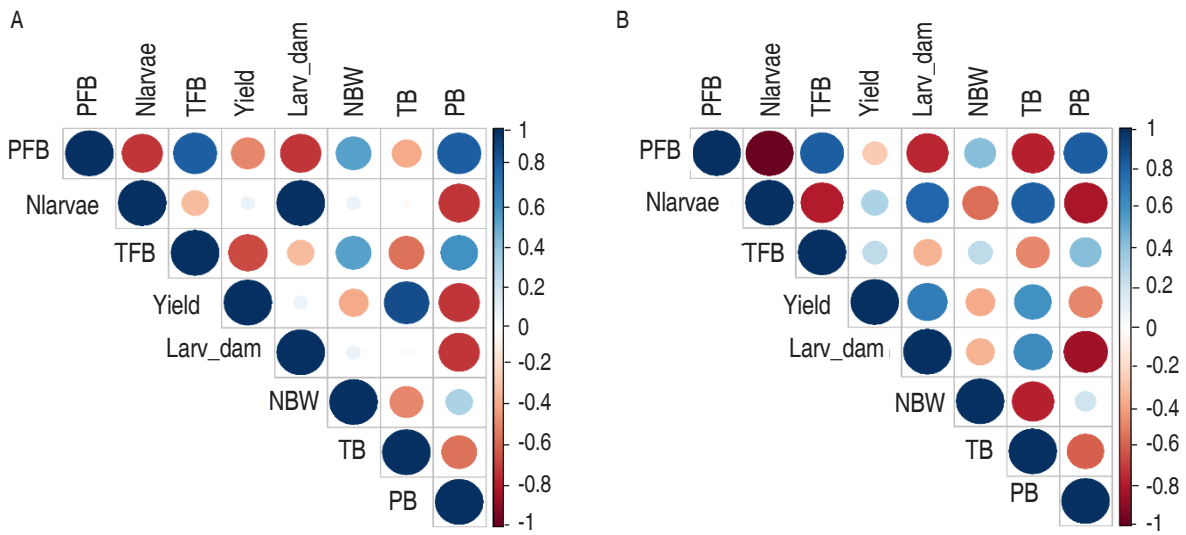

Figure 3. Correlation matrices that explain the relationship between yield and pests in cotton crop. A. DP90®. B. Fibermax1740B2F. *PFB*= Perforated Floral Buds, Nlarvae= Number of larvae, *TFB*= Total Floral Buds, Larv_damage= Larvae damage, *NBW*= Number of cotton boll weevil, *TB*= Total Bolls, *PB*= Perforated Bolls.

Figure 4 shows the results of the factorial analysis for mixed data (FAMD). In Figure 4A the biplot for qualitative categories is shown, while in Figure 4B, the circle of correlations is presented for the quantitative variables. The variability explained by the first two components of the FAMD was 82.6%. The principal component one (CP1), with 50.4%, shows a relationship of the *A. grandis* variables with the years. The variables *PFB* (0.98), *PB* (0.94), *TFB* (0.86) and *NBW* (0.66) were directly related to CP1, while *TB* (-0.68) was inverse. This explains that the number of healthy *TB* was inversely related to all the variables that exerted an insect damage including *TFB*. Likewise, in years, 2016 was related to a greater damage of *A. grandis*, compared to 2015.

The main component two (PC2) explained 32.2% and focused on the relationship of the larvae of the *Spodoptera* complex with the yield and the *TB*. The number of larvae and the damage were directly correlated to PC2 with 0.89 and 0.82 respectively, while the yield (-0.72) and *TB* (-0.59) were inverse. The above indicated the inverse relationship of *Spodoptera* spp. with the yield, which is explained by the joint presence of *Cry1Ac-Cry2Ab* proteins that has negatively affected the growth rate and feed conversion by the larvae confirming the antibiotic effects of GM crops on the development and survival of these larvae (Valencia-Cataño *et al.,* 2014). Further, the species of the *Spodoptera* complex in cotton are considered secondary pests in Colombia, although

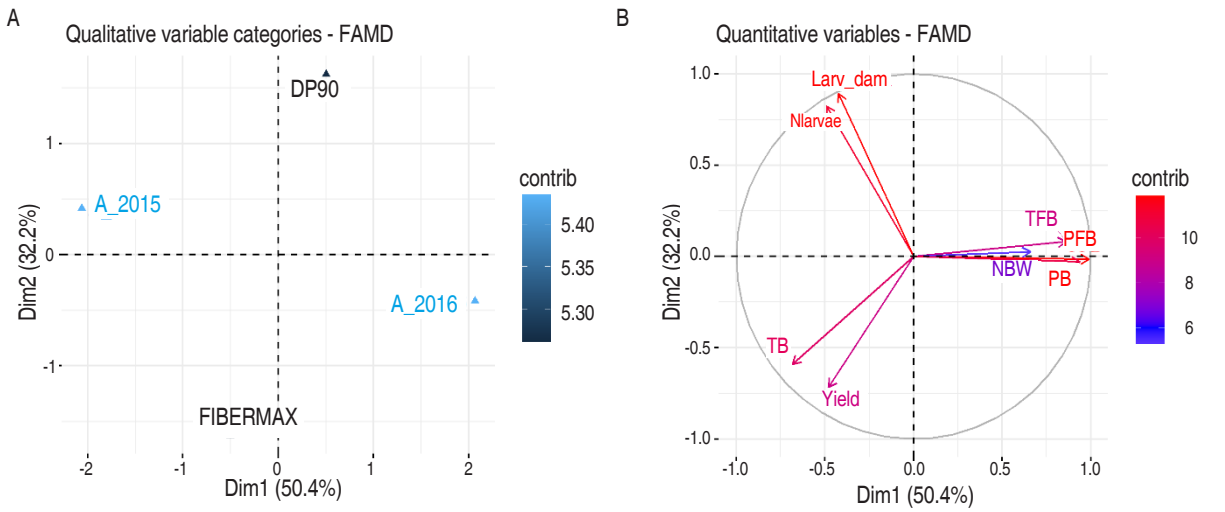

Figure 4. Factorial analysis for mixed data (FAMD). A. Biplot of categories. B. Circle of correlations. *PFB*= Perforated Floral Buds, Nlarvae= Number of larvae, *TFB*= Total Floral Buds, Larv_damage= Larvae damage, *NBW*= Number of cotton boll weevil, *TB*= Total Bolls, *PB*= Perforated Bolls.

the potential risk of this complex to go from secondary to primary pests in Cry1Ac cotton has been highlighted (Santos *et al.,* 2009).

Comparison of pest accumulation between year and variety

The maximum accumulation point of *TFB* did not show any statistical difference between varieties (Table 1); however, the DP90® variety showed a higher accumulation with 174.42±8.74 compared to FM1740B2F with 164.35±8.74. Between years, statistically significant differences were found. In 2016 with 186.15±8.73 and in 2015 with 156.67±8.73, respectively. Referring to *PFB* (Figure 1B), there were no statistical differences between varieties; but discrepancies were observed between years. In evaluation of Bt and non-Bt genotypes for *A. grandis*, it has been reported that there are no differences in their populations (Nava-Camberos *et al.,* 2018), which is explained because this pest is not targeted by the Bt cotton and the Bollgard II® cotton has no effectiveness against it (Showalter *et al.,* 2009). In 2015, the invasion of *A. grandis* was retarded, which was explained in a lower accumulation of *PFB* with 20.36±2.72, in contrast, in 2016, the insect achieved establishment and generalization within the crop, reflected in its accumulation (71.62±8.47). In 2016 with 186.15±8.73 and in 2015 with 156.67±8.73, respectively.

The accumulation of the number of *TB*, presented statistical differences between years and varieties. Higher *TB* accumulation was recorded in 2015 (168.94±5.72) compared to 2016 (147.54±5.72). Likewise, in *PB* (Figure. 1D), statistical differences were determined for years and varieties. The variety with Bollgard II® technology showed a lower maximum accumulation point (19.60±1.10), concerning the conventional variety (DP90®) 37.23±1.77. For years, 2015 presented 29.67±1.28 compared to 47.30±1.10 in 2016. In *NBW*, there were no statistical differences between years and variety; however, lower insect accumulation was observed in 2015 (6.45±1.46) compared to 2016 (10.14±2.17). The results show that in 2015 the establishment of the insect was delayed in time (±80 DAE), with higher accumulation of *TB*, lower accumulation of *PFB* and *PB*; whereas, in 2016, where the establishment was at ±60 DAE, a higher accumulation of *PFB*, *PB* and *NBW* was evidenced; consequently, lower accumulation of *TB*. Regarding the accumulation of *Spodoptera* average larvae (N_ larvae), there were statistical differences between years and variety. While between years, there was a higher accumulation in 2015, with 4.32±0.57 and 2016 with 1.08±0.16 in conventional and transgenic, respectively. As for varieties, DP90® recorded 4.08±0.58 and Fibermax1740B2F 1.32±0.12.

Table 1. Statistical differences in the variables evaluated.

Variable	Factor	F	P-value
Total Flower Buds (*TFB*)	Varieties	1.62	0.250 ns
	Years	10.15	0.019 *
Perforated Floral Buds (*PFB*)	Varieties	1.86	0.222 ns
	Years	33.2	0.001 *
Total Bolls (*TB*)	Varieties	15.63	0.008 *
	Years	10.76	0.018 *
Perforated Bolls (*PB*)	Varieties	32.84	0.012 *
	Years	385.32	<0.001 *
Number of cotton boll weevil (*NBW*)	Varieties	0.13	0.726 ns
	Years	3.71	0.102 ns
Number of larvae (N_Larvae)	Varieties	15.42	0.008 *
	Years	32.67	0.001 *

ns= non-significant differences, *significant differences.

In the yield variable, there were no statistically significant differences between years (F=5.35; gl=1; *P*=0.060); however, for 2015, an average yield of 3.16±0.06 t ha^{-1} was presented, compared to 2.57±0.25 t ha^{-1} in 2016. In varieties, statistically significant differences were found (F=21.41; gl=1; *P*=0.0036). The Bollgard II® variety technology presented higher yields with 3.46±0.23 t ha^{-1} on average, while DP90® registered 2.28±0.10 t ha^{-1}. This corroborates the results presented in Figure 4.

The correlations between climatic factors, with the parameters and productive variables, showed directly proportional relationships between the maximum rate of damage accumulation in flower bud (R=0.87; t=5.6438; *P*=<0.0001) and boll (R=0.95; t=9.3212; *P*=<0.0001) with the average temperature. In studies on a global scale, mean annual temperature has been reported as a significant variable influencing the potential global distribution of *Anthonomus grandis*. The adaptation of the pest remained at the highest level when the annual mean temperature was 23 °C, and the response curve declined until 30 °C to reach stability (Jin *et al.,* 2022). On the other hand, there were inversely proportional correlations between the maximum rate of damage accumulation in flower bud (R=-0.87; t=-5.6438; *P*=<0.0001) and boll (R=-0.95; t=-9.3212; *P*=<0.0001) with the average relative humidity. The productive variables did not show significant correlations (*P*>0.05) with the climatic parameters and factors. When correlations between the populations and climatic variables in different phenological stages were calculated, the following results were obtained: in the vegetative stage, only *Spodoptera* complex was evaluated, but without statistical significance (*P>0.05*) with the climatic variables. In reproductive and maturation stages, average temperature, and populations of *Spodoptera* presented significant statistically direct correlation in the non-Bt cotton with R=0.60 (*P*=0.0229) and R=0.63 (*P*=0.0150), respectively. In maturation, *PFB* showed inversely correlation (*P<0.01)* with average temperature (DP90®=-0.75; Fibermax1740B2F=-0.80) and positively (*P<0.01)* with relative humidity (DP90®=0.66; Fibermax1740B2F=0.70).

CONCLUSION

Larvae and damage of the *Spodoptera* complex were found on cotton plants with Cry1Ac + Cry2Ab endotoxins, indicating some range of resistance to plants with this endotoxin. The presence of larval populations and damage of the *Spodoptera* complex on Bt cotton plants requires increased monitoring and evaluations; in addition, refuge areas need to be established. In the case of *A. grandis*, no differences in its populations were recorded, which is explained by the fact that this pest is not a target of Bt cotton and Bollgard II® cotton has no efficacy against it. Appropriate management actions were recommended that incorporate optimal planting dates, constant monitoring of the *A. grandis* and *Spodoptera* complex and developing action plans that consider a baseline of resistance in the populations, establishing refuge areas.

ACKNOWLEDGMENTS

The authors thank the Ministerio de Agricultura y Desarrollo Rural (MADR) for funding, la Corporación Colombiana de Investigación Agropecuaria (Agrosavia), and the Nataima Research Center, in the project: Recomendaciones de manejo integrado de plagas y enfermedades, para los sistemas arroz-maíz-algodón en los valles interandinos y el Caribe.

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Research article

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Revista Facultad Nacional de Agronomía

UNIVERSIDAD NACIONAL DE COLOMBIA

Degradation dynamics of organophosphorus insecticides applied in stored soybean (*Glycine max* L.) during supervised trials.

Dinámica de la degradación de insecticidas organofosforados aplicados en soja almacenada (*Glycine max* L.) durante ensayos supervisados.

https://doi.org/10.15446/rfnam.v76n1.102472

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Keywords:
Chlorpyrifos-methyl residues
Dichlorvos residues
Dissipation kinetics
Half-life
Pirimiphos-methyl residues
Postharvest grains

ABSTRACT

During storage, soybean kernels can be attacked by insects, which are preventively controlled with insecticides. Information on the dissipation of insecticide residues is crucial to know their final concentrations in food and establish the waiting periods necessary for consumption without health risks, and to determine the minimum waiting period necessary to comply with national and international standards. The aims of this study were to quantify the residue levels of organophosphorus insecticides (dichlorvos, chlorpyrifos-methyl and pirimiphos-methyl) in stored soybean, establish the effect during the storage period, and model the dissipation dynamics. Insecticide residues in soybeans were analyzed at 2, 30, 60, 90 and 120 days after application. An analytical method based on QuEChERS extraction followed by gas chromatography mass spectrometry (GC-MS/MS) determination was validated, with mean recoveries of 82-105%, depending on the spiking levels. Residues decreased below 80% of the initial concentration at 60 days after application and below quantifiable levels at 120 days. Residues followed a pseudo-first-order dissipation dynamics [$C_t = C_0 \times \exp^{(-k.t)}$], with the dissipation constant (k) and half-lives being 0.538 and 1.3 days for dichlorvos, 0.018 and 38.8 days for chlorpyrifos-methyl, and 0.023 and 30.1 days for pirimiphos-methyl, respectively. These results allow concluding that, at the recommended dosage, these insecticides are safe for use on soybean grains stored under standard conditions commonly nowadays used in Argentina.

Palabras clave:
Residuos de clorpirifos-metilo
Residuos de diclorvos
Cinética de disipación
Vida media
Residuos de pirimifos-metilo
Granos de poscosecha

RESUMEN

Durante el almacenamiento, los granos de soja pueden ser atacados por insectos, que se controlan preventivamente con insecticidas. La información sobre la disipación de los residuos de insecticidas es crucial para conocer sus concentraciones finales en los alimentos y establecer los períodos de carencia necesarios para su consumo sin riesgos para la salud, así como para cumplir con las normas nacionales e internacionales. Los objetivos de este estudio fueron cuantificar los niveles de residuos de insecticidas organofosforados (diclorvos, clorpirifos-metilo y pirimifos-metilo) en soja almacenada, establecer el efecto de los días de almacenamiento y modelizar la dinámica de disipación. Los residuos de insecticidas en la soja se analizaron a los 2, 30, 60, 90 y 120 días después de la aplicación. Se validó un método analítico basado en la extracción por QuEChERS seguida de la determinación por cromatografía gaseosa acoplada a espectrometría de masas (GC-MS/MS), con recuperaciones medias del 82-105%, dependiendo de los niveles de adición. Los residuos disminuyeron por debajo del 80% de la concentración inicial a los 60 días de la aplicación y por debajo de los niveles cuantificables a los 120 días. Los residuos siguieron una dinámica de disipación de pseudoprimer orden [$C_t = C_0 \times \exp^{(-k.t)}$], siendo la constante de disipación (k) y las vidas medias de 0,538 y 1,3 días para diclorvos, 0,018 y 38,8 días para clorpirifos-metilo, y 0,023 y 30,1 días para pirimifos-metilo, respectivamente. Estos resultados permiten concluir que, a la dosis recomendada, estos insecticidas son seguros para su uso en granos de soja almacenados.

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Received: October 2, 2022; Accepted: November 30, 2022
Rev. Fac. Nac. Agron. Medellín 76(1): 10167-10175. 2023

ISSN 0304-2847 / e-ISSN 2248-7026

The cultivation of soybean (*Glycine max* L.) is widely spread around the world, with an estimated surface area of 123 million hectares in 2017 (FAOStat, 2020). Soybean production is an important activity due to the high nutritional properties of grains and the large variety of by-products. The crop has multiple uses for human and animal consumption as well as for industrialization. According to FAOStat (2020), the most important producers worldwide are the USA (119 million tons), Brazil (114 million tons) and Argentina (55 million tons). One of the main destinations of these grains is the export market and the soybean market plays a very important role in the world food consumption. In Argentina, 87% of soybean is exported to China.

In soybean production, pesticides are used to control pests and diseases in the field to increase crop yield, but also during grain storage. To prevent pest attacks, a large variety of pesticides, especially organophosphorus and pyrethroid insecticides, are applied frequently during the storage of grains (Arthur, 1996; Lorini, 2012; Abadia and Bartosik, 2014). Until recently, stored soybeans were treated on the surface of silos (i.e., top dress treatment), to prevent infestations by lepidopteran insects entering clean silos from the upper part (Abadia and Bartosik, 2014). Lorini *et al.* (2010) identified *Lasioderma serricorne* as a primary insect pest of soybean and *Oryzaephilus surinamensis*, *Cryptolestes ferrugineus*, *Ephestia kuehniella* and *E. elutella* as secondary pests. In Argentina, *Sitotroga cerealella* and *Acanthoscelides obtectus* have also been mentioned as pests of soybean (Abadia and Bartosik, 2014). These pests cause physical degradation of grains, a fact that imposes barriers to commercialization because of the zero-tolerance policy for live insects in the export market (Arthur, 1996; Lorini, 2012).

In some cases, the doses used during the application of pesticides during storage are higher than those recommended for commercial products, mostly because of the increasing insecticide resistance acquired by insects along the time (Abadia and Bartosik, 2014). As a result, the level of pesticide residues on grains at the moment of commercialization is higher than the maximum residue limits (MRLs) permitted by regulation. Along with the environmental risk, a high level of pesticide residues can affect the quality of the grains and processed products and it may ultimately reach the consumer and cause health hazards. Therefore, to prevent health risks, it is important to monitor the presence of pesticides and regulate their levels in stored soybean grains. To avoid the presence of residues in grains above the MRLs legally allowed (Abadia and Bartosik, 2014; Lorini *et al.*, 2010), pesticides should be appropriately applied, following Good Agricultural Practices. In the European Union, Regulation 396/2005/EC establishes the MRLs of pesticides permitted in products of animal or vegetable origin intended for human or animal consumption (Grimalt and Dehouck, 2016). The MRLs for pesticide residues in soybean grains mostly range between 0.01 mg kg^{-1} and 5 mg kg^{-1}, depending on the pesticide. The MRLs for dichlorvos, chlorpyrifos-methyl and pirimiphos-methyl are 0.01, 0.2, and 0.05 mg kg^{-1}, respectively (European Commission, 2022).

To measure these low concentrations, highly selective, sensitive and accurate analytical methods are needed in longitudinal experiments to monitor pesticide degradation along time, storage time is a crucial variable. The dissipation dynamics can be modeled by several kinetic models, being the pseudo-first-order kinetics one of the most chosen ones (Fantke and Juraske, 2013). This kinetic modeling allows inferring the half-lives of residues and the acceleration of the residue degradation process. This information is important to determine the residue concentrations that might be found in grains treated with insecticides during storage, and to establish the minimum waiting period required for safe food consumption. The dissipation of insecticide residues has been extensively studied in cereal grains (Afridi *et al.*, 2001; Alleoni and Baptista, 2001; Balinova *et al.*, 2006; El-Behissy *et al.*, 2001; Fleurat-Lessard *et al.*, 1998; Lucini and Molinari, 2011; Pal and Shah, 2008; Sgarbiero *et al.*, 2003, Yu *et al.*, 2014) but is still poorly known in soybean (Lalah and Wandiga, 2002; Zayed *et al.*, 2007; Zhao *et al.*, 2014).

Grains differ widely in the surface characteristics and physico-chemical properties of the tegument, and similar differences may occur among varieties stored under different environments. The lipid content of the grains also affects the retention of pesticides and the waiting period, mainly in oily grains more than in cereal

ones since most of the active ingredients are soluble fat. Specific degradation models are thus required to describe pesticide residue degradation for a grain species stored under particular conditions (Fleurat-Lessard *et al*., 1998). Based on the above, the aim of this work was to monitor the residue levels of dichlorvos, chlorpyrifos-methyl and pirimiphos-methyl applied to stored soybean grains along the storage period, under the standard storage conditions in Argentina.

MATERIALS AND METHODS

Experimental design

The experimental storage assays were performed at the Estación Experimental Agropecuaria of the Instituto Nacional de Tecnología Agropecuaria (INTA), in Manfredi, Córdoba province, Argentina. A completely randomized experimental design was applied, with three repetitions per treatment. The treatments were three insecticides and a control with no insecticide application. The experimental unit consisted of 13 kg of soybean grains, previously confirmed to be free of insecticide residues, and put in a plastic container (20 L) simulating regular storage conditions (20-25 °C daily mean temperature and 40-60% relative humidity) during a 120-day period. Grain moisture content was 14% m.c. Repetitions were three separate lots (13 kg) of treated soybean and five samples over time were taken from each lot of soybean. The organophosphorus insecticides used were dichlorvos (DDVP), chlorpyrifos-methyl+deltamethrin (CPM) and pirimiphos-methyl (PMM), formulated as emulsifiable concentrates. The concentrations (and rates in µg active ingredient per gram of grain) were 100% (20 µg g^{-1}), 14.5% + 0.65% (2.9 µg g^{-1}), and 50% (5 µg g^{-1}), respectively. Grains were spread on a 100-µm polyethylene sheet to obtain a homogeneous insecticide distribution. Insecticides were applied using a hand 1.5 L Giber sprayer (Giber SA, Buenos Aires, Argentina) and grains were vigorously mixed, placed inside the duly labeled containers, capped and stored. The lids were perforated to allow gas exchange. Samples (500 g) were collected from each experimental unit with a bag trier at 2, 30, 60, 90 and 120 days after insecticide application and placed in layered bags, which were then frozen at below -20 °C until analytical residue measurement.

Analytical method for pesticide residue analysis

Samples were processed in the Laboratory of Grain Quality at INTA Manfredi. Grains were milled using Oster blenders equipped with glass jars and stainless steel blades (400 watts). Insecticides were extracted from grains using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) technique (Anastassiades *et al*., 2003; Lehotay, 2007) adapted to dry matrices. The following solvents and reagents were used: acetonitrile (99.98% HPLC grade), toluene (99.5% HPLC grade), sodium chloride (99.7% analytical grade), anhydrous magnesium sulfate (>99.5% analytical grade), deionized water, PSA bonded silica Bulk (Supelco 40 µm) and Discovery DSC-18 SPE (Supelco 40 µm). For validation assays and quality control of the extraction process, the following high-purity analyte standards were used: dichlorvos (99.9%), chlorpyrifos-methyl (99.9%) and pirimiphos-methyl (99.5%) from Sigma Aldrich (USA); and the internal standards ethoprophos (93.1%) (Eto) and triphenylphosphate (99%) (TPP) (Sigma Aldrich, USA). Stock solutions of DDVP, CPM and PMM were obtained by weighing 50 mg of the certified material in a 50 mL flask and stoppering with toluene; the concentration was 1 mg mL^{-1}. Eto and TPP concentration was 2 µg mL^{-1} using a 25 mL flask. Working solutions (mixture of DDVP, CPM and PMM pesticides) were prepared by combining stock solution aliquots to obtain a final concentration of 40 µg mL^{-1} of each pesticide. The working solution for Eto had a concentration of 20 µg mL^{-1}, whereas that for TPP had a concentration of 2 µg mL^{-1}. To ensure correct sample processing and analytical performance, a double internal standard was used: Eto for the control of the extraction process and TPP as quality control to isolate variations derived from the chromatographic stage. The acceptance criterion was controlling that the TPP/Eto ratio peaks were within ± 2 standard deviations (SDs) for all the samples analyzed. Moreover, in each set of analysis, were included a quality control (acceptance criterion: recovery percentage between 70 and 120% and residual SD below 25%) and a reagent blank (acceptance criterion: absence of interferences and contaminants).

QuEChERS extraction. Each sample of milled soybean (5 g) was extracted in a centrifuge tube by adding water (10 mL) and acetonitrile (10 mL), and 150 µL of internal standard solution of Eto (20 µg mL^{-1}), 4 g of anhydrous magnesium sulfate and 1 g of sodium chloride. The extract was then homogenized and centrifuged

(RCF=5000 g, 5 °C for 5 min). An aliquot (2 mL) of the organic phase supernatant was transferred to a clean-up tube containing 0.3 g of anhydrous magnesium sulfate, 0.1 g of PSA bulk sorbent and 0.1 g of C18, shaken and centrifuged (RCF=5000 g, 5 °C, 5 min). An aliquot of the supernatant (1 mL) was transferred and evaporated to dryness under a stream of nitrogen with the water bath set at 35 °C. The sample was recovered with toluene (500 µL) and shaken; 300 µL was collected and placed in a vial. Then, 50 µL of TPP solution (2 µg mL^{-1}) and toluene (25 µL) were added.

Validations were performed using calibration tests, matrix effect studies and recovery experiments. The fortification levels used were 0.01, 0.05, 0.1, 1, 3 and 9 µg g^{-1}, using a matrix of organic soybean flour; three replicate tests per level were performed. Calibration curves were prepared by artificially contaminating an extract of organic samples and samples not containing matrix with known amounts of analytes. The relationship between the peak area of each analyte and the peak area of Eto was analyzed with respect to analyte concentration. These curves were used to detect the matrix effect and define the linear range.

Recovery experiments were used to calculate recovery, expanded uncertainty (U: a range around the reported result within which the true value can be expected to lie with a specified probability of 95% confidence level), and limits of detection (LoD) and quantification (LoQ). Expanded uncertainty was estimated using the data from the recovery experiment, with Equations 1 and 2:

$$RSD = SD_{R\%} / R\%_{mean} \quad (1)$$

$$U = RSD \times k \times C \quad (2)$$

Where RSD is the relative standard deviation, $SD_{R\%}$ is the recovery standard deviation, $R\%_{mean}$ is the mean recovery, C is the concentration of the fortified level and k is a statistical quantity (k is ~2 for a confidence level of 95 %). LoD is expressed as three times the SD of the lowest recovery level that meets the accuracy and precision criteria; LoQ was determined with respect to the lowest nominal concentration validated for accuracy and precision parameters. Recovery data were used to assess accuracy; the method precision was calculated and expressed as RSD (European Commission, 2009).

GC-MS analysis

Pesticide residues were determined and quantified using high-resolution gas chromatography-mass spectrometry (GC-MS) at the Food Technology Institute at INTA, Castelar, Buenos Aires, Argentina. The technique was applied using a Perkin Elmer Clarus 600 gas chromatograph coupled to a mass spectrometer with electron impact ion source and quadrupolar analyzer. A programmable temperature vaporizer injection system was used. The initial temperature was 35 °C (4 min), then increased to 290 °C (200 °C min^{-1} for 2.5 min), and finally decreased to 35 °C (60 °C min^{-1}). The injection volume was 25 µL. Fused silica guard column of 5 m x 0.25 mm (Supelco). Capillary column (Varian, Factor Four VF-5ms cat. CP8944), 30 m x 0.25 mm (id, 0.25 µm) of stationary phase, low bleed poly (5% diphenyl 95% dimethyl siloxane). High purity helium (99.999 %) was used. The initial pressure was 13,789 Pa (3.9 min), reached 172,369 Pa (at 172,369 Pa min^{-1} for 7 min), and then decreased to 103,421 Pa (at 172,369 Pa min^{-1}), which was maintained until the end of the assay (35 min).

The initial split flow was 100 mL min^{-1}, then closed at minute 3.9 (0 mL min^{-1}) and then set at 20 mL min^{-1} at minute 10. The initial oven temperature was set at 70 °C (8 min), increased to 170 °C (25 °C min^{-1}), then to 230 °C (5 °C min^{-1}), reaching a maximum of 290 °C (at 20 °C min^{-1}), which was held for 10 min. The transfer line in the detector was held at 290 °C and the collision cell at 200 °C. Chromatography data were obtained by ionization by electron impact (EI+), quadrupole analyzer and monitoring of specific ions (SIM, selected ion monitoring), one ion for quantitation and at least two for qualification for each analyte. For each analyte, the time of relative retention (TRR) of Eto and the ratio of relative abundance relation the quantifier ion and at least two qualifier ions were determined. For DDVP, TRR was 8.4 min, quantifier ion (m/z) 185 and qualifier ions (m/z) 220+145+109; for CPM, TRR was 14.1 min, quantifier ion (m/z) 286 and qualifier ions (m/z) 288+125+290; for PMM, TRR was 14.7 min, quantifier ion (m/z) 305 and qualifier ions (m/z) 290+276+233; for TPP, TRR was 20.9 min, quantifier ion (m/z) 326 and qualifier ions (m/z) 325+77+215; and for Eto, TRR was 17.7 min, quantifier ion (m/z) 126 and qualifier ions (m/z) 139+200+242. The results of the analyses of DDVP, CPM and PMM residues were determined and expressed in micrograms

of insecticides per gram of grains (µg g^{-1}). The laboratory performing the analyses of pesticide residues works under a quality system to ensure a consistent and reliable approach with the use of quality control measures.

Statistical analysis

Residue decline (considering a 100% value measured at 2 days after application) and daily dissipation rates (DDR) were calculated. A nonlinear mixed model was adjusted to evaluate the effect of days after application on the degradation of each insecticide. The model included a random effect to induce correlations among repeated measurements of the same experimental unit. The pseudo-first-order kinetic model was the model chosen to fit the dissipation kinetics according to equation 3:

$$C_t = C_0 \times e^{(-k.t)} \quad (3)$$

Where C_t is the residue concentration at time t, C_0 is the apparent initial concentration or nominal rate and k is the dissipation constant. The parameters estimated were used to calculate the average half-life (HL) of each insecticide in stored soybeans by using Equation 4:

$$HL = Ln(2)k^{-1} \quad (4)$$

Fisher LSD test was used to determine statistical differences between treatments. Analyses were performed using the statistical software InfoStat (Di Rienzo *et al.*, 2019).

RESULTS AND DISCUSSION

Validation of the QuEChERS+GC-MS methodology

The QuEChERS technique was validated for the insecticides DDVP, CPM and PMM applied to stored soybean grains. The values of average recovery, RSD, expanded uncertainty (%U), LoD (µg g^{-1}) and LoQ (µg g^{-1}), and the regression equations of the calibrations are shown in Table 1. An effect of the matrix was observed for all analytes and the quantification was performed through calibration curves, which were linear for the concentration range studied (R^2>0.98). Recovery values in the study range were acceptable for validation in pesticide analysis (70 to 120%), showing adequate reproducibility. These results are consistent with previous validation of QuEChERS in grains (Strada *et al.,* 2021; Mastovska *et al.*, 2010).

Table 1. Spiking level, recovery, relative standard deviation (RSD), expanded uncertainty (U), limit of detection (LoD), limit of quantification (LoQ) and regression equation for dichlorvos, chlorpyrifos-methyl, and pirimiphos-methyl.

Active Ingredient	Spiking level (µg g^{-1})	Recovery (%)	RSD (%)	U (%)	LoD (µg g^{-1})	LoQ (µg g^{-1})	Regression Equation	R^2
Dichlorvos	0.01	87.7	25.0	22.2	0.003	0.01	y=0.23x+0.01	0.98
	0.05	85.0	7.0	14.0				
	0.10	95.5	7.3	14.6				
	1.00	82.3	13.3	26.5				
	3.00	101.3	12.5	25.0				
	9.00	102.8	6.0	12.0				
Clorpyrifos-methyl	0.01	83.3	12.5	25.0	0.003	0.01	y=1.24x-0.03	0.99
	0.05	94.2	6.4	12.8				
	0.10	95.6	11.5	22.9				
	1.00	89.8	5.7	11.4				
	3.00	105.4	6.6	13.2				
	9.00	105.3	8.3	16.5				
Pirimiphos-methyl	0.01	94.2	10.0	20.0	0.006	0.01	y=0.89x-0.03	1
	0.05	92.8	17.0	34.0				
	0.10	87.3	10.3	20.7				
	1.00	97.4	10.2	20.3				
	3.00	105.5	8.4	16.8				
	9.00	90.5	12.5	25.0				

Insecticide residues in stored soybean

Residues of the active ingredients studied were found in all the samples treated with insecticide, whereas the control samples were negative for residues of the applied insecticides. Residue levels (mean ± SEM), residue decline (%) and DDR in treated soybean grains at 2, 30, 60, 90 and 120 days after applications are presented in Table 2.

The dissipation dynamics for PMM was well fitted with the following first-order kinetic model root function one and a half order, for CPM was half order and for DDVP the fitted models were second order. All insecticide residues decreased with time, as previously reported for these active ingredients in other matrices. In addition, insecticide residues below the LoQ were still found at 120 days (Table 2).

Table 2. Residues, daily dissipation rate (DDR) and residue decline for dichlorvos, chlorpyrifos-methyl and pirimiphos-methyl in stored soybean at 2, 30, 60, 90 and 120 days after application.

Variable	Active ingredient	Days after application				
		2	30	60	90	120
Residues† (μg g^{-1})	Dichlorvos	6.76±2.14 a	1.68±0.65 ab	0.41±0.21 bc	0.10±0.07 c	<LQ d
	Chlorpyrifos-methyl	3.57±0.53 a	2.32±0.52 a	1.29±0.25 ab	0.36±0.15 b	<LQ c
	Pirimiphos-methyl	5.17±1.73 a	3.12±0.80 ab	0.95±0.36 ab	0.69±0.30 b	<LQ c
DDR† (μg g^{-1} day^{-1})	Dichlorvos	0.181±0.098 a	0.130±0.140 a	0.010±0.006 b	0.003±0.002 b	---
	Chlorpyrifos-methyl	0.045±0.014 a	0.034±0.013 a	0.031±0.004 a	0.032±0.031 a	---
	Pirimiphos-methyl	0.120±0.006 a	0.072±0.038 ab	0.017±0.020 ab	0.023±0,01 b	---
Residue decline† (%)	Dichlorvos	---	70.5±22.3 b	93.5±3.4 c	98.7±0.8 c	100±0 c
	Chlorpyrifos-methyl	---	35.3±11.8 b	63.5±7.6 c	90.0±3.9 d	9.8±0 d
	Pirimiphos-methyl	---	55.1±8.3 b	81.6±3.6 c	84.9±9.5 c	99.9±0.1 d

<LQ= below limit of quantification.

† Per row, means with different letters are statistically different (P<0.05) according to Fisher LSD test.

Lalah and Wandiga (2002) reported that residues in oily matrices can be more persistent due to the liposoluble nature of these active ingredients, which generates a biochemical phenomenon of pesticide retention by the grain lipid content. However, in this study, storage for 120 days was found to be effective in reducing residue levels in soybeans.

The magnitude and variation of DDR values depended on each active ingredient and days after application, showing statistically significant effects (P<0.05) for DDVP and PMM (Table 2). For DDVP, the DDR values until 60 days after application were different from the values found at 90 days. For PMM, the DDR values were higher at 2 days after application and only different from those obtained at 90 days. By contrast, the DDR values for CPM were not statistically different. For DDVP and PMM, residues declined by more than 80% with respect to the nominal rates at 60 days after application (Figure 1), whereas for CPM, residues declined by 60%, with the decrease being statistically significant (P<0.05) for all active ingredients (Table 2). By contrast, other studies on the dissipation of insecticide residues in stored grains showed a slow decrease (Arthur, 1996; Holland *et al.*, 1994). For DDVP, some works determined declines in fresh matrices of over 95% at 30 days after application, depending on the initial residue (El-Behissy *et al.*, 2001), whereas Zayed *et al.*, 2007) found residues between 15 and 21% after 30 weeks of storage in soybean grains. With respect to CPM in soybeans, some authors determined a decline of 62% of residues at 112 days, with this decline being statistically significant. (Zhao *et al.*, 2014) and other researchers indicated a 91% reduction of residues 6 months after application (Pal and Shah, 2008). Other studies found that PMM declined by 71% at 120 days after application (Sgarbiero *et al.*, 2003), by up to 75% at 180 days after application in corn (Fantke and Juraske, 2013) and by 65% at 126 days after application in rice (Yu *et al.*, 2014).

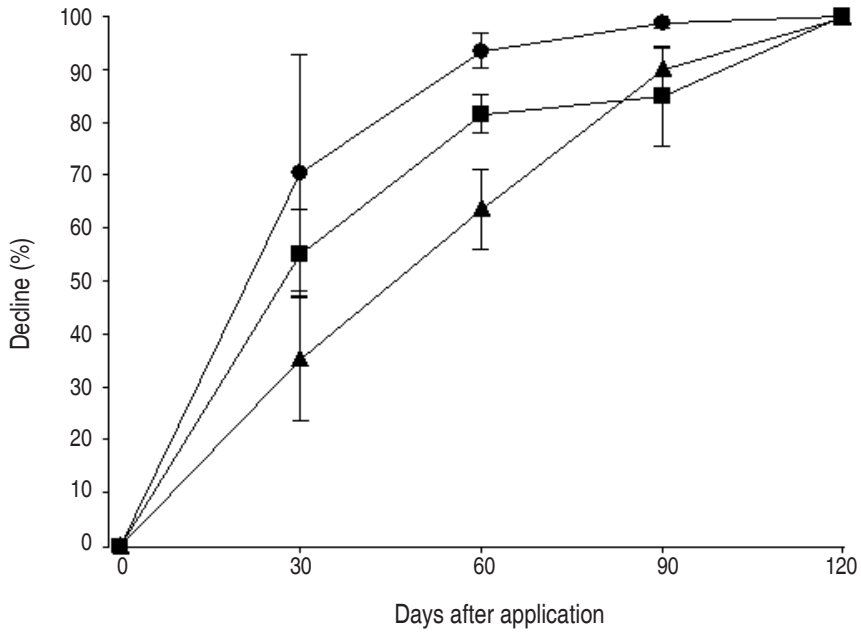

Figure 1. Decline (%) of residues of dichlorvos (dots), chlorpyrifos-methyl (triangles) and pirimiphos-methyl (squares) in stored soybean grains at 2, 30, 60, 90 and 120 days after application.

The dissipation kinetics has three phases: the "stripping phase", which is mainly due to mechanical factors not operating in storage trials (such as wind and rain); the "degradation phase", which is due to physical and chemical factors; and the "persistence phase", determined by residue retention in the material (Coscollá, 1993). In the present study, DDVP showed the highest k values (0.542), followed by PMM (0.023) and CPM (0.018). For DDVP, the first-order kinetics established the highest average k values of the three active ingredients. These high k values may respond to the high volatility characteristic of this insecticide, which provides short-term protection (15 days) (CASAFE, 2020). Thus, first-order kinetics should be used to predict the behavior of residues only at the "stripping phase" of dissipation. For CPM, some authors have found a similar model with a k value of 0.007 (Zhao *et al.*, 2014), whereas for PMM, in a work involving wheat and oats, no degradation kinetics was detected due to the persistence of residues (Lucini and Molinari, 2011).

Regarding the HL of organophosphorus pesticides, previous studies have found high variability (0.7 to 55 days) (Fantke and Juraske, 2013). In this study, HL was found to be greater for CPM (38.8 days) than for PMM (30.1 days) and DDVP (1.3 days); these differences were associated with the physico-chemical characteristics of each active ingredient (Balinova *et al.*, 2006; Lalah and Wandiga, 2002). Reported estimates of HL vary between two weeks for DDVP in wheat (Holland *et al*, 1994), between 70 and 169 days for CPM (Afridi *et al.*, 2001; Fleurat-Lessard, 1998), depending on the grain moisture, and 99 days for CPM in soybean (Zhao *et al.*, 2014). In addition, a slower dissipation has been reported for PMM, with a HL between 100 and 490 days in wheat (Afridi *et al.*, 2001; Holland *et al.*, 1994) and between 85 and 96 days in corn, depending on the dose (Alleoni and Baptista, 2001). The results of our study suggest that the HLs here observed in soybean are lower than those reported in other studies of soybean grains.

CONCLUSION

Insecticides residues in soybean decreased below 80% of the initial concentration at 60 days after application and below quantifiable levels at 120 days. Residues followed a pseudo-first-order dissipation dynamics [$Ct = Co \times exp^{(-k.t)}$], with the dissipation constant (k) and half-lives being 0.538 and 1.3 days for dichlorvos, 0.018 and 38.8 days for chlorpyrifos-methyl, and 0.023 and 30.1 days for pirimiphos-methyl, respectively. These results

allow concluding that, at the recommended dosage, these insecticides are safe for use on soybean grains stored under standard conditions commonly nowadays used in Argentina. The knowledge of the dissipation curves in these three insecticides allows us to predict the post-harvest interval necessary to attain a certain level of residues in order to comply with current legislation.

ACKNOWLEDGEMENTS

The authors thank to the Instituto Nacional de Tecnología Agropecuaria (INTA), Argentina, for supporting this work by the projects PE I 147 (2019/22-PE-E7-I-147-001), PNCER 023531 and PNCyO 1127022/21 and CONICET.

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Revista Facultad Nacional de Agronomía

UNIVERSIDAD NACIONAL DE COLOMBIA

Canangucha (*Mauritia flexuosa* L. f): A potential fruit in the colombian amazon

Canangucha (*Mauritia flexuosa* L. f): Un fruto potencial en la amazonia colombiana

https://doi.org/10.15446/rfnam.v76n1.100536

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Keywords:
Antioxidant capacity
Bioactive components
Chemical composition
Moriche palm
Morphometry

ABSTRACT

Canangucha is a palm from the Colombian Amazon of great industry interest, due to its expansion and ecosystem and food services it offers to communities. The objective of this work was to evaluate the morphometric, physical, and chemical characteristics, as well as the yield of the canangucha fruit in both EI and EII ecotypes, this characterization can become the starting point for the development of the value chain of the fruit. For the fruits and seeds, the longitudinal diameter (LD), equatorial diameter (ED), mass (g), and color (L*, a*, b*) were determined. The pulp was characterized based on pH, °Brix, moisture content, a_w, color, crude protein, total lipids, crude fiber, carbohydrates, minerals, total phenols (TP), antioxidant capacity (ABTS and DPPH methods), α-carotene, β-carotene and α-tocopherol. The fruit mass is comprised 61-65% seed, 15-17% pulp and 19-21% pericarp. The morphometric variables of the fruit and seed of ecotypes I and II showed a significant difference (P<0.05). The color of the fruit presented significant changes based on its ripeness, and the luminosity fluctuated between 26.8%-53.7%; while the pulp presented a yellow-orange hue with a variation in the color plane (a*, b*), being between (11.3, 5.1) and (23.4, 43.5). The EI ecotype pulp presented better quality attributes: total lipids (34.2%), crude fiber (22.2%), crude protein (6.8%), pH: 3.6-4.4, °Brix: 15-16, TP: 1467.3±146.5 mg GAE 100g^{-1}, DPPH: 2.5±0.1 mg TE g^{-1}, ABTS: 3.0±0.2 mg TE g^{-1}, β-carotene: 68.2±9.6 mg 100g^{-1}, and 11927.7 µg RAE. It was concluded that canangucha has an important nutritional value and compounds with physiological activity, which identifies it with great potential to be used in the food and pharmaceutical industries.

Palabras clave:
Capacidad antioxidante
Compuestos bioactivos
Composición química
Palma de moriche
Morfometría

RESUMEN

La canangucha es una palma de la Amazonia Colombiana de gran interés industrial debido a la expansión y a los servicios ecosistémicos que ofrece a las comunidades. El objetivo de este trabajo fue evaluar las características morfométricas, físicas y químicas, así como el rendimiento del fruto en los ecotipos EI y EII. Esta caracterización puede servir de punto de partida para el desarrollo de la cadena de valor del fruto. Para los frutos y semillas se determinó el diámetro longitudinal (DL), diámetro ecuatorial (DE), masa (g) y color (L*, a*, b*). En la pulpa se caracterizó el pH, °Brix, humedad, actividad de agua, color, proteína cruda, lípidos totales, fibra cruda, carbohidratos, minerales, fenoles totales (FT), capacidad antioxidante (métodos ABTS y DPPH), α-caroteno, β-caroteno y α-tocoferol. Se encontró que la masa del fruto está compuesta por un 61-65% de semilla, 15-17% de pulpa y 19-21% de pericarpio. Las variables morfométricas del fruto y semillas de los ecotipos I y II presentaron diferencia significativa (P<0,05). El color de la fruta presentó cambios significativos en función de la madurez y la luminosidad fluctuó entre 26,8-53,7%, mientras que la pulpa presentó una tonalidad amarillo-naranja con variación en el plano cromático (a*, b*) entre (11,3-5,1) y (23,4-43,5). La pulpa del EI presentó lípidos totales: 34,2%, fibra cruda: 22,2%, proteína cruda: 6,8%, pH: 3.6-4.4, °Bx: 15-16, FT: 1467,3±146,5 mg GAE 100g^{-1}, DPPH: 2,5±0,1 mg TE g^{-1}, ABTS: 3,0±0,2 mg TE g^{-1}, β-caroteno: 68,2±9,6 mg 100g^{-1} y 11.927,7 µg RAE. Se concluye que la canangucha tiene un importante valor nutricional y compuestos fisiológicamente activos que la identifican como una fuente potencial de uso en la industria alimentaria y farmacéutica.

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Received: June 21, 2022; Accepted: July 28, 2022
Rev. Fac. Nac. Agron. Medellín 76(1): 10177-10187. 2023
ISSN 0304-2847 / e-ISSN 2248-7026

Mauritia flexuosa is an oil palm found in the Colombian Andean Amazon region. It is a species recognized in Amazonian ecosystems for serving as a biological corridor for biodiversity and carbon sinking, in addition to its ecological and cultural value. Its fruits are covered by small scales that change color from reddish to violet when they reach maturity. Are oleaginous in character and are characterized by a high nutritional and medicinal value and are recognized by studies in the Brazilian Amazon as a potential source of carotenes, tocopherols, natural antioxidants, and phytosterols, also as one of the fruits with the most expressive nutraceutical properties (Barboza *et al*., 2022; De Oliveira and Franca, 2017); in Ecuador is considering highly nutritious (Abreu-Naranjo *et al*., 2020). In turn, they have high concentrations of oleic acid and five times more β-carotene than carrots, and their bioactive components possess important biological activity. The oil has photoprotective properties, healing and anti-inflammatory effects, and high oxidative stability. It is also a potential source of fatty acids with agro-industrial potential (Nastur *et al*., 2016). The characterization of food raw materials is the fundamental basis for establishing the qualities and possible use in the industry; in the Colombian Amazon there is little information of the canagucha that allow identifying its potential use and transformation.

Continuous consumption of fruits and vegetables is recommended for the high level of antioxidants, which limit the action of free radicals in the human body, and in turn, they prevent chronic diseases, such as: cancer, diabetes and cardiovascular and neurodegenerative diseases. Exotic fruits have an important industrial role because of components with physiological activity that contribute to preventing some diseases, in addition to formulation of new foods and cosmetic use (Araujo-Díaz *et al*., 2017).

Current consumer trends are oriented towards acquiring exotic products that also fit the context of functional foods. In this sense, the objective of the present work is to evaluate the morphometric, physical, and chemical characteristics, as well as the yield of the canangucha fruit in two ecotypes: I (EI) and II (EII) existing in the Colombian Amazon and contribute to the development of the value chain of the fruit.

MATERIALS AND METHODS

Collection of the fruits and pulp

Canangucha fruits (*Mauritia flexuosa* L. f) were collected at random from ninety adult individual trees in natural populations located in the municipalities of Florencia, Morelia, and Belén de los Andaquíes in the state of Caquetá – Colombia. The study area presented an altitude of 242 masl, a warm-humid climate characteristic of tropical humid forest, an average temperature of 28 °C and annual rainfall of 3840 mm (IDEAM, 2020). The fruits of ecotypes I (elliptical) and II (round) were selected according to the degree of maturity, identifying No. four (Sinchi, 2018). The pulp was obtained by immersing the fruits in water at room temperature for 12 hours to facilitate the detachment of the pericarp. The pulping was done manually, obtaining pulp, pericarp, and seed, and then, its percentage distribution was determined from 60 fruits per ecotype (6 replicates of 10 fruits). The pulp was cut into small pieces and stored in plastic bags at -18 °C until analysis.

Characterization methods

Morphometric characterization of the fruit and seed was performed based on the longitudinal diameter (LD) and equatorial diameter (ED) (100 samples for each ecotype) and a 78440 Staley digital Vernier caliper was used. The volume (V) and the sphericity (ϕ) were determined according to equations 1 and 2 respectively, described by Mc Cabe *et al*. (1993), in addition, the mass (g) was evaluated using a precision analytical balance (PCE-BSH 6000).

$$V = \frac{4\pi\, a\, b^2}{3} \qquad (1)$$

Where, V: Volume (cm^3); a: Larger diameter (cm); b: Minor diameter (cm).

$$\phi = \frac{6\ Vp}{Do\ Sp} \qquad (2)$$

Where, Φ: Sphericity; Vp: Particle volume (cm^3); Sp: Surface area for the particle (cm^2); Do: Equivalent sphere diameter.

Soluble solids were measured using a digital refractometer (Hanna Instruments Inc. Woonsocket RI-USA-96801),

and the results were expressed in °Brix. The pH was determined by direct reading on a benchtop pH meter (GPH503). Water activity (a_w) was determined using a dew point hygrometer (Aqualab 3TE series, Decagon, Devices, Pullman, WA, USA). The peroxide index (PI) was performed according to Ariza *et al*. (2011), leaving the sample in the dark for 5 min and then measuring the absorbance at 500 nm. The color was determined in fruits with pericarp and without pericarp (50 fruits for each ecotype), according to the CIE L*a*b* and CIE L*C*H* coordinates. These were derived using a sphere spectrophotometer (SP64, X-Rite Inc, MI, USA) under conditions of illuminant D65, 10° observer, and both including and excluding the specular component.

Total phenol content (TP) was determined using a Folin ciocalteu reagent and 0.07 N Na_2CO_3 in aqueous solution (7.44% w v^{-1}). The method described by Restrepo *et al*. (2010) measuring the absorbance at 760 nm in a spectrophotometer (Thermo scientific, Madison, USA), and the results were expressed in mg gallic acid equivalent (GAE) 100 g^{-1}. The antioxidant capacity was determined from the DPPH and ABTS methods, using analytical grade reagents: 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-Azino bis (3 ethilbenzothiazoline-6-sulfonic acid) (ABTS) from Sigma-Aldrich Co. (St. Louis, MO, USA). DPPH was determined according to the method described by Cerón *et al*. (2010) that was measured at 517 nm, where the results were expressed in mg equivalent Trolox (TE) g^{-1}. ABTS was determined according to the method described by Re *et al*. (1999) at 734 nm, expressing the results in mg TE g^{-1}. For DPPH and ABTS, the percentage inhibition formula was described by Koolen *et al*. (2013).

The carotene content (α and β) was determined according to the methodology described by Sierra *et al*. (2007) and Cândido *et al*. (2015). An Agilent 1100 series HPLC with DAD, C18 column in reverse phase 250 cm, 4.6 mm, 5 µm, with an acetonitrile mobile phase (0.05% triethylamine): methanol: ethyl acetate (95:5:0) was used for 20 min and then adjusted to a ratio of 60:20:20, respectively, until the end. A flow of 0.5 mL min^{-1} and an injection volume of 10 µL were used, reading at 450 nm at room temperature. α-Tocopherol was determined according to the methodology described by Sierra *et al*. (2007) using an Agilent 1100 series HPLC with DAD, C18 column in reverse phase 250 cm, 4.6 mm, 5 µm, mobile phase of methanol: water (95:5), flow of 0.5 mL min^{-1}, injection volume of 10 µL, and reading at 292 nm at room temperature. The mineral content (Ca, Cu, Fe, Mg, K, Na, and Zn) was performed by atomic absorption spectrometry method 985.35 (AOAC, 1980).

The bromatological characterization of the pulp was determined in triplicate in terms of moisture content, protein, ether extract, ash, and fiber, following the AOAC method, (1990) (920.151, 984.13, 920.39, 942.05, and 985.29 respectively).

Statistical analysis

Differences in morphological variables of fruits and seeds between the ecotypes were evaluated with *t* test (P<0.05) using Statgraphics 8.0 program. Principal component analysis was used to ordinate the samples using morphological variables of fruits and seeds. Biplot graphs were used to visualize the ordination and the morphological variables, using InfoStat version 2020 (Di Rienzo *et al*., 2008).

RESULTS AND DISCUSSION

Physical characteristics

The results corresponding to the morphometric variables of the EI and EII ecotypes are presented in Table 1. All the morphometric measures except the sphericity (ϕ) presented significant differences (P<0.05) with respect to the ecotype of the fruit. Ecotype EI presented higher values for all variables evaluated in comparison to Ecotype EII however, these values were lower than those reported by Dos Santos *et al*. (2015) and Carvalho *et al*. (2013) in Brazil. The morphometric measurements from ecotype II were similar to those reported by Quispe *et al*. (2009) in Perú and lower than those reported by Guerra *et al*. (2011) in the Colombian Orinoquía. On the other hand, the total weight obtained from the EI ecotype was similar to that reported by different authors with a small fluctuation, this difference can be attributed for the soils and development conditions of the palm.

The principal component analysis (PCA) of the morphometric measurements of the E1 and E2 ecotypes are presented in Figure 1. The differences can be attributed to the weight, which is conditioned by genetic and environmental factors and can generate

high variability in the fruits. On the other hand, agronomic and bioclimatic factors can influence the development of the palm and its ecotypes (Guerra *et al*., 2011). The variables LD, ED, weight, and V are positively related to higher values for EI in comparison to EII.

Table 1. Morphometric characteristics of canangucha fruits EI and EII.

Ecotype	LD (cm)	ED (cm)	Weight (g)	V (cm^3)	ϕ
EI	4.5±2.40 a	4.2±1.39 a	47.8±3.88 a	46.8±3.14 a	0.8±0.03 a
EII	4.1±1.65 b	3.6±1.11 b	34.6±3.45 b	32.3±3.21 b	0.9±0.04 a

Mean ± standard deviation, different letter in the same column indicates significance difference (P<0,05), according to t-test.

In general, the parameters allow for establishing the necessary quantities of raw material that are required in a transformation process and, in turn, choosing the most suitable transport inside and outside the plant. Likewise, sphericity allows for determining the best conditions for processing of the fruits.

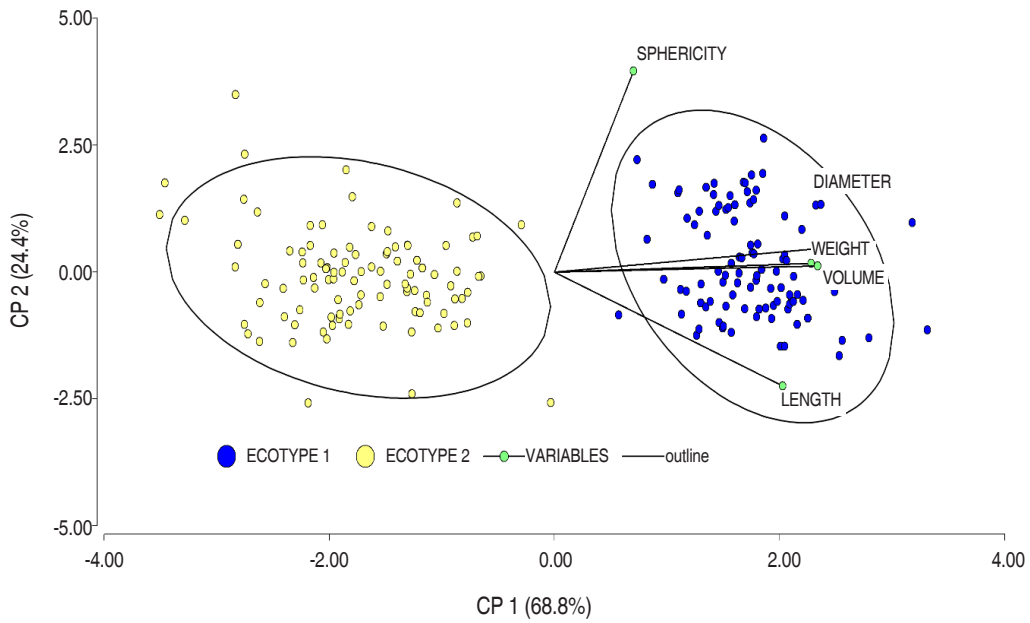

Figure 1. PCA morphometric measurements in EI and EII.

The percentage distribution of the fruit parts and their weights are presented in Table 2. It is observed that the greatest proportion is the seed, followed by the pericarp and finally the pulp. The values obtained from seeds in both ecotypes are higher than those reported by Quispe *et al*. (2009) in Perú and Imbrozio *et al*. (2010) in Brazil with 57.5%, and 49.51%, respectively. For the pulp, the values obtained in both ecotypes were lower than those reported by Quispe *et al*. (2009): 21.2% and Vásquez *et al*. (2010): 27.0%.

Various investigations report similar percentage distribution of the components of the canangucha fruit for EI with 62.5%, 17.6%, and 19.9% for the seed, pulp, and pericarp respectively. In Brazil, Imbrozio *et al*. (2010) reported higher values for pulp and pericarp with 24.2% and 22.0%, respectively. On the other hand, Quispe *et al*. (2009) reported similar values in the pericarp (21.3%) for EII and lower for ecotype I. For ecotype II, the investigation reported lower values in the seed (54.8%) similar in the pericarp (19.6%), and higher

Table 2. Percent distribution of the canangucha fruit and weights for ecotypes EI and EII.

Ecotype	Part of the fruit	Percentage (%)	Weight (g)
EI	Seed	65.2	31.2±1.7 a
	Pulp (mesocarp)	15.6	7.4±1.1 a
	Pericarp	19.2	9.2±1.8 a
	Whole fruit	100.0	47.8±3.8 a
EII	Seed	61.3	21.1±2.3 b
	Pulp (mesocarp)	17.2	5.9±1.4 b
	Pericarp	21.3	7.3±2.1 b
	Whole fruit	100.0	34.6±3.4 b

Mean ± standard deviation, different letter in the same column indicates significance difference (P<0.05), according to test t.

for the pulp (25.6%) (Dos Santos *et al*., 2015). Similarly, Batista *et al*. (2012) reported higher values for the pulp and pericarp with 63.8% and 25.1%, respectively, and lower values for the pulp (11.0%).

Regarding the weight of the different fruit parts, the seed was less than that reported by Guerra *et al*. (2011) and Imbrozio *et al*. (2010) with 43.5 g and 41.8 g, respectively. In turn, it was found that EI presented higher fruit and seed weight (%) and less pulp (%) and pericarp (%) than EII. The differences in the percentage distribution of the fruit components have been attributed to the fruit characteristics, climatic changes, and soil conditions, among other factors (Dos Santos *et al*., 2015; Guerra *et al*., 2011; Imbrozio *et al*., 2010).

The morphometric variables of the seeds from ecotypes EI and EII presented similar values for LD and ED. For these variables, Vásquez *et al*. (2010) and Freitas-Alvarado *et al*. (2011) report higher LD and lower ED values than those found by the present investigation. The weight of the seeds presented significant statistical differences (P<0.05) with respect to the ecotype, determining the EI ecotype to be higher than EII. It was also noted that the seed weight of ecotype I is higher than that found by Freitas-Alvarado *et al*. (2011) and lower for ecotype II. Figure 2 shows

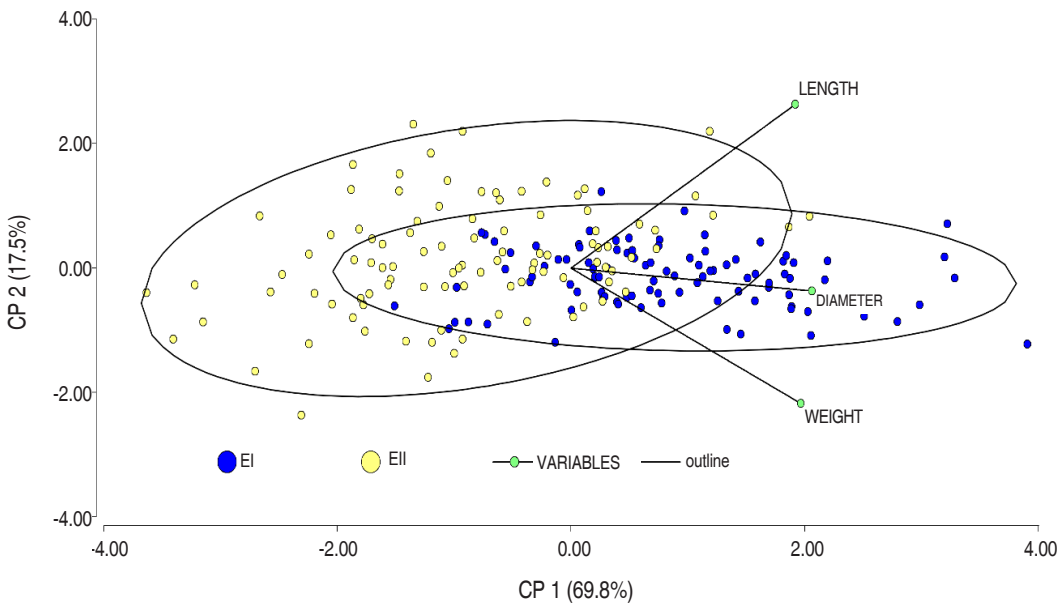

Figure 2. PCA seeds morphometry for ecotypes EI and EII.

the PCA for seed morphometry for EI and EII. It can be observed that the variables weight and LD are more related to ecotype EI and ecotype EII presented lower values for the afore mentioned variables.

The CIE-L*a*b* color coordinates for the whole fruit with pericarp and without pericarp (pulp) of ecotypes E1 and E2 are presented in Table 3. Significant differences (P<0.05) were found in all the variables regarding the sample type and the ecotype; however, it is observed that the color parameters of the fruit with pericarp did not present significant differences (P>0.05) with respect to the ecotype. The Canangucha pulp only shows significant differences (P<0.05) in the L* with respect to the ecotype.

Table 3. Color coordinates of the fruit and pulp of canangucha ecotypes EI and EII.

Variable	Pericarp fruit		Pulp fruit	
	EI	EII	EI	EII
L*	27.5±2.4 a	26.8±2.4 a	53.7±6.43 b	46.8±5.63 c
a*	13.4±2.6 a	11.3±2.2 a	23.9±3.11 b	23.4±3.22 b
b*	6.2±1.6 a	5.1±1.7 a	43.5±7.32 b	43.6±8.43 b
C*	14.7±2.9 a	12.5±2.4 a	45.8±6.63 b	49.0±10.52 b
H*	24.8±4.0 a	23.9±6.6 a	71.7±5.57 b	61.4±3.25 b

Mean ± standard deviation, different lowercase letter in the same row indicates significant difference (P<0.05), Tukey test.

The canangucha pulp presented greater clarity (>L*) than the fruit with pericarp, the latter being smooth. The pulp has a porous surface, which can present a greater homogeneity of the surface refractive index, due to the air content in the pores and less light absorption that makes it appear clearer (Carvalho *et al*., 2013). For the pulp, the EI ecotype presents greater L* than EII, which could be attributed to greater matrix content of dark pigments contributed by the carotenes (Cândido *et al*., 2015). These were enhanced and presented the most orange tone (<H*) (main contribution of carotene pigments) and the highest intensity or color saturation (>C*) for ecotype II.

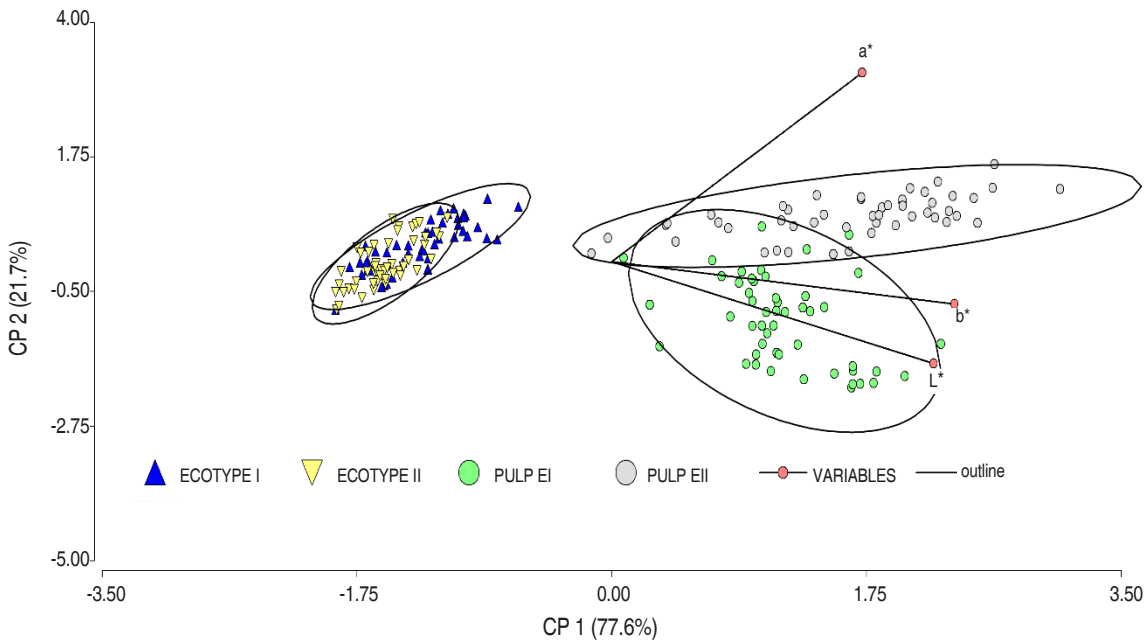

Figure 3. PCA color coordinates (L*, a*, b*) in canangucha ecotype EI and EII.

The pulp color coordinate is in the first quadrant of the chromatic plane a*b*, where the behavior or correlation of the color parameters is not very clear. This is because chromaticity b* is mostly associated with the carotene content and is similar in both ecotypes, a situation that confers the search for other factors, such as porosity, composition, among others.

The PCA of the color parameters for the fruit pericarp and pulp in the EI and EII ecotypes are presented in the Figure 3. The results illustrate that the fruit pericarp for both ecotypes were similar, where the pulp only denotes differences in the L* and was greater in the E1 ecotype. For their part, the values of a* were independent of L*. However, both a* and b* and b* and L* were positively related.

Chemical Characteristics

The pH presented significant differences (P<0.05) between ecotypes I and II, where the values fluctuated between 4.5±0.2 and 3.7±0.7, respectively. The °Brix did not present significant statistical differences (P>0.05), fluctuating between 16.4±1.4 and 15.2±1.0 respectively, and these results were similar to those reported by Vásquez *et al*. (2010). The pH value found for EI is higher than that reported by Guerra *et al*. (2011) (3.74) in Venezuela and lower than that reported by Milanez *et al*. (2018) (4.9) in Brazil. This acidic characteristic can give the fruit a greater advantage against bacterial deterioration, improving its conservation (Guerra *et al*., 2011). Some Amazon fruits have similar characteristics, such as arazá, cocona, copoazu, camu camu, and caimarona grape.

The proximal analysis of the Ecotype EI of the canangucha fruit is presented in the Table 4, this was selected due to the availability of fruit in the study area with respect to EII, observing that the fruit contains an ideal moisture content with its corresponding high a_w, which makes it a favorable substrate for microbial growth and deterioration reactions. The humidity of the fruit was similar to that reported by Vásquez *et al*. (2010) (63.96%) in the Shambo variety. On the other hand, it was higher than reported by Sinchi, (2017), Vásquez *et al*. (2010) and Quispe *et al*. (2009) with 57.0%, 51.3%, and 54.3%, respectively. The values found for the canangucha were within the range for oleaginous fruits, which range from 54 to 80% according to Vásquez *et al*. (2010).

Table 4. Proximal pulp analysis of canangucha EI.

Sample	Parameter	Mean ± SD (%) *
Pulp	Humidity	63.7±4.9
	a_w	0.95±0.03
	Acidity	1.3±0.2
	Ashes	4.1±0.7
	Lipids	34.2±2.2
	Fiber	22.2±1.6
	Protein	6.8±1.1
	Carbohidrates	32.7

*dry base (db).

The lipid content found for the pulp was similar to that reported by Sinchi (2017) for EI in the Caqueta foothills (37.0%). On the other hand, it was higher than that reported by Quispe *et al*. (2009) (18.1%) and Vásquez *et al*. (2010) (23.1%), and lower than that reported by Imbrozio *et al*. (2010) (38.0%). Lipid values can vary according to the ecotype, the stage of fruit maturity, and the seasonality of the harvest.

In Peru, Quispe *et al*. (2009) found that the main component of canangucha oil was oleic acid (78%), in turn, Milanez *et al*. (2018), mentioned that this oil has high concentrations of carotenes, tocopherols, and acids such as oleic and palmitic, which help prevent cardiovascular diseases. Similarly, some authors indicated that the oil has functional properties due to the high concentrations of monounsaturated fatty acids, conferring hypocholesterolemic action. Other studies have indicated that canangucha has higher content of omega 3, 6 and 9 fatty acids than sacha inchi (*Plukenetia volubilis*), which are important for the lipid metabolic process and decrease the risk of cardiovascular diseases (Sinchi, 2017).

Fiber is a component that has functional properties, preventing conditions such as constipation, irritable colon, and obesity. Therefore, the consumption of canangucha can be considered healthy. The result found was on the order of 22.2%, lower than that reported by Sinchi (2017) (37.7%) and higher than that reported by Quispe *et al*. (2009) (10.1%).

The pulp protein content was similar to that reported by Quispe *et al*. (2009) with 2.32%, and lower than that

reported by Sinchi (2017) with 3.62% and Vásquez *et al*. (2010) with 5.50%. Protein intake from the canangucha pulp is relatively low. However, it is a high-quality plant-based protein that contains aromatic amino acids, such as tyrosine and phenylalanine, and sulfur amino acids, such as cysteine, methionine, and tryptophan (De Oliveira and Franca, 2017).

For carbohydrates (32.7%), the value found was higher than that reported by Sinchi (2017), Vásquez *et al*. (2010) and Guerra *et al*. (2011) with 21.2%, 5.6% and 18.1%, respectively. Canangucha can be considered an energy source with respect to its carbohydrates and lipids (Sotero *et al*., 2013).

Minerals are important in the diet since they act as cofactors in different metabolic reactions that are carried out in the body. In turn, they are essential regulatory and structural components that must be consumed in the diet, according to Sotero *et al*. (2013). In this sense, the mineral content in the canangucha pulp is presented in Table 5, highlighting higher levels of Ca than that reported by Pereira *et al*. (2016) and lower than those reported by Sotero *et al*. (2013) and Vásquez *et al*. (2010) with 354 ppm and 1197 ppm, respectively, the Ca is important for the prevention of diseases, such as osteoporosis and rickets.

Table 5. Mineral content in El canangucha pulp.

Sample	Mineral	[ppm]*
Pulp	Ca	315
	Cu	5
	P	98
	Fe	4.5
	Mg	23
	P	97.5
	Na	450
	Zn	4

*dry base (db).

On the other hand, the Cu level was similar to that reported by Sotero *et al*. (2013) with 6 ppm, and it was higher than that reported by Vásquez *et al*. (2010) (4.6 ppm). In the case of P, Fe, and Mg levels, these were lower than those reported by Sotero *et al*. (2013) and Vásquez *et al*. (2010), and higher than those reported by Pereira *et al*. (2016). For the Na content, the values were higher than that reported by Vásquez *et al*. (2010) and Sotero *et al*. (2013), with 134 ppm and 126.9 ppm, respectively, therefore it´s consumption should be limited in hypertensive people. Regarding Zn, the values were lower than those reported by Sotero *et al*. (2013) and Vásquez *et al*. (2010) with 10.8 ppm and 726 ppm, respectively. This variability observed for the minerals in the canangucha fruit of the Colombian Amazon region when compared to those grown in Peru, Brazil, and Venezuela may be due to edaphoclimatic, genetic conditions, and the state of maturity of the fruit.

The results of TP, ABTS, DPPH, α-carotene, β-carotene and α-tocopherol in the canangucha pulp are presented in Table 6, in addition to relating the peroxide index value. These components conferring physiological activity are important because they protect the organism from the damaging effects of free radicals (Koolen *et al*., 2013) and counteract and prevent various cardiovascular diseases, in addition to slowing down lipid oxidation processes in food products.

Table 6. Antioxidant, peroxides, and bioactive components in canangucha pulp from El.

Parameter	Mean ± SD*
TP (mg GAE 100 g^{-1})	1467.3±146.5
DPPH (mg TE g^{-1})	2.5±0.1
ABTS (mg TE g^{-1})	3.0±0.2
α-carotene (mg 100 g^{-1})*	6.7±2.3
β-carotene (mg 100 g^{-1})*	68.2±9.6
α-tocopherol (mg 100 g^{-1})*	23.3±0.9
Peroxide Index (meq O_2 kg oil^{-1})	0.5±0.1

*dry base (db).

The TP content was higher than those reported by Best *et al*. (2022), Carmona-Hernández *et al*. (2021), Abreu-Naranjo *et al*. (2020), Milanez *et al*. (2018), Schiassi *et al*. (2018), Nogueira (2017), Cândido *et al*. (2015), Dos Santos *et al*. (2015), Vásquez *et al*. (2010), and Koolen *et al*. (2013) with 28.8, 235.9, 435.08, 110.7, 47.2, 270.6, 435.1, 281.0, 187.5, and 378.0 mg GAE 100 g^{-1}, respectively, in countries, such as Brazil, Peru, and Venezuela. Likewise, Koolen *et al*. (2013) mentioned that the antioxidant activity of the canangucha pulp is important, due to the phenolic

compounds. In turn, Tauchen *et al*. (2016) report 87 mg GAE g^{-1} in liquid extract obtained from canangucha pulp.

On the other hand, Resende *et al*. (2019) report TP contents in canangucha by product flours that vary between 93.2 to 934.6 mg GAE 100 g^{-1}, in other Amazonian fruits some authors reported, such as asai (*Euterpe oleracea* M), camu camu (*Myrciaria dubia* McVaugh), and acerola (*Malpighia emarginata* S) values of 454, 1176, and 1063 mg GAE 100 g^{-1}, respectively. Other investigations carried out by Koolen *et al*. (2013) with phenolic extracts of canangucha indicate that these possess a strong capacity to inhibit the growth of pathogens. In addition, Milanez *et al*. (2018) mentioned that it has high concentrations of antioxidants that prevent oxidative stress. For DPPH, the values found are higher those reported by Tauchen *et al*. (2016) with 0.131 mg TE g^{-1} in pulp extract and higher than that reported by Nogueira. (2017) with 0.58 mg TE g^{-1}. In other studies, Schiassi *et al*. (2018), and Koolen *et al*. (2013) reported 951.5 EC_{50} g fresh weight g^{-1} and 19.8 mg mL^{-1}, respectively.

For ABTS, the results were higher than those reported by Schiassi *et al*. (2018) with 1.51 mg TE g^{-1} and Nogueira (2017) with 2.18 mg TE g^{-1} in Brazil. However, they were lower than those reported by Cândido *et al*. (2015) with 8.26 mg TE g^{-1} in Brazil. In some Amazonian fruits, such as arazá (*Eugenia stipitata*), copoazú (*Tehobroma grandiflorum*), sacha inchi (*P. volubilis*), asai (*E. oleracea*) and camu camu (*M. dubia*) levels are found of 5.05 mg TE g^{-1}, 2.4 mg TE g^{-1}, 2.47 mg TE g^{-1}, 3.77 mg TE g^{-1} and 38.3 mg TE g^{-1}, respectively. Studies carried out by Camelo-Silva *et al*. (2021) in Brazil report values for ABTS of 293.7-411.4 µmol TE g^{-1}, and for DPPH of 0.07–10.45 µmol TE g^{-1}.

The present peroxide index for canangucha was higher than that reported by some authors in Brazil. These values are important due fat content can trigger oxidation reactions, and this can cause the product to deteriorate, generating an unpleasant taste and odor. For their part, Quispe *et al*. (2009) reported a value of 4.6 and 4.8 meq O_2 kg^{-1} for extraction of canangucha oil at 25 and 60 °C in Peru.

Physiologically active compounds (PAC) act as protectors of the immune system, participate in defense reactions, and improve biochemical and metabolic processes in the body, and these compounds include vitamins, antioxidants, minerals, among others, frequent consumption of these decreases the risk of suffering from some cardiovascular and chronic diseases (Milanez *et al*., 2018). The results obtained for α-carotene are high to those reported by Hamacek *et al*. (2018) (2.3 mg 100 g^{-1}) in Brazil, while α-carotene reached levels of 1.5 mg 100g^{-1}. Furthermore, Milanez *et al*. (2018) reported values of 28.8, 42.9, and 45.1 mg 100 g^{-1} in green, semi-ripe, and ripe canangucha fruits, respectively, while Sotero *et al*. (2013) reported 29.6 mg 100 g^{-1} in canangucha oil. For β-carotene, the results found are higher those reported by Abreu-Naranjo *et al*. (2020), Hamacek *et al*. (2018) Schiassi *et al*. (2018) and Sotero *et al*. (2013) with 19.5, 21.6, 17.0 and 10.4 mg 100 g-1, respectively, and close to than those reported by Cândido *et al*. (2015) and Vásquez *et al*. (2010), with 52.9, 34.2 mg 100 g^{-1}, respectively.

In Brazil, Cândido *et al*. (2015) indicated that, among the carotenes in canangucha, β-carotene (100% activity of pro-vitamin A) is the majority component (13.7 mg 100 g^{-1}), On the other hand, Dos Santos *et al*. (2015) also reported 65% for the β-carotene/oleic acid ratio when evaluating the canangucha pulp. These variations may be due to the degree of maturity, variety, agronomic factors, and extraction process. The results for α-tocopherol were lower than those reported by Hamacek *et al*. (2018) and Vásquez *et al*. (2010) with 44.9 and 68.3, respectively, these results can be due to genetic variation and development of the palm.

According to Beltrán *et al*. (2012), 1 µg of retinol equivalent activity (RAE) is equal to 12 µg of α-carotene and 6 µg of β-carotene; therefore, in the present study, canangucha has a contribution of 11,927.7 µg RAE 100 g^{-1} in dry base and 4449.2 µg RAE 100 g^{-1} on a wet basis. In turn, the value found is higher than reported by Hamacek *et al*. (2018) with 1899.33 µg 100 g^{-1} RAE. Some authors mentioned that canangucha is an important source of provitamin A, which could be used as a supplement in populations with deficiencies for this compound. In turn, Koolen *et al*. (2013) mentioned that these levels are 20 times richer in provitamin A than carrots, acerola, and papaya. For their part, some investigations proposed the consumption of canangucha as a supplement in the treatment of testoterone replacement therapy (TRT), due to its contribution in antioxidants, tocopherols, β-carotene, phytosterols, and oleic acid in patients with hypogonadism.

Finally, some authors indicated that the variety and the state of fruit maturity has great influence on the chemical composition, level of antioxidants and bioactive compounds of the canangucha, this fruit has the potential to application in functional foods and as a source of antioxidants and bioactive compounds (Abreu-Naranjo *et al*., 2020). Furthermore, the highest contents of TP antioxidants, and vitamin C were found in the green state, while β-carotene was more prevalent in the mature state. The results found for this study were specific to the Colombian Amazon.

CONCLUSION

The canangucha fruit of ecotype I presented higher LD, ED, mass, and volume with respect to ecotype II, which allows for establishing the differences between them. On the other hand, both present great morphometric variation with respect to fruits from Brazil and Peru, which can be attributed to the genetic variability, edaphoclimatic conditions of the growth zones, and the species. The ecotypes I and II pulp coordinates were in the first quadrant of the color plane with reddish-orange colorations corresponding to maturation degree four. The pulp percentage was higher for EI when compared to EII, which presented a higher percentage of seeds.

Canangucha is characterized by its contribution of lipids, fiber, total phenols, and β-carotene as a source of provitamin A, in addition to significant contribution of equivalent activity of retinol and these can be used as a supplement for people with vitamin A deficiency. Due to its nutritional and antioxidant characteristics canangucha is considered a species with great food and pharmaceutical potential, as well as a promising and sustainable value chain for the Colombian Andean Amazon region.

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Revista Facultad Nacional de Agronomía

Study of the physicochemical and mechanical stability of an edible leather of mango (*Mangifera indica*) and pineapple (*Ananas comosus*) pulp

UNIVERSIDAD NACIONAL DE COLOMBIA

Estudio de la estabilidad fisicoquímica y mecánica de una lámina comestible de pulpa de mango (*Mangifera indica*) y piña (*Ananas comosus*)

https://doi.org/10.15446/rfnam.v76n1.100730

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Keywords:
Drying
Edible Leather
Mango
Pineapple
Storage

ABSTRACT

Mango (*Mangifera indica*) and pineapple (*Ananas comosus*) are two important fruits with many industrial uses and excellent sensory, nutritional and functional characteristics. In this research work, the development of intermediate moisture edible leathers obtained by convective drying technology of the mixture of mango and pineapple pulp at 60 and 70 °C was carried out, evaluating their physicochemical characterization and stability under controlled storage conditions at 25 and 35 °C. The results showed that leathers subjected to drying at 60 °C and stored at 35 °C presented a significant increase in water activity. Leathers stored at 35 °C showed greater browning due to the effect of storage temperature. The highest resistance to cutting and tension was observed in edible leathers dried at 70 °C and stored at 25 °C. The Young's Modulus in tension varied between 1.317 and 2.22 MPa. The greatest degradation of vitamin C (57%) was found in leathers dried at 70 °C and stored at 35 °C. It was possible to conclude that the mango and pineapple pulp-based leathers stored for 4 weeks presented physical-chemical and techno-functional characteristics that make them suitable for consumption.

Palabras clave:
Secado
Lámina comestible
Mango
Piña
Almacenamiento

RESUMEN

El mango (*Mangifera indica*) y la piña (*Ananas comosus*) son dos importantes frutas con amplios usos a nivel industrial por sus significativas características sensoriales, nutricionales y funcionales. En la presente investigación se llevó a cabo el desarrollo de láminas comestibles de humedad intermedia obtenida por tecnología de secado convectivo de la mezcla de pulpa de mango y piña a 60 y 70 °C, evaluando su caracterización fisicoquímica y de estabilidad en condiciones de almacenamiento controladas a 25 y 35 °C. Los resultados mostraron que en las láminas secas a 60 °C y almacenados a 35 °C hubo un aumento significativo de la actividad del agua. Las láminas almacenadas a 35 °C presentaron un mayor pardeamiento por efecto de la temperatura de almacenamiento. La mayor resistencia al corte y tensión se observó en las láminas comestibles secadas a 70 °C y almacenadas a 25 °C. El Módulo de Young en tensión varió entre 1,317 y 2,22 MPa. La mayor degradación de vitamina C (57%), se encontró en las láminas secadas a 70 °C y almacenadas a 35 °C. Se pudo concluir que las láminas a base de pulpa de mango y piña almacenados durante 4 semanas presentaron características físico-químicas y tecno-funcionales que los hacen aptos para el consumo.

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Received: June 18, 2022; Accepted: August 04, 2022
Rev. Fac. Nac. Agron. Medellín 76(1): 10189-10200. 2023
ISSN 0304-2847 / e-ISSN 2248-7026

Mango (*Mangifera indica*) and pineapple (*Ananas comosus*) are two highly produced fruits worldwide, with quantities of 55.9 and 28.2 million tons, respectively, where Colombia shows the participation of 1% in mango production and 3.6% in pineapple production (FAOSTAT, 2019). Besides that these tropical fruits have excellent sensory properties and some good nutrients for health such as vitamin C, also have high production, preference and commercial availability, and high availability of nutraceutical components (Masibo and He, 2008).

An innovative processing alternative for the consumption of some fruits and vegetables is the production of edible leathers of intermediate moisture and flexible consistency since they could be considered ready for consumption. These emerging foods are the result of a process of decreasing water activity and are characterized by having sufficient moisture without allowing deterioration due to microbial effects (Offia-Olua and Ekwunife, 2015; Bravo, 2022).

The development of edible fruit-based leathers has been the object of study by various authors such as Sharma *et al*. (2016) in pineapple; Offia-Olua and Ekwunife (2015) in apple, banana, and pineapple; Vanegas and Parra (2012), and Da Silva *et al*. (2019) in mango and Torres *et al*. (2015) in apple and quince.

There are few studies on the development and agro-industrial production of edible leathers of intermediate ($0.6<a_w<0.85$) and flexible texture based on a mixture of mango and pineapple pulp; two fruits with high availability, mass consumption, important nutritional content, and high commercialization potential. Therefore, the objective of the study was to develop and analyze the physicochemical and techno-functional stability of edible leathers of intermediate moisture from the mixture of mango and pineapple pulps under controlled storage conditions.

MATERIALS AND METHODS

Raw Materials

Tommy Atkins Mango (*Mangifera indica*) with a maturity index of 3 according to NTC 5210-2003 (ICONTEC, 2003), honey-glow pineapple (*Ananas comosus*) with a maturity index of 5 according to NTC 729-1-1996 (ICONTEC, 1996), and sucrose (white sugar) were obtained from a local supplier from Medellín, Colombia. Carboxymethylcellulose (CMC), rapid citric pectin, unflavored gelatin, ascorbic acid, and citric acid were purchased from Tecnas S.A. (Medellín, Colombia).

Formulation Development

The fruits were washed and immersed in chlorinated water at 100 ppm, later they were peeled, chopped, and homogenized in an industrial blender (Javar-LC15 1F 15LT). Through preliminary tests of the formulation of edible leathers and their sensory acceptance evaluations on the characteristics of flexibility, color, smell, and taste, carried out on 15 untrained judges, the final formulation was established.

The preliminary tests for the formulations consisted of mixing mango and pineapple pulp in equal quantities, adding 1% CMC, 1% pectin, 1% unflavored gelatin, 0.1% ascorbic acid, 0.1% citric acid, and 4.5% white sugar. In total, 3 types of leathers were formulated, each with a different hydrocolloid. The leathers formulated with 1% CMC, 0.1% ascorbic acid, 0.1% citric acid, and 4.5% sugar —added in relation to the base of the pulp mixture— presented the best sensorial acceptance and flexibility; therefore, it was selected as the formulation to this study.

Characterization of the fruit suspension to be dried

Color: A SP60 sphere spectrophotometer - X-Rite - illuminant D65, a 10° observer as a reference, and with a CIE-L*a*b* scale, measuring the browning index (BI) and color difference (ΔE *) (Garzón-García *et al*., 2018). **Total soluble solids (TSS)**: refractometric method (AOAC 932.12/90), using a HANNA HI-96801 digital refractometer. **Water activity (a_w)**: using a dew point hygrometer (Aqualab series 3TE, Decagon Devices, Pullman, WA, USA) at 25 °C. **Moisture**: official method (AOAC, 1990) taking 1 g of sample to dry in an oven at 105 °C until constant weight for 16 h. **pH***:* potentiometer method (AOAC, 1990) by immersing the electrode (HANNA HI2211) in the prepared sample consisting of 1 g of sample and 30 mL of distilled water. **Titratable acidity**: titration method (AOAC, 2005) with NaOH solution (0.1N), using phenolphthalein as an indicator, expressing its value as a percentage of citric acid. **Vitamin C:** high performance liquid chromatography

(HPLC), according to the methodology proposed by Abe-Matsumoto *et al*. (2020), expressing its value as mg ascorbic acid (AA)/g dry matter (DM).

Drying of suspension

The formulated suspension was poured into a metal tray previously lined with aluminum foil and impregnated with food-grade unflavored glycerin until the mixture reaches a thickness in the tray between 7-8 mm. The convective drying was carried out at temperatures of 60 °C and 70 °C, and air velocity of 2 m s^{-1} (Universal Memmert INB 500). The process was finished when a moisture content between 20 to 22% w.b (wet basis) was reached.

Leather stability study

The leathers obtained were cut in dimensions of 12.5×4 cm and vacuum packed in low-density polyethylene bags with a thickness of 70 µm. The leathers were subjected to controlled conditions of temperature (25 °C and 35 °C), relative humidity of 80%, exposure to white light, and air circulation speed of 0.1 m s^{-1} (Memmert ICH260 climate chamber). The physicochemical and mechanical resistance determinations were carried out every week in triplicate during 4 weeks of storage.

Characterization of the fruit leathers

Physicochemical properties: The color (CIELAB method), total soluble solids, water activity (a_w), moisture content, pH, titratable acidity, and content of vitamin C were obtained according to the methods described above for the suspension. Data collection for each test was performed in triplicate.

Mechanical and Textural Properties: Leather cuts (12.5×4.0 cm) were subjected to tensile stress using a TA-XT2i universal texture analyzer (Stable Micro Systems, London, UK), following the ASTM E8 protocol with the modifications proposed in food according to the methodology given by Honikel (1998): test speed of 1 mm s^{-1}, pre- and post-test speed of 2 mm s^{-1}, and maximum deformation of 50 mm, where the Elastic or Young's modulus (YM) was obtained from the flow curve of the material. Regarding the texture, the Warner-Bratzler cutting blade (Stable Micro Systems®) was used, with the same operating conditions used in the stress test. The results were analyzed using the Texture Analysis Software (Stable Micro Systems Ltd., Godalming, Surrey, UK). Data collection for each test was performed in triplicate.

Statistical analysis

A 2×2 factorial design was carried out, drying temperature (DT) (60 and 70 °C) and storage temperature (STE) (25 °C and 35 °C) repeated in time. The data were analyzed by multifactorial ANOVA (α=5%) using the Statgraphics Centurion XVI.I software.

RESULTS AND DISCUSSION

The physicochemical characterization of the formulated mixture and the leathers obtained during convective drying is observed in Table 1.

Leathers that were dried at 60 °C and 70 °C, reached an intermediate humidity in 11 and 8.75 h of process, respectively. Braga *et al*. (2019) reported a moisture content of 84.2% w.b in mango pulp, while the pineapple pulp reaches moisture of 87.3% w.b. These results are slightly higher than those found in this study (82.24±0.203 w/w) for the mixture intended for convective drying. This difference can be explained by the addition of low moisture powders (CMC and sucrose) and by physiological differences inherent to the vegetable product. The water activity (a_w) value of the suspension was higher than leathers dried at 60 and 70 °C (P<0.05). This characteristic could allow increasing the stability of the fruit leathers against any type of microbiological deterioration.

The soluble solids of the mixture for drying (18.533±0.197 °Bx) are high due to the addition of sucrose to the mixture. The total soluble solids of the leathers dried at 60 °C and 70 °C are significantly higher than those determined in the formulated mixture (P<0.05). These results may be due to the concentration of the components as a consequence of dehydration, an aspect that improves the sensory quality and stability of the fruit leathers.

For the pH value, there was no statistically significant difference (P>0.05) between the pulp mixture and fruit leathers. The acidity of the suspension expressed as % of citric acid (0.650±0.018) was significantly lower (P<0.05) than the acidity of the leathers dried at 60 °C (2.663±0.038) and 70 °C (2.634±0.103) which could be due to the concentration of acids.

Table 1. Characterization of the formulated mixture and the leathers obtained by drying.

Parameter	Fruit suspension	Leathers 60 °C	Leathers 70 °C
Moisture (% w.b)	82.240±0.203 a	20.990±0.303 b	20.565±0.343 b
Water activity (a_w)	0.987±0.002 a	0.586±0.012 b	0.570±0.021 b
Degrees Brix (°Bx)	18.533±0.197 a	83.300±1.218 b	83.417±0.768 b
pH	4.120±0.041 a	3.973±0.036 a	3.962±0.039 a
Acidity (% citric acid)	0.650±0.018 a	2.663±0.038 b	2.634±0.103 b
Vitamin C (mg ascorbic acid g^{-1} dry matter)	3.108±0.088 a	2.995±0.381 a	2.047±0.503 b
Loss of vitamin C (%)		3.643±1.497 a	34.131±1.776 b
L*	52.763±0.932 a	58.727±3.220 b	61.833±2.502 b
ΔL		5.964±2.076	9.070±1.298
a*	2.687±0.416 a	13.515±1.412 b	13.088±1.358 b
Δa*		10.828±0.914	10.401±0.887
b*	29.713±0.785 a	38.992±0.448 b	37.953±1.156 b
Δb*		9.279±0.616	8.240±0.970
ΔE*		15.457	16.073
BI	82.431	118.111	105.470
Shear Failure Force (SFF) (N)	-	83.386±13.994 a	93.712±25.438 a
Tensile Failure Force (TFF) (N)	-	34.078±4.020 a	37.442±3.432 a
Young's Modulus (YM) (MPa)	-	1.547±0.316 a	1.990±0.324 a

Mean values with the same letter are not significantly different $P<0.05$

The vitamin C for the suspension (3.108±0.088 mg ascorbic acid g^{-1} dry mass (DM)) was similar to the content of vitamin C in the mango pulp and pineapple pulp mentioned by Chakraborty *et al*. (2015). Regarding the retention of vitamin C, it is observed that it was higher at 60 °C, presenting retention in relation to the suspension of 96.3%; while at 70 °C, the retention of vitamin C was 65.9%. The vitamin C in the suspension, as well as that of the leathers dried at 60 °C, was significantly higher ($P<0.05$) concerning the leathers obtained at 70 °C. This result could be explained by the high thermal sensitivity of this micronutrient against temperatures above 60 °C.

According to color coordinates, the L*, a*, and b* values of the drying mixture were significantly lower ($P<0.05$) in relation to the values for leathers obtained at 60 °C and 70 °C. These results show that the dried leathers displayed a tendency to yellow (+b) and red (+a) tones. According to Badjona *et al*. (2019), carotenoid pigments such as β-carotene, which are present in mango and pineapple pulp, are responsible for these shades.

The ΔE* value for the leathers obtained at 60 and 70 °C indicates that the color difference is easily visible. In addition, the high value of the browning index (BI) obtained in the dried leathers indicates a trend of darkening with respect to the base suspension. These results can be attributed to the concentration of pigments due to the evaporation effect of water through drying and their degradation by the action of heat. In a similar study carried out by Shende *et al*. (2020), the greatest color difference between mango leathers subjected to tray drying at 60 °C and fruit puree was ΔE*=35.75.

According to Table 1, the leathers at 60 and 70 °C did not present a statistically significant difference ($P>0.05$) in tensile failure force (TFF), shear failure force (SFF), and elastic modulus (YM), behavior that could be

explained by the similar moisture content. Da Silva *et al*. (2019) found higher values of shear failure force (SFF) and Young's modulus (YM) in mango puree leathers (6.37% w/w).

Storage stability

The leathers dried at 60 and 70 °C had a thickness of 1.94±0.28 mm. Figure 1 shows the moisture content during the storage period under drying conditions, where the shortest drying times were achieved at 70 °C due to the higher evaporative capacity of the process. Sharma *et al*. (2016) reported moisture of 20% w/w in pineapple leathers dried at 60 °C by direct solar treatment. Vanegas and Parra (2012) found similar drying times of 9 h for mango pulp in convective drying at 70 °C, reaching final moisture in the leather of 17.04% w/w. Azeredo *et al*. (2006) achieved moisture of 17.2% w/w in mango leathers after drying at 80 °C. Da Silva *et al*. (2019) and Offia-Olua and Ekwunife (2015), reached moisture content (<5% w/w) for leathers made from mango and pineapple pulps using cast-tape (80 °C) and solar drying (80 °C), respectively.

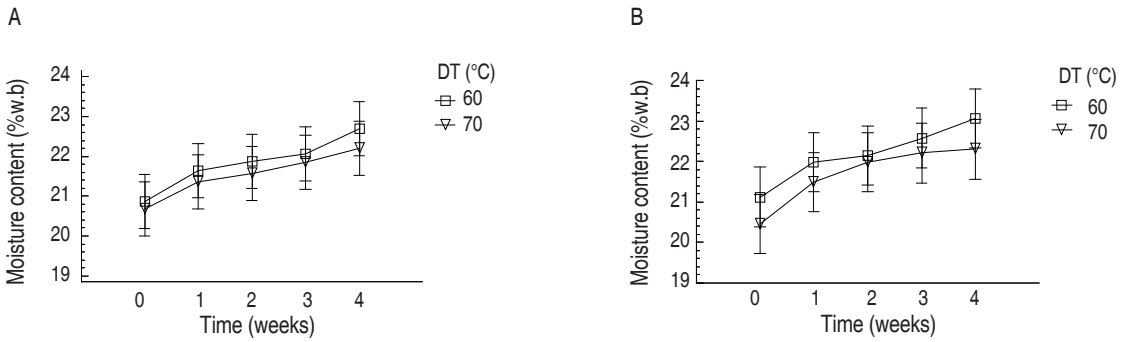

Figure 1. Moisture content of the leathers: A. Storage at 25 °C; B. Storage at 35 °C

The ANOVA did not show statistically significant differences ($P>0.05$) for the interaction of the drying temperature (DT) and storage temperature (STE) factors on the moisture content. The moisture content variations are less than 2% w/w; however, the product shows a certain degree of hygroscopicity. This low moisture gain could be explained by the presence of CMC which could have created a surface barrier between hygroscopic particles. In the same way, vacuum packaging in low-density polyethylene bags could also have become a barrier to water vapor from the environment.

Figure 2 shows that during storage the variation in water activity was $0.552<a_w<0.672$. Vanegas and Parra (2012) reached an a_w value of 0.603 in mango leather. Offia-Olua and Ekwunife (2015) report an a_w value of 0.8 in leather made from pineapple puree. Torres *et al*. (2015) reached values between $0.56<a_w<0.69$ in apple sauce and quince leathers. Da Silva *et al*. (2019) report water activity values between $0.419<a_w<0.463$ in mango leathers. According to the ANOVA, the drying temperature and the storage temperature have a statistically significant individual effect ($P<0.05$) on the water activity. In Figure 2B, it is observed that the leathers dried at 60 °C and stored at 35 °C increased their water activity (a_w) during the storage time in a more pronounced way compared to the leathers dried at 70 °C and stored at 25 °C (Figure 2A). The water activity of the leathers dried at 60 °C was always higher than those found for the samples subjected to 70 °C, this trend was more marked in the samples stored at 35 °C (Figure 2B). These variations could be a consequence of both the permeability of the polyethylene bags to high relative humidity (80%), and the acceleration of the mass transfer phenomenon into the packaging caused by a storage temperature higher than 35 °C.

Merino (2006) argued that foods subjected to high drying temperatures are prone to a displacement of solutes towards the surface promoting the formation of a hard surface layer or crust with waterproof properties known as shortening. The higher a_w observed in the samples

stored at 35 °C is because in this condition there is a higher vapor pressure during storage, which increases water activity compared to samples stored at 25 °C. According to Krapf and Gantenbein-Demarchi (2010), greater water activity brings with it an increase in food instability, leading to darkening reactions, rancidity, as well as greater susceptibility to the probable attack of microorganisms.

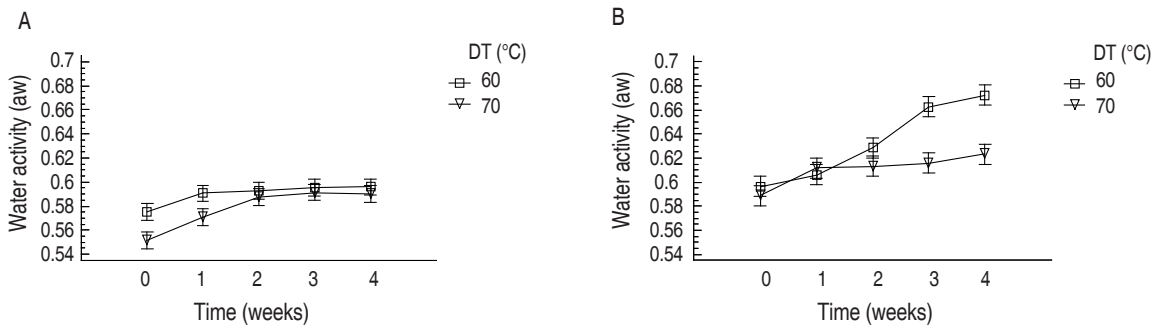

Figure 2. Behavior of a_w during stability: A. Storage at 25 °C; B. Storage at 35 °C.

The interaction between the drying temperature (DT) and storage temperature (STE) factors did not present a statistically significant effect (P>0.05) on the TSS, pH, and acidity values. The high content of total soluble solids for the leathers (82 °Bx – 84 °Bx) is due to the concentration as a result of the drying process (Figure 3).

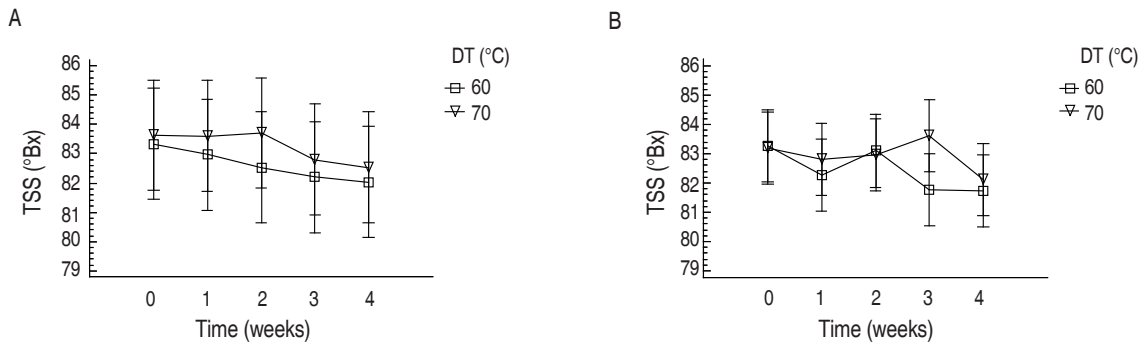

Figure 3. TSS behavior during stability: A. Storage at 25 °C; B. Storage at 35 °C.

The value found in this study was higher than that reported by Torres *et al*. (2015) for apple puree leathers (70.7 °Bx), and quince puree (76.2 °Bx), with moisture of 15.9 and 17.2% w w^{-1}, respectively. This difference may be due to the inequality of moisture in the leathers, the addition of sucrose made in the present study, and the characteristics of the fruits studied.

The pH values (Figure 4) are similar to those reported by Siller-Cepeda *et al*. (2009) for mango pulp (3.6<pH<4.3), and by Chutintrasri and Noomhorm (2015) for pineapple pulp (3.72). Azeredo *et al*. (2006) and Torres *et al*. (2015) report similar pH values in mango (3.8) and apple (4.05) leathers. Offia-Olua and Ekwunife (2015) report a pH value greater than 6.03 in leathers of apple, pineapple, and banana puree mixture. These differences are caused by the addition of acids in the formulation and by the physical-chemical characteristics of the products used. The pH values during storage at 25 °C were similar to those obtained during storage at 35 °C, ranging from 3.90 to 4.00. Similarly, the total acidity (Figure 4, C and D) of the edible leathers stored at 25 and 35 °C during the 4 weeks of storage did not present a statistically significant difference (P>0.05). These results could mean an advantage in terms of microbiological stability during storage of the edible leathers thanks to their low pH and high acidity.

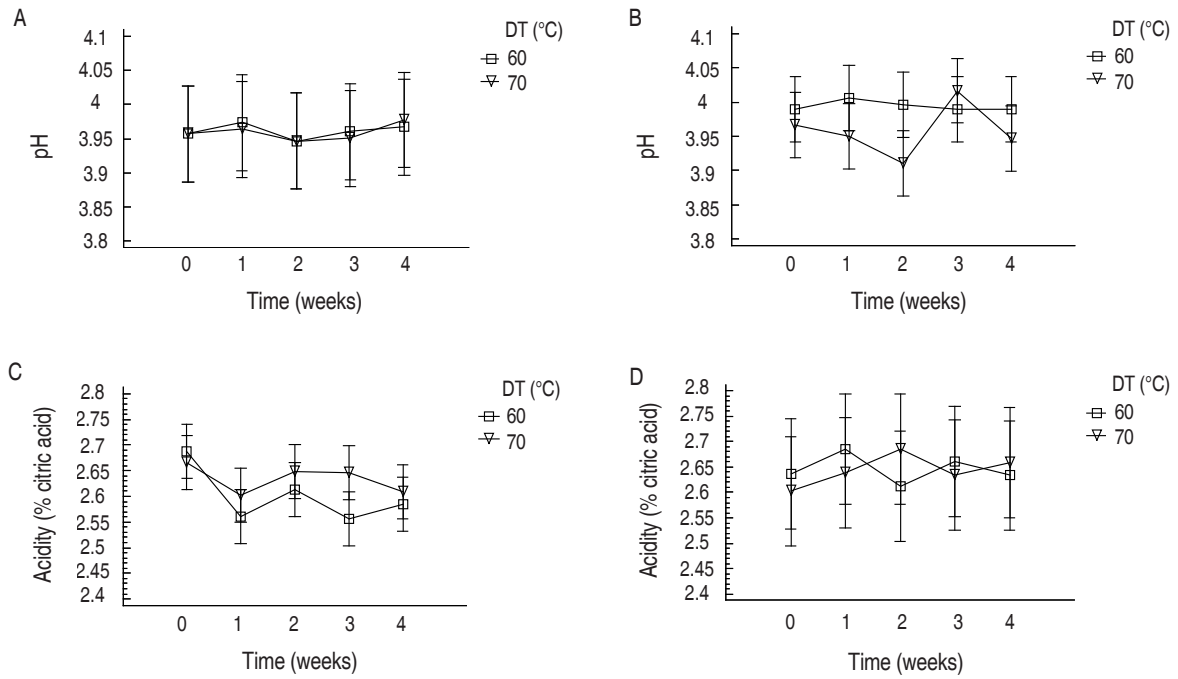

Figure 4. pH (A, B) and acidity (C, D) Values of the leathers during the stability study: (A, C) Storage at 25 °C; (B, D) Storage at 35 °C.

The shear failure force (SFF) varied between 62.8 N and 116.7 N (Figure 5), while the tensile failure force (TFF) varied between 25.3 N and 40.2 N (Figure 6, A and B). The highest shear failure force of 116.8 N and tensile force of 39.7 N were found in the leathers dried at 70 °C and stored at 25 °C. Therefore, under these conditions, there is a greater stress requirement during the chewing process compared to leathers dried at 60 °C and stored at 35 °C. The ANOVA showed a statistical effect in the interaction between DT and STE on TFF (P<0.05). The force necessary to reach the point of failure was statistically lower in the leathers dried at 60 °C and stored at 35 °C (Figures 5 and 6 (A and B)). This result can be attributed to the possible shortening present in the leathers dried at 70 °C, increased rigidity, and the increase in moisture and a_w over time during storage at 35 °C. Regarding the shear

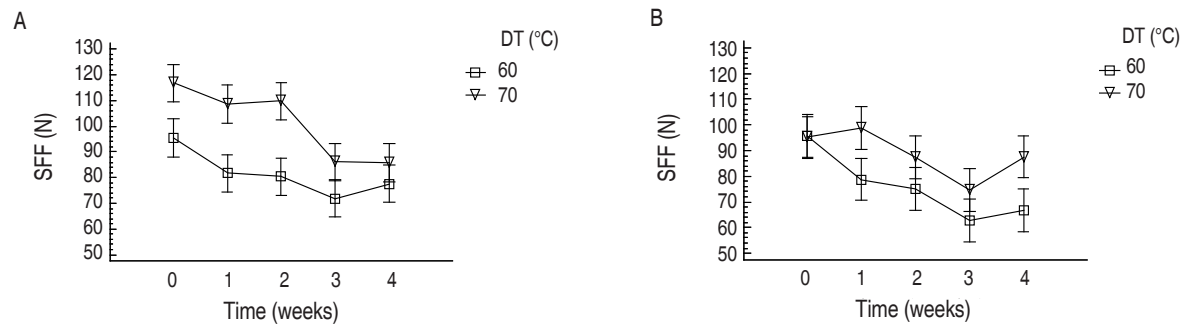

Figure 5. Shear failure stress of the leathers during stability study: (A) storage at 25 °C; (B) storage at 35 °C.

failure force (SFF), this is higher the lower the storage temperature (25 °C). This result could be explained due to the increase in the water activity and moisture content of the leathers during the 4-week study period. During the storage time, there is a tendency to decrease the shear force (SFF) (Figure 5), which may be due to the increase in moisture in food during storage. The edible leathers subjected to drying at 60 °C and storage

at 25 and 35 °C, showed a lower shear failure force and tensile failure force, which could indicate a soft mouth grinding process, also allowing a potential use as food for the elderly and infants.

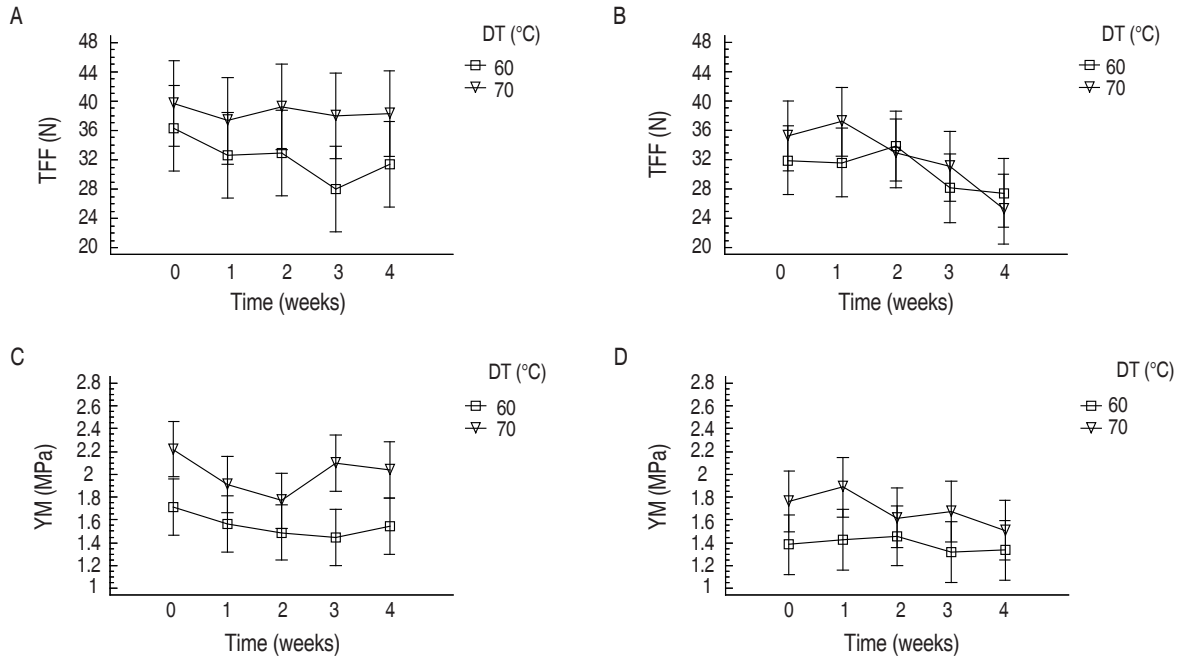

Figure 6. Force to failure in tension (A, B) and Young's Modulus (C, D) behavior of edible leathers during storage at 25 °C (A, C) and storage at 35 °C (B, D).

According to Figure 6 (C, D), the elastic modulus (YM) varied between 1.31 MPa and 2.22 MPa. The YM values found in this study refer to a product with low rigidity or a soft texture. Da Silva *et al.* (2019) found an elastic modulus (YM) of 9.8 ± 1.7 MPa in mango puree leathers, which was presented due to the low moisture content (5.4%-6.4% w/w), an aspect that could confer greater rigidity and a lower percentage of deformation to the leather.

According to the ANOVA, the interaction of the DT and STE factors did not affect the YM value (P>0.05). In Figure 6 (C, D) it is observed that rigidity was higher in the leathers dried at 70 °C. This may be because at high drying temperatures less deformation is achieved at the point of failure of the material. Roos (1995) stated that the increase in temperature and water content (main plasticizer), can significantly affect the mechanical properties (decrease in the elastic modulus) during processing or storage. This allows the change in the viscoelastic properties of the product above the glass transition, due to the loss of the vitreous state (rigidity) and consequent tendency to the liquid state: the increase in storage temperature gives rise to molecular expansion suggesting low viscosities (greater fluidity), that is, loss of rigidity. This statement agrees with that obtained in the present study given that the elastic modulus (YM) decreased as the storage temperature and the water content increased. The tendency to decrease Young's Modulus (Figure 6, C and D) suggests that through storage time, edible leathers suffer a loss of rigidity, which could be translated into a greater ease for oral processing of food (less force to achieve the chewing) (Roos, 1995).

According to the ANOVA, there is no statistically significant interaction of DT and STE factors on the L* and a* variables. However, the double interaction with the b* coordinate did occur, with higher values in the leathers dried at 60 °C and stored at 25 °C. In Figure 7B, it is observed that the value of L* decreases significantly during the storage time, the drying temperature, and the storage temperature. In Figure 7A, it is observed that during storage at 25 °C, there was no significant difference (P>0.05) during the storage time between leathers dried at 60 °C, the same occurred in leathers dried at 70 °C. At time zero, the values of luminosity L* (Figure 7B) and chromaticity b*

(Figure 7F) of the leathers dried at 60 °C and 70 °C were significantly higher than those obtained in week 4 during storage at 35 °C. A similar situation occurred in the value of a* during storage at 25 °C (Figure 7C). During storage at 35 °C, the value of a* (Figure 7D), a statistically significant difference (P>0.05) was not observed during the storage time, however, there was a slight tendency to decrease. This same behavior was observed in the values of b* during storage at 25 °C (Figure 7E). These results could have been due to the presence of CMC which could have delayed the darkening process (Sánchez *et al*., 2018). Da Silva *et al.* (2022), found a similar behavior (tendency to decrease) of the CIE-L*a*b* parameters in strawberry leathers stored at 25°C with a relative humidity of 22.5 and 52.3% for 90 days. Sánchez *et al.* (2018) obtained similar values of L* (40-45), a* (12.10-13.96), and b* (18.57-24.48) in mango leathers made with CMC, gum arabic, and citric slow pectin. The results indicate that during the stability period at 35 °C, the leathers presented a greater tendency to darken than leathers stored at 25 °C, which could be due to the degradation of pigments, possibly influenced by the stability temperature, exposure time of the leathers to direct light and increased a_w. Since the degree of discoloration of food is due to the availability of oxidizing agents and, the fact that enough energy is communicated (in the form of light) for the degradation reaction to take place, the loss of vacuum of the packaging and the constant exposure to white light during storage can lead to thermal degradation, photodegradation, and acidification of carotenoids (Mora *et al*., 2018).

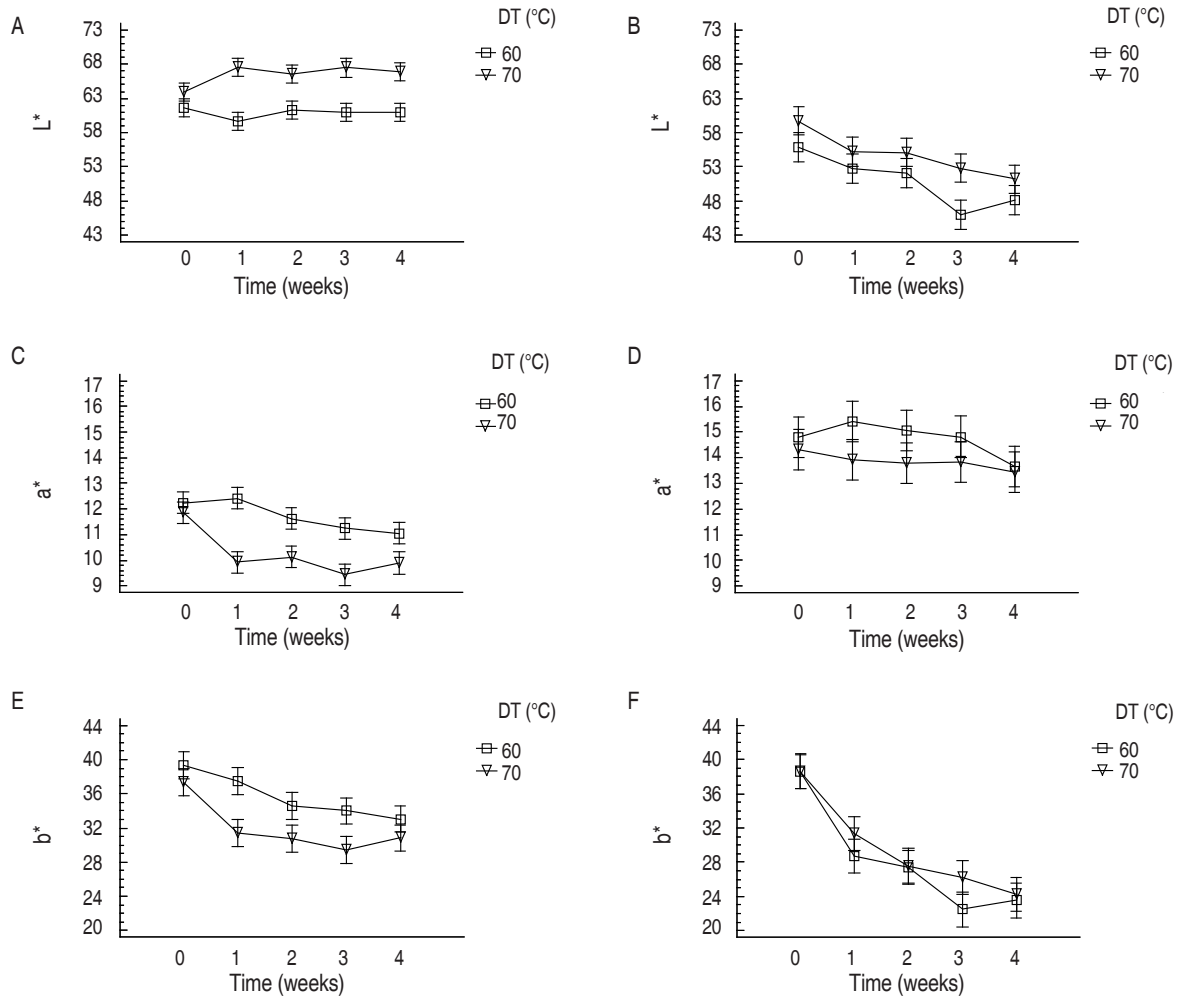

Figure 7. CIE-L*a*b* parameters of edible leathers during stability: (A, C, E) and (B, D, F) correspond to storage temperatures of 25 and 35 °C, respectively.

The presence of ascorbic acid ranged from 3.371 to 0.692 g g^{-1} DM. This result is higher compared to those reported by Sharma *et al.* (2016) in leathers of pineapple puree, and to the values obtained by Offia-Olua and Ekwunife (2015) in low moisture leathers (<5% w/w) of apple, pineapple, and banana puree. These differences could be explained by the different matrices and processes used in the elaboration and the addition of ascorbic acid in the formulation of the leathers in the present study.

The ANOVA showed a statistical significance of DT and STE on the content of vitamin C (P<0.05). Figure 8 shows that the content of vitamin C was lower when the DT and STE values were increased. The product at 60 °C presented a higher content of vitamin C. The highest degradation of vitamin C (57.5%) was obtained for DT=70 °C and STE=35 °C, which shows the thermolability of this micronutrient. Regarding the individual factor for STE, there is no statistical effect on the content of vitamin C (P>0.05); however, in the leathers stored at 35 °C there is a slightly greater loss.

Figure 8A, indicates that 25 °C storage temperature with exposure to direct light was sufficient to achieve vitamin C degradation during the 4 weeks of storage, however, the degradation values were lower than those observed in storage at 35°C (Figure 8B). Similarly, in Figure 8, it is observed that the fruit leathers subjected to drying at 70 °C, presented a greater loss of vitamin C before (week 0) and after (week 4) the stability study was completed. However, although vitamin C degradation was observed during the present study, the edible leathers retained a considerable amount of vitamin C, being up to 1.7 mg ascorbic acid g^{-1} dry matter in leathers dried at 60 °C and stored for 4 weeks.

The degradation of vitamin C or ascorbic acid could be due to the effect of temperature, light, presence of oxygen, presence of enzymes, and increases in water activity (Phillips *et al.*, 2016). The loss of vitamin C due to high temperatures is associated with the opening or closing of the lactone ring (isomerization of L-isomers to D-isomers) and/or the formation of chiral compounds when the vitamin is exposed to high temperatures (Aguilar *et al.*, 2019). Regarding the degradation of vitamin C by exposure to light, ascorbic acid is photo-oxidized to form dehydroascorbic acid, and this oxidation increases as light increases (Duncan and Chang, 2012). Ascorbic acid degradation is also related to the presence of ascorbic acid oxidase and peroxidase enzymes; the former catalyzes the oxidation of ascorbic acid in the presence of oxygen resulting in dehydroascorbic acid and water; the second catalyzes the reduction of hydrogen peroxide by ascorbic acid giving rise to the production of water and dehydroascorbic acid (Dbrowska *et al.*, 2007). According to Phillips *et al.* (2016), the acidic pH (found in this study) can lead to the inactivation of enzymes such as ascorbic acid oxidase, which reduces degradation. Saipei and Hwa (2014) mention that the degradation of vitamin C can decrease when sugar is added, which suggests, for the present study, that the addition of sucrose in the formulation was of great importance to achieve the considerable amounts of vitamin C obtained in the fourth week of storage.

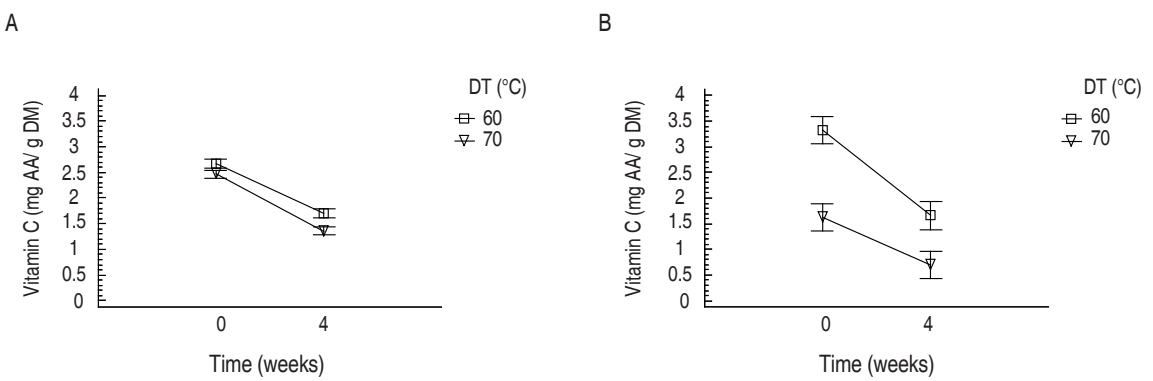

Figure 8. Behavior of vitamin C content during stability: (A) and (B) correspond to storage temperatures of 25 and 35 °C, respectively.

CONCLUSION

The intermediate moisture edible leathers made from Tommy Atkins mango and pineapple pulp showed slight changes in the physicochemical and mechanical characteristics during the storage period. Vitamin C content decreased with storage time and drying temperature. The highest percentage of loss of vitamin C occurred in leathers dried at 70 °C. During the stability period at 35 °C, the leathers showed a tendency to dark colors, possibly as a result of carotenoid degradation. The mechanical properties presented a behavior slightly dependent on the variation of moisture content during the storage. The changes observed in the Tommy Atkins mango and pineapple leathers show that they presented physical-chemical and techno-functional characteristics that make them suitable for human consumption after during weeks of storage. It is necessary to study new formulations, other methods, and drying conditions together with packaging conditions that minimize the loss of vitamin C.

ACKNOWLEDGGMENTS

The authors express their gratitude to the Universidad Nacional de Colombia, Sede Medellín where the development of this research was carried out, which is part of the Master's Thesis: "Desarrollo y caracterización fisicoquímica de una lámina comestible a base de pulpa de mango (*Mangifera indica*) y piña (*Ananas comosus*)." Also, the authors are immensely grateful to Laboratory of Fruits and Vegetables and Agricultural Processes of Universidad Nacional de Colombia-Sede Medellín which provided facilities, equipment, and personnel.

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Development and characterization of a fermented dairy beverage from permeated and concentrated sweet whey sweetened with tagatose

UNIVERSIDAD NACIONAL DE COLOMBIA

Desarrollo y caracterización de una bebida láctea fermentada a partir de permeado y concentrado de lactosuero dulce edulcorado con tagatosa

https://doi.org/10.15446/rfnam.v76n1.100958

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ABSTRACT

Keywords:
Beverage
Dairy product
Ultrafiltration
Whey

Membrane separation technology in the dairy industry has become a basis for the innovation and processing of new products, as well as an alternative for the use of co-products, contributing to the reduction of the environmental impact that this industry generates. The objective of this work was to develop a fermented dairy beverage using sweet whey ultrafiltration permeate (UFP) and whey protein concentrate (WPC), evaluating its effect on physicochemical and techno-functional characteristics under storage with controlled conditions. The experimental design was a simplex centroid mixtures with WPC (2-5%), UFP (51-58%), and milk (40-47%) where a non-hydrolyzed (NHFDB) and hydrolyzed (HFDB) (> at 85%) beverage was formulated. Optimum beverages were obtained by minimizing syneresis and maximizing protein content, and overall product acceptability. The results of the multiple response desirability analysis showed the following formulation: WPC (5%), UFP (52.2%), and milk (42.8%) for the non-hydrolyzed fermented dairy beverage (NHFDB) and WPC (5%), UFP (51%) and milk (44%) for the hydrolyzed beverage (HFDB). The developed beverages presented a non-Newtonian behavior (pseudoplastic) and gel-like characteristics for the non-hydrolyzed beverage, high sensory quality, acidity (0.55-0.68% lactic acid), pH (4.18-4.45), and syneresis (10.7-13.2%). The non-hydrolyzed fermented dairy beverage was more stable over storage time in terms of physicochemical characteristics and syneresis than the hydrolyzed fermented dairy beverage.

RESUMEN

Palabras clave:
Bebida
Producto lácteo
Ultrafiltración
Suero

La tecnología de separación por membranas en la industria láctea se ha convertido en una base para la innovación y procesamiento de nuevos productos, así como una alternativa de aprovechamiento de coproductos, aportando a la disminución del impacto ambiental que esta industria genera. El objetivo de este trabajo fue desarrollar una bebida láctea fermentada usando permeado (UFP) y concentrado proteico (WPC) de ultrafiltración de lactosuero dulce evaluando en condiciones de refrigeración, su efecto en las características fisicoquímicas y tecno-funcionales. El diseño experimental fue un diseño de mezclas centroide simplex, variado WPC (2-5%), UFP (51-58%) y leche (40-47%) donde se formuló una bebida sin hidrolizar (NHFDB) e hidrolizada (> al 85%) (HFDB). Las bebidas óptimas se obtuvieron al minimizar sinéresis, maximizar cantidad de proteína y aceptabilidad general del producto. Los resultados del análisis de deseabilidad por múltiples respuestas mostraron la siguiente formulación: WPC (5%), UFP (52,2%) y leche (42,8%) para la bebida láctea fermentada sin hidrolizar (NHFDB) y WPC (5%), UFP (51%) y leche (44%) para la bebida hidrolizada (HFDB). Las bebidas desarrolladas presentaron naturaleza no newtoniana (seudoplástica) y de características tipo gel para la bebida no hidrolizada, alta calidad sensorial, acidez (0.55-0.68% de ácido láctico), pH (4,18-4,45), y sinéresis (10,7-13,2%). La bebida láctea fermentada sin hidrolizar fue más estable en el tiempo de almacenamiento en términos de características fisicoquímicas y sinéresis, que la bebida láctea fermentada hidrolizada.

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Received: June 08, 2022; Accepted: August 17, 2022
Rev. Fac. Nac. Agron. Medellín 76(1): 10201-10212. 2023

ISSN 0304-2847 / e-ISSN 2248-7026

The world production of cheese for the year 2020 amounted to approximately 21.69 million metric tons, being the European Union the main producer of cheese in the world with a production volume of around 10.35 million metric tons (FAO, 2020). Whey constitutes approximately 90% of the total volume of milk after cheese making. It contains more than half of the soluble elements such as sugars, salts, proteins, and fats, among others (Asas *et al.,* 2021). The nutritional value of whey is given by the amount of proteins, minerals, and amino acids present in it, among the most abundant proteins found in this co-product, are β-lactoglobulin and α-lactalbumin, which correspond to 10 and 4% of all milk protein, respectively; among the most notable amino acids are tryptophan, leucine, and lysine, which are essential amino acids; and in terms of minerals, potassium, calcium, phosphorus, sodium, and magnesium stand out, in addition to the B vitamins, being pantothenic acid and ascorbic acid found in greater quantity with concentrations of $3.4*10^{-3}$ g L^{-1} and $2.2*10^{-3}$ g L^{-1}, respectively (Maticorena *et al.,* 2018).

The final disposal of whey constitutes an environmental problem because it is usually discarded in water sources, and due to the high load of organic matter it contains, it causes a relatively high oxygen demand to be produced, ranging from 30,000 mg O_2 L^{-1} to 50,000 mg O_2 L^{-1} (Agualongo *et al*., 2022). Membrane filtration is a technology used to separate one or more compounds from a solution, and in the case of whey, it has been used to separate proteins and sugars, among others, with various applications in the food industry and other sectors (Iltchenco *et al*., 2018). Whey permeate obtained by ultrafiltration, containing lactose and minerals, can be considered a raw material to be used in food products, particularly for fermented dairy beverages, because lactose is found in significant quantities as a structural carbohydrate, which allows the growth and multiplication of lactic acid bacteria.

The Food and Agriculture Organization (FAO, 2020) defines a fermented dairy beverage as a milk product obtained through the fermentation of milk, which may have been made from products obtained from milk with or without modifications in composition, through the action of suitable microorganisms and resulting in a reduction in pH with or without coagulation (isoelectric precipitation). Montesdeoca *et al*. (2017) and Santos *et al.* (2008) have shown that by substituting milk for sweet whey in percentages between 30% and 40%, dairy beverages with high acceptability are produced, both in sensory and physical-chemical tests, showing optimal characteristics for its possible consumption and commercialization. Different authors have also used several types of flour and fruit in whey-based dairy beverages to obtain better viscosity and consistency (Gavilanes *et al*., 2018; Marulanda *et al*., 2016; Rodríguez and Hernandez, 2017; Muñoz *et al.,* 2019). Pescuma *et al*. (2010) used whey, WPC, and lactic acid bacteria for the elaboration of a functional beverage, finding that it had improved characteristics, such as reduced content of β-lactoglobulins and increase in essential branched-chain amino acids.

It is currently estimated that two-thirds of the world's adult population suffer from lactose intolerance (Alcalá *et al.,* 2021). Enzymatic hydrolysis through the enzyme β-galactosidase is commonly used in the dairy industry, causing the breakdown of lactose, and achieving easy digestion of these foods, thus, this population can consume dairy products. In addition, this splitting not only solves this problem but also increases the power of sweetness in the food.

The sugars used in the food industry define the degree of sweetness of the final product and also affect the physicochemical and techno-functional properties of various food matrices. Thereon, tagatose is a monosaccharide sugar remarkably similar in its structure to fructose, which can be an alternative to replace sucrose in various applications, presenting a sweetening power of 92% and 38% fewer calories than sucrose (Alvarez and Molina, 2017). Various entities such as the FAO, the WHO, and the European Union accepted it as a safe food ingredient because it has prebiotic effects and helps in the treatment of diseases related to obesity and diabetes (Manzo *et al.,* 2019).

At present, there is little or no development of fermented dairy beverages using simultaneously milk, sweet whey permeate and ultrafiltration concentrate subjected to hydrolysis process. The objective of this study was to develop a hydrolyzed and non-hydrolyzed fermented dairy beverage from ultrafiltration permeate, whey protein

concentrate, and whole milk sweetened with tagatose, evaluating its physicochemical and techno-functional stability over time under controlled refrigeration.

MATERIALS AND METHODS

Raw Materials

Whole milk: it was obtained from the dairy facilities of the Paysandú farm, property of the Universidad Nacional de Colombia (Medellín Campus). *Ultrafiltration Permeate* (UFP) *and Protein concentrate* (WPC): These products were obtained from 250 L of whey ultrafiltration using a PERINOX membrane filtration system equipped with a semi-permeable polyethersulfone spiral membrane with a 10 kDa cut-off size and the following operating conditions: pressures of 1 and 3 bars at the outlet and inlet, respectively; a temperature of 48 °C, and a concentration factor of 18 (Vargas, 2017). Both the UFP (240 L) and WPC (10 L) obtained were stored under refrigeration at 4 °C until the dairy beverage was prepared. *Starter culture:* DRI-SET 432, a lyophilized microbiological culture provided with *Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus salivarius subsp. thermophilus* lactic acid bacteria, provided by the company VIVOLAC. *Tagatose:* Tagasweet was acquired from the company BIOFOODS. *Starch:* Thermflo (waxy corn base) E 1440 was provided by the company INGREDION. *Stabilizer:* Pectin LM 35 from the company INGREDION and Sodium Citrate from the company TECNAS.

Raw materials characterization

For whole milk, the content of total solids (ICONTEC, 2001), proteins (AOAC, 1996), fat (AOAC, 2000), acidity as a % lactic acid (ICONTEC, 1999), density (AOAC, 1995), cryoscopic point (ICONTEC, 2013), and pH (ICONTEC, 1999).

The UFP was characterized by measuring the pH according to the method (AOAC, 2000), acidity (% lactic acid) (AOAC, 1997), total soluble solids (AOAC, 2000), lactose by liquid chromatography (HPLC), proteins (AOAC, 2009), minerals (ICONTEC, 2003), and ash (AOAC, 1990). The WPC, total soluble solids (AOAC, 2000), proteins (AOAC, 2009), α-lactalbumin and β-lactoglobulin were determined by liquid chromatography (HPLC) using a Shimadzu chromatograph and following the methodology proposed by Elgar *et al.* (2000), and density (AOAC, 1995). Table 1 presents the corresponding characterization.

Table 1. Physicochemical parameters of milk, ultrafiltration permeate (UFP), and whey protein concentrate (WPC) as raw material for the preparation of the fermented dairy beverages.

Milk	Mean value	UFP	Mean value	WPC	Mean value
Density (g L^{-1})	1.0313±0.0004	Acidity (% lactic acid)	0.08±0.003	Total Solids (°Bx)	13.05±0.58
pH	6.68±0.04	pH	6.53±0.067	Protein (%w w^{-1})	8.36±0.31
Acidity (% lactic acid)	0.17±0.01	Soluble solids (°Bx)	5.40±0.085	α-lactalbumin (g L^{-1})	1.47±0.42
Fat (%)	3.7±0.1	Lactose (g L^{-1})	50.052±0.134	β-lactoglobulin (g L^{-1})	4.30±1.11
Protein (g L^{-1})	3.25±0.06	Protein (%)	$\leq$ 2.5	Density (g mL^{-1})	1.08±0.00
Non-fat solids (%)	8.70±0.08	Ashes (%w w^{-1})	0.481±0.032	Humidity (%)	83.0±0.01
Total solids (% w w^{-1})	12.37±0.13	Calcium (mg kg^{-1})	283.063±27.472	Water activity	0.97±0.00
Cryoscopic point (°C)	0.514±0.003	Potassium (%w w^{-1})	0.14±0.10		

Formulation of the dairy beverages

The beverages formulation was carried out through a simplex-centroid mixture design: 40% <milk<47%, 51%<UFP<58% and 2%<WPC<5% (Table 2). This design was conducted for both the hydrolyzed beverage (HFDB) and the non-hydrolyzed beverage (NHFDB) with total soluble solids of 11%, in which sucrose was replaced by TagaSweet Tagatose (Biofoods) to achieve these values.

In each treatment, the whole liquid milk was mixed with the UFP and WPC, in the case of the HFDB, commercial lactase 0.4 g L^{-1} was added to these mixtures at 4 °C for 24 h. The NHFDB and the HFDB,

it was subjected to a heat treatment at 42 °C, adding starch (1.3%), sodium citrate (0.2%), and the stabilizer (0.3%). Subsequently, the mixture was homogenized at 17.23 MPa and pasteurized at 85 °C with 15 min of retention time and cooled to 45 °C when the process of inoculation with biological culture (DRI-SET 432) was realized. The mixture was incubated at 42 °C until reaching 0.52% lactic acid (Imbachi, 2018) and stored at 4 °C to sweetening with Tagasweet under continuous stirring.

Table 2. Experimental design to produce of fermented dairy beverages (HFDB and NHFDB).

Formulation	WPC (%)	UFP (%)	MILK (%)
F1	2	58	40
F2	5	55	40
F3	2	55	43
F4	2	53	45
F5	3	57	40
F6	3	54	43
F7	3	52	45
F8	4	53	43
F9	2	51	47
F10	4	56	40

Physicochemical analysis in the fermented dairy beverages

Acidity was measured by titration (ICONTEC,1999). The pH was determined with an OHAUS STARTER 3100 potentiometer (ICONTEC, 1999). The syneresis index was performed by taking 20 g of sample and subject to centrifugation at 1250 rpm for 20 min at 4 °C, the supernatant was separated by pouring, weighed, and recorded as syneresis (g $100g^{-1}$ of sample) (Amaya *et al*., 2008). The determination of viscosity was established employing the flow curves at 4 °C using a Brookfield DV-III ultra rheometer with the SC4-27 spindle (Ramírez and Vélez, 2013). Viscoelastic behavior within the linear viscoelasticity range was determined utilizing a frequency sweep (0.01-100 Hz) using an Anton Paar rheometer (MCR-302) coupling a cone/plate geometry with temperature control at 4 °C. The protein was determined by the Kjeldahl method (AOAC, 2009). Soluble solids were expressed as Brix degrees (°Bx) and quantified using a digital refractometer (HI 96801) (Baldasso *et al*., 2011). For the percentage of hydrolysis, glucose, galactose, and lactose carbohydrates were measured by means of HPLC agilent technologies Series 1200 with an aminex HPX-87H ion exchange column (300 x 7.8 mm), and as a mobile phase solution of H_2SO_4 0.008 N at a constant flow of 0.6 mL min^{-1} (Beltrán and Acosta, 2012).

Sensory analysis in fermented dairy beverages

The sensory analyzes were carried out in two stages: the first one was performed by 20 expert technicians belonging to the Laboratory of Dairy Products of the Universidad Nacional de Colombia at Medellín campus, which was effected using a 5-point Hedonic scale, where the general acceptability of the 10 hydrolyzed fermented dairy beverages (HFDB) and the 10 non-hydrolyzed fermented dairy beverages (NHFDB) was evaluated, information that was used for choosing the best hydrolyzed and non-hydrolyzed beverage. In the second stage, a sensory profile was implemented by multidimensional approximation for the fermented dairy beverage samples according to the methodology indicated by NTC 3932-1996 (ICONTEC, 1996). This analysis was conducted by a panel of expert judges made up of 5 people between the ages of 25 and 60 years old. The relevant descriptors on the sensory attributes were identified and selected, assessing the intensities on a rating scale from 0 to 5 for all the descriptors except for overall quality, where a scale from 1 to 3 was used, 3 being the highest value and 1 the lowest value.

Stability of the fermented dairy beverage

In this phase, a batch was prepared for the best HFDB and another for the best NHFDB from the optimization of the statistical mixture design. The samples were packed in polystyrene containers —commercial presentations of 200 g — with a screw cap and left in storage at 5±1 °C. Measurements were made on days 0, 7, 14 and 21 for the variables pH, lactic acidity %, and syneresis index.

Statistical analysis

Phase I: A mixture design was carried out, evaluating the results with an ANOVA at 5% using the statistical program Statistica (StatSoft, version 12), with adjustment to a special cubic model (this model was used because presents the best goodness of fit with R^2=0.98). The optimization process was performed using the multiple response desirability technique. Phase II (Stability study): the factor was the type of HFDB and NHFDB, performing four readings for a study time of 21 days. The results were analyzed using an ANOVA (α=5%) and mean difference test over time using Fisher's LSD method (α=5%).

RESULTS AND DISCUSSION

The physicochemical results of the non-hydrolyzed (NHFDB) and hydrolyzed (HFDB) fermented dairy beverages of the mixture design are shown in Table 3 and Table 4, respectively. For the analysis of the mixture design, optimization was performed by minimizing the response variable syneresis and maximizing general acceptability and protein content variables (Figure 1A and B). Optimization analyzes showed values of WPC (5%), UFP (52.2%), and milk (42.8%), for the NHFDB (R^2=0.9772). For HFDB, the optimal formulation under the same conditions (R^2=0.9798) was WPC (5%), UFP (51%) and milk (44%).

Table 3. Physicochemical characteristics and sensory evaluation of non-hydrolyzed dairy beverages under the different formulations (NHFDB).

Run	pH	Acidity (% Lactic Acid)	Syneresis (%)	Soluble solids (°Bx)	Total protein (%)	Overall acceptability
F1	4.39±0.05	0.55±0.01	13.16±0.69	11.00±0.00	1.05±0.071	3.7±0.5
F2	4.45±0.02	0.57±0.01	10.74±0.72	11.00±0.00	1.70±0.000	4.4±0.7
F3	4.35±0.02	0.56±0.00	13.19±0.57	11.00±0.00	1.15±0.071	3.5±0.8
F4	4.42±0.02	0.59±0.01	12.42±1.02	11.00±0.00	1.75±0.071	3.4±0.7
F5	4.44±0.01	0.58±0.00	10.86±0.11	11.00±0.00	1.60±0.000	3.6±0.7
F6	4.52±0.02	0.57±0.01	13.22±1.87	11.70±0.17	1.45±0.071	3.7±1.2
F7	4.44±0.01	0.60±0.00	12.34±0.20	11.40±0.17	1.85±0.071	3.3±0.8
F8	4.30±0.01	0.58±0.01	14,78±0.47	11.00±0.00	1.50±0.000	3.7±0.8
F9	4.37±0.02	0.61±0.01	10.81±1.29	11.00±0.00	1.55±0.071	3.7±0.7
F10	4.29±0.02	0.58±0.01	12.82±0.35	11.00±0.00	1.45±0.071	3.7±0.7

Table 4. Physicochemical characteristics and sensory evaluation of hydrolyzed dairy beverages under the different formulations (HFDB).

Run	pH	Acidity (% Lactic acid)	Syneresis (%)	Soluble solids (°Bx)	Total protein (%)	Hydrolysis (%)	Overall acceptability
F1	4.32±0.03	0.59±0.00	14.96±1.02	11.00±0.00	1.45±0.07	84.98	3.6±0.5
F2	4.35±0.03	0.68±0.01	10.84±0.65	11.00±0.00	1.85±0.07	93.11	4.0±0.0
F3	4.32±0.02	0.60±0.01	10.75±1.09	11.00±0.00	1.50±0.00	97.16	3.6±0.5
F4	4.35±0.02	0.66±0.01	11.96±0.76	11.00±0.00	1.70±0.14	97.81	4.4±0.5
F5	4.30±0.01	0.60±0.01	12.84±0.38	11.40±0.17	1.65±0.07	93.13	3.8±0.8
F6	4.30±0.00	0.63±0.00	13.22±1.87	12.00±0.00	1.80±0.14	93.39	3.6±0.8
F7	4.19±0.01	0.64±0.01	12.34±0.20	13.00±0.00	1.70±0.00	93.57	3.0±0.7
F8	4.21±0.01	0.60±0.01	14.78±0.47	13.00±0.00	1.80±0.00	97.60	3.8±0.4
F9	4.18±0.01	0.64±0.01	13.31±1.28	12.00±0.00	1.85±0.07	96.04	3.8±0.4
F10	4.31±0.01	0.64±0.01	12.82±0.35	11.00±0.00	1.75±0.07	92.22	3.6±0.5

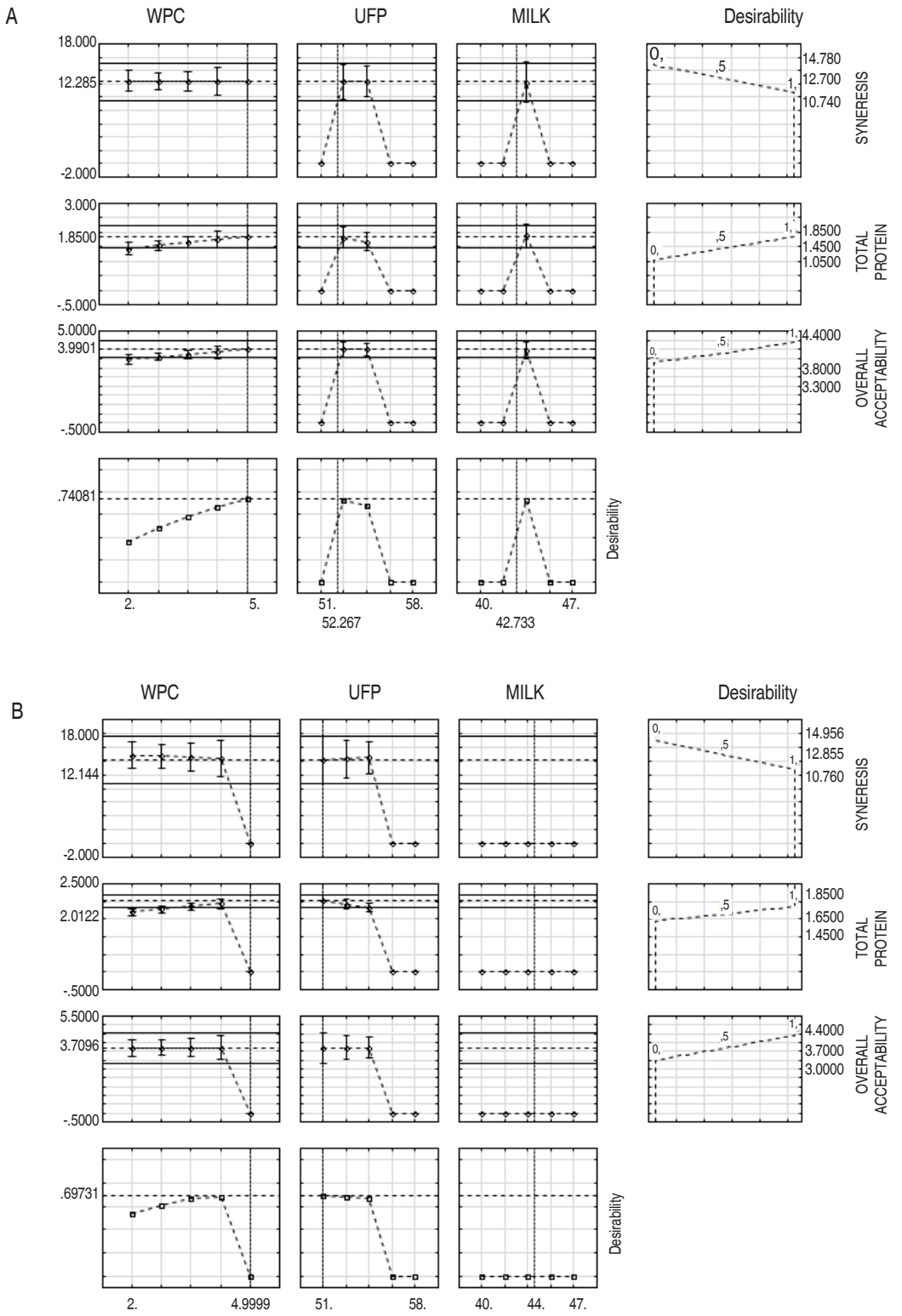

Figure 1. Multiple response optimization: A. NHFDB and B. HFDB.

Characterization of selected beverages

The formulations selected through the mixture design were experimentally validated (Table 5). Regarding the value of total soluble solids (11.375 °Bx), they were similar to the sweet whey-based dairy beverages reported by Gavilanes *et al*. (2018) whose value was 12.87 °Bx. Nogueira *et al*. (2016) reported a soluble solid value of 10 °Bx for a dairy beverage fermented with Kefir. The pH and acidity of the dairy beverages developed in this study presented values similar to those reported for fermented dairy beverages developed by Gavilanes *et al.* (2018): 4.42<pH<4.68, 0.63<acidity<0.68% expressed as lactic acid. Miranda *et al.* (2014) reported acidity values of 0.63% (expressed as lactic acid) and a pH value of 4.36. The protein (1.74% for both beverages) coincides with the values reported by Almeida *et al.* (2001) of 1.94% for a fermented dairy beverage made with 50% sweet whey. Also, the syneresis values of 10.69% for NHFDB and 10.72 for the HFDB are similar to those reported by Zambrano and Zambrano (2013), who found a value between 9.90-10.10% using 30% whey in the beverage formulation.

The rheological results showed a pseudoplastic behavior with the best fit using the power law model (R^2>0.99) where the apparent viscosity decreases with the increase in shear rate (Figure 2A). This behavior (non-Newtonian) was also observed by Pacheco *et al*. (2017) in beverages with whey, skim, and whole milk, indicating a continuous reorganization of the molecular weight structure, resulting in less resistance to flow due to the presence of a high molecular weight substance such as corn starch. This rheological result has also been found by Colominas *et al*. (2019), on a fermented beverage of whey, mango pulp, and rice flour.

Table 5. Characterization of HFDB and NHFDB.

Parameter	NHFDB	HFDB
Acidity (% lactic acid)	0.64^{a}±0.0057	0.63^{a}±0.0057
pH	4.27^{a}±0.0435	4.27^{a}±0.0251
Soluble solids (°Bx)	10.80^{a}±0.0000	10.80^{a}±0.0000
Syneresis (%)	10.69^{a}±0.8936	10.72^{a}±0.9896
Protein (%)	1.74^{a}±0.1414	1.75^{a}±0.0707
Fluidity index (n)	0.1406^{a}±0.017	0.199^{a}±0.0378
Consistency index, K (Pa.s^{n})	16.950^{a}±2.150	18.180^{a}±3.200

Means with the same letter are not significantly different (*P*>0.05)

For the optimal dairy beverages (Table 5) there were no statistically significant differences (*P*>0.05) in the variables evaluated. The consistency index (K) and the fluidity index (n) found in this study are similar to those reported by de Castro *et al*. (2009) who evaluated oligofructose in probiotic dairy beverages, reporting values of 17-20 Pa.s^{n} for the consistency index and between 0.11-0.22 for the fluidity index expressing the pseudoplastic behavior of the material. Additionally, evaluating the apparent viscosity at a shear rate of 50 s^{-1}, since this value would represent the approximate viscosity felt in the mouth (Díaz, 2018), viscosity values of 0.587 Pa.s were obtained in the HFDB and 0.7919 Pa.s for the NHFDB, qualitative values that coincide with the sensory evaluation of the product (Figure 3). Moreira *et al.* (2014) found values from 0.5338 to 0.6609 Pa.s, under 50 s^{-1} conditions, with pseudoplastic behavior in a dairy drink with soy milk and lecithin. Pachekrepapol *et al*. (2021) for a non-hydrolyzed and hydrolyzed beverage, presented a higher apparent viscosity for the non-hydrolyzed beverage because of the larger particle size of galactooligosaccharides tends to destabilize the protein matrix and lower the viscosity, which agrees with the results obtained.

Figure 2B shows the viscoelastic behavior of the beverages, indicating the destruction of the gel network of the product due to the effect of hydrolysis, which means less stability (higher degree of syneresis) for the HFDB beverage. Additionally, the gel behavior (G´>G") for the NHFDB beverage is observed, which gives greater stability to the product.

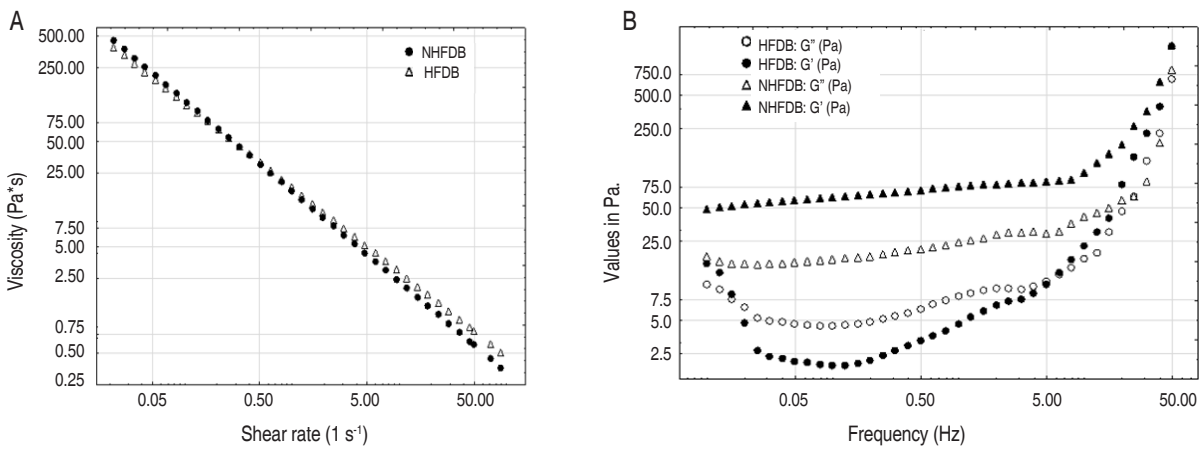

Figure 2. Rheology of fermented dairy beverages: A. Rheogram; B. Viscoelastic behavior (frequency sweep at 4 °C).

Sensory analysis

Figure 3 shows the multidimensional sensory analysis for HFDB and NHFDB. The results indicated a slight variation in the odor and acid taste, this is related to the results obtained in the physicochemical characterization where the NHFDB presented a higher acidity, although not significant. Additionally, no significant difference in viscosity was shown, although NHFDB had a higher viscosity because galacto-oligosaccharides are formed due to its hydrolysis (Gómez and Sánchez, 2019). According to Pachekrepapol *et al.* (2021), these oligosaccharides weaken the gel network by interfering with the protein network, which can cause a decrease in viscosity. Both drinks were rated by the expert judges as high-quality drinks.

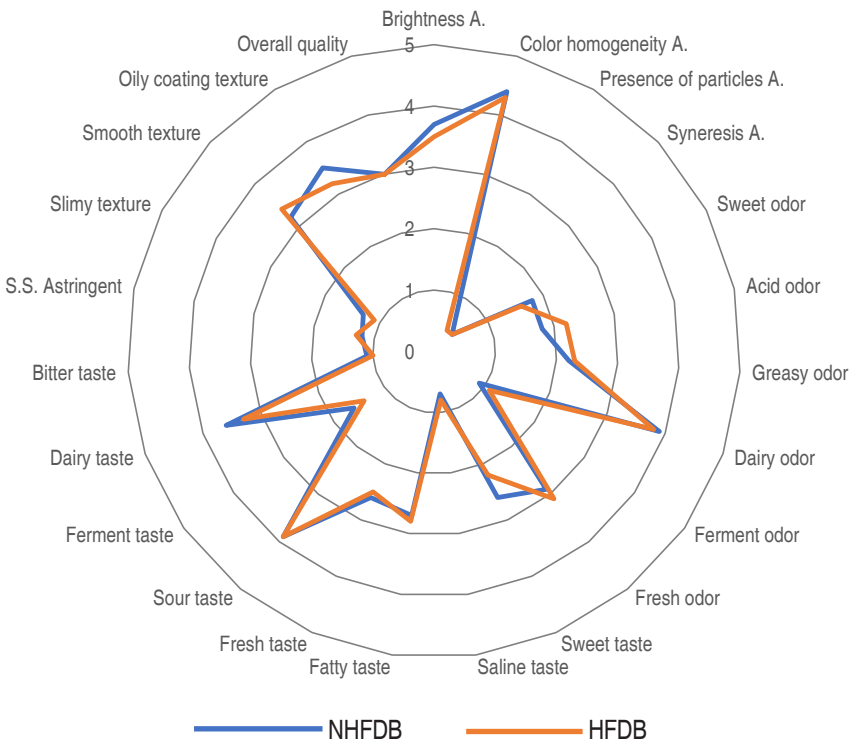

Figure 3. Sensory profile of hydrolyzed fermented dairy beverages (HFDB) and non-hydrolyzed (NHFDB).

Stability Beverage

Fermentation is one of the key operations in yogurt-type fermented dairy beverage technology. When the mixture is inoculated with *S. thermophilus* and *L. bulgaricus* bacteria, it begins a lactic acid fermentation where enzymatic hydrolysis of lactose occurs, reaching glucose and galactose monosaccharides as a result and hence the glucose is finally decomposed to lactic acid (Cabrera and Villa, 2020). This production of lactic acid causes a decrease in pH and an increase in acidity, which takes place not only during incubation but also during storage of the fermented dairy beverage because the microorganisms remain viable, although the decrease is less marked due to the effect of low temperature (Figure 4 A and B). Such behavior may be due to the production of galactooligosaccharides formed during enzymatic hydrolysis and cause instability in the gel network formed during fermentation, leading to a higher percentage of syneresis at the end of the storage time.

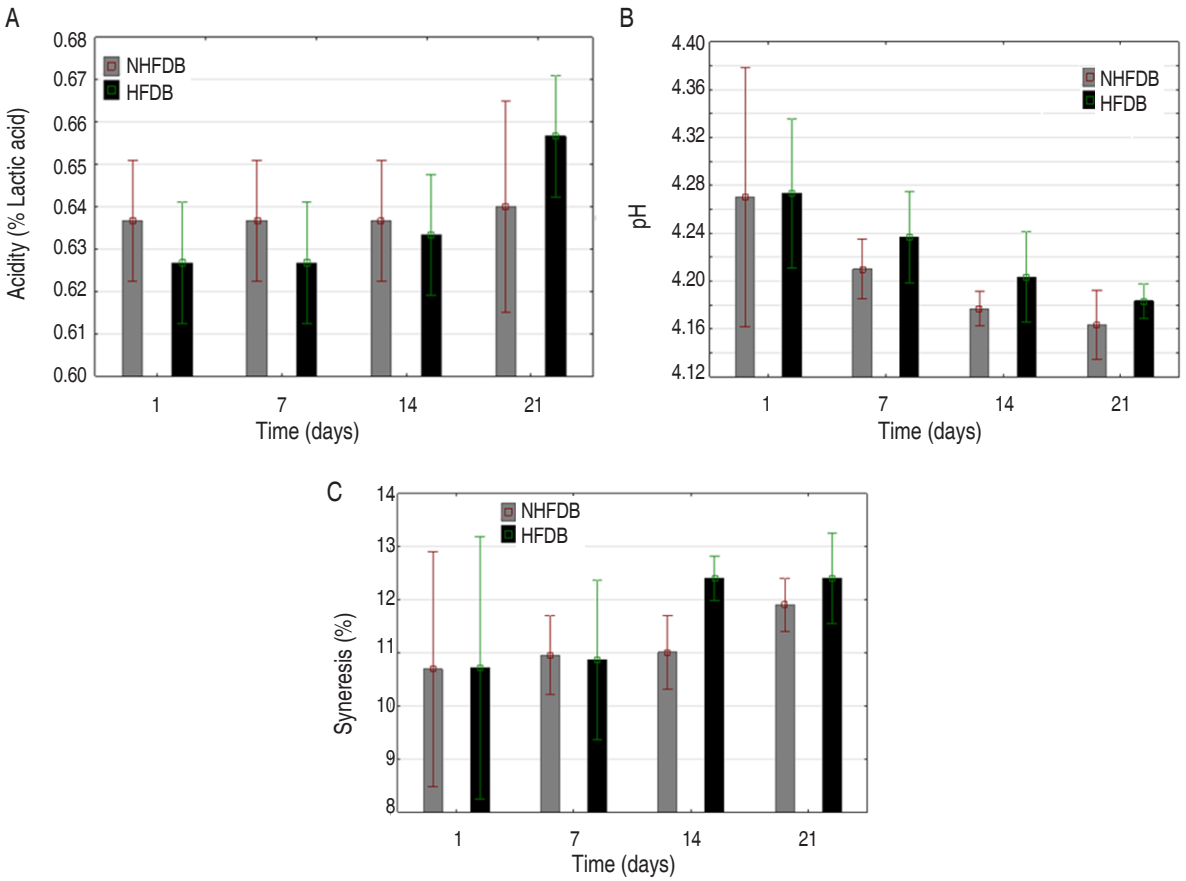

Figure 4. Stability study of fermented dairy beverages (95% confidence intervals for the mean: Fisher LSD Method): A. Acidity; B. pH; C. Syneresis (%).

Figure 4A shows that total acidity tends to increase for both beverages. There are no statistically significant differences between NHFDB and HFDB, but over time there is a significant difference for HFDB between day 1 and day 21 since lactic acid bacteria have the characteristic of producing acids that increase the concentration of H^+ in the medium. Different authors comment on the proteolytic activity of *Lactobacillus* spp, in which amino acids and small acid peptides that induce a reduction in pH are produced, additionally, S. *thermophilus* generates metabolites such as formic acid and carbon dioxide that also reduce the pH value (Zapata *et al.*, 2015). Other authors have reported this same effect, reaching an acidity of up to 1.44 (% lactic acid) at the end of storage (Hussain *et al.*, 2009; Parra, 2013). According to the NTC 805-2005 standard and the

Codex Alimentarius STAN 243-2003, corresponding to fermented milk-yogurt, the acidity value must be at least 0.6% expressed in lactic acid, thus, the titratable acidity values of the different acidity treatments comply with both norms (ICONTEC, 2005; FAO, 2003).

Figure 4B shows the decrease in pH during storage for both beverages. Between the NHFDB and the HFDB, there are no statistically significant differences, but over time there is a statistically significant difference for the HFDB between day 1 and day 21 of storage. This behavior can be attributed to the fact that microbial activity by the lactic acid bacteria present in the fermented dairy beverage occurred during storage under refrigeration conditions (Simijaca *et al.,* 2018; Zhi *et al.,* 2018). Gomes *et al.* (2017) sweetened yogurt with honey and stored it for 28 days at 4 °C, where the pH decreased over time, relating this behavior to the degradation of lactose into lactic acid. In addition, in both beverages where sucrose was replaced by tagatose, and the pH was found within the acceptable ranges, thus maintaining the microbiological quality. This agrees with works reported by Torrico *et al.* (2019), who carried out different replacements of tagatose in yogurt, they found that Physicochemical quality associated with titratable acidity, pH, viscosity, and soluble solids was not affected by tagatose replacements in yogurts.

Figure 4C shows a tendency to increase the percentage of syneresis with storage time, which is due to the loss of stability and elimination of water from the components of the fermented dairy beverage due to possible structural modifications in the gel network (Simanca *et al.*, 2013). The above behavior may be due to the production of galactooligosaccharides formed during enzymatic hydrolysis and cause instability in the gel network formed during fermentation, leading to a higher percentage of syneresis at the end of the storage time. Although the maximum level reached in this study was 13%, close to those reported by Lobato *et al.* (2014) between 9.7 and 23.6, it can be concluded that the gel-like structure formed for HFDB tends to be lower concerning to the NHFDB beverage, which coincides with the viscoelasticity analysis performed. Tamime (2006) reports syneresis values greater than 42%, which according to the author is indicative of loss of structure and high phase separation that deteriorates the physical and techno-functional quality of the product.

CONCLUSION

The use of conjugated systems based on ultrafiltration permeate and protein concentrate of sweet whey and milk makes it possible to obtain fermented dairy beverages with adequate sensory characteristics and physicochemical quality. However, the effect of hydrolysis is notorious in the stability of the emulsions, since the stability of the product is reduced, presenting a higher degree of syneresis for hydrolyzed beverages. Both beverages presented sensory characteristics of a high-quality fermented beverage, with a fresh and milky taste and smell; therefore, it can be stated that tagatose as a sweetener does not affect the sensory perception of these beverages. In relation to stability over time, there is a tendency towards a decrease in the pH value and an increase in acidity and syneresis, the latter being the most noticeable characteristic for HFDB, showing a marked weakness in the gel structure.

ACKNOWLEDGMENTS

This research was supported partially by Secretaría de Agricultura y Desarrollo rural de la Gobernación de Antioquia (Colombia), Auralac SA and Ministerio de Ciencia, Tecnología e innovación (Minciencias/ Colombia) under award number 80740-007-2019.

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Revista Facultad Nacional de Agronomía

Catalytic properties of purified alpha amylase from *Aspergillus flavus* cultivated on low-cost agricultural substrate

UNIVERSIDAD NACIONAL DE COLOMBIA

Propiedades catalíticas de alfa amilasa purificada de *Aspergillus flavus* cultivada en sustrato agrícola de bajo costo

https://doi.org/10.15446/rfnam.v76n1.100842

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ABSTRACT

Aspergillus flavus isolated from fermented millet flour produced a crude enzyme, which was purified via ammonium sulphate precipitation and subsequent chromatographic techniques. The biochemical characteristics of the purified amylase were thereafter investigated showing activity in a wide range of pH and temperature, with optimal conditions of pH 6.0 and 50 °C. The enzyme retained even 89% of its activity after 1 h at 50 °C and 2 h at pH 6.0. The purified enzyme was stimulated by Ca^{2+}, Zn^{2+} and Co^{2+}, while Hg^{2+} and EDTA caused mild inhibition of α-amylase activity. The kinetic indices (K_m and V_{max}) and molecular weight of the enzyme were estimated in 1.71 mg mL^{-1}, 2.133 µmol min^{-1} mL^{-1} and 45 kDa respectively. The catalytic properties of α-amylase from *A. flavus* makes it a promising candidate for use in various starch processing industries.

Keywords:
Alpha amylase
Characterization
Fermented cereals
Organic composite
Purification
Stability

RESUMEN

Aspergillus flavus aislado de harina de mijo fermentada produjo una enzima cruda, que se purificó mediante precipitación con sulfato de amonio y técnicas cromatográficas. Posteriormente se investigaron las características bioquímicas de la amilasa purificada mostrando actividad en un amplio rango de pH y temperatura, con condiciones óptimas de pH 6,0 y 50 °C. La enzima retuvo incluso el 89% de su actividad después de 1 h a 50 °C y 2 h a pH 6,0. La enzima purificada fue estimulada por Ca^{2+}, Zn^{2+} y Co^{2+}, mientras que Hg^{2+} y EDTA causaron una leve inhibición de la actividad de la α-amilasa. Los índices cinéticos (K_m y V_{max}) y peso molecular de la enzima se estimaron en 1,71 mg mL^{-1}, 2,133 µmol min^{-1} mL^{-1} y 45 kDa respectivamente. Las propiedades catalíticas de la α-amilasa de *A. flavus* la convierten en un candidato prometedor para su uso en diversas industrias de procesamiento de almidón.

Palabras clave:
Alfa amilasa
Caracterización
Cereales fermentados
Compuesto orgánico
Purificación
Estabilidad

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Received: June 21, 2022; Accepted: August 10, 2022
Rev. Fac. Nac. Agron. Medellín 76(1): 10213-10225. 2023

ISSN 0304-2847 / e-ISSN 2248-7026

The applications of enzymes for various bioproducts formulation for human needs is the driving force behind the evaluation of microorganisms for different extracellular enzymes (Kwatia and Dzogbefia, 2018). The main focus is on starch-degrading enzymes, mainly α-amylase (α-1,4 glucan-glucanohydrolase) which represent more than 60% of the enzyme market due to their usefulness in several industrial processes (Ali *et al*., 2017). Alpha amylase breaks down the α-1,4 glucosidic bond in starch-containing substrate resulting in monosaccharides such as glucose, short-chain dextrin, and oligosaccharides (Ali *et al*., 2017).

Being an extracellular enzyme amylase can be extracted from plants, animals, and certain microorganisms. However, more attention has been paid to bacterial and fungal amylases because of their thermostability, ease of cultivation, and higher productivity in industrial applications (Ali *et al*., 2017, Ahmad *et al*., 2019). Amylases from fungi are documented to be more commercially consistent than amylases from bacterial sources. They also have a highly accepted GRAS (generally recognized as safe) status. As a result, investigations have been carried out on the process parameters of promising fungal strains (Lim *et al*., 2020). *Aspergillus* spp. has been reported to possess some unique enzyme machinery with exceptional catalytic properties making them suitable candidates for industrial operations (Karim *et al*., 2018). For instance, *A. oryzae* serves as a host for heterologous protein production and possesses the capacity to produce large quantities of relevant industrial enzymes that are of great economic value. Also, *Aspergillus niger* highly opposes contamination and is acid-tolerant (Gurung *et al*., 2013). Solid substrate fermentation (SSF) has been considered a promising technique for the production of enzymes due to its economic and engineering advantages (Razdan and Kocher, 2018). In recent years, the SSF process has been developed and extensively used for the synthesis of bio-products, especially when it involves fungi (Kwatia and Dzogbefia, 2018). SSF cultivated microbes have tight contact with insoluble substrates letting a higher nutrient utilization from them and achieving high enzyme yields during fermentation. Numerous advantages are attributed to SSF these include higher volumetric production, utilization of simple fermentation media, require less water, generate, fewer effluents, no laborious control of process parameters, bacterial contamination is reduced, and requires less capital involvement for further processing (Ahmad *et al*., 2019).

With the high demand for novel amylases, getting a cheap, rich, and the readily available suitable substrate is key to meeting the high demand with excellent catalytic efficiency. Hence, cheaper and readily available starchy foods such as cereals (rice, corn, wheat, and millet) and tubers (yam, cassava, potatoes, and cocoyam), could be adopted as an additional use in starch enzymatic breakdown to produce reducing sugars. The enzymatic is inexpensive in terms of energy consumption and the use of a simpler process than the conventional that utilizes pregelatinized starch as substrate (Kwatia and Dzogbefia, 2018).

Purification is an important aspect after enzyme production to obtain a purified component from the crude extract. Without purification, enzyme activities and associated proteins might be difficult to characterize accurately due to the impurities in the crude solution, resulting in unreliable data and information (Lim *et al*., 2020). Searching for a novel fungal strain with high catalytic properties at a low cost, the present study isolated and cultivated an amylolytic fungus using an economic substrate for amylases production, purification, and characterization. The fungal strain purified amylases demonstrated some biochemical properties making them promising candidates to use in different industries processing starch.

MATERIALS AND METHODS

Collection of samples

Grain samples namely: rice (O*ryza sativa*), wheat (*Triticum aestivum*), corn (*Zea mays*), and millet (*Pennisetum glaucum*) were purchased at Oja-oba market, Akure, Nigeria. Debris and unwanted particles were sorted out from the grains. They were washed, ground into fine particles, packaged in transparent plastic bags, labeled, and transported to the laboratory for further microbial and biochemical analysis.

Fungal isolation and identification

Fungi were isolated from fermented cereals using potato

dextrose agar (PDA) by standard pour plate techniques and incubated at 30 °C. The fungal colonies were sub-cultured to purify the isolates. The isolates were prepared on slides and stained with lactophenol cotton blue to identify their morphology and compare them with a standard fungal atlas (Pitt and Hocking, 2009).

Qualitative screening of isolates for amylase production

Alpha-amylase producing isolates were screened on starch-agar medium (soluble starch 5 g L^{-1}; peptone 5 g L^{-1}; yeast extract 5 g L^{-1}; $MgSO_4 \cdot 7H_2O$ 0.5 g L^{-1}; $FeSO_4 \cdot 7H_2O$ 0.01 g L^{-1}; NaCl 0.01 g L^{-1}; agar 15 g L^{-1} plates. The isolate was inoculated at the center of the plates, at an appropriate temperature and then was stained with Lugol solution. The colonies forming the largest halo zone were selected for further studies (Yalcın and Çorbacı, 2013).

Genetic identification

The genetic identification of the isolates was performed by extracting DNA following the standard technique of Xian *et al*. (2015). Then, using PCR the partial internal transcribed spacer (ITS) region genes were amplified with the corresponding primer sets that target ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC CC GCT TAT TGA TAT GC-3') (Xian *et al*., 2015). The amplification protocol adopted was: 40 PCR cycles with denaturation at 95 °C for 2 min, annealing at 58 °C for 2 min, and extension at 72 °C for 2 min, with the final extension for 10 min. The sequences of the amplicon were compared with the sequences in the GenBank using a BLASTN search and aligned with related species according to Xian *et al*. (2015).

Fungal inoculum

Spore suspension of the amylase-producing fungal isolates was prepared by scrapping off fungal spores which were dissolved in 40 mL of distilled water until the final volume of 60 mL and vortexed. The SSF production medium was inoculated with 2 mL fungal spore suspension (Kwatia *et al*., 2017). The mineral salt medium (MSM) contains 0.1 g KH_2PO_4, 0.25 g NaCl, 0.01 g $MgSO_4 \cdot 7H_2O$, 0.01 g $FeSO_4.7H_2O$, and 0.10 g yeast extract in 100 mL of distilled water.

Amylase production in SSF

To choose the best substrate in the SSF for amylase production, commercial starch was substituted with corn, rice, wheat, and millet as selected organic substrates. The organic substrate was used singly; 100% of corn (C); rice (R); wheat (W); millet (M) or in a mixture at different ratios: 1:1 of corn and rice (CR); corn and wheat (CW); corn and millet (CM); rice and wheat (RW); rice and millet (RM); wheat and millet (WM); 1:2:1 corn, rice, and wheat (CRW); rice, corn, and millet (RCM); millet, wheat and rice (MWR); wheat, millet, and corn (WMC); 1:1:1:1 corn, rice, wheat, and millet (CRWM). 4 g of each combination was dissolved in 10 mL of MSM and sterilized at 121 °C for 15 min. At room temperature, the dissolved substrate was inoculated with 2 mL of the fungal spore suspension, thoroughly mixed, and incubated aerobically at 30 °C for 96 h (Kwatia *et al*., 2017).

Enzyme extraction

The extracellular crude enzyme was extracted from the fermentation medium using 0.05 M citrate phosphate buffer (pH 6.0). 50 mL of the buffer was dispensed into the 4 days old enzyme production medium, homogenized, and kept in a rotary shaker (250 rpm) at 30 °C for 30 min. The resulting mixture was filtrated using a cheesecloth and the filtrate was centrifuged (3600 xg, 15 min at 4 °C). The clear crude extract had crude enzymes for amylolytic activity evaluation (Kwatia and Dzogbefia, 2018). The composite substrate with the highest α-amylase production was considered a potential substrate.

Amylase assay

The amylase activity was determined following the protocol of Ahmed *et al*. (2020). A mixture of 0.5 mL of crude extract and 0.5 mL of soluble starch was incubated at 40 °C in a water bath for 15 min, the reaction was stopped with 1 mL of 3, 5 dinitrosalicylic acid, boiled for 5 min and cooled to measure the reducing sugars and estimate the enzymatic activity according to the method of Miller (1959). The commercial substrate, i.e., soluble starch was prepared by dissolving 1% (w/v) of the substrate in 0.05 M citrate phosphate buffer (pH 6.0). The absorbance was taken at 540 nm against a substrate blank using the UV Spectrophotometer (Axiom 721 vis spectrophotometer). The quantity of reducing sugars released from the enzyme-catalyzed starch was estimated using a glucose standard curve. A unit of

amylase activity was indicated as the amount of enzyme needed to liberate 1 µmol of reducing sugars (maltose/glucose) per minute under standard assay protocols (Ahmed *et al*., 2020).

Determination of protein concentration in the crude extract

The protein contents in the crude extract were evaluated by the Bradford method (1976). Different concentrations (0.1-1.0 mg mL^{-1}) of Bovine Serum Albumin were prepared and used as a standard. Finally, the absorbances of the samples were measured at 595 nm.

Crude amylase purification and molecular weight

The crude enzyme was purified by three purification steps: ammonium sulphate precipitation, ion exchange, and gel filtration chromatography (Abdulaal, 2018). After purification, the biochemical characteristics of the purified enzyme, kinetic parameters, and molecular weight were determined.

Ammonium sulphate precipitation

Solid ammonium sulphate was continuously and gently dissolved in crude enzyme solution until 60% of saturation. Allowing the salt precipitation in the mixture at 4 °C for 12 h, the saturated solution was centrifuged for 20 min at 14,000 rpm to obtain the pelletized proteins, which were dissolved in 10 mL of 0.05 M citrate phosphate buffer (pH 6.0). Furthermore, the protein was dialyzed in the same buffer for 96 h (Hassan *et al*., 2018).

Dialysis

The precipitated protein was aseptically poured into a dialysis bag and dialyzed in the same buffer for 96 h in refrigeration until releasing the sulphate from the solution, replacing the buffer in the bag embedded. After dialysis, the protein content and α-amylase activity were subsequently determined (Hassan *et al*., 2018).

Ion-exchange chromatography

The dialysate free of salt was loaded on a DEAE Sephadex a-50 column (2.5×40 cm), previously treated with 0.05 M citrate phosphate buffer pH 6.0. The protein was eluted using the same buffer at a flow rate of 2 mL min^{-1}. The unbound inactive protein was eluted from the column, followed by the elution of the bound proteins by a linear salt gradient (0.1–1.0 M NaCl). The protein content eluted was determined by reading the absorbance at 280 nm and the α-amylase activity was assayed following the prescribed protocol. The fractions showing enzyme activities were concentrated with 4 M sucrose solution (Hassan *et al*., 2018).

Gel filtration

The concentrated enzyme was further purified using a Sephadex G–100 column (2.5×75 cm) previously treated with 0.05 M citrate phosphate buffer pH 6.0. The protein was eluted using the same buffer at a flow rate of 20 mL h^{-1}. The protein content of each fraction was monitored by reading the absorbance at a wavelength of 280 nm and α-amylase activity was assayed as previously described. The pooled fractions were stored at 4 °C and used for biochemical characterization (Hassan *et al*., 2018).

Biochemical characterization of the purified α-amylase

The biochemical properties of the purified enzyme and kinetic parameters, such as K_m and V_{max}, were studied. The influence of temperature and pH on the activity and stability of purified α-amylase and the effect of metal ions and inhibitory agents on enzyme activity were also determined.

Influence of temperature and pH on the catalytic activity and thermal stability of purified α-amylase

To determine the optimal temperature for α-amylase activity, equal volumes of enzyme and substrate were incubated at different temperatures, from 40 to 90 °C. The thermal stability of the enzyme was evaluated by taking samples every 30 min for 2 h, and the residual activity was evaluated following standard assay protocol (Abdulaal, 2018).

The influence of pH on the catalytic activity of purified α-amylase was determined by incubating at different pH in 50 mM buffer system: glycine-HCl buffer (pH 3.0), sodium acetate buffer (pH 4.0–5.0), phosphate buffer (pH 6.0–7.0), Tris- HCl buffer (pH 8.0–9.0) and glycine-NaOH (pH 10.0–12.0). The stability of the purified enzyme was monitored by incubating in the appropriate buffer solutions for 2 h and taking an aliquot for enzymatic assay every 30 min (Abdulaal, 2018).

Influence of metal ions and inhibitors on the catalytic activity of purified α-amylase

The influence of different concentrations of metal ions and enzyme inhibitors (5 and 10 mM) on the catalytic properties of the enzyme was determined by incubating the mixture enzyme/substrate with the metal ions (Ca^{2+}, Cu^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Hg^{2+}, Na^{+}, Zn^{2+}, K^{+}, and K_3Fe (CN) or the inhibitors (EDTA and urea). After incubation, the enzymatic activity was determined following standard assay procedures (Abdulaal, 2018).

Estimation of kinetic parameters of the enzyme

The initial rate of the amylase-substrate reaction was measured using soluble starch (1-10 mg mL^{-1}) as substrate. The kinetic parameters (K_m and V_{max}) of the purified enzyme were estimated from the double reciprocal plot of Lineweaver and Burk (Abdulaal, 2018).

Determination of molecular weight of purified α-amylase

The homogeneity and subunit molecular weight of the purified α-amylase was determined by 10% gel sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using a wide range protein molecular weight marker as standards from 11 to 180 kDa and stained with Coomassie brilliant blue (Abdulaal, 2018).

RESULTS AND DISCUSSION

Presumptive identities of fungal isolates from corn, rice, wheat, and millet cereal flour

The isolated fungi from the cereal samples of wheat, rice, corn, and millet were tentatively identified as *Aspergillus niger, A. flavus, Rhizopus stolonifer, Fusarium poae*, *Penicillium chrysogenum*, and *Cladosporium* sp. From all the samples, *Aspergillus* spp. occurs frequently followed by *Penicillium* spp. The result is similar to that of Hussain *et al.* (2018) who also identified five fungal genera from cereals of wheat, barley, corn, and rice and documented *Aspergillus* as the most predominant. The predominance of *Aspergillus* on fermented cereals could be that they are degraders and common spoilage organisms of carbohydrate-containing (Hussain *et al.*, 2018).

In addition, the genetic identity of the best α-amylase producer resulted in the fungus as *A. flavus* with 97% similarity. The amylase activities of the fungal isolates on different organic substrates are presented in Figure 1.

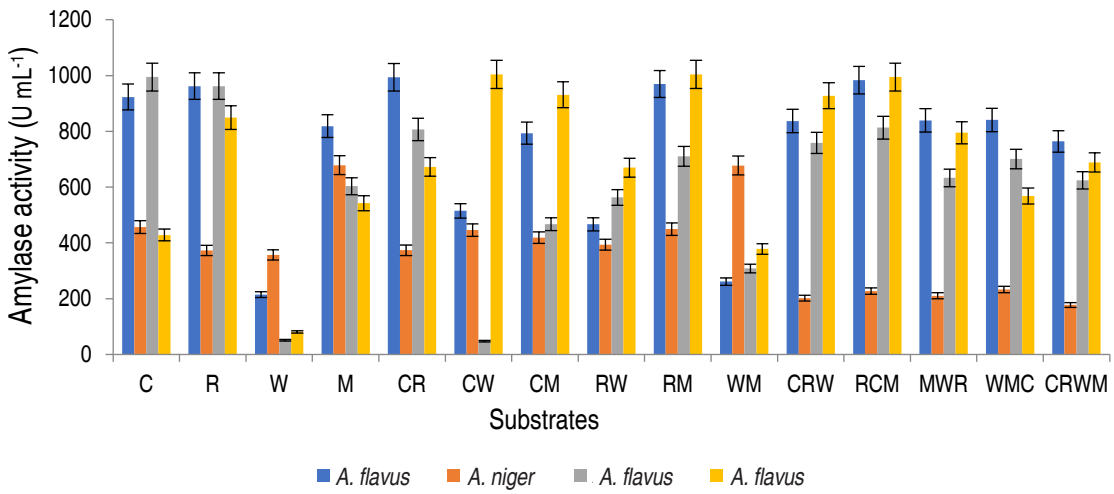

Figure 1. Amylase activity of the amylolytic fungi on different organic substrates viz; 100% corn (C); rice (R); wheat (W); millet (M); 1:1 corn and rice (CR); corn and wheat (CW); corn and millet (CM); rice and wheat (RW); rice and millet (RM); wheat and millet (WM); 1:2:1 corn, rice and wheat (CRW); rice, corn and millet (RCM); millet, wheat and rice (MWR); wheat, millet and corn (WMC); 1:1:1:1 corn, rice, wheat and millet (CRWM).

Aspergillus flavus isolated from millet had the highest amylase activity of 1.004 U mL^{-1} on the combination of rice and millet (RM), *A. flavus* from rice was observed to have the highest amylase activity on rice and millet, with values of 0.814 U mL^{-1}, while *A. niger* from corn had the best activity on millet only. Based on α-amylase activity, *A. flavus* from millet was selected for purification and characterization studies. The production of α-amylase on different substrates from fungal strains using SSF has been studied. Kwatia *et al*. (2017) and Sadhasivam *et al*. (2018) produced amylase from *A. niger* and *A. luchuensis* BS1 by SSF using agro-residues. Abdulaal (2018) and Balakrishnan *et al*. (2021) also separately produced α-amylase from *Trichoderma pseudokoningi* and *A. oryzae* via SSF using orange peels and edible oil cakes, respectively. In SSF, the selection of a suitable culture medium and a suitable microbial strain are key factors for the production of α-amylases (Razdan and Kocher, 2018). This study revealed appreciable amylase production by *A. flavus* in the composition of rice and millet. This study is in agreement with Ali *et al*. (2017) who documented higher amylase by *A. flavus* on mandarin peels. Oppose to this study, Oshoma *et al*. (2017) found maximum α-amylase yield in *A. niger* using different agricultural wastes via SSF. These variations might be due to the differences in physiological status, and genetic makeup of the organisms leading to varied abilities in utilizing the solid substrates for α-amylase production (Badejo *et al*., 2021).

Purification summary of amylase

The elution profile of α-amylase from *A. flavus* on the ion exchange column is presented in Figure 2.

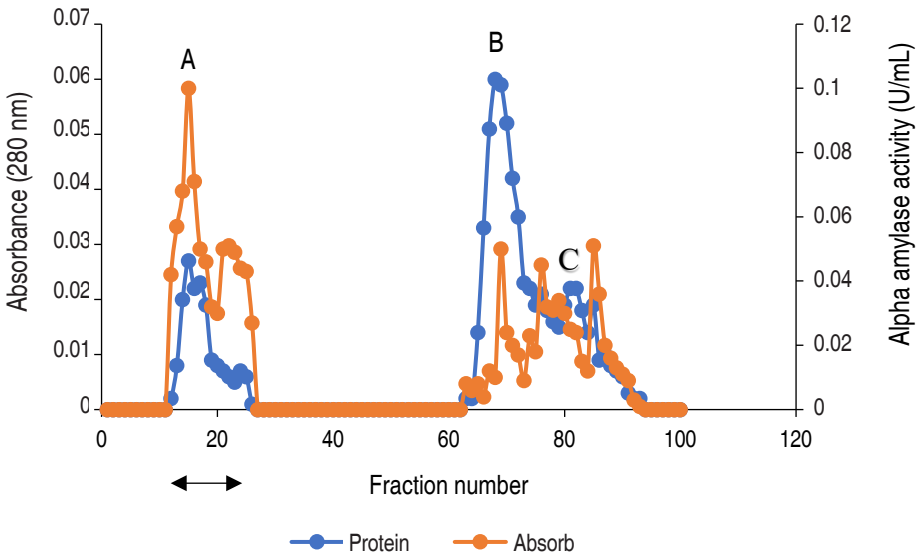

Figure 2. Chromatogram of α-amylase produced by *A. flavus* on ion-exchange column (2.5×40 cm) of DEAE Sephadex.

The profile revealed three (3) activity peaks labeled as A, B, and C. Each peak was pooled together to form the three fractions. The fractions forming peak A had an activity of 0.497 U mL^{-1}, peak B of 0.169 U mL^{-1}, and peak C constitutes the lowest activity of 0.024 U mL^{-1}. The major active peak A was pooled together and was further subjected to purification by gel chromatography. The elution profile of α-amylase from *A. flavus* via gel filtration is shown in Figure 3.

The profile revealed two (2) activity peaks (A and B). Peak B had the highest activity hence, fractions from peak B were pooled together and used for the characterization.

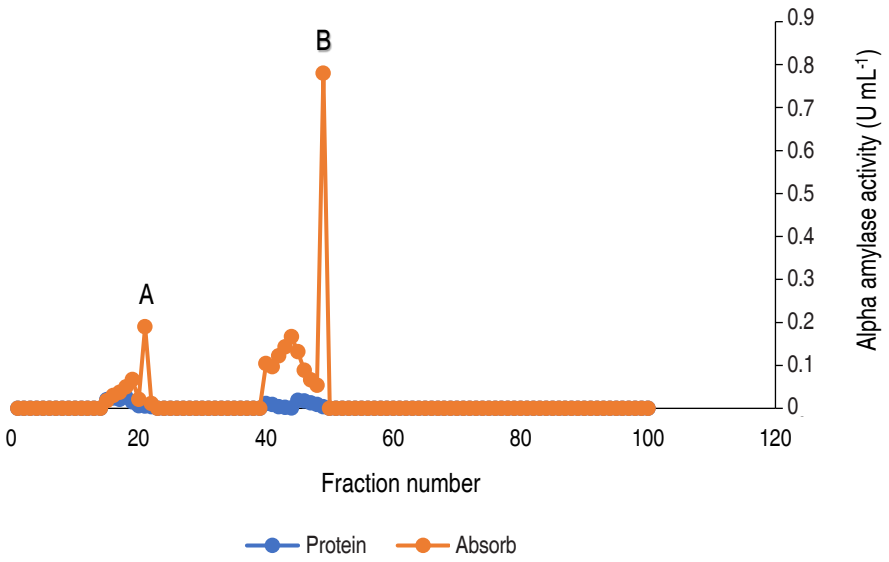

Figure 3. Elution profile of α-amylase via gel filtration with two activity peaks. Pooled fractions were represented with black arrow.

Table 1 has a summary of the processes and the techniques used for the obtaining homogeneity of the enzyme. Alpha amylase from *A. flavus* was purified to homogeneity with a purification yield of 4.3% and a fold of 7.9. Similar to this result, Nithya *et al.* (2017) recorded a purification fold of 7.06, while Hassan *et al.* (2018) and Kwatia *et al.* (2017) reported a lower purification fold of 4.78 and 4.48, respectively. Higher purification folds of 18 and 16.6% were documented by Abdulaal (2018) and Gbenga *et al.* (2017) for purified α-amylase from different fungal isolates. The differences in the purification yield and fold by different authors is due to the type of resins used and other factors such as buffers and purification conditions (Badejo *et al.*, 2021). Fold purification indicates the purification status of the enzyme; an increase in fold purification reveals an increase in the purity of the enzyme subjected to purification when compared with the crude enzyme (Badejo *et al.*, 2021).

Table 1. Summary of purification of α-amylase from *A. flavus.*

Steps	Volume (mL)	Protein concentration (mg mL^{-1})	Total protein (mg)	Activity (U)	Total activity (U mL^{-1})	Specific activity (U mL^{-1})	Yield (%)	Purification fold
Crude extract	500	0.259	0.130	0.681	0.341	2.629	100	1
$(NH_4)_2SO_4$	31	0.109	0.003	0.608	0.019	5.529	5.521	2.103
DAEA Sephadex	18	0.066	0.002	0.497	0.009	7.417	2.614	2.820
Sephadex G-100	34	0.021	0.001	0.432	0.015	21.000	4.317	7.987

Physicochemical properties of a purified amylase

Figure 4 shows the catalytic activity of the purified amylase. The highest temperature was considered as 100% and the rest of the temperature was estimated relative to the maximum activity. The relative activity of purified α-amylase increased from approximately 45% at 30 °C and reaches its peak at 50 °C with the value of 100% relative activity, beyond 50 °C, a steady decrease in relative activity was observed, at 90 °C, the activity decreased to 14%.

In this current study, the activity of purified α-amylase reached optimum at 50 °C. Abdulaal (2018) also recorded maximum α-amylase from *Trichoderma*

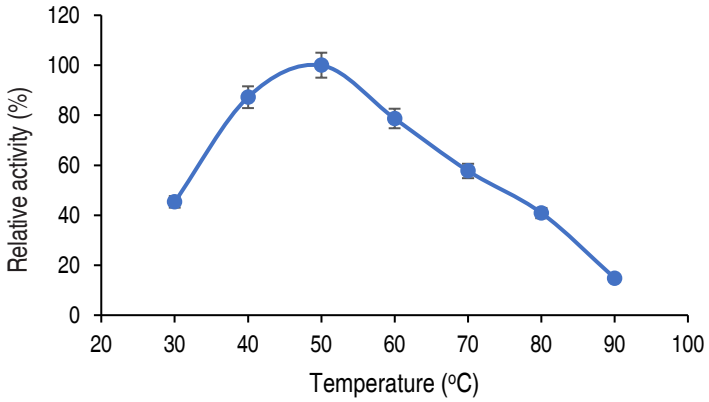

Figure 4. Effect of temperature on the activity of purified α-amylase from *A. flavus.*

sp. at 50 °C. Similarly, El-Sayed *et al.* (2019) also documented optimum amylase activity from *Bacillus mojavensis* at 55 °C. Higher and lower temperatures than those obtained in this study had been recorded from other studies; Hassan *et al.* (2018) showed optimum amylase activity from *E. coli* at 30 °C, while Wang *et al.* (2018) showed that the amylase activity from *Pseudoalteromonas* sp. was 25 °C. Sadhasivam *et al.* (2018), Trabelsi *et al.* (2019) and Wang *et al.* (2018) in separate studies documented optimal activities at 60 °C for purified α-amylase from *A. luchuensis, B. subtilis* and *Thermomyces dupontii* (expressed in *Komagataella phaffii,* respectively. The decrease in α-amylase activity beyond 50 °C might be connected to the disruption of various bonds in the amylase (Wang *et al.*, 2018). While the variations observed in the optimal temperatures from different studies could be due to the source of the microbial enzyme and differences in the genetic makeup of the organisms (Badejo *et al.*, 2021).

Thermal stability of the purified α-amylase

The infuence of temperature on the stability of the enzyme is shown in Figure 5.

The enzyme has a residual activity of approximately 81%

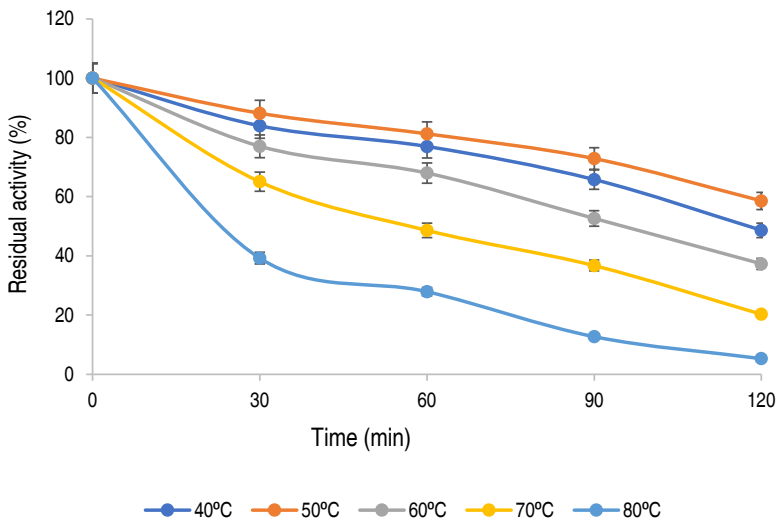

Figure 5. Effect of temperature on the stability of α-amylase from *A. flavus.*

after 1 h at 50 °C and 58% of its activity was retained after 2 h, while 76%, 67%, and 48% of residual activities were retained at 40 °C, 60 °C, and 70 °C, respectively after 1 h of incubation. The purified α-amylase from this study was stable and retained 81%, 67% and 48% of its activity at 50 °C, 60 °C and 70 °C for up to 60 min respectively. Karim *et al*. (2018) similarly documented thermo-stable α-amylase from *A. flavus* NSH9 at 50 °C, retaining 87% of its residual activity after 60 min of incubation. The decline in enzyme activity beyond the period of stability could be due to hydrolysis of peptide chain or aggregation, thus causing abnormality in the conformation of the enzyme (Karim *et al*., 2018).

Influence of pH on purified α-amylase activity

The influence of pH on purified α-amylase is presented in Figure 6.

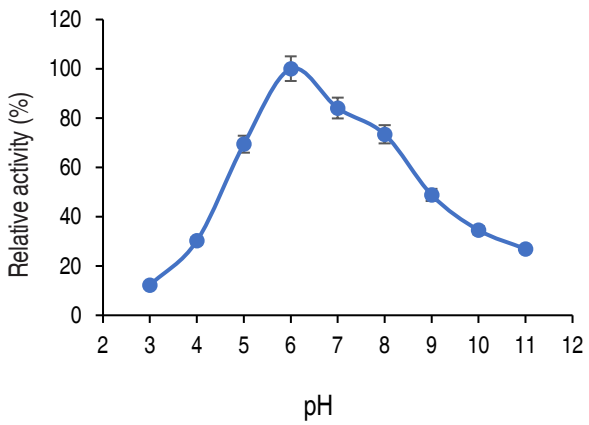

Figure 6. Effect of pH on the activity of α-amylase by *A. flavus*

The relative activity of purified amylase increased from pH 3.0 and reached its optimum at pH 6.0 and then decreased. At pH 8.0 and 9.0, the relative activities were approximately 73% and 48% respectively.

The purified α-amylase from this study was active both in acidic and alkaline regions (5.0 to 8.0), where an optimum pH was recorded at pH 6.0. The purified α-amylase from different fungi have pH optimum ranged from acidic (3.0) to alkaline region (11.0) (Abdulaal, 2018). Different studies have reported various pH optimal for purified α-amylase. Doss and Anad (2012) reported 6.0 as the best pH for optimum α-amylase activity from *A. flavipes* and *A. wentii*. Trabelsi *et al*. (2019) similarly recorded a pH of 6.0 for maximum purified α-amylase activity from different sources. On contrary, a pH of 7.0 was optimum for amylase from *A. nidulans* and *A. versicolor* (Sethi *et al*., 2016). Hammami *et al*. (2018) and Priyadarshini and Ray (2019) reported an optimum α-amylase from *Bacillus mojavensis* SA and a *Bacillus* sp. at pH 9.0. The variations in the optimal pH observed in different studies could occur as a result of the microbial origin of the enzyme as well as modification of the genetic make-up of the isolate (Badejo *et al*., 2021). Also, the activity of the purified α-amylase over a wide range of pH shows the versatility of the α-amylase for several processes. The decline in α-amylase activity at pH 4 and 10 might be due to the denaturation of essential co-factors or dissociation of the enzyme structure (Badejo *et al*., 2021).

pH stability of the purified α-amylase

The influence of pH on the stability of the purified α-amylase is presented in Figure 7. The purified α-amylase was 100% relatively stable at pH 6.0 for 30 min, and after 2 h of incubation, 68% residual activity was retained. The enzyme retained more than 40% of its residual activity after 90 min at pH 8.0. The stability of this enzyme fulfills the requirement for its application in many industrial operations especially for starch processing.

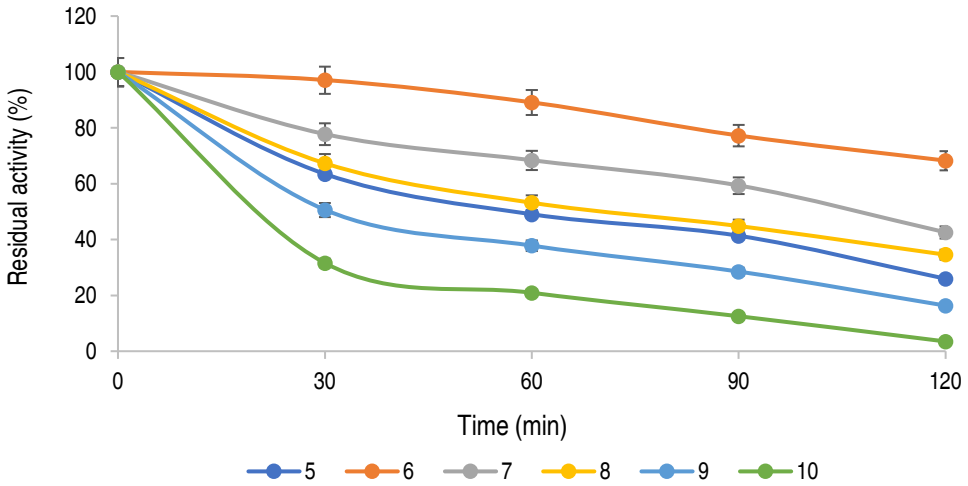

Figure 7. Effect of pH on the stability of purified α-amylase by *A. flavus.*

Influence of metal ions and inhibitors on α-amylase activity

The effect of different metal ions and enzyme inhibitors on the purified α-amylase from *A. flavus* is presented in Figure 8.

The α-amylase activity was stimulated in the presence of Ni^{2+}, Mn^{2+}, Cu^{2+}, Ca^{2+}, Na^{+}, Zn^{2+}, Co^{2+} and urea at 5 mM, while Hg^{2+} and EDTA mildly inhibited α-amylase activity at concentration dependent manner.

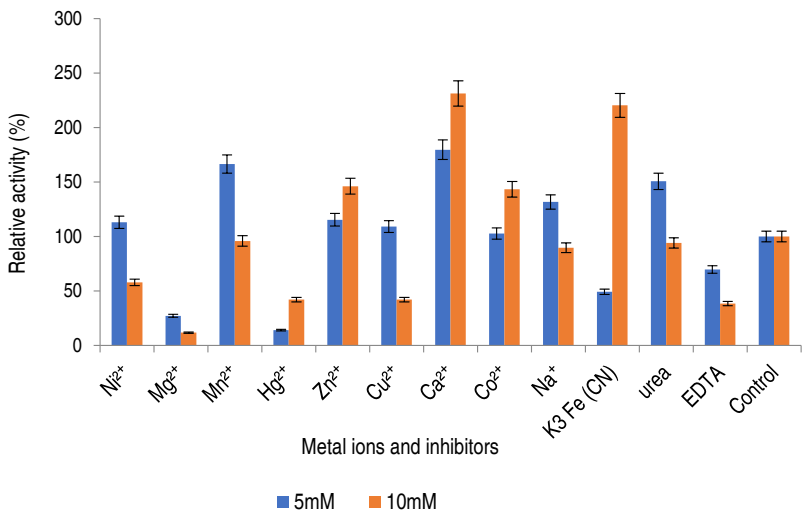

Figure 8 Effect of metal ions and inhibitors.

In this study, the purified enzyme was stimulated by some salts, notably calcium ion. Many of these metal ions are known to enhance enzyme activity, some metal ions act as cofactors increasing the activity of amylase for starch hydrolysis (Lim *et al*., 2020). From this study, the addition of Ca^{2+}, Co^{2+} and Zn^{2+} positively modulated purified α-amylase at both concentrations of 5 and 10 mM, amylase activity was only activated by Na^{+}, Cu^{2+}, Ni^{2+} and Mn^{2+} at 5 mM while the enzyme activity was inhibited in the presence of EDTA, and Hg^{2+}. A similar observation was documented by Kwatia and Dzobgefia (2018) and Aladejana *et al.* (2020) when α-amylase from *B. subtilis* was incubated with Ca^{2+}. Also, Bashir *et al.* (2014) documented stimulation of α-amylase from

B. lichiniformis on the addition of Zn^{2+}, Co^{2+}, and Mn^{2+}. Ca^{2+} activated α-amylase suggest that calcium ion might act as an activator and stabilizer of α-amylase and that requirement for Ca^{2+} differs for different α-amylases (Maalej *et al*., 2021).

In contrary with this study, the inhibitory action of Ca^{2+} at 10 mM against purified amylase was documented by Babu and Satyanarayana (1993). EDTA had an inhibitory effect on the α-amylase activity. Chelating agents such as EDTA are known to inhibit enzymes by either detaching metal ions from the enzyme or by binding to the enzyme to form a ligand (Aladejana *et al*., 2020).

The findings of this study suggests that the enzyme from *A. flavus* is a metalloenzyme and that the enzyme contains metallic ions which are removed by the chelating agent, forming an active complex with EDTA and consequent loss of activity (Aladejana *et al*., 2020). In general, variations from different studies occurs because the metal-binding ability of α-amylases, were found to be dependent on the enzyme sources (Maalej *et al*., 2021).

Kinetic parameters of purified α-amylase

The kinetics parameters, maximum velocity (V_{max}) and Michaelis-Menten constant K_m are showed in Figure 9.

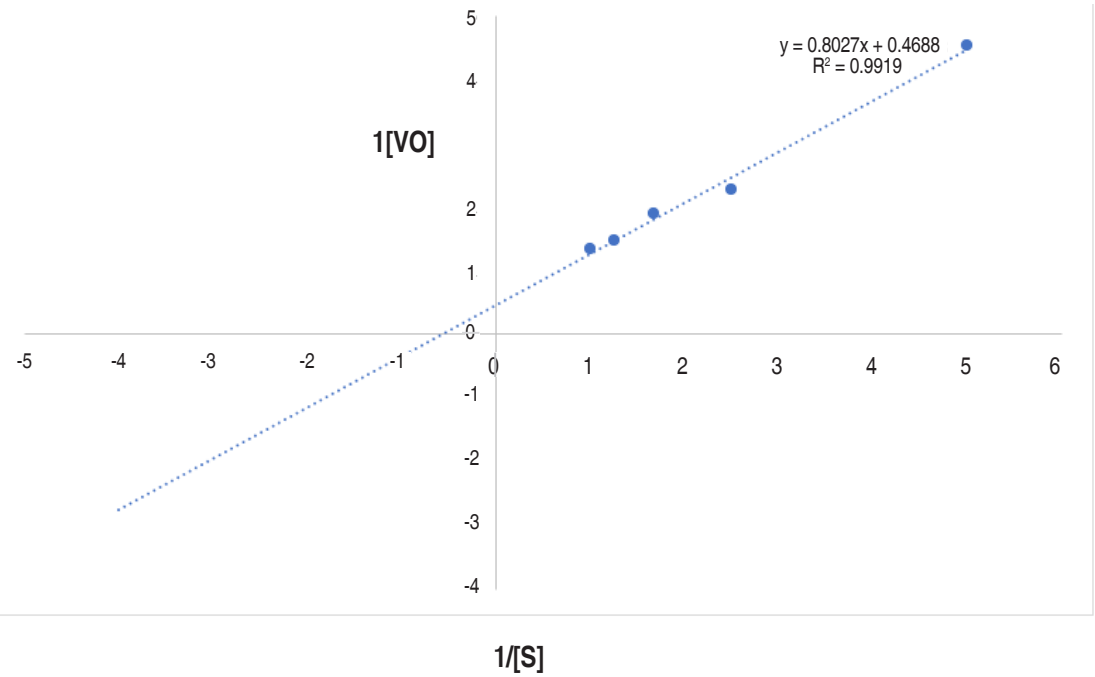

Figure 9. Line weaver-Burk plot of reaction velocity against substrate concentration for purified α-amylase using starch as substrate.

This research documented the apparent K_m and V_{max} of the purified α-amylase to be 1.71 mg mL^{-1} and 2,133 µmol min mL^{-1} respectively. Higher K_m and V_{max} of α-amylase from *Bacillus* sp. MB6 were documented as 5.45 mg mL^{-1} and 24.15 µmol min mL^{-1} (Paul *et al*., 2017). Abdulaal (2018) also reported the K_m and V_{max} values for purified α-amylase from *Trichoderma pseudokoningii* to be 4.0 mg mL^{-1} and 0.74 µmol min mL^{-1}. K_m measures the affinity of the enzyme for substrates i.e., the enzyme substrates complex, while the V_{max} of an enzyme is the measure of the maximum rate of reaction. The K_m of an enzyme, relative to the concentration of its substrate under normal assay conditions allows prediction of whether or not the rate of product formation, will be affected by substrate concentration (Badejo *et al*., 2021). From this study, the higher V_{max} and lower K_m values suggest a reasonable bonding between the substrate and enzyme. It also confirms the efficiency of the purified α-amylase for diverse industrial applications (Badejo *et al*., 2021) such as in food industries.

Molecular weight estimation

The most active fraction from the purified amylase subjected to SDS-PAGE for molecular weight determination was estimated to be 45 kDa. SDS-PAGE is considered to determine the molecular mass of the purified enzyme as well as a method to indicate the purity of an enzyme.

Maalej *et al*. (2021) similarly documented 45 kDa in the study of a novel digestive α-amylase from Blue Crab. Xian *et al*. (2015) reported higher molecular weight of 70 kDa from purified α-amylase obtained from *Talaromyces pinophilus*. The variations in molecular weights obtained from different organisms might be related to isozymic properties as well as types of amino acid residues of the enzyme (Maalej *et al*., 2021).

CONCLUSION

The fungus *A. flavus* isolated from fermented cereals cultivated on low-cost feedstock via SSF demonstrated α-amylase activity with excellent biochemical properties. The biochemical properties of the purified α-amylase showed that it is acidophilic and thermotolerant with a high affinity for the substrate. These are the properties required in a wide range of industries. Therefore, the combination of rice and millet as substrate serves as a good medium for higher amylase production for various industrial applications.

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Review article

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Revista Facultad Nacional de Agronomía

Bioprospecting in food production: an approximation of the current state in Colombia

UNIVERSIDAD NACIONAL DE COLOMBIA

La bioprospección en la producción de alimentos: aproximación del estado actual en Colombia

https://doi.org/10.15446/rfnam.v76n1.101705

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Keywords:
Biodiversity prospection
Biofertilization
Biopreservation
Bioremediation
Environmental pollution

ABSTRACT

Microbial bioprospecting is the study and classification of microorganisms with industrial value. Different researches word wide are focusing on the study of natural molecules that can be used for medicine, agriculture and the environment, among others. In Colombia, marine bioprospecting has become highly relevant. Also, different universities and institutes are working on the study of the biodiversity and its applications. The aim of this review was to compilate the most important laws and decrees related with the use of the resources with commercial purposes. Also, the elucidation of the current state of bioprospecting in Colombia and the principal applications of microorganisms in the food production chain. The special focus of this review is to show the potential use of bioprospection on agricultural development of the country in order to change the conventional practices to eco-friendly process in food production. Also, this review proposes the bioprospecting of lactic acid bacteria as an alternative to use their biomass and metabolites for food preservation.

Palabras clave:
Bioprospección
Biofertilización
Biopreservación
Biorremediación
Contaminación ambiental

RESUMEN

La bioprospección microbiana es el estudio y clasificación de microorganismos con valor industrial. Diferentes investigaciones a nivel mundial se están enfocando en el estudio de moléculas naturales que pueden ser utilizadas para la medicina, la agricultura y el medio ambiente, entre otros. En Colombia, la bioprospección marina ha cobrado gran relevancia. Asimismo, diferentes universidades e institutos están trabajando en el estudio de la biodiversidad y sus aplicaciones. El objetivo de esta revisión fue recopilar las leyes y decretos más importantes relacionados con el uso de los recursos con fines comerciales. Asimismo, la elucidación del estado actual de la bioprospección en Colombia y las principales aplicaciones de los microorganismos en la cadena productiva de alimentos. El enfoque especial de esta revisión es mostrar el uso potencial de la bioprospección en el desarrollo agrícola del país para cambiar las prácticas convencionales a procesos ecológicos en la producción de alimentos. De esta manera, esta revisión propone la bioprospección de bacterias ácido-lácticas como una alternativa para utilizar su biomasa y metabolitos para la conservación de alimentos.

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Received: October 09, 2022; Accepted: November 04, 2022
Rev. Fac. Nac. Agron. Medellín 76(1): 10227-10246. 2023
ISSN 0304-2847 / e-ISSN 2248-7026

In recent decades, awareness of has increased for the depletion of natural resources has increased in parallel with the exponential growth of the food industry. For the agricultural sector, achieving high yields in crops production is required to meet the increasing food demand, which influences negatively sustainable production goals (Tyagi *et al*., 2022). This has resulted in the excessive use of fertilizers and pesticides, which affects the production chain and causes serious environmental problems. Production chain of food depends on the relationship between microbial diversity and plants. For example, microorganisms maintain the soil health in different ways. They participate in the cycling of nutrients and process the waste (not only biological, but also chemical) using their enzymes. Some factors as high levels of toxic molecules (Shuaib *et al*., 2021) or changes in pH (Zhang *et al*., 2015) causes the loss of microbial diversity caused by chemical pest control and fertilization. This reduces the possibility to obtain the nutrients and minerals delivered by the microbial metabolism. Therefore, sustainable agriculture that protects the environment is needed, nowadays many researches around the world are studding new ways to produce food using ecofriendly process. They take advantage of existing microbial communities in different ecosystems, studying the microbial role in the biogeochemical cycles and develop natural products for the benefit of food production.

The investigation of the metabolism of microorganisms has shown their ability to contribute in soil and crop remediation processes. Bioprospecting as a tool to search new alternatives for fertilization or biological control of pathogens could minimize the use of chemical substances and in the long term could contribute with preserving the environment. In Colombia, microbial bioprospecting is very important in the environmental, industrial and veterinary medicine sectors, with research focused on the potential of microorganisms (Melgarejo, 2013). The environmental advantages of using microorganisms are evident in agricultural activities. The metabolic products decontaminate soils and degrade polluting residues by improving the structure of soils and promoting nitrogen fixation or solubilizing phosphates (Montenegro *et al*., 2019). For example, herbicides as Quinclorac have been used in the word, also in Colombia for controlling undesirable grasses in the rice fields (Shi *et al*., 2017). Residues of Quinclorac in the soil can cause serious phytotoxicity in sensitive plants and for the recovery of the soil health it can be used techniques such as bioremediation. It has been reported that strains of different genera as *Bacillus megaterium* Q3, *Burkholderia cepacia*, *Bordetella* sp. strain HN36, *Alcaligenes* sp. *Pantoea* sp. QC06 can metabolize this herbicide. Biomass cultured in liquid medium of the isolates strains was applied directly over samples quinclorac-contaminated soil and de biodegradation was performed. Some possible ways to do this transformation are through the reduction of carboxyl, or through dechlorination of quinclorac (Liu *et al*., 2014). For development of those products, the biomass is produced in bioreactors obtaining homogeneous cell suspensions produced under optimum conditions that must keep the viability after application on field (Tyagi *et al*., 2011).

Additionally, these microorganisms can protect crops from pests and pathogens since several microbial metabolite's present antimicrobial activities, serving as potential biological controllers. Therefore, bioremediation, biological control, biostimulation, and bionutrition promote more productive, nutritious and healthy crops with fewer negative effects on the environment (Carranza, 2017). Bioprospecting offers possible alternatives through the use of biofertilizers based on microorganisms (microalgae, bacteria or fungi) applied to soil and plants to partially or totally replace synthetic fertilization. These microorganisms perform functions such as decomposition of organic matter, detoxification of pesticides, stimulation of plant growth and development, supplying nutrients to plants and soil, secondary metabolites and production of bioactive compounds such as vitamins and hormones that protect against pathogens (Vimal *et al*., 2017).

Application of products obtained from isolated strains with biofertilization or bioremediation traits reduce agrochemicals that become environmental pollutants in soil and water as a result of agricultural work (Armenta *et al*., 2010). Additionally, it can reduce costs for producers by using natural products obtained locally, increasing crop yields and offering the final consumer healthier products free of agrochemical residues, resulting in sustainable agriculture. Likewise, natural

products reduce the application of toxic additives by providing alternatives for food biopreservation. The objective of this review was present some important laws that govern the use and exploitation of genetic resources in Colombia. Also, we elucidate the current state of bioprospecting in Colombia and the principal applications of microorganisms in the food production chain, focusing on agricultural uses of biofertilization and bioremediation and on biopreservation of food.

MATERIALS AND METHODS

Different scientific articles were reviewed in the databases of universities such as Universidad Nacional Abierta y a Distancia and Universidad Nacional de Colombia, along with articles in Scopus and search engines such as Google Scholar. Documents available from the Ministry of the Environment and other regulatory bodies were also reviewed. Decrees and laws of different years were reviewed independently of the date. The articles and thesis reviewed belong to a span of 20 years. According with the literature we divide the information in sections that deal with topics like regulations, applications and perspectives of the bioprospecting in Colombia.

RESULTS AND DISCUSSION

Bioprospecting or biological prospecting is a systematic process of searching for genes, natural compounds, or organisms in nature that have the potential to develop a product that benefits man (Oyemitan, 2017). Specifically, microbial bioprospecting is defined as the search for microorganisms with desirable characteristics that can be used in various industries to impart commercial value through the development of process and products in sectors such as cosmetics, biotechnology, pharmaceuticals, medicine, food, and agriculture, among others (Duarte and Velho, 2009; Beattie *et al*., 2010). This activity is based on access, characterization, and transformation for biological and genetic resources, obtaining products that can contribute to the solution of environmental, social, and various types of problems without affecting biodiversity.

Regulations

Because of the effect that resource exploitation has on humanity, bioprospecting has been addressed in international treaties and legal agreements to control biopiracy, which is the use of resources without the approval of original peoples or obtaining profits without respecting international treaties (Beattie *et al*., 2010). Access to genetic resources represents a development and conservation issue for the diversity of microorganisms and natural sources.

Once microorganisms have been obtained from different sources, they must be identified to have a detailed record of the different types of microorganisms in each sampled area. Then, a collection of microorganisms must be developed and studied, introducing the most viable alternative to sustainable industrial and agro-industrial processes. The storage site guarantees the conservation of microorganisms for important biological resources and genetic information. When determining areas with microbial diversity, conservation or exploration strategies can be developed, generating greater economic importance for the areas (DNP, 2011).

Colombia has different genebanks; the main one is located in the Corporación Colombiana de Investigación Agropecuaria (Agrosavia). Other entities such as the Instituto Colombiano Agropecuario (ICA), Pontificia Universidad Javeriana, Instituto de biotecnología at the Universidad Nacional de Colombia, and Instituto Humboldt also have collections of great interest. In Colombia, these banks are divided into three subsystems: animals, plants and microorganisms. The Instituto Humboldt is linked to the Ministry of the Environment and is the regulatory body for the conservation of genetic diversity for different species. Genetic resources are the property of the Colombian state; they are inalienable, imprescriptible and unseizable (Rojas *et al*., 2016). Access in the form of genes and derived products is regulated by Andean Decision 391, called the Common Regime on Access to Genetic Resources, which includes Peru, Ecuador and Bolivia (Álvarez-Tafur, 2014).

Andean Decision 391 states that the Andean community must guarantee a fair and equitable sharing of benefits derived from the use of resources and associated traditional knowledge, laying the foundation for the recognition and valuation of genetic resources and their derived products, promoting the conservation of diversity, and developing scientific, technological and technical capacities (Álvarez-Tafur, 2014). Table 1 describes some important laws used in Colombia in order to regulate the prospection activities in the country.

Table 1. Laws and decrees related with the bioprospecting activities.

Law	Description
Decree 2811 of 1974	It is the National Code of Renewable Natural Resources and Environmental Protection. Article 9 presents the fundamental principles for the use and exploitation of natural resources. There is mentioned that the exploitation of resources will be protecting the rights of the community, therefore the physical limits must be respected, avoiding the depletion or deterioration of exploited resources. This exploitation must be justified through the urban and rural development of the country.
The Political Constitution of 1991	Chapter 3 in its articles 79 and 80 recognizes the obligation of the state to protect the environment and the resources of its exploitation and commercialization and to generate the necessary laws to ensure this protection. Likewise, the state is committed to planning for the management and use of resources. Article 81 establishes that the government must create rules to regulate the entry and exit of genetic material into the country. Also, establishes that the country's environmental management will be decentralized, democratic and participatory.
Law 99 of 1993	It establishes that biodiversity is a national patrimony and of interest to humanity, therefore it determines the creation of the Ministry of the Environment for the management of the environment and renewable natural resources. That Ministry has as responsibilities the regulations over the recovery, conservation, protection, ordering, management, use and exploitation of renewable natural resources and the environment of the Nation, in order to ensure sustainable development. The foundations of the Colombian environmental policy were established, which stipulates that the economic and social development process of the country will be guided by the universal principles and sustainable development contained in the Rio de Janeiro Declaration of June 1992 on Environment and Development. In addition to mentioning the rights of peoples to live in harmony with nature and use sustainable resources. It emphasizes moorland and sub-moor areas, water sources and aquifer recharge areas as objects of special protection. It also seeks scientific evidence that justifies the exploitation of a resource and establishes that in the event of a significant environmental danger, its use will not be allowed. Non-governmental environmental protection organizations must be created and environmental impact studies must be declared mandatory.
Andean Decision 391 of 1996	The Common Regime on access to genetic resources is approved, establishing that Member Countries exercise sovereignty over their genetic resources and their derivative products, therefore they have the power to determine the conditions of their access. It is also established that each member country will regulate the standards for the conservation and sustainable use of genetic resources and their derivative products, in accordance with the principles and provisions contained in the Convention on Biological Diversity. Likewise, it is established that genetic resources and their derived products are assets or patrimony of the Nation or of the State of each Member Country and are inalienable, imprescriptible and not attachable, without prejudice to the property regimes applicable to the biological resources that contain them, the property on which they are located, or the associated intangible component.

Table 1. Laws and decrees related with the bioprospecting activities.

Law	Description
Decree 3570 of 2011	Establishes the objectives of the Ministry of Environment and Sustainable Development as manager of the environment and renewable natural resources, regulator of the environmental planning of the territory. It must define the norms for the recovery, conservation, protection, ordering, management, use and sustainable exploitation of the renewable natural resources and the environment of the Nation. Therefore, it must formulate the national environmental and renewable natural resources policy. It must evaluate the scope of large-scale projects on the environment and sustainability of the nation. Therefore, it must monitor the Regional Autonomous Corporations and intervene in the face of environmental deterioration that may arise from the execution of development activities or projects, as well as from the exploration, exploitation, transport, benefit and use of renewable and non-renewable natural resources. Also, it must order the competent national body for the issuance of environmental licenses under the Ministry of Environment and Sustainable Development, the suspension of work or activities when it be necessary.
Decree 1375 and 1376 of 2013	This decree regulates the biological collections in the national territory, the rights and obligations of the holders of the collections and the registration procedure of the same before the Institute of Biological Resources Research "Alexander von Humboldt". The functions of the collections at the service of the nation related to research, care of specimens and loan for academic purposes are established. Provisions are established for the execution of contracts for access to genetic resources with the Institute.
Resolution 1348 of 2014	Contains the regulations related to the collection permits for specimens of wild species of biological diversity for non-commercial scientific research purposes. It establishes who must manage the permits and which the entities that do not require it are. It also decrees that the contract for access to genetic resources and/or derivative products must be carried out to access genetic resources and/or derivative products, for industrial, commercial or biological prospecting purposes, of the specimens collected within the framework of a collection permit.

To avoid biopiracy, Colombia protects its genetic potential through legislation. The Instituto Humboldt is responsible for promoting, coordinating and conducting research on the conservation and sustainable use of biodiversity. Shaping the national biodiversity inventory and facilitating the efficient and permanent management of biodiversity information that is useful for the processes of information analysis and the generation of information products. Also, the institute take the decision-making and defines of policies related to activities of access and exploitation of genetic resources in Colombia.

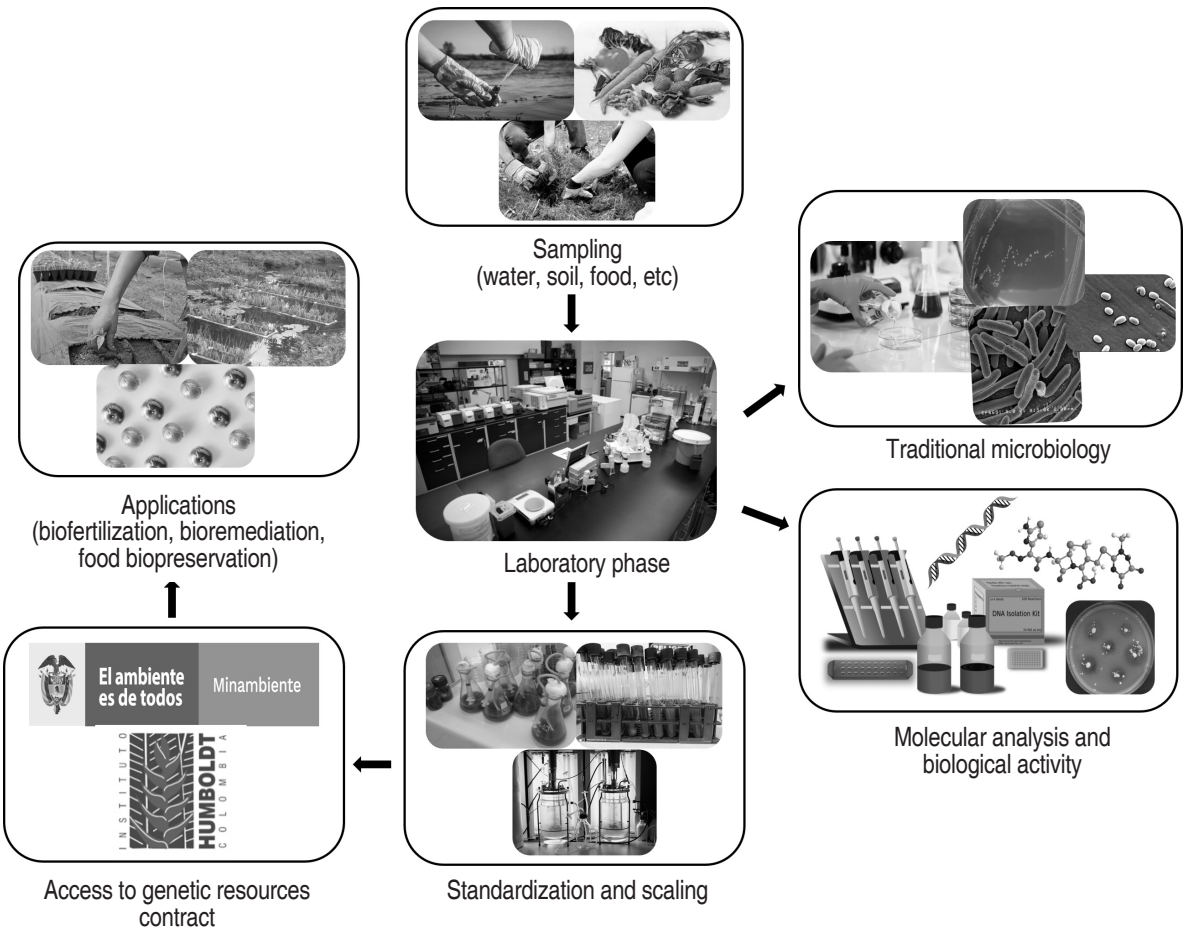

Figure 1. Procedures during a bioprospecting process in search of product development with applications in the food industry.

National and international focus

At the international level, bioprospecting focuses mainly on secondary metabolites because of the economic impact on the pharmaceutical industry. This process has greatly developed this industry and has set guidelines for the innovation and application of technologies using metabolites with characteristics applicable to new products. Additionally, the search for biological molecules with biological activity has revealed the potential for applications in food and uses in different industries and for crop protection through the application of biological inputs. These developments are of great economic and environmental importance for the preservation of life (Melgarejo, 2013). Applications in food production are fundamental because of the magnitude of environmental impacts that agricultural practices and food processing have. In Central and publications and to increase. Figure 2 shows the results in Scopus using the word "bioprospecting" as search criteria. According to the results, there are currently 2426 publications worldwide on bioprospecting, published up to November of 2022. 64 publications were carried out in Colombia, where records have been kept since 2006. The main area of interest is agriculture, a fundamental part of the food production chain. Pioneering entities for these publications include the Universidad Nacional de Colombia, Universidad de la Sabana, Universidad de Caldas and Universidad de Antioquia.

Bioprospecting in Colombia

Microbial bioprospecting is an expanding topic, where the benefits for economic development and environmental sustainability can be explored. It will be an important component in studies on ecological diversity in this

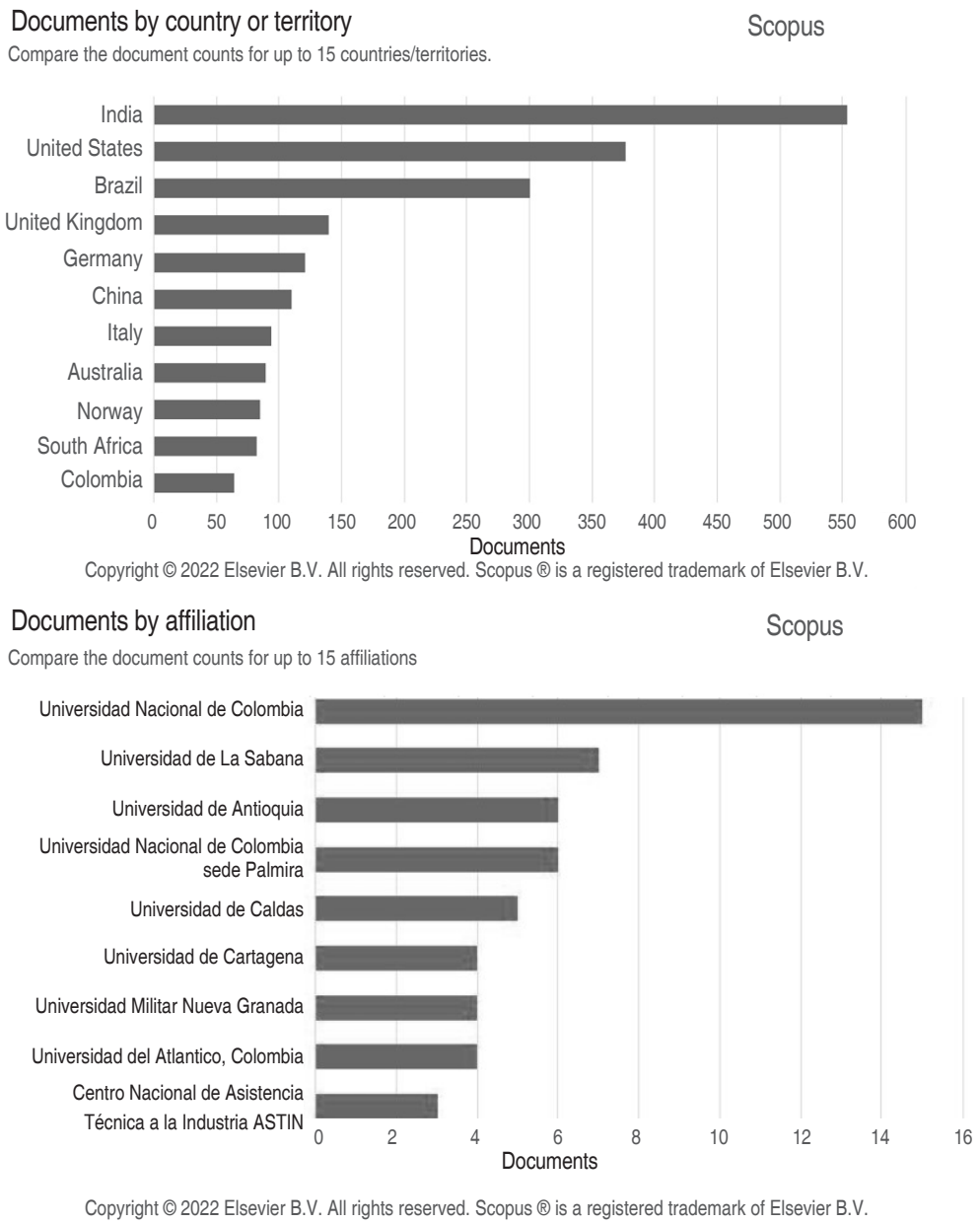

Figure 2. Analysis of the search results of publications on bioprospecting in Scopus. November 2022.

country, as well as for knowledge on the structure of microbial populations. There are potential applications for the food production chain. Sustainable agricultural production systems can be developed, ensuring conservation of the country's biological and microbial diversity. Colombia has great diversity in species of fauna, flora and microorganisms, which represent an opportunity for research on microbial bioprospecting.

Research groups specialized in bioprospecting have conducted studies focused on bioactive principles in plants, bacteria, hive products and marine organisms. *Beauveria bassiana* is a fungus that has been used to control the coffee borer and has demonstrated usefulness for the food industry in Colombia (Antia *et al.*, 1990). This form of biological control is still used today and is promoted by the coffee research center

(Cenicafé). Likewise, there are studies on the isolation of volatile substances from vegetables and essential oils, on the identification and characterization of fungi, and on biological control of agricultural pests and diseases (Duarte, 2011), as well as in biopreservation of food. Another biological way to control pathogens are the application of metabolites of some bacteria, for example the bacteriocins is an option for replace synthetic preservatives (Jutinico-Shubach *et al*., 2020). On the other hand, these studies have contributed to alternatives for the recovery of contaminated areas through bioremediation practices and for reducing the use of agrotoxins applied during the food production in Colombia. INVEMAR (José Benito Vives de Andréis Marine and Coastal Research Institute) has been a pioneering research center for bioprospecting aquatic environments. This institute has 6 megaprojects, including two that are currently focused on bioremediation of contaminated environments. On the other hand, the research group "Studies on the use of Natural Marine Products and Fruits of Colombia" at the Universidad Nacional has as an area of interest that includes marine invertebrates, corals, sponges, algae, microorganisms and some species of plants obtained from marine environments as sources of bioactive compounds. This group has almost 30 research projects for the search and application of bioactive compounds for the cosmetic and health industries. The Bioprospecting Research Group of the Universidad de la Sabana focuses on the search, isolation, characterization, evaluation and scaling at an industrial level of genes, proteins and metabolites in macro and microorganisms obtained in different environments of Colombia. This group has 11 research projects currently underway that are dedicated to the search for bioactive compounds and their modification with biological and industrial potential. The Universidad de Antioquia has two lines of research related to bioprospecting. One of them is called "Ecology of populations, communities and ecosystems" and has the research group "Microbial Ecology and Bioprospecting". In this line, projects have been developed in biological control, molecular biology of fungi, microorganism-habitat interaction, pollinator-plant and microbial decomposition of litter. The second line is "Biotechnology" whose work is based on the use of Colombian biodiversity (plants, animals and microorganisms) with basic and applied studies. Their work is developed in 3 research groups: Agrobiotechnology, Biotechnology and Biocontrol, and Environmental Microbiology. The center BioInc belongs to Universidad Icesi works to collaborate with the colombian industrial sector to promote the development of bioprocesses based on circular economy and proper use of biodiversity. They have two active projects, one of them "Meeting policy challenges for a responsible biodiversity based bio-economy in Colombia" and "Towards a sustainable bio-economy in Colombia: Organic residue valorization and bioprocessing". Also, they had finalized five projects which the mayor goal is to take advantage of the diversity to improve the agriculture in Colombia. The Centro de Investigación de Agricultura y Biotecnología (CIAB) at the Universidad Nacional Abierta y A Distancia (UNAD) has a line of research called Agricultural and environmental biotechnology with sublines for biofertilization, bioprospecting and bioremediation with 7 projects. The Bioprocess and Bioprospecting Research group at the Instituto de Biotecnología of the Universidad Nacional de Colombia has lines of research in Agricultural Microbiology and Bioprocesses with four biofertilizer products obtained through bioprospecting processes that have been patented and are currently commercialized. The Colombian company Ecopetrol created the Colombian Petroleum Institute 37 years ago in Piedecuesta (Santander) together with universities, research centers and national and international technology-based companies. Its focus is on the development of new processes and innovations, experimental tests and development of engineering and technological products. Its technological and scientific infrastructure consists of 9 laboratories comprising more than 40 experimental and analytical areas and 36 pilot plant units. The institution supports undergraduate and master's students nationwide with scholarships mediated by the Ministerio de Ciencia y Tecnología. Some of the research topics they develop are biotechnology, microbiology and molecular biology, bioprocesses and biofuels.

Agriculture

The so-called green revolution promoted the use of chemically synthesized fertilizer as a response to the demand for food worldwide that stemmed from uncontrolled population growth. This solution increased the use of fungicides and fertilizers that, by stimulating crop productivity, caused serious environmental

problems such as soil salinization, loss of fertility and loss of organic matter (Medina, 2018). To care, protect and foment the development of sustainable agriculture, the application of beneficial microorganisms that improve production and control crop pests are part of the solution for the environmental pollutions. Several microorganisms have been identified and tested as beneficial elements for agriculture. At the Universidad Militar Nueva Granada, there are about 120 rhizobacterial isolates (*Pseudomonas*), five with biocontroller potential against *Fusarium oxysporum*. On the other hand, in Agrosavia, there is a collection of 303 filamentous fungi and 249 yeasts with biocontrolling potential for pest insects and soil, foliar and post-harvest phytopathogens, with a collection of 45 bacteria and five mycorrhizae with biofertilizing potential (Cotes *et al*., 2012).

According to a study carried out by MADS (Ministry of Environment and Sustainable Development), the IDEAM (Institute of Hydrology, Meteorology and Environmental Studies) and the U.D.C.A (University of Applied and Environmental Sciences) soils have lost quality and microbial diversity, generating lower agricultural production. According to the FAO (Food and Agriculture Organization of the United Nations), for 2015, 40% of Colombian soils had some type of erosion, with a reduction of land suitable for agricultural cultivation from 12% in 1961 to 8.1% in 2014 (Medina, 2018). Biofertilization takes advantage of beneficial microorganisms to improve soil conditions and colonizes the rhizosphere or the interior of plants where their metabolic processes benefit agricultural crops and pastures, which reduces or eliminates the need to use chemical fertilizers (Figure 3). Application of natural fertilizer decrease the adverse effects of remaining chemicals in food that affect the health of consumers. Also protects the environment meanwhile increase the food production, which are part of the global agenda for Sustainable Development Goals (SDGs). For example, one crop yield can increase by 10–40%, also the continuous application of biofertilizer of the land for years can retain fertility, keeping the added microbial community. Because they are environmentally friendly can be more economic using natural ingredients for the formulations (Seenivasagan and Babalola, 2021).

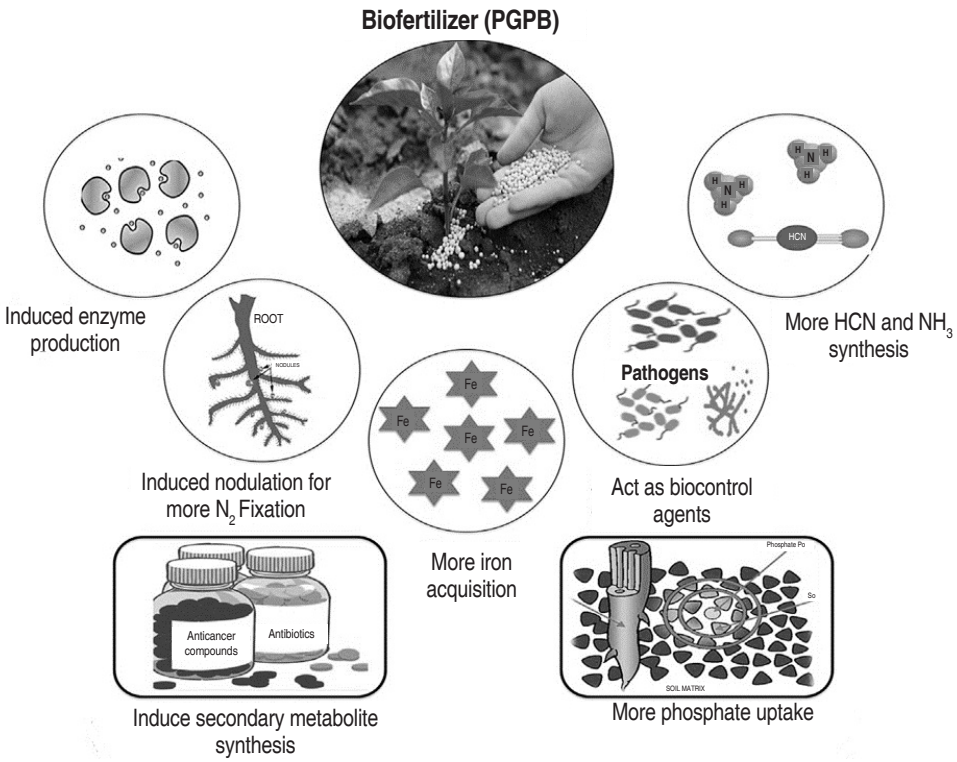

Figure 3. Benefits of biofertilization, use of plant growth promoting bacteria (PGPB). Source: modified from Singh *et al*., 2019

Several research has sought to develop products and processes that allow fertilization to be carried out naturally. The application of nitrogen fixators enhances crop production and growth of beneficial microorganisms for the soil (Carvajal and Benavidez, 2010). This has been demonstrated with N2-binding cyanobacteria strains such as *Anabaena* sp. UTEX 2576 and *Nostoc muscorum* (UTEX 2209S), and a polyculture of *Chlorella vulgaris* (UTEX 2714) and *Scenedesmus dimorphus* (UTEX 1237) in crops such as rice (Jochum *et al*., 2018), demonstrating that biofertilization benefits agriculture, the environment and health. Nitrogen is a limiting factor for rice crops growth, since producers must add inorganic N but it was not efficient. Authors demonstrate that biomass of the microalgae cultivated in a photobioreactor can improve the plant height under greenhouse conditions.

In addition to bacteria, fungi are also a fundamental part of the microbial biodiversity associated with plants. Mycorrhizae, a symbiotic association between a fungus and the roots of a plant, are also effective methods for biofertilization and bioprotection of crops (Carvajal and Benavidez, 2010). Arbuscular mycorrhizal fungi represent a promising microbiological resource for the development of sustainable agriculture (Guerra-Sierra, 2008). Biofertilizers that possess nitrogen fixation and phosphorus solubilization traits have been reported to have the greatest potential to improve crop yield, indicating the great potential of arbuscular mycorrhizal fungi (AMs) as one of the biofertilizer for most crops and climatic situations (Schütz *et al*., 2022). Table 2 shows the genera of microorganisms with potential use in biofertilization.

Table 2. Species of microorganisms used in biofertilization.

Category	Species	
AMs (arbuscular mycorrhizal fungi)	*Glomus mosseae* *Gigaspora rosasea* *Arthrobacter chlorophenolicus* *Penicillium bilaii* *Pseudomonas aeuriginosa, P. argentinenses, P. cepacea* *Serratia marcescens* *Staphylococcus saprophyticus*	*Entrophosphora colombiana* *Glomus caledonium, G. clarum, G. etunicatum* *Rhizophagus irregularis* *Bacillus firmus, B. megaterium, B. musilaginous* *Enterobacter asburiae, Microbacterium arborescens* *Paenibacillus* sp.*, P. polymixa*
Nitrogen fixers	*Beijerinckia indica, B. japonicum* *Brevundimonas diminuta* *Burkholderia vietnamensis.* *Gluconacetobacter diazotrophicus* *Herbaspirrillum seropedicae* *Mesorhizobium ciceri, Rhizobium leguminosarum*	*Anabaena azollae, A. cylindrica, A. variabilis, A. turulosa* *Aphanothece ssp., Aulosira fertilissima* *Azolla caroliniana, Azospirrilluim brasilense, A. lipoferum* *Azotobacter brasilense, Bacillus polymyxa, B. subtilis* *Staphylococcus* sp.*, Tylopthrix tenius*
Nitrogen fixers plus phosphorus solubilizers	*Bacillus. polymixa* *Enterobacter* sp.	*Bacillis megaterium*
Other biofertilizers	*Aspergillus niger, A. tubingensis* *Ochrobactrum antrophic, O. ciceri* *Penicillium brevicompactum, P. solitum*	*Actinomycetes* *Bacillus circulan, B. mycoides., B. pummitus, B. simplex* *Piriformopora indica, Rhodobacter capsulatus* *Rhodopseudomonas* sp.*, Thiobacillus* sp.*, T. tihioxidans*

Source: Cubides (2021)

According with Ortiz-Moreno *et al*. (2022) in Colombia exist a diversity of product based on native fungi with agriculture applications. That products are the result of the biotechnological development and years of evaluating the biological activity of the native isolated fungi. Table 3 summarizes the examples mentioned by the authors.

Table 3. Examples of colombian companies and their products with application in the agriculture industry (Ortiz-Moreno *et al*., 2022).

Company	Product	Fungi	Action
Agrosavia	Trichotec	*Trichoderma koningiopsis* Th003	Biocontrol of *Fusarium oxysporum, Rhizoctonia solani, Sclerotinia sclerotium, S. minor* and *Botrytis cinérea.* In tomato, rice, lettuce and red fruit crops. Also, plant growth-promoting action.
Biocultivos S.A.	Fosfobiol	*Penicillium janthinellum*	Phosphorus solubiliser. In cotton, rice, coffee, sugar cane, corn, pastures and soybeans.
	Trifesol	*Trichoderma viride*	Biocontrol agent (mycoparasite), plant growth promoter, and cellulose degrader.
	Residue treatment	*Penicillium pinophillum* and *Pleurotus ostreatus* consortium	Cellulose and lignin degraders for the management of plant biomass residues.
Natural Control company	Anisagro	*Metarhizium anisoplae*	Bio controllers, plant growth promoters, plant protectors and soil improvers applicable to different crops.
	Vercani	*Lecanicillium lecanii*	
	Bassar	*Beauveria bassiana*	
	Fitotripen	*Trichoderma harzianum, T. koningii* and *T. viride*	
	Mycorrhizagro	*Glomus, Acaulospora, Scutellospora* and *Entrophospora*	
	Safelomyces	*Purpureocillium lilacinum* and *Cordyceps fumosorosea*	

(Ortiz-Moreno *et al*., 2022).

Bioremediation as an alternative for the recovery of environments contaminated by agricultural practices and other industries

Environmental pollution due to the inadequate and excessive use of agrochemicals and fertilizers is a problem that is already being part of the agenda of world organizations. In 2012, the FAO established the Global Soil Partnership (GSP), which promotes sustainable soil management in agricultural tasks for food security and improved nutrition, adaptation, and mitigation of climate change and sustainable development (FAO, 2022). One of the alternatives for soil care is bioremediation. This is a low-cost technology that uses the metabolic traits of the microorganisms to decontaminate or recover an environment altered by polluting substances (Jochum *et al*., 2018). One of the advantages of this technology is applications in natural environmental conditions, guaranteeing decontamination and recovery of soils, with an economic, social and environmental sustainability benefit (Senthil *et al*., 2017).

Water, air and soil are affected by contamination with heavy metals such as mercury (Hg), arsenic (As), lead (Pb), cadmium (Cd), zinc (Zn), nickel (Ni) and chromium (Cr), products of activities such as agriculture and mining (Tchounwou *et al*., 2012). These metals can be found at different concentrations in fish, meat, milk and vegetables as a result of bio-accumulation, putting at risk not only human health but also food safety and the environment (Reyes *et al*., 2016). Cadmium is a heavy metal found naturally in the soil, but it is also introduced to the environment anthropogenically through phosphate fertilizers, increasing the normal load of the ore. This causes toxic effects on the health of humans, plants and animals. Once cadmium is absorbed by the body, it is housed in vital organs such as the kidneys and liver, remaining in the body for several years (Kumar *et al*., 2012). Cadmium and lead are heavy metals with the greatest tendency to accumulate in plants, causing severe imbalances in the processes of nutrition and water transport.

Several studies have demonstrated the ability of lactic acid bacteria (LABs) for water and soil treatments. *Pediococcus* sp., *Leuconostoc* sp. and *Lactobacillus* sp. have demonstrated their ability to reduce contaminants such as chromium, cadmium and lead (Petrova *et al*., 2022). It has also been shown that lactobacilli species such as *Lactobacillus amylovorus, Lb. reuteri* and *Lb. dextrinicus* are efficient at decontamination of sludge with CD and PB, providing a possible solution for decontamination of heavy metals in food and water (Kirillova *et al*., 2017). A study using *Lb. plantarum* MF042018 isolated on the shores of the Mediterranean Sea demonstrated great potential for the treatment of heavy metals derived from the manufacture of batteries, presenting biosorbent characteristics for the removal of heavy metals in industrial wastewater (Ameen *et al*., 2020). This type of work is fundamental for knowledge on the behavior of bacteria with potential in bioremediation. Likewise, it has been shown that there is greater effectiveness with a consortium of bacteria than with pure cultures, showing the metabolic pathways followed by different species of bacteria, which can have synergistic effects for the degradation of toxic compounds in certain environments (Zhang and Zhang, 2022). A study carried out with strains of *Viridibacillus arenosi* B-21, *Sporosarcina soli* B-22*, Enterobacter cloacae* KJ-46 and *E. cloacae* KJ-47, where parameters such as optical density, pH, urease activity, calcite production, tolerance to heavy metals and impermeability were monitored, showed that bacterial mixtures had greater resistance and efficiency for the remediation of heavy metals than the use of a single strain (Khan *et al*., 2016). The presence of heavy metals in soils, food and water sources is a global concern because decontamination processes can generate large costs; bioremediation is an economic and sustainable alternative applied in several countries (Yadav *et al*., 2017).

The control of plant species that compete with crops is carried out with herbicides that contaminate agricultural soils, river systems, and aquifers and change the structure and function of soil microbial populations. Herbicides directly or indirectly impact organisms other than weeds, even humans (Pileggi *et al*., 2020). Glyphosate is the most used chemical worldwide in agriculture; it is an herbicide that acts on all plant species by inhibiting the activity of enzymes that synthesize aromatic amino acids (Choque and Nogales, 2019). Those amino acids are essential for protein synthesis and are precursors for some secondary metabolites required for plant growth (Tzin and Galili, 2010). Several studies have reported residues of this herbicide in soils, water and food for human consumption, which can cause effects on health and the environment (Ruuskanen *et al*., 2020). A bioremediation study in glyphosate (GP) contaminated soils, comparing native strains and two introduced strains recognized as degrading: GP *Achromobacter* sp. Kg 16 (VKM B2534D) and *Ochrobactrum anthropi* GPK 3 (VKM B-2554D), demonstrated the efficiency of the strains introduced in the first phase in the laboratory. In the field, the introduced strains had greater efficiency in the biodegradation of GP (between 49.5% and 65.8%) than native strains that did not exceed 11% for GP biodegradation (Ermakova *et al*., 2010). This shows the great potential of bioprospecting activities to obtain strains with higher degradation activities that can be introduced into environments in a controlled manner. On the other hand, bacteria such as *Bacillus pumilus*, *Streptomyces* sp., *Serratia marcescens*, *Alcaligenes* sp., *Penicillium* sp. and *Pseudomonas putida* have been reported as organophosphate degraders. Among these species, *B. pumilus* has demonstrated the ability to degrade 300 mg L^{-1} of TCP chlorpyrifos (3,5,6-trichloro-2-pyridine) in 8

days. Additionally, it has been reported that molds such as *Aspergillus terreus* have degraded the same amount in 24 hours, which shows that this fungus has a greater ability to degrade organophosphates (Hernández-Ruiz *et al*., 2017). Figure 4 shows an outline of the impact of these chemicals.

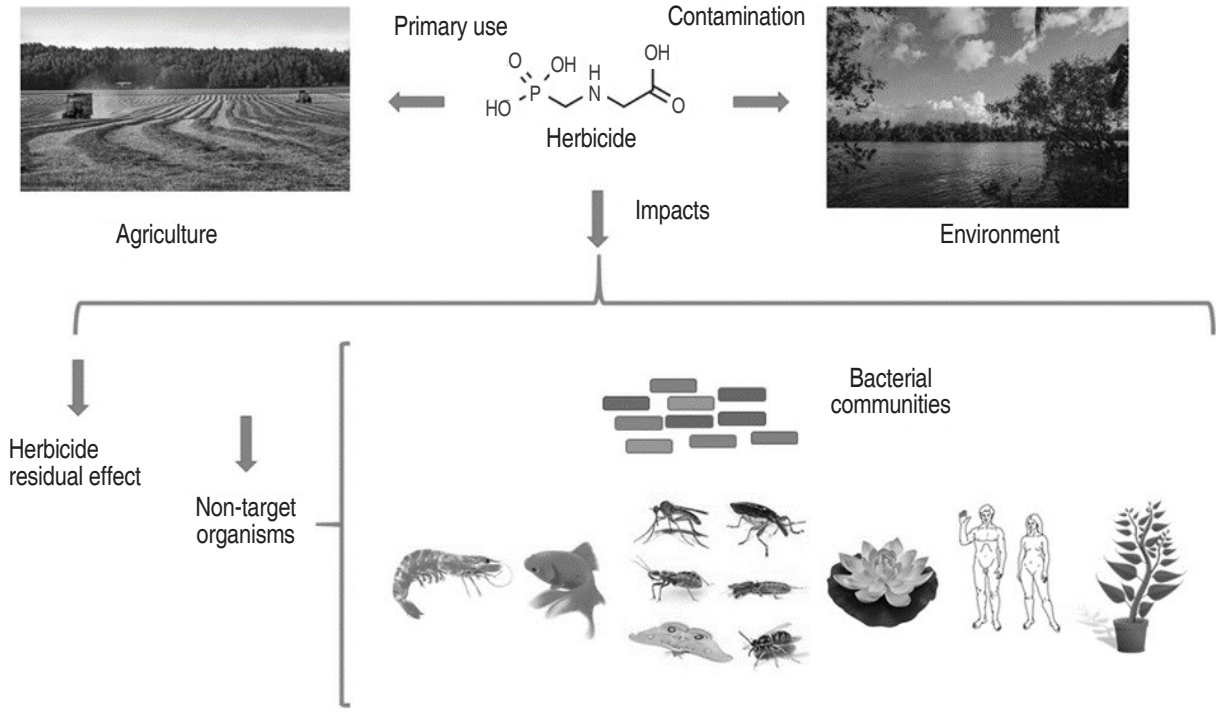

Figure 4. Impact of the use of agrochemicals in food production. Source: Pileggi *et al*., (2020).

On the other hand, economic losses caused by insect pests and nematodes in crops has forced the use of pesticides. Organochlorine compounds are fat-soluble, water-insoluble and highly toxic to plants and animals. These pesticides are absorbed by the soil and are difficult to degrade (Gregory *et al*., 2015), which is why they have been banned or restricted in several countries according to the Latin American Observatory of Environmental Conflicts. Organochlorines (alpha and beta endosulfans) can be found accumulated in different layers of soil. In one study, total degradation of these compounds was achieved using a bacterial consortium with the genera *Bordetella petrii* I GV 34, *B. petrii* II GV 36 and *Achromobacter xyloxidans* GV 47 (Odukkathil and Vasudevan, 2016).

The ability to assimilate agrochemicals of some species of fungi has been also demonstrated. One study demonstrated the degradation of heptachlor and heptachlor epoxide through the use of the macroscopic fungi *Phlebia tremellose, P. brevispora* and *P. acanthocystis*, achieving between 71 and 90% heptachlor removal and between 16 and 25% heptachlor epoxide (Dar *et al*., 2019). Species such as *Penicillium miczynskii, P. Raistrickii, Aspergillus sydowii, Trichoderma* sp., and *Bionectria* sp., obtained from marine sponges, have also demonstrated a capacity for biodegradation of organochlorine compounds (Parte *et al*., 2017).

Organophosphates are also highly toxic compounds that control pests such as insects, mites and nematodes (Badaii and Varela, 2008). These compounds can be found in everyday consumer products such as dairy, leading to public health problems. In one study was evaluated the degradation capacity of organophosphates by LAB during milk fermentation using different combinations of species of the genus *Lactobacillus* (*Lb.*

plantarum 1.0317, *Lb. plantarum* 1.0624, *Lb. plantarum* 1.0315, *Lb. brevis* 1.0209, *Lb. helveticus* 1.0203, *Lb. helveticus* 1.9204, *Lb. lactis 4*.0611, *Lb. bulgaricus* L6 and *Streptococcus thermophilus* 3.0503), some with strong degrading activity and others weak. They observed that when combining strains that individually presented weak activity, there is synergism, and organophosphate pesticides are degraded more rapidly (Zhang *et al*., 2014).

Bioremediation in Colombia

In recent decades, water quality studies in Colombia have shown higher concentrations of heavy metals from increases in populations, industrialization and mining activities. There are reports of large concentrations of cadmium and lead in the Negro, Bogotá, Cararé, Marmato, Cauca la Pintada, Achi and Pinillos rivers (Reyes *et al*., 2016). On the other hand, Colombian soils have a large amount of heavy metals from agricultural work that takes place throughout Colombia, which becomes an important problem, since these compounds represent a risk for health and the environment. In the municipality of Codazzi (Cesar), large quantities of chemical inputs have been used in cotton crops for several years. In a study conducted by Kopytko *et al*. (2017) in this region, a recovery process for soils was carried out using varieties of native strains of the genera *Pseudomonas, Aeromonas, Burkholderia, Bacillus* and *Enterobacter*, which are reported in the literature as microorganisms with a high potential to degrade organochlorine compounds. Authors applied the bioaugmentation method to remove 56.2% of DDT (dichloro-diphenyl-trichloroethane), 17.1% DDD (dichlorodiphenyldichloroethane) and 44.5% DDE (chlorophenylethylene) for 8 weeks (anaerobic treatment) and 46.5% removal of DDT, 17.8% DDD and 8.5% DDE after 20 weeks (aerobic treatment).

In Colombia, the development of bioremediation processes began more than 25 years ago. Studies carried out by research groups from universities such as the Center for Microbiological Research (CIMIC) of the Universidad de los Andes, Universidad Nacional de Colombia in Medellín, and Universidad de Antioquia have compiled published studies on bioremediation in Colombia. In a study carried out in the municipality of Manaure (Guajira), a mixed culture strategy was developed to isolate halophilic bacteria with antibacterial and cytotoxic activity. The strain *Vibrio diabolicus* A1S was isolated and its production of bioactive metabolites was evaluated with biodegradable polyhydroxybutyrate (PHB) polymer. This material has plastic-like characteristics and has potential applications in different industries. The *V. diabolicus* A1S genome was sequenced to establish the genes responsible for the production of PHB and the metabolic pathways of this microorganism, where bioprospecting isolated at least 600 different microorganisms with potential industrial applications (Conde, 2019).

In one study, some actinobacteria were isolated from water and sediments of the Guaviare River with antimicrobial capacity over bacterial as *Chromobacterium violaceum*, *Bacillus subtilis*, *Acetobacter baumannii* and *Klebsiella pneumonia* and fungi as *Colletotrichum gloeosporioides*. These isolated strains represent a potential for future research with valuable contributions to medicine and agribusiness (Pastrana *et al*., 2016). In another study carried out in two riparian areas of the Arauca River, it was found that the combination of physicochemical pretreatments and the use of techniques such as MALDI-TOF MS facilitated the detection of low abundance actinobacteria with potential use as a source of antimicrobial agents (Arango *et al*., 2018). Another study reports the analysis of samples of deep marine sediments from the Caribbean Sea from which were isolated representatives of microbial communities that contribute to global carbon recycling. Also, in this study microorganisms with antimicrobial activity were isolated, evaluating their resistance to methionine, and 78 of these bacteria belonged to the Streptomycetaceae family. Three phylogenetic groups were found: Proteobacteria, Actinobacteria and Firmicutes. This information gives an estimation of the phylogenetic diversity and provides a first step to the creation of taxonomic inventories from existing microbial populations with bioremediation capacity (Blandón *et al*. 2022).

Applications of microbial bioprospecting in food processing

The processing of food after harvest called transformation, require preservation techniques because organic matter is susceptible to deterioration by microorganisms. Therefore, extending the shelf-life of food has been a challenge, which is why a large number of preservatives have been developed (Amit *et al*., 2017). These preservatives in many cases can be toxic due to their continuous consumption,

causing in some cases long-term diseases. Which is why the consumption of less processed foods with fewer preservatives or from natural sources is a current tread (Gutiérrez-Cortés and Suarez, 2014).

Biotechnology is focused on food with alternatives for conservation. Bioactive molecules obtained from plants (essential oils, secondary metabolites, extracts), hive products or bacteria are capable of inhibiting or slowing down the growth of microorganisms that cause deterioration (Ferreira *et al*., 2021). One of the main groups of bacteria that are sought after for applications in food biopreservation are LABs because they are considered as GRAS (Generally recognized as safe), which allows them to be applied directly to food or in separate products. Figure 5 shows some sources of LAB used in bioprospecting processes.

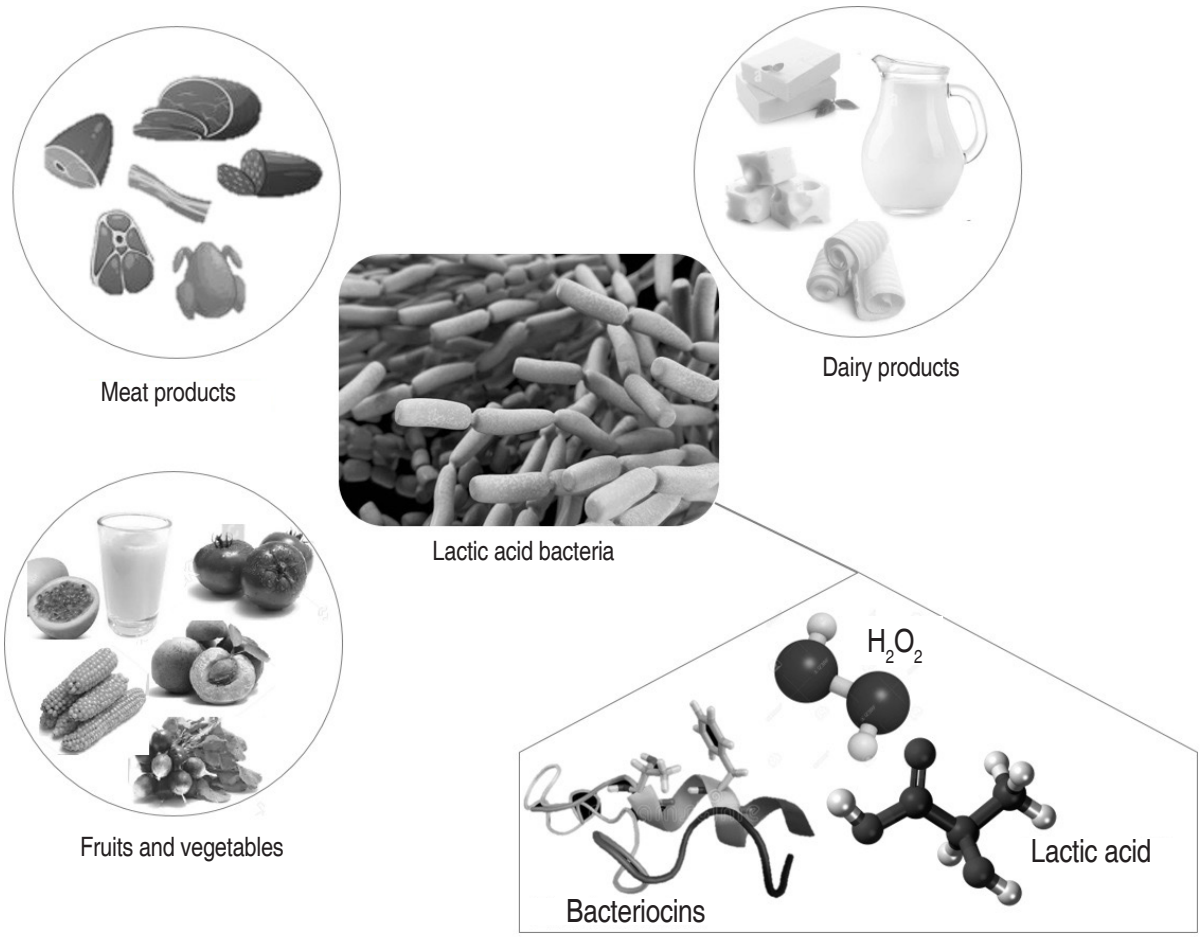

Figure 5. Main sources of LAB and main antimicrobial metabolites with biopreservation potential.

Because of the ubiquity of LABs, it is useful to characterize the microbiota of fermented food producing regions through bioprospecting protocols for a natural source of genes to develop new initiating strains with antagonistic traits of great interest in food (Topisirovic *et al*., 2006). In general, the use of LAB as biopreservatives is an alternative of direct preservation since they eliminate competing microbiota, improving the characteristics of the product and extending the shelf-life without carrying out processes of separation of antimicrobial compounds. However, acidification of the fermented products or the flavors produced because the microbial metabolites, sometimes are not desirables, so another alternative is purification, which can be added directly, such as lactic acid and bacteriocins (Gutiérrez-Cortés *et al*., 2018).

The use of LAB directly in food can give probiotic characteristics to the product (Giraffa, 2012), which must be done in the logarithmic or stationary phase at a concentration between 10^8 and 10^9 CFUs when consumed. They must be able to survive the storage conditions of the product, as well as the pH, water activity, carbon, nitrogen, minerals and oxygen (Rivera-Espinoza and Gallardo-Navarro, 2010). LABs are widely known for their presence in dairy products; however, there are other food matrices that can be fermented or be vehicles of probiotic biomass. In the case of fermented meats, LABs must withstand high salt concentrations (15% - a_w: 0.85 – 0.86) and temperatures during production (4 to 7 °C during preparation, 18 to 24 °C during fermentation, and 12 to 15 °C during maturation) and low pH values (4.6 to 5.1) (Ammor and Mayo, 2007).

The LAB species most commonly used as initiators in meat fermentation are *Lb. sakei, Lb. curvatus, Lb. plantarum, Lb. pentosus, Lb. casei, Pediococcus pentosaceus* and *P. acidilactici,* which cause pH reductions below 5.1, preventing the growth of sensitive microorganisms, extending shelf-life, and modifying some sensory properties. LABs reduce pH by coagulating proteins, giving texture to the product and eliminating accompanying microbiota. Later, moisture is reduced by 70–80%, decreasing the aw, which also reduces the microbial load (Ravyts *et al*., 2012). Table 4 shows some examples of LAB applications in the processing of meat and plant-based products. A common denominator of these studies is the release of lactic acid and bacteriocins for the control of food pathogen populations.

Table 4. Examples of some applications of LAB and its metabolites in food.

	Product	Microorganism or metabolite	Pathogen	Reference
Animal origin	Meat	*Lb. sakei*	*L. monocytogenes* and *E. coli* 0157:H7	Pragalaki, Bloukas and Kotzekidou, 2013
	Chicken	*Lactococcus lactis* subsp. *lactis*	*S. aureus* (10^4 – 10^5)	Akbar and Anal, 2014
	Casein plus sorbitol edible film on meat chunks	*Lb. Sakei*	*L. monocytogenes*	Gialamas *et al*., 2010
	Sausage casings	Nisin	*Clostridium sporogenes*	Wijnker *et al*., 2011
	Salmon fillets	Bacteriocins de *Lb. pentosus* 39	*L. monocytogenes* y *Aeromonoas hydrophila*	Anacarso *et al*., 2014
	Vacuum-packed and refrigerated cachama hybrid fillets	Bacteriocin produced by *Lb. plantarum* LPBM10	Mesophiles, psychrotrophs, total and fecal coliforms, reducing sulfite spores and *Salmonella*.	Suárez *et al.,* 2008
	Gluten-activated low-density polyethylene films in sausages	Bacteriocins lactocin 705 and lactocin AL705 produced by *Lb. curvatus* CRL 705	*L. innocua* y *Lb. Plantarum*	Blanco *et al*., 2014
Vegetal origin	Ready-to-eat artichokes	*Lb. paracasei* LMGP22043	*L. monocytogenes, S. enterica* subsp. *enterica*, and *E. coli*	Valerio *et al*., 2013
	Raw vegetable salad: spinach, paprika, coriander, cabbage, turnip, radish, betel leaves, mushrooms, cucumber, cabbage, tomato, carrot, soybeans and radish	Bacteriocin HKT-9 (2,5 kD) of *Lc. Lactis* spp. *Lactis* HKT-9	*Aeromonas* sp. and *S. aureus* ATCC 9144	Kumar *et al*., 2012

Likewise, these matrices are used as a vehicle for probiotic microorganisms as an alternative to dairy products. Sheehan *et al*. (2007) evaluated the viability of *Lb. salivarius, Lb. paracasei, Lb. rhamnosus, Lb. casei*, and *Bifidobacterium animalis* in heat-treated orange juice (pH 3.65) (90 and 76 °C) and pineapple

juice (pH 3.4). Pineapple juice inhibited the growth of *Lb. rhamnosus* and *Lb. casei* (2 and 1 log CFU mL^{-1} respectively) more than orange juice because of its lower pH; however, *Lb. paracasei* ssp. *paracasei* was not affected. *Lb. salivarius* was the most sensitive to acidity, demonstrating the possibility of introducing some strains of LAB in acidic fruit juices to preserve viability, improve the probiotic quality, and extend shelf-life (Sheehan *et al*., 2007). There are several studies that have shown the potential of fruit juices to convey probiotic bacteria while maintaining viability. Most studies were carried out with commercial strains; however, these matrices could be used in post-bioprospecting processes for the development of new products that contain this type of bacteria without using milk. Acevedo-Martínez *et al*. (2018) demonstrated the viability of *Lb. casei* in mango nectar using FOS 5% as a prebiotic substance and the acceptance of the product by a panel of consumers. Bernal-Castro *et al*. (2019) evaluated the viability of a commercial strain of *Lb. casei* in a drink prepared with red fruits and 1% inulin.

Hive products such as propolis have also been used as bioconservative substances in different food matrices. Studies demonstrated how propolis can replace synthetic preservatives to increase the shelf-life of fish sausages and fillets (Gutiérrez-Cortés and Suarez 2014; Suarez *et al*., 2008). Propolis can also be used in microencapsulates for edible films that prolong the shelf-life of fish fillets, as demonstrated in a study by Piedrahita *et al*. (2018). In addition, a mixture of microencapsulated propolis and chitosan were also used to prolong the shelf-life of packaged pork, using electrospun polycaprolactone in linear low-density polyethylene (Vargas *et al*., 2021).

CONCLUSION

Faced with the global targets for sustainable development, it is very important to take into account the role of bioprospecting throughout the food production chain. Food production increasingly requires new technologies and increases in capacity. That increment has to be developed in a responsible way, then the reduction of agrochemicals and additives is a goal. Taking into account that agriculture activities are responsible of an important part of pollution of soils and water as a result of the pest control or fertilizations, it is necessary the use of natural substances that recover soil and bodies of water and controlling organisms that affect crops and that cause post-harvest deterioration. There are more and more applications of native microorganisms and bioactive compounds in the production and transformation of food, which is why, in Colombia, this initiative is increasing. While there have been advances in bioprospecting in Colombia, these efforts also need to be directed towards the agricultural industry.

CONFLICT OF INTEREST STATMENT

All authors declare that they have no conflicts of interest

ACKNOWLEDGMENTS

This work was supported by the Departamento Administrativo de Ciencia, Tecnología e Innovación – (MINCIENCIAS), call No. 848 of 2019 for postdoctoral fellowship developed in the Instituto de Ciencia y Tecnología de Alimentos (ICTA) of the Universidad Nacional de Colombia. Also, this article is a derivation of the undergraduated thesis of Claudia Patricia Cubides Guerra entitled “La bioprospección como alternativa para la disminución de la contaminación ambiental producida por agroquímicos: aproximación del estado actual en Colombia (2021) for the Envirnomental Engineering program of the Universidad Nacional Abierta y A Distancia (UNAD).

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Revista Facultad Nacional de Agronomía

UNIVERSIDAD NACIONAL DE COLOMBIA

Space-time analysis of scientific research on *Brosimum alicastrum* Swartz

Análisis espacio temporal de la investigación científica sobre *Brosimum alicastrum* Swartz

https://doi.org/10.15446/rfnam.v76n1.101008

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Keywords:
Bibliometric analysis
Co-authorship networks
Mayan nut
Mexico
Ramón tree
Silviculture

ABSTRACT

Brosimum alicastrum is a forest species of broad natural distribution in southeastern Mexico, with high potential for animal and human diets, although with incipient forest management. The objective of this study was to analyze the spatial-temporal evolution of basic and applied research where *B. alicastrum* was the object of study; through a bibliometric analysis of the texts available in the main editorial houses; to identify research areas that are not developed. In 308 texts found from 1883 to 2020, spatial-temporal evolution showed an exponential growth that concentrated the highest productivity from 2002 to 2020 (222 texts) in countries of the Americas. For the case of Mexico, it was found that the research was focused the southeast, which coincides with the natural distribution of the species. However, this research had a low impact (measured by the number of bibliographic citations) as a result of the publication in journals edited in Spanish, while impact journals are led by English-speaking countries, in English. Therefore, the research about *B. alicastrum* in Latin America has a broad margin of improvement through the publication of texts in English and in journals of greater impact, through the development of research areas that have been slightly explored such as silviculture of the species with special emphasis on its propagation, management in nursery, and forest plantations, which can contribute to food security in each country by ensuring the prime material of an emerging food agro-industry.

Palabras clave:
Análisis bibliométrico
Redes de coautoría
Nuez Maya
México
Árbol Ramón
Silvicultura

RESUMEN

Brosimum alicastrum es una especie forestal de amplia distribución natural en el sureste de México, con alto potencial para la alimentación animal y humana, pero con incipiente manejo silvícola. El objetivo de este trabajo fue analizar la evolución espacio-temporal de la investigación básica y aplicada donde *B. alicastrum* fue objeto de estudio; mediante un análisis bibliométrico de los textos disponibles en las principales casas editoriales; para identificar áreas de investigación no desarrolladas. Se encontraron 308 textos de 1883 a 2020 cuya evolución espacio-temporal mostró un crecimiento exponencial que concentro la mayor productividad de 2002 a 2020 (222 textos) en países de América. Para el caso de México se encontró que la investigación se focalizó en el sureste, que coincide con la distribución natural de la especie. Sin embargo, esta investigación tuvo un bajo impacto (medido por el número de citas bibliográficas) como resultado de la publicación en revistas editadas en español, cuando las revistas de impacto están lideradas por países anglosajones, en inglés. Por lo que la investigación sobre *B. alicastrum* en Latinoamérica tiene un amplio margen de mejora a través de la publicación de textos en inglés y en revistas de mayor impacto, mediante el desarrollo de áreas de investigación poco exploradas como la silvicultura de la especie con especial énfasis en su propagación, manejo en vivero y plantaciones forestales, lo que puede contribuir a la seguridad alimentaria en cada país al garantizar la materia prima de una agroindustria de alimentos emergente.

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Received: October 12, 2022; Accepted: November 06, 2022

Rev. Fac. Nac. Agron. Medellín 76(1): 10247-10261. 2023

ISSN 0304-2847 / e-ISSN 2248-7026

Brosimum alicastrum Swartz, known commonly as Ramón, ojite, ojuch, mojú and ox (Maya term), is a tree that is native to Mesoamerica and the Caribbean with broad distribution in Mexico (Peters and Pardo-Tejeda, 1982). It is appreciated because it has foliage with high nutritional content, primarily for bovine and caprine livestock, and because of its availability during drought periods (Rojas-Schroeder *et al.,* 2017). *B. alicastrum* represents an ecologically important element in the floristic composition of low and medium tropical forests in southern Mexico (Gutiérrez-Granados and Dirzo, 2009).

In Mexico, the Maya culture cultivated and consumed the fruits *of B. alicastrum*, which were even known as the Maya maize due to the importance in the diet represented by this species for the culture (Meiners *et al.,* 2009). The seed, foliage, latex and wood of *B. alicastrum* has a high economic potential, both for the diet (animal and human) and medicinal and cultural uses (Ramírez-Sánchez *et al.,* 2017; Domínguez-Zarate *et al.,* 2019). The forage (leaves) has 14% crude protein, 3.9% ether extract or fat, 13% ash or minerals and 39% crude fiber. In human nutrition, Ramón seed has proven to be a food high in protein 11.5% and dietary fiber 13% (Sarmiento-Franco *et al.,* 2022). However, even with this importance, currently the species is distributed mostly naturally, with virtually no forest management (Santillán-Fernández *et al.,* 2021a).

Due to its properties in the restoration of degraded soils, *B. alicastrum* was included in 2019 as a priority species in Mexico's federal program called Sowing Life (Sembrando Vida) which is coordinated by the National Forestry Commission (Comisión Nacional Forestal) and whose objective is to propagate the species with the purpose of reforestation (CONAFOR 2021). In addition, facing a context of climate change and food security, *B. alicastrum* has become a widely used local resource for animal and human diets (Ramírez-Sánchez *et al.* 2017). Faced with these scenarios, the demand for specimens (plant and fruit) of *B. alicastrum* has increased, and with that, the need to generate research regarding forestry topics of the species (Santillán-Fernández *et al.* 2020, Pedraza-López 2021).

According to Santillán-Fernández *et al.* (2021b), bibliometric techniques are the most adequate to detect research gaps of a topic in particular where new knowledge must be generated, since they allow generating indicators and mathematical models to characterize the development and evolution of the frequency and quality of texts published around the topic of interest (Malesios and Arabatzis, 2012). The publication of a scientific text is the most effective way to transmit knowledge acquired as a consequence of the research, and its visibility is important for the researchers themselves, the institutions where they work, and for the organizations that finance the research (Sanz-Valero and Wanden-Berghe, 2017).

In the forest sector, bibliometric studies have been conducted for specific topics such as silviculture (Polinko and Coupland, 2020), community forest development (Bullock and Lawler, 2015), use of drones in the determination of forest biomass (Raparelli and Bajocco, 2019), and even to evaluate national forest systems as in the case of India (Hazarika *et al.,* 2003) and Bangladesh (Miah *et al.,* 2008). In addition, evaluating the scientific productivity of forest researchers in Tanzania (Sife *et al.,* 2013) and India (Parabhoi *et al.,* 2017), and of high impact journals in the Journal Citation Reports as Forests (Uribe-Toril *et al.,* 2019).

In Mexico, studies have used bibliometric techniques, such as those by Martínez-Santiago *et al.* (2017) in forest models; Vargas-Larreta *et al.* (2017) in forest biometry for the integral management of forests; Reyes-Basilio *et al.* (2020) in the evaluation of growth rings to estimate the carbon fixation potential; Gallardo-Salazar *et al.* (2020) in the use of drones for forest management; and Ayala-Montejo *et al.* (2020) who identify the research needs about carbon and nitrogen dynamics in agro-forestry systems.

However, these studies analyze broad topics in the forestry sector, and they are not centered on the evaluation of a species in particular. The objective of this study was to analyze the spatial-temporal evolution of basic and applied research about *B. alicastrum* through the bibliometric analysis, to identify areas of opportunity in research that have not been developed.

MATERIALS AND METHODS

Origin of the Information

In this study, only research where the species *B. alicastrum* was the object of study were considered. The studies where

the species mentioned but there was not an analysis or description of were omitted. Elsevier, Springer and Scopus were reviewed, databases of free access journals articles (Latindex, Scielo, Redalyc, Clarivate Analytics, Periodica, Directory of Open Access Journals, and Conricyt), and the free access search engine Google Scholar. The information compiled was complemented with the references available in the book "Publications about *Brosimum alicastrum*" by Vergara-Yoisura *et al.* (2014). The texts were gathered from February to May 2021, taking into account the texts available until 2020.

The keyword used in the search was *Brosimum alicastrum* identifying it in titles and keywords of the publications. In addition, the "snowball" technique was used to obtain the remaining texts, from the list of references of studies found initially (Leipold, 2014). However, since the snowball technique is considered to be a non-probabilistic technique it can present biases in text recovery, since it is more likely that the studies in English are cited (Streeton *et al.,* 2004); it was decided to use the scientific name of the species as a keyword, which allows capturing most of the relevant publications.

Bibliometric Indicators

The variables analyzed from each of the texts were: editing institution, country of editing, the language of publication, and for the case of the scientific articles the name of the journal was also considered, which served to determine the profile of the institutions that publish similar studies to the topic of *B. alicastrum*. The variables: first author and collaborators served to understand the network of authors involved in the research; year to place the information in a temporal line; institution of the first author and country of origin of the first author to evaluate the frequency of publications of the institutions by country.

The postal code of the institution of the first author served to determine the geographic location of the institution of origin of the information, and in the cases where the postal address did not appear, the name of the institution was found Google Earth® tools, and in the official webpages of the institutions. The title, abstract and keywords were used to categorize the topic that addresses the publication according to the classification of the National Consortium of Scientific and Technological Resources (CONRICYT) (CONACYT, 2021a) for *B. alicastrum*. Finally, the impact of the publications was determined the number of citations.

For the classification of the texts by area of research, the topics were arranged into nine categories: 1) Ethnography: where texts that relate the species with the Maya culture, studies of paleontology, and history were included; 2) Rural development: texts where the localities have achieved some growth from the use of *B. alicastrum*, value chain, productive reconversion, economy, and sustainable use; 3) Potential industrial uses: such as the generation of ethanol, medicines, biopolymers, and quality of the wood; 4) Botany: taxonomic, genetic and physiological description of the species; 5) Ecology and anthropic impact: conservation of ecosystems, floristic composition of ecosystems, effects of the species in the soils, impact of anthropogenic activities, resilience of the species, and environmental services (carbon capture, water balance, and temperature regulation); 6) Animal diet: in domestic species such as cattle, sheep, pork, goat, rabbit and chicken; 7) Forestry: viability and storage of *B. alicastrum* seeds, evaluation of the species in different nursery conditions, silviculture, plant tissue culture, evaluation of pests and diseases, plantations and harvest, agro-silvo-pastoral, wood technology, and reforestation; and, 8) Human diet, gastronomy and beverages.

Finally, the texts were also classified according to the type of academic product (CONACYT, 2021a), into: 1) Scientific article, which included texts published in journals with ISSN (International Standard Serial Number); 2) Book, texts published by editorials with ISBN (International Standard Book Number); 3) Thesis: Undergraduate, Master's and Doctorate's; 4) Manual, including those documents without ISSN or ISBN where instructions are set out for the collection, dissemination and harvest of the species; 5) Complete congress proceedings; 6) Dissemination work, which included newspaper articles, interviews and online texts where opinions or advances are expressed that have not been subjected to a process of scientific review; and 7) Report, which included studies that only describe topics around the species, and were reported as products of research projects.

The capture of variables for the bibliometric analysis was done in a spreadsheet. The original language of each

of the texts was respected. During the capture of all the information, some records were standardized, because the information available in the texts was sometimes incomplete or presented with variables (Aguado-López *et al.,* 2009). In addition, special characters were eliminated to ease the analysis, such as: ñ (for n), accents, superscript, subscript, ®, ©, among others.

Analysis with text mining

With the help of the RcmdrPlugin.temis complement of the statistical software R (Bouchet-Valat and Bastin, 2013), the number of texts and bibliographic citations were obtained by year, type of text, category of research, and country of origin of the first author. For the case of Mexico, the frequency of texts by the institution of the first author was also obtained.

Network Analysis

With the Sci2tool software (Börner, 2011), the interactions present between the first authors and collaborators were analyzed with the aim of understanding the consistency in the researcher's work; that is, evaluating if he/she has published only one year or else has been publishing constantly through time, which gives an idea of his/her consolidation in the topic of *B. alicastrum*. The syntax used in the Sci2tool software was Extract bipartite Network, and for its visualization the Gephi software was used (Bastian *et al.,* 2009).

Finally, of the variable postal code and Google Earth® tools, the geographic coordinates were obtained in tenth degrees (longitude, latitude) of the institution of the first author of each of the texts analyzed. The spatial representation of the number of articles per country was carried out with the geographic package ARGIS® (ESRI, 2021). For the case of Mexico, the potential distribution area of *B. alicastrum* obtained by Santillán-Fernández *et al.* (2021a) was spatially associated with the frequency publication of the first author's institutions and the research category per institution.

RESULTS AND DISCUSSION

Scientific production at the international level where the *B. alicastrum* species was object of study

From 1883 to 2020 a total of 308 texts were published where the forest species *B. alicastrum* was the topic of analysis; this scientific production gave rise to 9622 bibliographic citations (Figure 1). The first study recorded dates from the year 1883; however, since the year 2000 a growing production was found for the topic of *B. alicastrum*. The period of greatest productivity was from 2002 to 2020 with 72.08% of the total (222 texts), which contributed to an exponential trend in the increase of publications (R^2=0.648). The most cited studies where the ones published in the period 2002-2012 which as whole represented 5594 bibliographic citations (58.14% of the total).

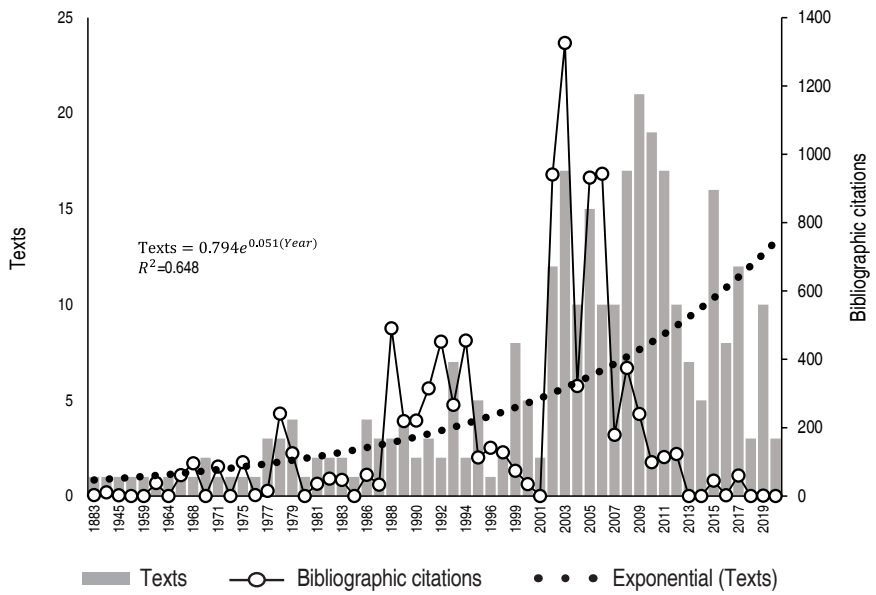

Figure 1. Temporal evolution of scientific texts and bibliographic citations where the species *B. alicastrum* was object of study from 1883 to 2020.

Vergara-Yoisura *et al.* (2014) attribute this growth in the number of publications to the diversity of uses of the species particularly in the animal and human diets, which has caused *B. alicastrum* to be a recurring research topic in southeastern Mexico and Central America. In this regard, Santillán-Fernández *et al.* (2020) found that within a context of food security, *B. alicastrum* represents an alternative for food generation. However, Santillán-Fernández *et al.* (2021c) found that more research is required about the silviculture of the species to ensure constant production of foods, since it is currently a species that is distributed mostly naturally with null forest management.

Regarding the country of origin of the first author in scientific texts, 308 studies were originated in 23 countries. Of them, 84.74% (254) were concentrated in six countries: Mexico (43.83%, 135 texts), USA (United States of America, 25.65%, 79), Guatemala (4.55%, 14), Costa Rica (2.92%, 9), Honduras (2.92%, 9) and El Salvador (2.60%, 8). The fact that the countries with the greatest scientific production are the countries of Latin America where the species *B. alicastrum* is native stands out (Peters and Pardo-Tejeda, 1982) (Figure 2). Figure 2 also shows that research has been developed around the species in Europe, Africa and Oceania, which helps to explain that 47.40% (146 texts) are published in English and 52.60% (162) in Spanish. This fact is interesting because according to Santillán-Fernández *et al.* (2021b), the researchers in Latin America publish mostly in Spanish, which lowers the impact of the publications (measured by the number of bibliographic citations).

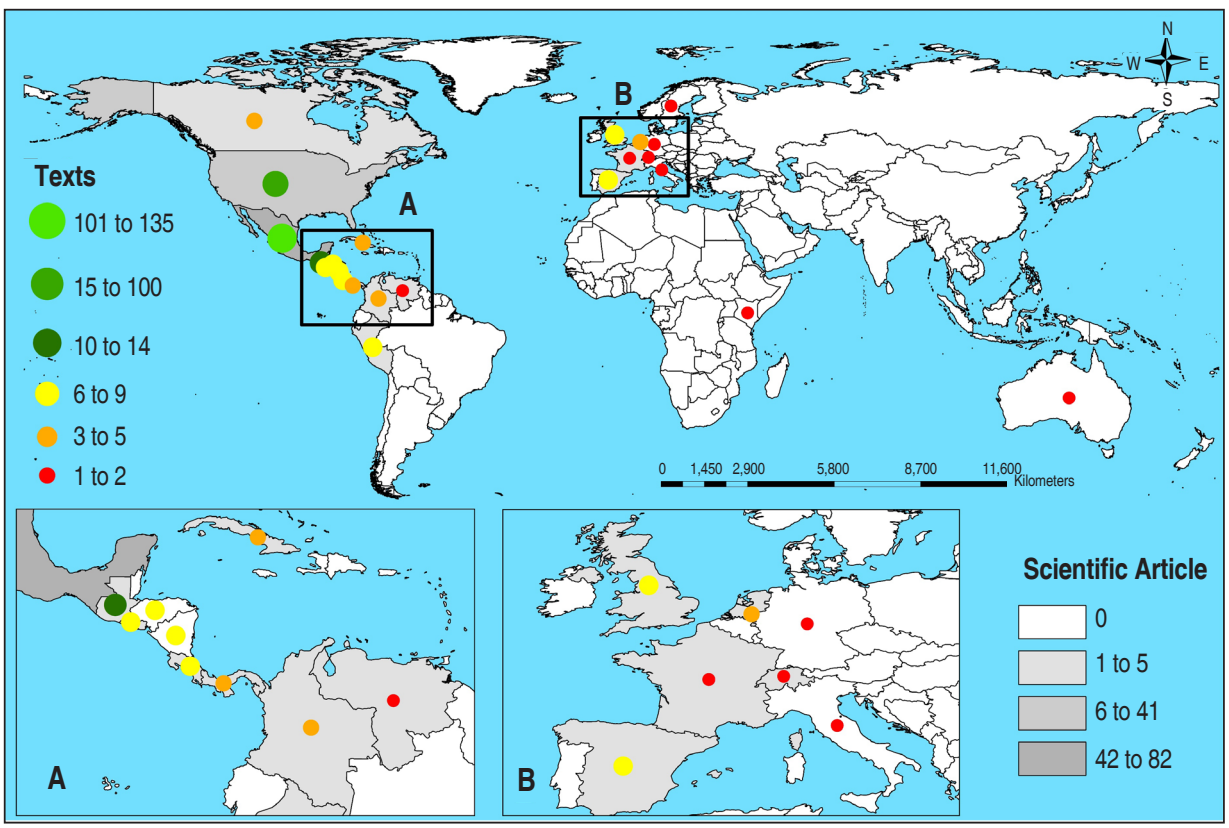

Figure 2. Spatial location at the international level of the productivity of texts where the species *B. alicastrum* was the object of study from 1883 to 2020. A: Latin American countries; B: European countries.

In the 308 texts analyzed, 251 different first authors were found, and between the first author and the coauthors they added 491 different individuals. The network of authors and coauthors (Figure 3) was made up of 491 nodes (authors) and 411 corners (links). The links in a co-authorship network analysis are important because through them an author can reach certain ideas, knowledge and information that are socially distant for

him (Granovetter, 1973). The density of the network had a value of 0.002, which implies that for the topic of *B. alicastrum* there is not much collaboration between authors. The density is an indicator in the co-authorship network analysis that implies that the nodes interact between each other (they are linked); mathematically it is a value within the interval [0 to 1], and the closer it is to 1 the interaction in the network is higher (Aguilar-Gallegos *et al.,* 2016).

The low connection of the authors in the research network of *B. alicastrum* was exposed when a co-authorship mean of 1.59 was found and a mode (139) of one author per text; in addition, 56 texts presented two authors, and only 23 texts were developed by six authors or more. In addition, 185 institutions (from 308 texts) were found, pointed out as the adscription of the first author. According to Santillán-Fernández *et al.* (2021c), the low connection between authors is explained by the null forest management of the species, which limits the knowledge about its potential uses and promotes the development of the focalized study since it is an emerging topic.

The institutions with the highest frequency ($\geq$10 scientific texts) were institutions in Mexico, located in the south of the country, where the highest abundance of the species is concentrated (Santillán-Fernández *et al.,* 2021a): 1) UNAM (Universidad Nacional Autónoma de México), 17 texts, principal author Gomez_Pompa_A, who developed studies about the botanical description of *B. alicastrum*; 2) UADY (Universidad Autónoma de Yucatán), 14, Sarmiento_Franco_L, whose studies describe potential uses of the species in the animal diet; and 3) CICY (Centro de Investigaciones Científicas de Yucatán), 13, Larque_Saavedra_FA, whose studies describe potential uses of the species in the human diet. It should be highlighted that Larque_Saavedra_FA was the author with the most contributions with a total of 15 (four as principal author).

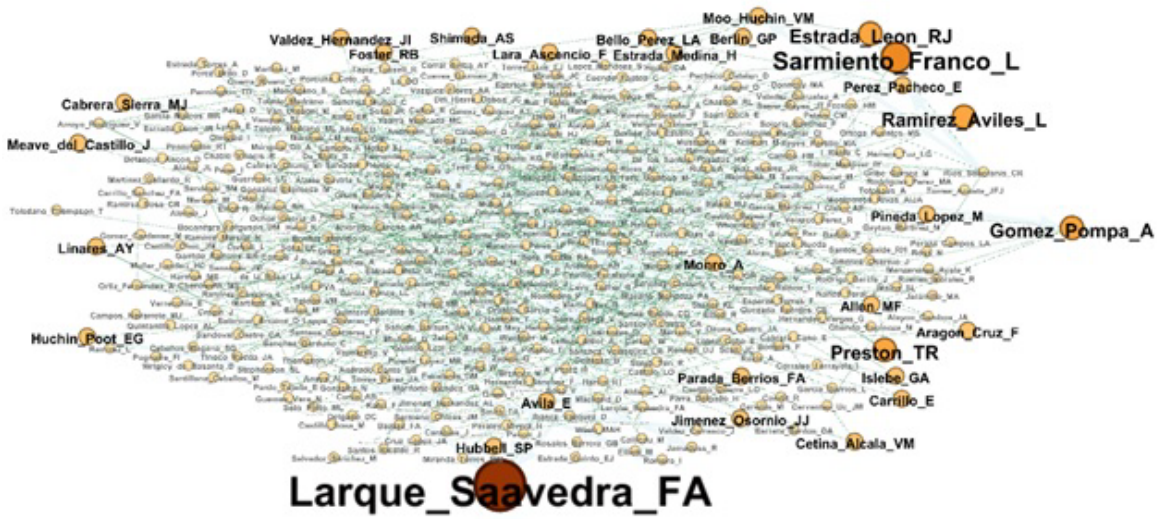

Figure 3. Network of authors and coauthors at the global level who have developed studies where the species *B. alicastrum* was the object of study from 1883 to 2020. The size of the node corresponds to its productivity.

Bibliometric Indicators

From the 308 texts analyzed, 49.68% (153) were scientific articles that as a whole reached 89.95% (8655) of the total bibliographic citations (Table 1). According to Bravo-Vinaja and Sáenz-Casado (2008), from the statistical validation of the studies and the peer review that gives feedback with constructive criticism of the research, scientific articles have a higher probability of being taken as reference to generate new knowledge. Table 1 shows that the first published study about the topic of *B. alicastrum* was a book in 1883. Since 1935, a constant production of scientific articles was observed, in contrast of texts such as manuals and reports. Santillán-Fernández *et al.* (2021c) attribute

this phenomenon to the incipient research developed about the species, which has made researchers seek to publish their findings as scientific articles to generate a greater impact their results.

Table 1 also shows that the studies with the highest number of bibliographic citations per type of text were published in English. According to Li and Zhao (2015) the publication of scientific texts in a language other than English limits the number of bibliographic citations, since English is the language adopted as universal by the scientific community. The fact stands out that none of the studies most frequently cited by type of text was developed by researchers in Mexico, where the species *B. alicastrum* is native and widely distributed (Peters and Pardo-Tejeda, 1982). Santillán-Fernández *et al.* (2021b) found that the low relevance of the research in Mexico (measured by the number of citations) is due mostly to the publication of the studies in Spanish.

From the studies with the highest number of bibliographic citations by type of text **(**Table 1), it was found that the scientific article (532 bibliographic citations) that corresponds to a study where the benefits of the species *B. alicastrum* for reforestation of degraded spaces are described. For the case of the manual (343), methodologies about the sexual propagation of the species are described; and regarding Doctorate Thesis (158), potential uses of the species in the animal and human diets are addressed. Vergara-Yoisura *et al.* (2014) found that there is a broad margin of action to develop research about the species *B. alicastrum*, particularly in topics of forest management, because presently the distribution of the species is completely natural with practically null forest management.

The research studies where more knowledge regarding the species *B. alicastrum* has been developed were ecology (18.5%, 57 texts), forestry-reforestation (15.26%, 47), botany (13.31%, 41), animal diet (12.01%, 37), human diet (11.04%, 34) and potential uses (11.04%, 34) (Table 2). These results agree with what was reported by Santillán-Fernández *et al.* (2021c), who found that the topics related ecology and botany of the species have been the most developed given its incipient forest management.

However, when analyzing the temporality of the studies (Table 2), it was found that the topics associated animal and human diets, as well as the description of potential uses in medicine and as fuel, were the first research topics developed (1883-2020); this fact is explained by the influence that *B. alicastrum* had as food in the flourishing of Maya culture that settled in southeastern Mexico (Vergara-Yoisura *et al.,* 2014). On the contrary, the topics silviculture and nursery were the most recent research areas to be developed (1987-2018), because the species is distributed mostly naturally (Santillán-Fernández *et al.,* 2021a), which according to Santillán-Fernández *et al.* (2021c) are presented as areas of opportunity to generate new knowledge about forest management of the species.

Scientific Articles

From the 308 texts analyzed, 153 were scientific articles that were published in 97 scientific journals. Of the scientific articles, 28.76% (44) were concentrated in 10 scientific journals, which also represented 30.97% (2681) of the bibliographic citations for scientific articles (Table 3). Among these 10 main journals, 9 were from American countries: Mexico (4), USA (4), and Venezuela (1). For the case of the journals in Mexico and Venezuela, they mostly published in Spanish and did not have JCR (Journal Citation Reports) impact factor or had a low factor that placed them in the categories Q3 and Q4. The journals with the highest number of bibliographic citations published in English and showed JCR impact factors higher than 1, which allowed them to be placed in categories Q1 and Q2 (WoS, 2021).

Table 3 shows that in journals of Latin American countries, topics associated with forestry and livestock production topics have been published, and in journals of English-speaking topics related the ecology of the species. Santillán-Fernández *et al.* (2020) found that the current research about *B. alicastrum* is focused on evaluating its properties and potential uses, which is why the development of research in forestry topics constitutes an area of opportunity (Santillán-Fernández *et al.,* 2021c), to guarantee the prime material of an emergent livestock agroindustry around *B. alicastrum* due to its high potential in the diet of porcine, bovine, ovine, poultry and aquatic species (Rojas-Schroeder *et al.,* 2017) within the

Table 1. Frequency and bibliographic indicators of the most cited studies per type of text where the species *B. alicastrum* was the object of study from 1883 to 2020.

Category	Frequency		Citations		Period	Most cited study					
	Number	%	Number	%		References	Year	Language	Institution of first author	Country	Citations
Scientific Article	153	49.68	8655	89.95	1935-2020	Padilla and Pugnaire (2006)	2006	English	Consejo Superior de Investigaciones	Spain	582
Manual	12	3.90	343	3.56	1970-2010	Peters (1994)	1994	English	New York Botanic Garden	USA	343
Book	18	5.84	214	2.22	1883-2018	Langman (2018)	2018	English	University of Pennsylvania	USA	67
Doctorate Thesis	6	1.95	193	2.01	1989-2009	Zahawi (2003)	2003	English	University of Illinois	USA	4
Master's Thesis	10	3.25	118	1.23	1968-2015	Puleston (1968)	1968	English	University of Pennsylvania	USA	6
Reports	41	13.31	76	0.79	1984-2016	Benavides (1999)	1999	Spanish	FAO	Costa Rica	74
Dissemination Studies	35	11.36	21	0.22	1945-2014	Orwa *et al.* (2009)	2009	English	World Agroforestry Centre	Kenya	21
Congress Proceedings	6	1.95	0	0.00	2006-2015	Undefined	----------		----------	----------	----
Undergraduate Thesis	27	8.77	2	0.02	1949-2018	Turcios and Castañeda (2010)	2010	Spanish	Escuela Agricola Panamericana	Honduras	2
Total	308	100.0	9622	100.0							

Table 2. Temporality and frequency per type of text and area of research of studies where the species *B. alicastrum* was the object of study from 1883 to 2020.

Category	Ecology	Botany	Ethnography	Diet		Potential Uses	Rural Development	Forestry				Total	
				Human	Animal			Reforestation	Silviculture	Nursery	Others	Number	%
	1962-2019	1945-2020	1971-2017	1935-2019	1949-2020	1883-2019	1979-2016	1970-2020	1987-2018	2002-2016	1945-2020		
Scientific Article	35	23	9	10	24	13	3	20	8	5	3	153	49.68
Reports	5	3	0	7	4	1	8	12	1	0	0	41	13.31
Book	3	3	0	3	0	6	0	1	1	0	1	18	5.84
Manual	2	3	0	0	0	0	1	6	0	0	0	12	3.9
Congress Proceedings	1	0	0	0	1	0	0	3	1	0	0	6	1.95
Doctorate Thesis	2	1	1	0	0	0	1	0	1	0	0	6	1.95
Undergraduate Thesis	2	2	0	7	5	5	2	1	1	2	0	27	8.77
Master's Thesis	1	2	0	0	3	2	1	0	1	0	0	10	3.25
Dissemination Studies	6	4	2	7	0	7	2	4	2	0	1	35	11.36
Total (Number)	57	41	12	34	37	34	18	47	16	7	5	308	
Total (%)	18.51	13.31	3.91	11.04	12.01	11.04	5.84	15.26	5.19	2.27	1.62		100

framework of food security (Ramírez-Sánchez *et al.* 2017) and climate change (Santillán-Fernández *et al.,* 2021a).

Among the 10 most frequently cited studies about the species *B. alicastrum*, seven belong to a first author whose

Table 3. Bibliometric indicators of the main journals that published scientific articles at the international level where the species *B. alicastrum* was the main topics of study from 1883 to 2020, ordered according to the number of articles published.

Name	Country	Institution	(WoS 2021)	Topics	Language	Articles	Citations	
						Number	Number	%
Journal of Tropical Ecology	United Kingdom	Cambridge University Press	1.163 (Q4)	Ecology	English	6	491	5.67
Biotrópica	USA	Association of Tropical Biology and Conservation	2.091 (Q2)	Ecology	English	5	619	7.15
Oecología	USA	International Association for Ecology	2.654 (Q2)	Ecology	English	5	610	7.05
Acta Botanica Mexicana	Mexico	Instituto de Ecologia	0.35 (Q4)	Botany	Spanish	5	182	2.10
Tropical and Subtropical Agroecosystems	Mexico	Universidad Autónoma de Yucatán	0.16 (Q4)	Forestry	Spanish	5	12	0.14
American Antiquity	USA	Society for American Archaeology	1.988 (Q1)	Archaeology	English	4	207	2.39
RCSCFA*	Mexico	Universidad Autónoma Chapingo	0.441 (Q3)	Forestry	Spanish/English	4	25	0.29
Revista Mexicana de Ciencias Forestales	Mexico	INIFAP**	Not available	Forestry	Spanish/English	4	20	0.23
Ecological Applications	USA	Ecological Society of America.	4.248 (Q1)	Ecology	English	3	469	5.42
Zootecnia tropical	Venezuela	Instituto Nacional de Investigaciones Agrícolas	Not available	Livestock	Spanish/English	3	46	0.53
Others (87)						109	5974	69.02

*Revista Chapingo Serie Ciencias Forestales y del Ambiente; ** Instituto Nacional de Investigación Forestal, Agricola y Pecuaria

institution of adscription is in the USA and only one study corresponds to a researcher in Latin America (Panama); they have all been published in English and in journals of English-speaking origin with impact factors higher than 2 (Q1 and Q2) (WoS 2021) (Table 4). However, the fact stands out that the study areas are located spatially in Latin America: Mexico (4), Panama (2), Guatemala (1) and Costa Rica (1), where the species presents a broad natural distribution (Peters and Pardo-Tejeda, 1982).

According to Gersbach and Schneider (2015), countries with consolidated economies such as the USA invest more in their research centers, which allows them to develop studies outside their borders and to achieve greater technological development, compared to underdeveloped economies such as the Latin American where the investment in research is lower. Therefore, strengthening international co-authorship networks constitutes a viable option to generate new knowledge in regions of interest with external investments (Aguado-López *et al.,* 2009).

Table 4. Bibliometric indicators of the main texts where the species *B. alicastrum* was a topic of study from 1883 to 2020, ordered according to the number of bibliographic citations.

Scientific Article						First Author			Area of Study
Title	Topics	Journal	(WoS, 2021)	Institution*	Citations	References	Institution	Country	
The role of nurse plants in the restoration of degrade environments	Forestry-Reforestation	Frontiers in Ecology and the Environment	9.295 (Q1)	Ecological Society of America	582	Padilla and Pugnaire (2006)	Consejo Superior de Investigaciones	Spain	Review
Structure and floristic composition of the lowland rain forest of Los Tuxtlas, Mexico	Botany	Journal of Vegetation Science	2.698 (Q1)	International Association for Vegetation Science	408	Bongers *et al.* (1988)	University of Utrecht	Netherlands	Mexico
Sustainable harvest of non-timber plant resource in tropical moist forest: an ecological primer **	Forestry-Silviculture			New York Botanic Garden	343	Peters (1994)	New York Botanic Garden	USA	Undefined
Light gradient partitioning by tropical tree seedlings in the absence of canopy gaps	Forestry-Nursery	Oecología	2.654 (Q2)	International Association for Ecology	352	Montgomery and Chazdon (2002)	University of Connecticut	USA	Costa Rica
Recruitment near conspecific adults and the maintenance of tree and shrub diversity in a neotropical forest	Ecology	American Naturalist	3.855 (Q1)	The American Society of Naturalists	332	Condit *et al.* (1992)	Smithsonian Tropical Research Institute	USA	Panama
Folkecology, Cultural Epidemiology, and the Spirit of the Commons	Ethnography	Current Anthropology	2.037 (Q1)	University of Chicago Press	333	Atran *et al.* (2002)	University of Michigan	USA	Guatemala
The impact of hurricane Gilbert on trees, Litterfall, and woody debris in a dry tropical forest in the northeastern Yucatán Peninsula.	Ecology	Biotropica	2.091 (Q2)	Association for Tropical Biology and Conservation	255	Whigham *et al.* (1991)	Smithsonian Environmental Research Center	USA	Mexico
Annual and spatial variation in seedfall and seedling recruitment in a neotropical forest	Forestry-Seed	Ecology	4.733 (Q1)	Ecological Society of America	207	Wright *et al.* (2005)	Smithsonian Tropical Research Institute	Panama	Panama
Impacts of early-and late-seral mycorrhizae during restoration in seasonal tropical forest, México.	Forestry-Reforestation	Ecological Applications	4.248 (Q1)	Ecological Society of America	171	Allen *et al.* (2003)	University of California	USA	Mexico
Recovery of biomass following shifting cultivation in dry tropical forests of the Yucatán.	Ecology	Ecological Applications	4.248 (Q1)	Ecological Society of America	170	Read and Lawrence (2003)	University of Virginia	USA	Mexico

*For all the cases, the country of the journal was USA and the publication language English; **The study corresponds to a Manual.

Scientific production on the topics of *B. alicastrum* in Mexico

From 1949 to 2020 Mexican researchers published 135 scientific texts about the topic of *B. alicastrum*. This productivity represented 43.83% of the total texts found (308), which agrees with the fact that Mexico is the place where the greatest wealth of the species is found in Latin America (Santillán-Fernández *et al.,* 2021a). For the 135 texts, 1763 bibliographic citations were recorded, which represented 18.32% of the total citations (9622), 44 texts did not present bibliographic citations. The texts, 69.63% (94) were published in Spanish and the rest, 30.37% (41), in English. The low level of research (measured by the number of bibliographic citations) that is developed in Mexico has been documented by Santillán-Fernández *et al.* (2021b), Martínez-Santiago *et al.* (2017) and López-Leyva (2011), who found that elements such as the language of publication (Spanish) and the priority in the publication of studies whose authors belong to the same institution that edits the journal, restrict the constructive criticism of peer review and reduce the visibility of the publications.

From the 135 texts, 72 (53.33%) are developed in eight out of 41 institutions, taking as reference the institution of adscription of the first author; 60.74% (82) were scientific articles, and 39.26% (53) other types of texts. The institutions with the highest productivity were: UNAM (16 studies), UADY (15), CICY (13), INECOL (Instituto de Ecologia A. C., 7), UdeG (Universidad de Guadalajara, 6), UV_Tuxpan (Universidad Veracruzana campus Tuxpan, 5), ColPos_Ver (Colegio de Postgraduados campus Veracruz, 5) and ColPos_Camp (Colegio de Postgraduados campus Campeche, 5) (Table 5). According to CONACYT (2021b), these institutions have strengthened their postgraduate programs in the biological and agro-silvo-pastoral sciences in the National Register of Quality Post-Graduate Programs (Padrón Nacional de Posgrados de Calidad, PNCP), which has allowed them to take initiatives for the development of research on the species *B. alicastrum*.

Table 5. Main research institutions in Mexico that published scientific texts on the species *B. alicastrum*.

Institution	Texts			Bibliographic Citations	
	Scientific Article	Others	Total	Number	%
UNAM	11	5	16	636	36.07
UADY	9	6	15	145	8.22
CICY	8	5	13	174	9.87
INECOL	7	0	7	202	11.46
UdeG	1	5	6	59	3.35
UV_Tuxpan	3	2	5	108	6.13
ColPos_Ver	5	0	5	21	1.19
ColPos_Camp	5	0	5	16	0.91
Otras (33)	33	30	63	402	22.80
Total (41)	82	53	135	1763	100.00

The spatial distribution of institutions with productivity of scientific texts on the species *B. alicastrum* (Figure 4) allowed establishing that the study of this topic is located in southeastern Mexico and agrees with the natural distribution area of the species. In contrast with research topics such as transgenic maize (Santillán-Fernández *et al.,* 2021b), estimation of forestry biomass species in forests (Vargas-Larreta *et al.,* 2017), and forest models (Martínez-Santiago *et al.,* 2017), where the research institutions were not spatially located in the areas where the studies were conducted, it was found that this gap made the transference of technology difficult and affected the quality of the studies. According to Santillán-Fernández *et al.* (2021c) for the case of *B. alicastrum*, the fact that the institutions were located in the distribution area of the species can be a factor of success for the generation of new knowledge to have a greater impact.

The spatial representation also allowed differentiating the topics of specialization by research institutions. Vergara-Yoisura *et al.* (2014) consider that with the creation of the CICY in 1979 the development of research around the species *B. alicastrum* was potentiated, which allowed developing areas regarding its potential uses in animals (UADY, UV_Tuxpan, ColPos_Ver and ColPos_Camp) and human diets (CICY), which complemented the research about the botany of the species (UNAM). Rojas-Schroeder *et al.* (2017) and Ramírez-Sánchez *et al.* (2017) consider that the framework of food security of institutions in southeastern Mexico had to develop studies about the local plant resources with alternatives for animal and human diets, and one of the species with the greatest potential is *B. alicastrum*.

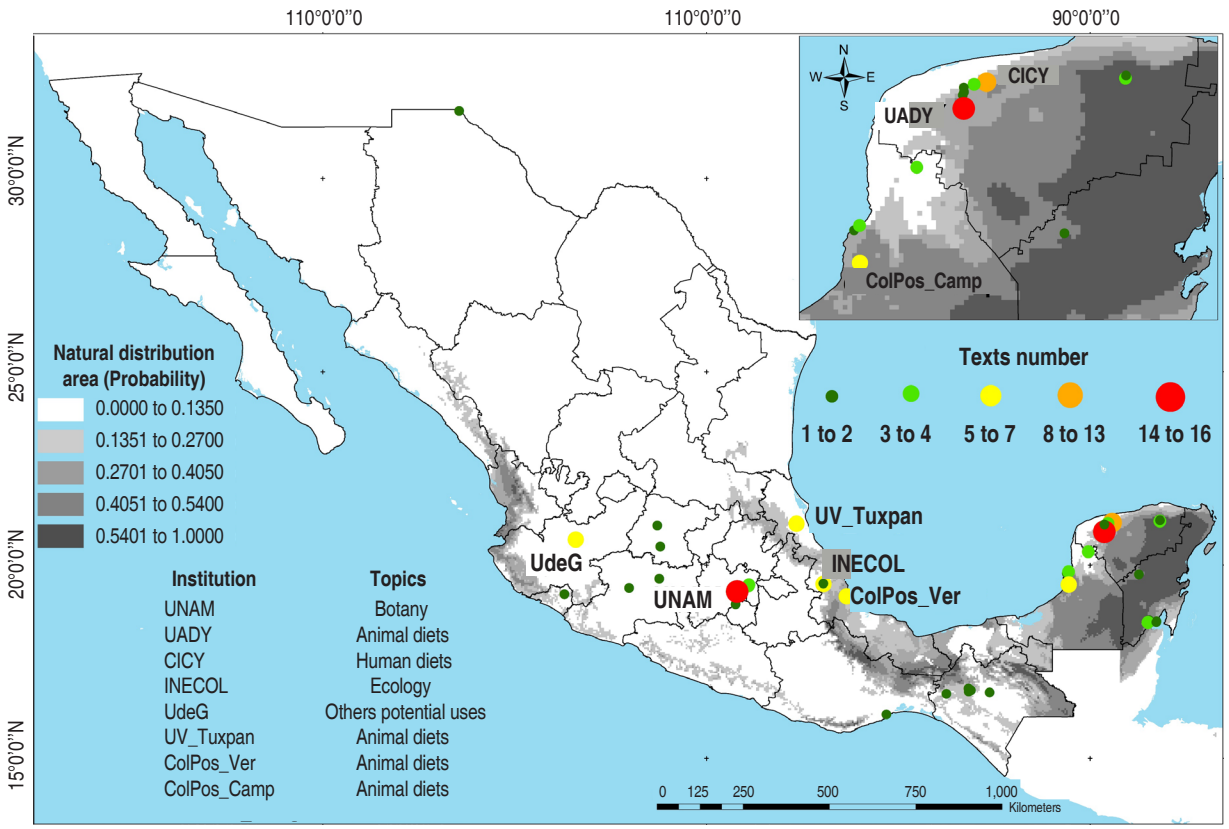

Figure 4. Spatial relationship of the academic and research institutions in Mexico that have developed studies where the species *B. alicastrum* was the object of study from 1883 to 2020, with the potential probability of natural distribution of *B. alicastrum* taken from (Santillán-Fernández *et al.,* 2021a).

However, Santillán-Fernández *et al.* (2021c) consider that the development of research around the species of *B. alicastrum* for the topics of botany, and its uses in animal and human diets, should be complemented with the generation of new knowledge about sexual and asexual reproduction of the species, management in nursery, silviculture, and development of plantations, with an agroindustry of foods where the prime material *B. alicastrum* is guaranteed.

CONCLUSION

The spatial-temporal evolution of scientific production showed an exponential growth of scientific texts worldwide where the forest species *B. alicastrum* was a research topic from 1883 to 2020. The principal productivity was concentrated in countries of the Americas where Mexico (43.83%, 135 articles) and the USA (25.65%, 79) dominated. However, in contrast with the studies developed in the USA, those from Mexico did

not have a relevant impact (measured by the number of bibliographic citations) as a result from the publication in journals edited in Spanish, when the impact journals are led by English-speaking countries, in English. The topics of greatest relevance were those related to ecology (18.51%), reforestation (15.26%), botany (13.31%), and uses in animal diets (12.01%), and human diet (11.04%). This evidenced a research void in topics related with silviculture of the species with special relevance in their propagation, management in nursery and forest plantations. For the case of Mexico, it was found that the research about this topic was focalized in southeastern Mexico, and it was led by UNAM topics of botany, UADY (animal diet) and CICY (human diet). The spatial location of the main research institutions in Mexico coincided with the area of the natural distribution of the species, which can be a factor for success for the generation of new knowledge to have a greater impact, by facilitating the transference of technology, particularly if it is considered that the research around the topic of *B. alicastrum* is incipient.

ACKNOWLEDGMENTS

This study is part of the Master's Thesis of the first author in the BIOSAT Program of the Colegio de Postgraduados campus Campeche, México; and to Project number 364. Sustainable productive reconversion for the development of rural producers in Campeche, assigned to the correspondence author by the Consejo Nacional de Ciencia y Tecnologia (CONACyT). And thanks to the CONACYT for the support through the program to National Laboratories.

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Review article

https://revistas.unal.edu.co/index.php/refame

Revista Facultad Nacional de Agronomía

Abaca: a general review on its characteristics, productivity, and market in the world

Abacá: una revisión general sobre sus características, productividad y mercado en el mundo

https://doi.org/10.15446/rfnam.v76n1.101710

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ABSTRACT

The abaca also known as cañamo of manila, produces a natural fiber that is known as the most resistant worldwide natural fiber, that has properties like resistance to salinity, mechanical strength, flexibility, and durability. The unique characteristics of fiber have caused an increase in its use in many industries. However, the cultivation of abaca is threatened by the presence of *Fusarium oxysporum* f. sp. *cubense*, one of the most important diseases which has been considered the most lethal, due to the scarcity of existing control methods. The article aims to highlight recent data about the characteristics of the plant and its fiber, crop management, productivity, and the market as an approach updating the current knowledge regarding the abaca. It was found that some characteristics of abaca fiber such as strength and physical properties depend on the position of its leaves, on the maturity of the plant, and also on the fiber extraction system. Regarding fiber extraction, it is mentioned that there are two types of methods (stripping and decortication). Concerning productivity, there exists a considerable difference between the ways of bundle extraction, for instance, the hand stripping technique produces about 20 kg of fiber bundles per day. It is concluded that abaca has great potential as a crop that could boost the markets of various countries. Though the information available on this crop is scarce, it is necessary to increase research about its production and management to promote greater use.

Keywords:
Crop management
Extraction
Fiber
Polymeric matrix
Varieties

RESUMEN

El abacá también conocido como cáñamo de manila, produce una fibra natural que es conocida como la más resistente del mundo, tiene propiedades como resistencia a la salinidad, resistencia mecánica, flexibilidad y durabilidad. Las características únicas de la fibra han provocado un aumento de su uso en muchas industrias, sin embargo, el cultivo de abacá se ve amenazado por la presencia de la marchitez por *Fusarium oxysporum* f. sp. *cubense,* una de las enfermedades más importantes y que ha sido considerada la más letal debido a la escasez de métodos de control existentes. El objetivo del artículo es resaltar datos recientes sobre las características de la planta y su fibra, manejo del cultivo, productividad y mercado como un enfoque para actualizar los conocimientos actuales sobre el abacá. Se encontró que algunas características de la fibra de abacá, como resistencia y propiedades físicas, dependen de la posición de sus hojas, de la madurez de la planta y también del sistema de extracción de la fibra. Respecto a la extracción de fibras se menciona que existen dos tipos de métodos (pelado y decorticación). En relación con la productividad, existe una diferencia considerable entre las formas de extracción de haces, por ejemplo, la técnica de pelado manual produce alrededor de 20 kg de haces de fibras por día. Se concluye que el abacá presenta un gran potencial como cultivo que podría potenciar los mercados de diversos países, sin embargo, la información disponible en torno a este cultivo es escasa, por lo que es necesario aumentar la investigación en referencia a su producción y manejo para promover un mayor aprovechamiento.

Palabras clave:
Manejo del cultivo
Extracción
Fibra
Matriz polimérica
Variedades

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Received: October 25, 2022; Accepted: November 24, 2022
Rev. Fac. Nac. Agron. Medellín 76(1): 10263-10273. 2023

ISSN 0304-2847 / e-ISSN 2248-7026

Musa textilis Nee also known as cañamo of manila or abaca by its common name, is a crop with the capacity to produce a natural fiber, extracted from the leaf sheath that surrounds the stem (Lalusin and Villavicencio, 2015; Ferrín and García, 2013). This plant belongs to the same family as the commercial banana and it is native to the Philippines, although it is possible to be found in tropical countries (Barba *et al*., 2020). The best conditions to grow abaca are temperatures of 22 °C to 28 °C, with precipitation between 1800 mm to 2500 mm during the year, an altitude of 350 masl to 450 masl, and high relative humidity (78-85%) (Zambrano, 2015).

The abaca fiber is the most resistant worldwide natural fiber and it has properties such as a huge resistance to salinity, mechanical strength, brightness, flexibility, durability, and a considerable fiber length (Armecin *et al*., 2014; Ojeda, 2012). The unique characteristics of this fiber have increased its use in the automotive industry, as a substitute for fiberglass in reinforced plastic components. Besides, it works as a replacement for the synthetic fiber that is in the vehicle which generates a weight decrease and a cost reduction (Llanes-Cedeño *et al*., 2019).

The economic importance of this crop started around the year 1820 when the first export took place to the United States from the Philippines. However, the abaca trade did not become important until after 1850 and it was not until 1887 that the crop passed sugar as the leading Philippine export (Spencer, 1951). Approximately, during a century, the monopoly of worldwide production belonged to the Philippines. However, since 1921, the USDA began the cultivation of this *Musa* in Guatemala, Honduras, Costa Rica, and Panamá (Lalusin and Villavicencio, 2015).

Currently, the cultivation of abaca and other *Musaceae* is threatened by the presence of *Fusarium oxysporum* f. sp. *cubense* (Heck *et al*., 2021) this is one of the most important diseases and has even been considered the most lethal due to the few existing control methods and their persistence in soils (Martínez, 2019), which has led countries like Costa Rica to decree, even internally, a ban on the racking of banana plants and seeds as well as other *Musaceae* to prevent the spread of the plague between countries and additionally, between the different producing farms of the country (Decree No. 42392-MAG, 2020). The decree was initially valid for one year. However, an extension was approved until the pest enters the country and is controlled, or until it is officially controlled internationally and therefore does not present a threat to national production (Decree No. 43109, 2021).

According to scientists, there are more than 40 different species of abaca in the Philippines, but not all the species are marketable; the more common species are Bungalanón, Tangongón, and Maguindanao (Zambrano, 2015; Jiménez Moreira and Landy Campos, 2013). In Ecuador, there are two varieties: Bungalanón and Tangongón (Pera, 2019). However, in the case of Costa Rica, Guatemala, and Panamá, no data were found on the cultivated varieties.

This review is intended to highlight recent data about the characteristics of the plant and its fiber, crop management, productivity, and the market as an approach for updating the current knowledge regarding the abaca.

Plant characteristics and fiber composition

The botanical classification of abaca plants corresponds to: division *Spermatophyta*, subdivision *Angiospermae*, class *Monocotyledonae*, order *Zyngiberales*, family *Musaceae* and genus *Musa* (Mandegani, Sumarto and Perdana, 2016).

This plant is a perennial herb of approximately 8 m tall that grows in a clump, has cylindrical short corms, and bearing buds that develop short rhizomes with slender adventitious roots that extend about 2-3 m (Hillman, 2004). The plant is composed of a central core that is wrapped in up to 30 sheaves forming the protostome, its stalk is between 2 cm and 6 cm tall and 9 to 30 cm in diameter (Mamun *et al*., 2015).

Abaca plant stems are made up of 93% water and 1.3-5% fiber (Hillman, 2004). In addition, the abaca fibers are composed mainly of combinations of basic polymers such as hemicelluloses, celluloses, and lignin (Table 1) and present a high crystalline index (more than 65%) (De Souza and d´Almeida, 2014).

Table 1. Chemical composition of abaca fiber

Component	Proportion (wt %)
Cellulose	63-68
Hemicellulose	19-20
Lignin	5-6
Pectin	<1
Fat and wax	<1
Water-soluble	1-2

Source: Mamun *et al.* (2015).

The abaca fiber is thermally stable until 250 °C, this property is desirable when one foresees its use in thermoplastic matrix composites. On the other hand, its tensile properties put the fiber at a high strength with a–medium elasticity modulus (De Souza and d´Almeida, 2014). This means that the fiber of abaca can be used with a high bending stress and with a medium elasticity requirement (Muthu and Gardetti, 2020).

Also, Mamun *et al.* (2015) mentioned that some characteristics of abaca fiber such as strength, physical properties, and composition depend on the extraction technique used and the position of its leaves. For instance, those leaves close to the center are characterized by being softer, finer, and whiter.

It has been observed that in terms of the weight of the sheets, there is no significant difference between the use of internal (young) or external (mature) sheets, however, when using external sheets, fibers with greater resistance and elasticity are obtained than those obtained from the internal leaves, characteristics that are attributed to the maturity of the fiber (Alemania, Santiago and Gloria, 1982). The application of the mechanical properties and a comparison with other natural fibers are shown in the next section of this review.

Uses of the abaca fiber

The abaca plants and banana plants despite being *Musaceaes* manifest some differences. For example, they have different uses and their transcendental distinction is that the fruits obtained from the banana can be eaten, but those obtained from the abaca cannot (Pera, 2019; Cerón, 2006). Despite this, the abaca leaves can become fiber, which is used in some industries. In addition, the use of fibers takes more importance worldwide, especially for the advantages that arise for the environment, since they constitute a natural and renewable resource (Pontón and Guerrero, 2010).

Punyamurthy *et al.* (2012) and Haque *et al.* (2010) mentioned that abaca has different applications and uses, characteristics such as being inexpensive, durable, resistant to salt water, cheap to produce, abundant, biodegradable, and its potential reinforcement in polymers, have made it, that over the years the fiber is utilized for the manufacture of nets used in fishing, elements of dress, and upholstery. Although it is used mainly for the production of tea and coffee bags, sausage casings, paper, napkins, machine filters, conduction cables, electrical and vehicle coating, money, gloves, caps, it is also a substitute for the bark of trees, being also once the main source of the manufacture of fabrics (Pera, 2019; Cárdenas, 2016). Also, Pontón and Guerrero (2010) mentioned that abaca fiber has industrial applications because it is being used in the field of polymeric matrix composite materials for the manufacture of automobile interior parts.

But this is not the only application in polymeric matrices, there are currently many research teams looking at the possibilities of creating polymers reinforced with abaca fiber, regarding that, Barba *et al.* (2020) mention that the use of abaca in composite of polymers have benefits for the environment but also economic benefits since they are cheaper than other alternatives such as fiberglass, but in contrast, they present disadvantages like the heterogeneous structure of the fiber, the variation of its physical characteristics which are influenced by crop management, this in addition to their hydrophilic

characteristic, which makes them incompatible with a variety of hydrophobic matrices.

Regarding this, it is mentioned that the use of abaca to reinforce materials and generate polymeric matrices mainly influences the mechanical characteristics corresponding to the modulus of elasticity and the bending stress, obtaining improvements in the elasticity and rigidity of the matrix, thus, when using short abaca fibers, a greater modulus of elasticity is obtained, while when using longer or continuous fibers, the greatest increases in flexural stress are obtained (Pontón and Guerrero, 2010).

The data are reinforced by the studies by Llanes-Cedeño *et al*. (2019), Karthik and Arunachalam (2020) and Widnyana *et al*. (2020) in which they evaluate the same parameters for polymers reinforced with abaca, with cabuya, and with coconut fibers. In this case, the data in Table 2 shows that the use of abaca fibers stands out in the modulus of elasticity, but also provides an improvement in bending stress.

Table 2. Mechanical properties to bending of different composite and materials.

Matrix material	Reinforcement material	Maximum bending stress (MPa)	Modulus of elasticity of bending (MPa)
Polyester	-	56.62	1,867.82
Polyester	Abaca	100	10,000
Polyester	Cabuya	51.39	2,355.58
Polyester	Coconut	122.7	1,328.5

Source: Llanes-Cedeño *et al.* (2019), Karthik and Arunachalam (2020), Widnyana *et al.* (2020).

Abaca varieties

Despite its cultivation in many countries around the world, abaca genetics are relatively unknown and variations due to differences in phenotypic expression in the field and the subjective nature of morphological characterization schemes further complicate the matter. There is not enough scientific evidence of the existing varieties in Costa Rica, it is believed that there are at least three different varieties that were brought from the Philippines. However, it is estimated that there are around 40 varieties of abaca plants in the Philippines (the world's leading producer of this crop) (Chamba *et al*., 2017). Besides, it is important to mention that not all varieties are commercialized. Some abaca varieties examples are Laylay, Inosa, Linawaan, Sinamok, Abuab, Putian, Libuton, Tangongon, Bungulanon and Maguindanao, being the last three the most common ones (Sinon *et al*., 2011; Galvez *et al*., 2021; Hidalgo, 1952).

For this reason, research and development in a variety of identification techniques are important, among which it can be mentioned the use of Simple Sequence Repeat (SSR) Markers to evaluate genetic diversity (Yllano *et al*., 2020); as well as the identification of resistance and susceptibility to diseases like the Bunchy Top Virus using microsatellites markers. Because of this technique, it was possible to identify 18 accessions that were resistant to the Bunchy Top Virus native from Palawan Island in the Philippines (Boguero *et al*., 2016); in addition, screening is used for molecular characterization and resistance detection of putative mutants lines generated from gamma irradiation (Descalsota *et al*., 2015).

In general, the use of molecular techniques has been associated with the identification of important traits such as resistance to diseases, however, the molecular characterization directed towards the identification of the different varieties and species has not been reported.

Abaca diseases

Abaca plants can be affected by numerous diseases, one of them being the Panamá disease or *Fusarium* wilt, which causes a yield reduction and damages in

plantations between 5% and 65% (Purwati *et al*., 2008). This pathogen can survive a long time in the form of a mycelium among the infected plant debris or in the form of *chlamydospora* in the soil, which makes the control more difficult (Purwati *et al*., 2008).

Abaca Bunchy Top Disease (ABTD) is another infection, in this case, the disease is produced by a virus of the *Babuvirus* genus, specifically, the Abaca Bunchy Top Virus which the transmission is done by the banana aphid, the *Pentalonia nigronervosa* (Sharman *et al.,* 2008). A plant with this disease can be recognized for symptoms such as stunting, bunched and rosette leaves, dark green flecks or vein clearing of the minor leaf veins, and up-curling and chlorosis of leaf margins (Sharman *et al.,* 2008).

Banana Bract Mosaic Virus (BBRMV) is another disease caused by a virus of the Potyvirus genus transmitted by the aphid *Pentalonia nigrovenosa* and it is capable of producing losses in production up to 40% (Manzo-Sánchez e*t al.,* 2014). The symptoms in petioles are yellowish spots or streaks, on the leaves symptoms may or may not be visible and it is possible to observe dark stripes on the pseudostem but the typical symptom that gives the disease its name is a characteristic mosaic in the floral bracts (Manzo-Sánchez e*t al.,* 2014).

Fiber extraction

It is important to mention that the abaca fiber is extracted from petioles of abaca leaves by techniques such as stripping or decortication (this implies a blade of decorticator which removes the primary and secondary fibers from the sheath) and it takes place as soon as the stems are cut, while they are still moist. (Radoor *et al*., 2020; Mamun *et al*., 2015).

To extract the abaca fiber by stripping technique, two methods called tuxying and stripping are necessary. The first one involves a special knife (tuxying knife) which is inserted between the inner and outer layers of the leaf sheath to separate the fiber, while the second one, is done for cleaning the fibers (Radoor *et al*., 2020). The technique used to extract the fiber affects directly the fiber quality and investment as shown in Table 3.

Table 3. Fiber quality, production rate, and investment.

Technique	Capacity (kg day^{-1})	Fiber quality	Investment cost (euro)
Hand stripping	10-20	Poor	4-6
Spindle stripping	100-200	Good	1,700-2,300
Multi-fiber decorticator	80-100	Poor	1,200-1,300
Auto-fed decorticator	600-800	Poor	6,800-7,000

Source: Sinon (2008).

In addition, different extraction techniques allow for obtaining short, long, or continuous fibers that vary in their characteristics and application possibilities (Llanes-Cedeño *et al*., 2019; Karthik and Arunachalam, 2020).

These extraction techniques include hand stripping, the spindle (machine stripping), and decortication. In general terms, the spindle provides a whiter and more resistant fiber (Muthu and Gardetti, 2020). However, poor handling of the machinery generates a significant loss of fiber, as well as a decrease in its quality (Alemania, Santiago and Gloria, 1982).

Muthu and Gardetti (2020) refer to the three types of extraction. First, hand stripping is a process that involves a large amount of work and lower yields, and the quality of the fiber depends on the skill of the worker, as well as the quality of the tools, mainly the density of the teeth of the blades. Meanwhile, the spindle is a process carried out by machines that are designed to apply the necessary pressure and with blades of adequate tooth density to achieve the highest possible yields and quality, also reducing effort and increasing production efficiency. Finally, decortication is the technique that provides the greatest processing capacity in exchange for a lower quality than the

spindle, but being a mechanized technique also reduces the effort of the workers.

With this, the spindle method seems to be the most suitable for the stripping of abaca fibers, however, it should be considered that using this method requires an initial investment on the part of the producers, therefore, if the production is not high enough, in some cases it is not profitable and hand scraping continues to be used. Also, if the market needs a large amount of fiber of regular quality, the decortication process can be used.

Crop management and productivity

Theoretically, there are four types of planting material (seeds, corms, suckers, and tissue cultured plants), however, seeds are no longer used and this method is only used in breeding research (Göltenboth and Mühlbauer, 2010). Planting material such as suckers, corms, or pieces of corm with a vegetative bud can be used, however, suckers are rarely used because of the difficulty of transport, so the most common way is the cultivation by corms, which is used in major zones and farmers because of its facility and cost (Jones, 2018).

Abaca plants or "seed pieces" are put in distances between 2.5 m and 3.0 m apart in holes and covered with 5–10 cm of soil. Young plants may be partially shaded for protection from excessive heat (Jones, 2018). It is mentioned that abaca plants commonly grow in loamy soils with a high content of sand, which provides better drainage, and as a consequence, the plant thrives (Spencer, 1951). According to Jones (2018), the time of harvesting depends on some factors such as the cultivar, the soil conditions, and climate, although commonly the plants can be harvested from 18 to 24 months after planting and two or four pseudostems can be harvested from each mat every 4–6 months.

According to Waller and Wilsby (2019) a considerable difference between the ways of bundle extraction exists, for instance, using the hand stripping technique produces about 20 kg of fiber bundles per day, while the spindle stripping technique produces between 80 kg and 120 kg per day, while the decortication technique produces 140 kg per day being the most efficient on terms of quantity but with a lower quality than the spindle method.

As previously mentioned, some conditions are necessary to grow abaca in a better way, this includes temperature, precipitation, altitude, and relative humidity, yet some studies mention that it is also important to know how the influence of shade, water, nutrient availability, and other factors affect the productivity of the crop (Bande *et al*., 2013; Kumar *et al*., 2017). Bande *et al.* (2013), conclude that an increase in light intensity, nutrient supply, and water does not affect the quality of the fiber, however, factors such as reducing the irradiance by applying 50% of shade have a significant impact on the physiological performance of the plant, which means that this directly affects the crop yield. Supporting this, according to the analysis of Araya *et al.* (2022*)* the use of a shadowing system shows a potential capacity for increasing proficiency and productivity of the abaca due to the morpho- and physiologic adaptations caused by the variations in the solar radiation perceived by the plant.

On the other hand, it was demonstrated that in crop fields where abaca was cultivated for many years without proper fertilization of the soil, the productivity can decrease, giving bad parameters of fiber extraction per hectare (ha) planted (Romel *et al.,* 2011). To avoid this, an NPK fertilizer can be used to improve the quality of the fibers (Bande *et al*., 2013)**.**

Regarding crop management for local production, as well as research and development, there is a great lack of information in countries such as Kenya and Indonesia. Regarding Equatorial Guinea, El Ministerio de Agricultura y Bosques, mentioned in 2012 that, during the 1990-2000 decade, there was interest in resuming cultivation, but no concrete action was generated, although they recommend the recovery of the product in the long term, additionally, El Banco Internacional de Reconstrucción y Fomento (2019) affirms that abaca is a commercial crop with great potential, but that the country does not have a transformation industry to take advantage of it.

For their part, Costa Rica and the Philippines were part of the diversification and expansion project of the sustainable Abaca initiative, which consisted of supporting small producers during the years 2017-2020 with training, disease control, monitoring of results, as well as support in other issues related to the production process. As a result of this, there was an important growth in cultivation and

production (GIZ, 2018) and thus, increase their importance in the market, as detailed in the next section.

Abaca fiber market

The abaca has a high potential as a crop, since sowing it can take from two to three years to have a biannual harvesting using few resources of pesticides and fertilizers which gives way to a non-expensive treatment of the crop (Lacuna-Richman, 2002). Furthermore, according to Global Market Insights (2021), the global market size of the abaca fiber is going to present a strong increment over 2021-2027, due to the accelerating adoption of abaca-reinforced hybrid materials because of its multiple uses and its sustainability. The world production of abaca is valued at $60 billion per year (FAO, 2021). In 2020 the Philippines was the world leader in abaca production followed by Ecuador and Costa Rica. This year, the Philippines produced around 67,388 t of abaca Ecuador produced around 36,634 t as shown in Table 4.

Table 4. Main country producers of abaca from 2016 to 2020 and its production in ton.

Country	2016	2017	2018	2019	2020
Philippines	68,965	67,967	67,579	67,483	67,388
Ecuador	36,210	36,619	36,754	36,528	36,634
Costa Rica	1,242	1,246	1,242	1,242	1,242
Indonesia	579	572	546	565	561
Equatorial Guinea	240	236	236	237	237
Kenya	48	50	51	52	52

Source: FAO, 2022.

Regarding the demand, Waller and Wilsby (2019), mention that productivity is not sufficient to meet the demands of fibers and fiber products on the international market. The gap between the supply and the demand by 2019 year was 25,000 t, this means that with the production of fibers in 2018 (32,000 t) an 80% increase in the supply is needed to meet the demand for 2018 (Waller and Wilsby, 2019).

The Philippine Statistics Authority (2021) mentions that among the countries with the highest imports of abaca fiber are, in descending order, Japan, the People's Republic of China, the Republic of Korea, Saudi Arabia, and Iran, among other minor import countries. However, abaca is presented as a highly exploitable non-traditional crop option according to the study by Ponce (2015), it is possible to produce abaca with a profit of up to $1982.25 per cultivated ha with a production performance of up to 1456.56 kg, which shows it as a highly profitable crop. Furthermore, during 2020 only in the Philippines, the production of abaca produced an income of USD 1.62 billion in 140,688 ha, which positions this crop as one of the most economically important for this country (Philippine Statistics Authority, 2021; FAO, 2022). Meanwhile, Ecuador produces 27,576 ha of land, which generates approximately USD 36.81 million, being the second largest producer (Torres, 2021; FAO, 2022). Besides, in 2020, Costa Rica harvested 1,070 ha, Indonesia 688 ha, and Equatorial Guinea 1,783 (FAO, 2022), these being minor producers, but with the potential to develop the crop.

It should be noted that Equatorial Guinea harvested a larger area than Costa Rica and Indonesia, however, its production is lower because the yield is 1,329 hg ha^{-1}, while Costa Rica's yield is 11,607 hg ha^{-1} and that of Indonesia reaches 8,154 hg ha^{-1} (FAO, 2022).

Plant breeding

The genetic pool of abaca is highly diverse as indicated by the high Shannon diversity index in some Philippine gene banks, which indicates that it could be a huge resource for breeding. However, there are no publicly available assembled draft abaca genomes for genome comparison studies of the varieties (Galvez *et al*., 2021). The conventional breeding methods are coupled with the new advances in biotechnology and molecular biology to come up with better solutions to the problems

that the abaca industry faces. These methods are used to develop different varieties that possess specific characteristics like good fiber quality, high fiber yield, and a high degree of resistance to major diseases of this plant family (Lalusin and Villavicencio, 2015).

In a study by Kumar *et al.* (2015), it is mentioned that the necessity to overcome the limitations of the low resistance to ABTV (Abaca Bundry Top Virus) has caused some strategies like transgenic approaches based on the pathogen-derived resistance (PDR) to be explored, strategies using ABTV DNA-R gene or satellite DNA (DNA-S4) resulted in partial resistance to ABTV. Also, mutation breeding using gamma-irradiation has been explored to induce genotyping and phenotyping variation for ABTV resistance (Kumar *et al.,* 2015).

In this sense, Purwati, Harran, and Sudarsono (2007) mention that the improvement of abaca using ethyl methanesulphonate is capable of offering resistance to infection by *F. oxysporum*. Also, it is important to mention that technological progress that began with breeding, and continued with mechanization, the use of synthetic fertilizers and the tools of genetic engineering to improve plant characteristics must continue, for the natural fiber industries to remain competitive (Townsend, 2020).

Despite the possibilities offered by molecular techniques, the most recent advances in improving quality and yield in abaca plantations occur through crop management and nutrition, light, hydration, and weed management conditions (Araya *et al*., 2022; Bande *et al*., 2016).

CONCLUSION

In this review, it was covered information generated over a long period that is related to the cultivation of abaca in diverse aspects, description of abaca as a plant, its uses, and applications, its production and markets, its characteristics as a product but also as a crop, the management that is given to it, as well as the diseases that affect it and the techniques and technologies that have been used to provide resistance to them.

Abaca fiber has great potential to replace other natural and synthetic fibers used in the market such as fiberglass, coconut, and cabuya, however, it is necessary to continue the study around its production since the known information about productivity is a bit sparse and this could be one of the reasons the cultivation is not expanding. Furthermore, there are many knowledge gaps in aspects such as molecular detection and crop management, but also in the information about the crop in countries like Indonesia, Equatorial Guinea, and Kenya.

Also, recent research and development are directed toward the formulation of fiber-reinforced polymeric matrices of abaca rather than breeding the crop itself. With this, abaca fiber is considered a material that can be given a greater number of uses, but at the same time leaves aside the interest in the development of the crop at the field level to improve its quality and yield. The latter allows to consider that for the next few years, around a growing global economy and in search of new production possibilities, producers from different Latin American countries such as Guatemala, Honduras, Costa Rica, and Panamá, should consider abaca as a non-traditional crop that could be highly exploited, but not only this, once this crop expands, it could even reach new countries that meet the necessary conditions for its growth. For this reason, this review will serve as a reference point on the current knowledge about the cultivation of abaca and the production of its fiber and is encouraged research around the great potential that it presents and for this, greater collaboration with research projects by universities is required. To advance more efficiently and increase national productivity, in this regard, the Costa Rican Technological Institute is the only academic institution that is doing research in abaca in the country, and it's open to collaboration with other national and international universities, and that could contribute significantly to the market and the economy of a variety of countries.

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