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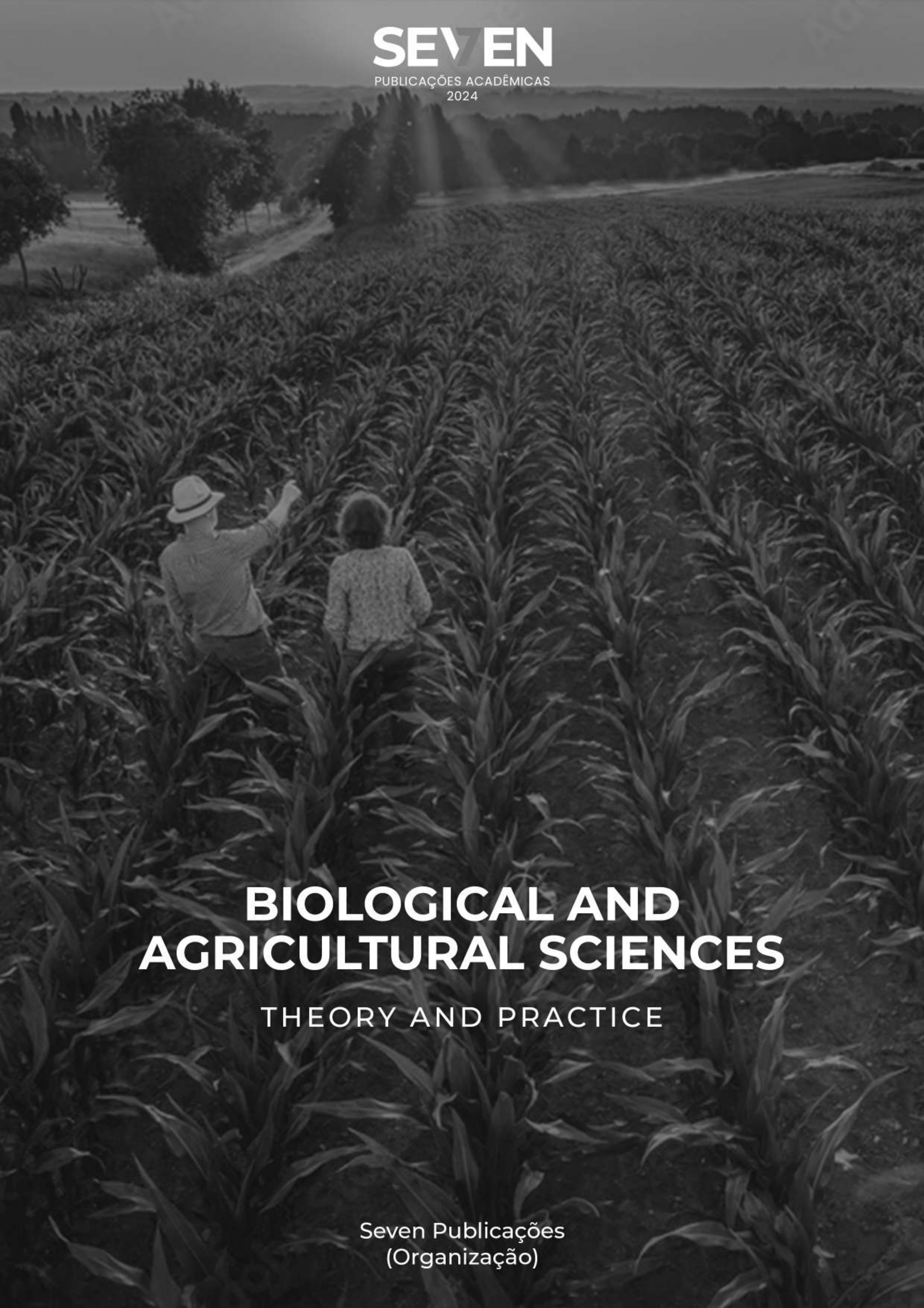

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SUMMARY

Photosynthetic microorganisms producing polyhydroxyalkanoates: Production, extraction, biosynthesis and alternative application in active packaging incorporated with essential oils

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ABSTRACT
Biopolymers have vast applicability, besides being biodegradable sources and presenting relatively shorter life cycles when compared to fossil energy sources. Some of these biopolymers are polyhydroxyalkanoates (PHAs), a class of polymers with the ability to form plastic membranes, similar to petrochemical plastics. Several studies suggest that microalgae/cyanobacteria are types of photosynthetic microorganisms that can be used to obtain PHAs at a lower cost because they have minimal nutritional requirements for growth and are naturally photoautotrophic, meaning they use light and CO 2 as their main energy sources. Furthermore, microalgae have potential for high productivity, are tolerant to changes in environmental conditions, and can be cultivated in areas unsuitable for agriculture. These PHA plastic membranes produced by these photosynthetic microorganisms can be an alternative for constructing a functional film with great antimicrobial characteristics when incorporated with essential oils, the famous active packaging, the future of packaging industries. This work demonstrates the production, extraction, biosynthesis, and application perspectives of these biopolymers in packaging industries, such as films incorporated with essential oils.

Keywords: Microalgae, Cyanobacteria, Bioplastic, Biopolymer, Polyhydroxyalkanoate, Essential oils.

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INTRODUCTION

Polymers have a variety of applications, which make them a necessary tool in society. Lowering production costs, minimizing energy consumption and environmental pollution, as well as reducing the generation of gases harmful to the environment. In addition to being versatile, with protective and preservative properties (electrical, acoustic and thermal) (Jamnongkan et al., 2022; Rosenboom et al., 2022). According to Wu et al. (2017), some polymers have the ability to form very thin films and films, which are attractive in production and application in food packaging.

These polymers, called polyhydroxyalkanoates (PHAs), are a class of polyesters that can be produced by a diversity of microorganisms, including photosynthetic microorganisms (Silva and Houllou, 2022), as alternative, ecological, important and sustainable sources of monomers for the production of bioplastics (Coppola et al., 2021; Nandal et al., 2022). The biosynthesis of these bioplastics is generally carried out under nutrient-limiting conditions, such as nitrogen, phosphorus, sulfur and excess carbon. However, the most interesting thing is that under aerobic conditions, several microorganisms can degrade these PHA films and generate carbon dioxide and water, while under anaerobic conditions they can generate methane and water (Surendran et al., 2020).

In the biosynthesis of PHAs, the main enzymes involved are PHA synthase (phaC) and are divided into gene classes I, II, III and IV. Class II enzymes are responsible for the synthesis of Mcl-PHA (medium chain length), while the rest (I, III and IV) synthesize Scl-PHA (short chain length) (Jia et al., 2016) . The yield of PHAs in photosynthetic microorganisms can vary between 1.0-70% (w/w) and the main tools for obtaining the polymer currently are still the use of sodium hypochlorite/chloroform/methanol (García et al., 2021; Panda et al., 2005;Sharma and Mallick, 2005; Bhati and Mallick, 2015; Ansari and Fatma, 2016). However, the prospect of applying these polymers in the formation of PHA films incorporated with essential oils (OE) to form active packaging (EA) is a major innovation in the packaging industry (Giaquinto et al., 2017).

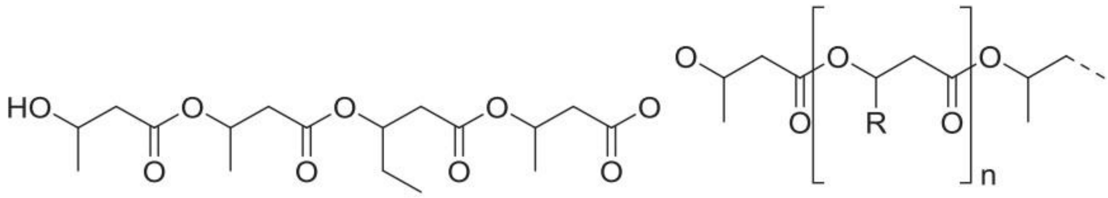
The purpose of this work is to demonstrate that photosynthetic microorganisms can be a low-cost and promising tool in the production of polyhydroxyalkanoates, showing extraction methods, polymer biosynthesis and future perspectives for the application of these PHAs in the formation of thin films incorporated with essential oils, the famous active packaging, the future of the packaging industry.

POLYHYDROXYALKANOATE (PHA)

Large quantities of non-degradable plastics are among the main global problems currently known. Biodegradable biopolymers produced by microorganisms are potential substitutes for plastics of petrochemical origin (Amadu et al., 2021). Polyhydroxyalkanoates (PHAs) are a class of natural esters secreted by microorganisms as intracellular granules under nitrogen-limiting conditions along with excess carbon source. This class of natural esters presents high variability as it covers more than 150 types of monomers and weighs up to 100,000 Da (Dalton). The polymer remains amorphous inside cells due to its stability contributed by phospholipids while it appears crystalline after extraction. Its composition was enunciated by Lemoigne in 1925, although Beijerinck reported its occurrence in 1888 (De Koning, 1993; Pal et al., 1999).

The basic structure of PHAs contains repeating units of aliphatic polyesters (Figure 1) ranging from 600 to 35,000 (Albuquerque and Malafaia, 2018). PHA with n=1 represents a class of poly(3-hydroxyalconates) while n=2 represents poly(4-hydroxyalconates). The type of derivative attached to the side chain (R) of the unit determines the functionality of the monomer. Depending on the number of carbon atoms in a monomeric chain unit, PHAs are classified into three types: short-chain monomers (3 to 5 carbon atoms), medium-chain monomers (6 to 15 carbon atoms), and long chain (more than 15). The type of PHA produced depends on the biochemical pathways inherently (or genetically modified) present in microorganisms (Arumugam, 2020).

Figure 1 - Repeating units of the Polyhydroxyalkanoate monomer.



Source: Reproduced from Koller et al., 2017.

PHA-PRODUCING PHOTOSYNTHETIC MICROORGANISMS

Several photosynthetic microorganisms are producers of PHAs, however, several modeling techniques in the cultivation medium are applied to accumulate this biopolymer inside the cells, among which we can highlight the reduction in the levels of nitrogen, phosphorus , iron and the increase in carbon source demand. Costa et al. (2018A) evaluating the production of PHA from the microalgae *Chlorella minutíssima* and the cyanobacteria *Synechococcus subsalsus* and *Spirulina* sp. LEB-18, in standard media using only salts and with 70% limitation of the inorganic source of nitrogen ($NANO_3$), found that *C. minutíssima* did not produce the biopolymer, however, cyanobacteria managed to produce PHA, and with greater intensity when the nitrogen source was limited. The results show that nitrogen reduction causes notable changes in the biochemical composition of cells, degrading proteins and photosynthetic pigments, but can favor the accumulation of other biomolecules such as lipids and polyhydroxyalkanoates. Costa et al. (2018B) also found that the cyanobacterium *Spirulina* sp. LEB-18 in open raceway culture using Zarrouk medium was also able to produce PHA. Roja et al. (2019) also managed to produce PHA in four different photosynthetic microorganisms: (i) *Chlorella* sp., (ii) *Oscillatoria salina* , (iii) *Leptolyngbya* *valderiana* and (iv) *Synechococcus elongatus* . García et al. (2021) evaluated the production of PHA from the microalgae *Scenedesmus* sp. under conditions of nutrient deficiency. Sixteen different types of modified culture media were prepared by varying the concentrations of nitrogen, phosphorus, iron, salinity and added carbon source (glucose). All media produced the biopolymer, with emphasis on the culture medium composed of: glucose (1 g L^{-1}), nitrogen (17.6 mM), phosphorus (0 mM), iron (0.021 mM) and salinity (0. 5 g L^{-1}). Table 1 shows the production of PHA from various photosynthetic microorganisms. Mourão et al. (2020) also verified the production of PHA from the microalgae *Stigeoclonium* sp. B23 in BG-11 medium supplemented with sodium acetate and sodium bicarbonate as source and carbon, or with carbon/nitrogen deficiency. Silva and Houllou (2022) also managed to obtain the production of PHA using the microalgae *Chlorella vulgaris* and *Tetradesmus obliquus* using Bold's Basal medium supplemented with agro-industrial waste corn.

Table 1 - PHA yield in different types of algae

Seaweed	Performance (%)	References
Anabaena sp.	2.3%	Lama et al., (1996)
Arthrospira subsalsa	14.7%	Shrivastav et al., (2010)
Very fertile aulosira	10%	Samantaray and Mallick (2012)
Botryococcus braunii	16.4%	Kavitha et al. (2016)
Calothrix scytonemicola TISTR 8095	25.2%	Kaewbai-ngam et al., (2016)
Calothrix sp.	6.4%	Bhati et al., (2010)
Microalgae consortium	31%	Rahman et al. (2015)
Nostoc muscorum	8.7 - 69%	(Panda et al., 2005; Sharma and Mallick, 2005; Bhati and Mallick, 2015; Ansari and Fatma, 2016)
Oscillatoria jasorvensis TITR 8980	15.7%	Kaewbai-ngam et al., (2016)
Phaeodactylum tricornutum	10.06%	Hempel et al. (2011)
Phormidium sp. TIST 8462	14.8%	Kaewbai-ngam et al., (2016)
Scenedesmus sp.	0.831 – 29.92%	García et al. (2021)
Scytonema sp.	7.4%	Bhati et al., (2010)
Spirulina sp. LEB-18	12 – 30.7%	(Costa et al., 2018A; Coelho et al., 2015)
Synechococcus elongates	7.02 – 17.15%	Mendhulkar and Shetye (2017)
Synechococcus MA19	55%	Nishioka et al., (2001)
Synechococcus subsalsus	16%	Costa et al. (2018C)
Synechocystis PCC6803	4.1 – 26%	(Khetkorn et al., 2016; Panda and Mallick, 2007; Wu et al., 2001)
Synechocystis saline	5.5 – 6.6%	Kovalcik et al. (2017)
Chlorella sorokiniana SVMIICT8	29.5%	Kumari et al. (2022)

Source: Author.

METHODS FOR EXTRACTING POLYHYDROXYALKANOATES FROM PHOTOSYNTHETIC MICROORGANISMS

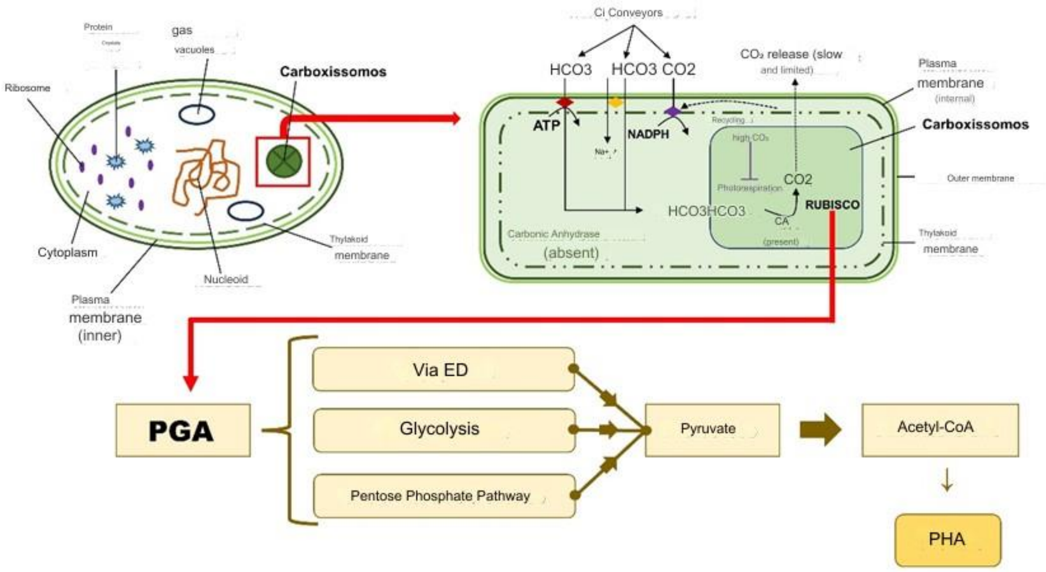
Several extraction methods are applied to obtain PHA polymers. Costa et al. (2018) obtains the biopolymer from an extraction using sodium hypochlorite at a final concentration of 4%, the sample is then incubated at 45 °C for 20 minutes, followed by centrifugation, extraction of the polymer using chloroform and followed by precipitation with methanol. Roja et al. (2019) uses the same extraction method mentioned above to extract the polymer from four species of algae, only modifying the extraction time (30 minutes). In turn, Silva and Houllou (2022) obtained two extracts from two microalgae using simultaneous extraction with 4% hypochlorite + chloroform, followed by centrifugation, collection of the organic phase, evaporation and degreasing with hexane. In studies by García et al. (2021) the dry biomass was first washed with distilled water and ethanol, then the centrifuged pellet was subjected to polymeric extraction with chloroform, and the solution was passed through a glass fiber filter (0.45μm) and the chloroform it is then evaporated in a rotoevaporator, ultimately obtaining the polymer. Kovalcik et al. (2017) obtained the biopolymer from the cyanobacterium *Synechocystis salina* , first using an ultrasonic bath of the biomass in ethanol and acetone, to extract the pigments. Then, PHA was recovered by Soxhlet extraction using hot chloroform, and subsequently precipitated with ice-cold ethanol. Finally, Morais et al. (2015B) evaluated three extraction methods: i) 4% sodium hypochlorite; ii) trichloromethane and methanol precipitation; iii) trichloromethane with pre-treatment with 4% hypochlorite. In general, extractions of polyhydroxyalkanoates from photosynthetic microorganisms are carried out with sodium hypochlorite, chloroform and methanol precipitation.

BIOSYNTHESIS OF POLYHYDROXYALKANOATES

Photosynthetic microorganisms are photoautotrophic organisms that produce primary biomass. These microorganisms have the ability to use minimal inorganic nutrients (CO_2) that are readily available, as well as sunlight and water, demonstrating high photosynthetic efficiency, which gives them the title of "microbial factory", and can generate several products of industrial interest (Rahman et al., 2013, 2014; Singh and Mallick, 2017), such as PHA. Several studies suggest that photosynthetic microorganisms produce several basic materials that can generate plastic biofilms. Poly-3-hydroxybutyrate (PHB) is a type of PHA that can be widely produced by microalgae and cyanobacteria, has a wide application and can be a sustainable alternative for the plastic industry, as it is a bioderived and biodegradable polymer (Costa et al., 2019; Mendhulkar and Shetye 2017;

Under autotrophic conditions, cyanobacteria fix the carbon source in the Calvin-Benson-Bassham (CBB) cycle through ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCO). RuBisCO is responsible for assimilating most of the carbon available on Earth, due to its high efficiency in capturing CO_2. Inorganic carbon transporters (Ci) present in the cell wall of photosynthetic microorganisms transport atmospheric CO_2 and help maintain the local carbon concentration for RuBisCO. The Calvin cycle output, glyceraldehyde-3-phosphate after its conversion to 3-phosphoglycerate (PGA), can then enter any of the three pathways for sugar metabolism, i.e. Entner-Doudoroff (ED) pathway, glycolysis , via pentose phosphate and be finally converted into acetyl-CoA to be used in the PHA synthetic pathway (Singh and Mallick, 2017; Figure 2).

Figure 2 - PHA conversion from carbon accumulation and $CO_{2\ fixation}$ in cyanobacteria



Source: adapted from Afreen et al. 2021.

ESSENCIAL OILS

Essential oils (EOs) consist of secondary metabolites that can protect plants against environmental threats, pathogenic microorganisms, among others, being mixtures of phenols, monoterpenes, sesquiterpenes and other aromatic plant compounds (Ballester-Costa et al., 2017; Sangha et al., 2017). Regarding the physical aspect, EOs are aromatic oily liquids derived from plants and can be extracted from different matrices such as leaves, buds, flowers, seeds, bark, roots, twigs, wood and fruits (Ghabraie et al., 2016; Lee et al. , 2018). They have traditionally been used in natural therapy, food preservation, as complementary medicines in various treatments and as culinary flavorings, given their organoleptic characteristics with great acceptability among consumers (Ballester-Costa et al., 2017; Fratianni et al., 2010). Currently, approximately 3,000 EOs are known and some of them are commercially important, being used in the agricultural, cosmetics - especially perfumery -, chemical, pharmaceutical and food industries due to their bioactive potential, highlighting their antimicrobial potential against different strains bacterial and fungal (Cutillas et al., 2018; Ghabraie et al., 2016; Lagha et al., 2019).

These compounds are generally recognized as safe (GRAS) (Ballester-Costa et al., 2016), and some are approved by the *Food & Drug Administration* (FDA) for use as food additives, such as lemon balm, EOs of basil, coriander, cloves, thyme and vanilla (FDA | US *Food & Drug*

Administration). Therefore, they are gaining interest for their potential as natural preservatives (Ballester-Costa et al., 2016), while their potential as antimicrobial and antioxidant agents provide the basis for many applications in the preservation of processed and raw foods (Ballester-Costa et al., 2013).

APPLICATION OF ACTIVE PACKAGING INCORPORATED WITH ESSENTIAL OILS

Polyhydroxyalkanoates (PHAs) are attractive sources for the polymer industry due to their great properties, such as their high biodegradability and processing versatility, and their potential to replace petrochemical plastics (Bugnicourt et al., 2014) . The use of packaging in the food processing industries is essential to maintain food quality and is being increasingly improved nowadays. Alternatives for the application of PHA packaging incorporated with essential oils (OE), the so-called active packaging (EA), are the most recent biotechnological innovations, and these packagings with antimicrobial characteristics provide great technological application, in addition to improving the mechanical characteristics of polymer matrices (Wani et al., 2014; Muppalla et al., 2014; Giaquinto et al., 2017).

In studies by Basnett et al. (2020) an msc-PHA was produced from the bacteria *Pseudomonas mendocina* using a cheap carbon substrate, sugar cane molasses. Characterization analyzes confirmed that it was a copolymer called P(3HO-co-3HD), which was incorporated with lemon essential oil (LEO) and its antimicrobial action capacity was evaluated. The antimicrobial properties of the film manufactured and incorporated with LEO against *Staphylococcus aureus* and *Escherichia coli* showed high activity against gram-positive bacteria. Storage studies also demonstrated that after one year the films showed a reduction in LEO content, however, they still showed activity against *S. aureus* . These so-called packaging guarantee quality, hygiene, safety and increase the shelf life of food, protecting food from internal and external environmental factors (Gouvêa et al., 2015, Wrona et al., 2015). The main components present in EOs that promote these antimicrobial properties in polymeric matrices are aldehydes, phenols and oxygenated terpenoids. Furthermore, the hydrophobicity of EOs allows lipids found in the membrane of bacterial cells to interact with each other, making the microbial cell wall less stable and permeable, allowing cellular components and ions to escape, which can cause cell death (Ju et al. al., 2017; Khaneghah et al., 2018).

In the analyzes by Silva and collaborators (2020), new antimicrobial films made of polyhydroxybutyrate (PHB) added with polyethylene glycol (PEG) and clove essential oil (CEO), with antimicrobial activity against three bacteria (*E. coli* , *E. aerogenes* and *S. aureus*) were evaluated. The main component found in CEO was eugenol (72.96%), a phenolic molecule found in several aromatic plants. The addition of EO provided greater flexibility and reduced intermolecular interactions between the polymer matrix structures, obtaining less crystalline and consequently more elastic films. The influence of orange essential oil (OEO) on PHB/PEG blends with additives was also evaluated against the bacteria *S. aureus* and *E. coli* , showing that the main component of EO (d-limonene) makes the films more resistant and flexible. mechanically and also has antimicrobial activity (Alves et al., 2021).

In turn, Giaquinto et al. (2017) also evaluated the antimicrobial activity of PHB films, however, incorporated with canola essential oil (CAOE). Mechanical analyzes also showed that films containing CAOE presented greater flexibility, and thermal analyzes showed that the addition of oil also changes the thermal properties of PHB, such as melting and crystallization temperatures, relative crystallinity and maximum crystallization rate. According to Basnett et al. (2020), the feasibility of EOs conferring antimicrobial activity to PHA films may lead to an expansion of the application of these biofilms in various industrial sectors, including implantable materials, allowing these antimicrobial materials to be used in the regeneration of soft tissues (skin).

CONCLUSION

This work demonstrates that photosynthetic microorganisms can be a viable and low-cost alternative for the production of biopolymers (PHAs) with great industrial application, with production similar to those found in the literature by bacteria, with biosynthetic pathways still to be better studied and elucidated, and with great prospects for the application of these biopolymers in the formation of thin films incorporated into essential oils, active packaging, with antimicrobial characteristics and great applicability in the packaging industry.

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Soil fertilization with the use of basalt and animal residues and their effects on soybean yield

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ABSTRACT
The use of basalt used with animal residues can be an efficient fertilizer alternative for soybean crops. The objective of this study was to evaluate the effect of doses of basalt powder with cattle manure and chicken litter on soybean yield, using soluble chemical fertilization (NPK) as a reference. The experiment was carried out in DBC with factorial arrangement (5 x 2) + 1, in four replications, using five basalt doses (0, 4, 8, 12 and 16 t ha-1) associated with cattle manure and chicken litter and an additional treatment with soluble chemical fertilization (NPK). The following parameters were evaluated: number of pods per plant, number of grains per pod, weight of one thousand grains and yield. The yield of soybean cultivated with basalt and chicken litter was on average 3,462.8 kg ha-1 and with cattle manure it was 3,439.2 kg ha-1. There was no effect of basalt rock dust and animal residues on any of the soybean yield components, but even so, expressive values were obtained, maintaining good yield results. The components of soybean production and yield found with the application of basalt associated with the residues were the same as those found with the use of soluble chemical admixture.

Keywords: Fertilization, Chicken litter, Agronomic characteristics, Remineralizing cattle manure.

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INTRODUCTION

The expansion of soybean cultivation and productivity is associated with technological advances in production, especially management and fertilization. Currently, the most common form of fertilization in agriculture is through industrialized sources of nutrients, which are basically soluble fertilizers such as NPK (a mixture of different concentrations of nitrogen, phosphorus, and potassium), in addition to other micronutrients specific to each type of soil and crop (PEIXOTO *et al*., 2018; TOSCANI; CAMPOS, 2017).

Brazil imports about 85% of the fertilisers it uses in agriculture. This reality shows a high level of external dependence and leaves the Brazilian economy vulnerable to fluctuations in the international fertilizer market (BRASIL, 2021).

Given this agricultural scenario, research has been advancing to propose alternative sources of fertilizers, with remineralizers/rock dust, which are ground rocks that have minerals capable of providing nutrients to plants, meeting their needs (ALMEIDA JÚNIOR *et al*., 2022).

Among the rocks that can be used in agriculture, basalt has a good potential due to its rich chemical composition, as it has variable amounts of nutrients that can be presented with greater or lesser ease of solubilization, thus contributing to the residual effect for a long period (FERREIRA *et al*., 2009; THEODORO *et al*., 2012).

As basalt rock dust has a low solubilization, one of the alternatives to increase its dissolution is the association with materials that have great biological activity, such as animal waste such as chicken litter and cattle manure. Because they have great biological activity, they can increase the rate of solubilization of minerals and favor the release of nutrients that are associated with the composition of the rock (SILVA *et al*., 2012), in addition to being sources of nutrients and having the potential to improve soil quality.

There are few studies that use basalt rock dust in association with chicken litter and cattle manure in the cultivation of grains and research needs to be intensified. Thus, the objective of this study was to evaluate the effect of doses of basalt rock dust associated with chicken litter and cattle manure on soybean yield, using soluble chemical fertilization (NPK) as a reference.

MATERIAL AND METHODS

The work was carried out in 2022, at the Professor Alcibíades Luiz Orlando Experimental Station located in the municipality of Entre Rios do Oeste-PR, belonging to the State University of Western Paraná - Campus Marechal Cândido Rondon-PR (UNIOESTE/MCR). The geographical coordinates are 24º40'32, 66" south latitude and 54º16'50.46" west longitude, at 244 meters altitude.

The soil of the experimental area is classified as a typical Eutroferric Red NITOSOL, with a very clayey texture, with smooth undulating relief (SANTOS et al., 2018) and presented the following results at a depth of 0.00-0.20 m: 28.5 mg dm-3 of P; 2.3 cmolc dm-3 of K; 5.9 cmolc dm-3 of Ca; 1.8 cmolc dm-3 of Mg; 14.1 g dm-3 of CO; 5.3 pH (CaCl2); 4.6 H+Al; as well as granulometry: 706.8 g $^{kg-1}$ of clay, 182.9 g $^{kg-1}$ of silt, 166.3 g $^{kg-1}$ of sand.

According to the Köppen climate classification, the climate of the region is of the subtropical humid mesothermal type (Cfa), with hot summers, average temperatures above 22ºC and winters with average temperatures and below 18ºC and an average annual rainfall of 1600-1800 millimeters (CAVIGLIONE et al., 2000).

The experiment was conducted in randomized blocks (DBC) in a factorial arrangement (5 x 2) + 1, with four replications. Five doses of basalt powder (0, 4, 8, 12 and 16 t $^{ha-1}$) were tested combined with two sources of animal residues: cattle manure and chicken litter. The treatment consisted of the use of soluble chemical fertilization in the soybean sowing furrow at a dose of 300 kg $^{ha-1}$ of the commercial formulation 02-20-18 (NPK). The plots had eight sowing rows with a total area of 40 m2 (5x8 m) and a useful area of 28 m2.

Prior to soybean sowing, basalt rock powder was manually applied at the studied rates and 5.0 t $^{ha-1}$ of animal residues on the soil surface and in the total area of each plot.

Soybean sowing was carried out mechanically in October 2020. The cultivar M 5947 IPRO was used, with a spacing of 0.50 m between rows, and approximately 15 seeds/linear meter were distributed. The cultivar has an indeterminate growth habit, with an early cycle and a maturation group of 5.9.

After sowing, constant monitoring was carried out in order to carry out the necessary cultural treatments of the crop. To control spontaneous plants, manual weeding was performed. Monitoring was carried out with a beating cloth to assess the presence of pests and perform phytosanitary control when necessary.

The evaluation of the production components (Number of pods per plant, Number of grains per pod) was carried out in ten plants of the useful area of each plot when they were at the R8 stage. The number of pods per plant was determined by quantifying all pods with grains, calculating the average number of pods per plant. The number of grains per pod was determined by counting the viable grains per pod. The average number of grains per plant was calculated by multiplying the average value of the number of grains per pod by the number of pods found per plant.

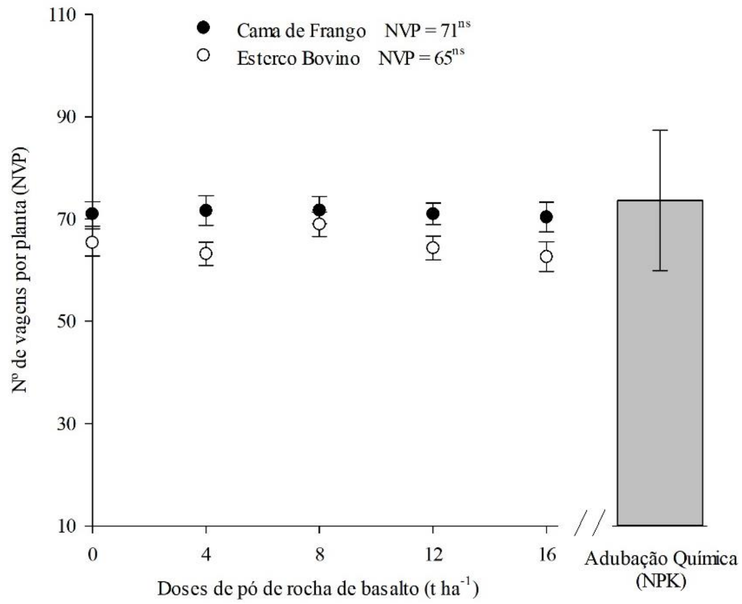
The yield (kg $^{ha-1}$) of soybean was determined when the crop was at the R8 stage (physiological maturation). The plants were collected from the useful area of the plot, being threshed and weighed on scales. The moisture content of the grains was determined and their weight was corrected to 13% moisture content and the results were expressed in kg $^{ha-1}$.

Once the assumptions were met, analysis of variance (ANOVA) was performed at the level of 5% significance for test F. For the doses within each residue, the data were submitted to polynomial regression analysis, and the model that best fit the investigated phenomenon was chosen. To compare the treatments (doses of rock dust and animal residues) with the additional control (soluble fertilization), the Dunnett test (5% probability of error) was applied, where the Dunnett DMS was calculated, which was added and subtracted from the mean value of the control to obtain a margin of comparison.

RESULTS AND DISCUSSION

The doses of basalt rock dust and animal residues did not promote significant changes in the number of pods per plant (NVP), number of grains per plant (NGP), thousand grain mass (MMG) and soybean yield, as well as the values of these variables were equal to those obtained in soluble chemical fertilization (Figures 1, 2, 3 and 4 respectively).

Figure 1 - Number of soybean pods per plant (NGV) as a function of doses of basalt rock dust and animal residues. ns: Not significant for polynomial regression fitting. Notes: Dot bars indicate the average error. Chemical Fertilization Bar indicates Dunnett's DMS (5% error).

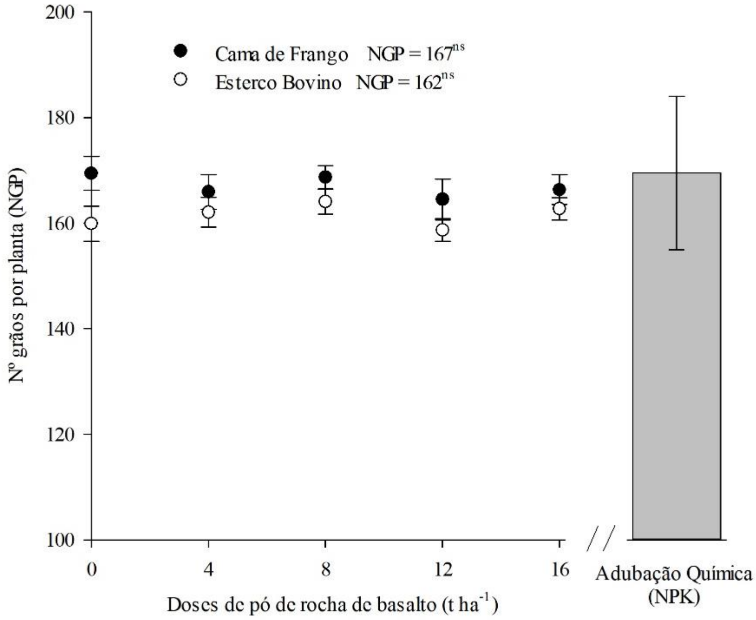


Alovisi et al. (2020), working with the application of rock dust doses ranging from 0 to 10 t ha-1, did not find a significant effect of the doses on NVP, obtaining an average of 74 pods per plant. Different results were observed by Sustakowski (2020), in this case with the basalt rock dust dose of 6.9 t ha-1 presented the highest NVP (85.25); that is, about 17 pods more than the amount obtained without the application of rock dust, which gave an increase of 25%.

For Bárbaro et al. (2006), the number of pods per plant is one of the most important for determining grain yield, and Carpentieri-Pípolo et al. (2005) found that plants with a higher number of pods also had higher seed weight per plant, which can interfere with the final yield.

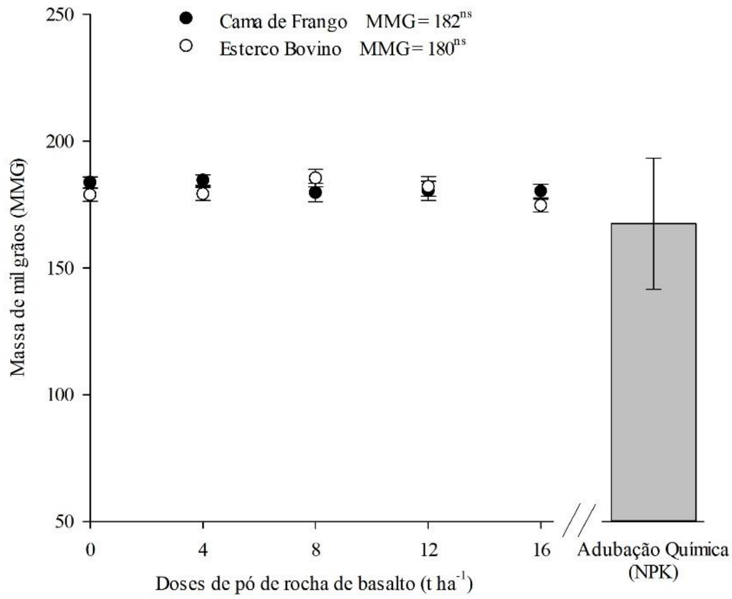
Despite the absence of effect of the treatments on the NGP, it presented considerable and close mean values (Figure 2).

Figure 2 - Number of soybean grains per plant (NGP) as a function of doses of basalt rock dust and animal residues. ns: Not significant for polynomial regression fitting. Notes: Dot bars indicate the average error. Chemical Fertilization Bar indicates Dunnett's DMS (5% error).



The mass of one thousand grains for the cultivar of SOYBEAN M 5947 IPRO is on average 170 g, and values above the average were found in this study with the use of chicken litter (182 g) and cattle manure (180 g) in the different basalt doses tested, which is a good result that even though there was no effect of the treatments on this variable, however, considerable MMG values were obtained from soybean (Figure 3).

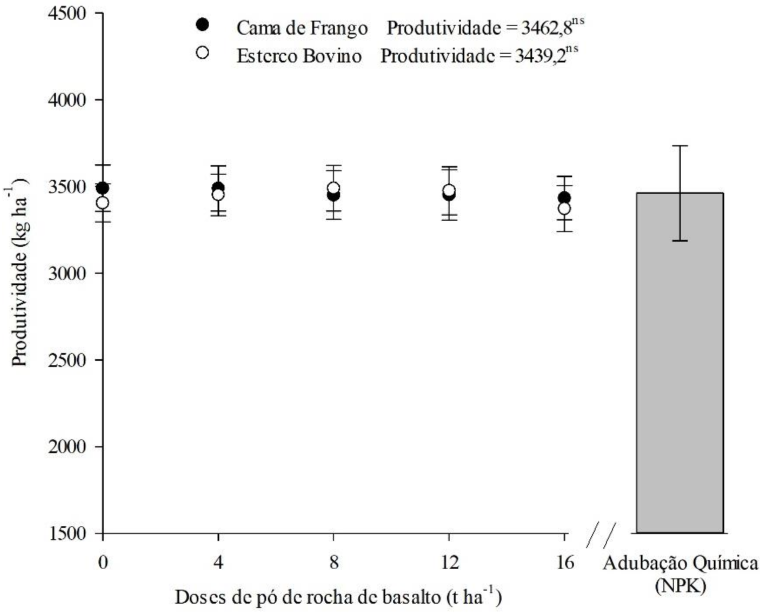
Figure 3 - Mass of one thousand grains (MMG) of soybean as a function of doses of basalt rock dust and animal residues. ^{ns}: Not significant for polynomial regression fitting. Notes: Dot bars indicate the average error. Chemical Fertilization Bar indicates Dunnett's DMS (5% error).



Almeida Junior et al. (2020) when evaluating the application of different doses of rock dust did not find a significant effect on the mass values of one thousand grains, however the values found by the authors are higher than those found in the present study. In general, the authors found an average weight of 1,000 grains of 197.5 g for the doses of 3, 6, 9 and 12 t $^{ha-1}$. On the other hand, Silva et al. (2019) also observed the effect of rock dust doses on the mass of one thousand grains, however the highest mass was at the dose of 6 t $^{ha-1}$, with a value of 155 g per thousand grains.

The yield of the crop was close to the national average of the 20/21 harvest (3,527 kg $^{ha-1}$) and that of the State of Paraná (3,535 kg $^{ha-1}$), being obtained in the cultivation of soybean subjected to basalt doses with chicken litter a productivity of 3,462.8 kg $^{ha-1}$ and with cattle manure of 3,439.2 kg $^{ha-1}$ (Figure 20).

Figure 4 - Soybean yield as a function of doses of basalt rock dust and animal residues. ns: Not significant for polynomial regression fitting. Notes: Dot bars indicate the average error. Chemical Fertilization Bar indicates Dunnett's DMS (5% error).



The productivity of the treatments used did not differ from that found with the use of soluble chemical fertilization, a very positive fact that reinforces the idea that the use of these inputs can favor the yield of the crop as much as the chemical fertilizer, and can be an alternative to complement or even substitute use over time. Silva et al. (2020), who for two consecutive seasons evaluated the effect of basalt powder doses (5, 10, 20, 40, 60, 80, 120, 160 and 200 t $^{ha-1}$) and chemical fertilization (NPK) on the black bean crop, found that doses from 5 to 60 t $^{ha-1}$ had an equivalent action on productivity compared to chemical fertilization.

Although it did not provide significant answers for the polynomial regression models tested, the average results obtained from the yield components were positive and within the reference values found for the crop, a reflection that may have been influenced by the nutrient contents in the soil and in the leaf and that contributed in an essential way to the yields obtained.

According to Lana et al. (2003), there is a direct relationship between soil fertility and soybean yield, which is directly dependent on the available concentration of nutrients in the soil solution. Therefore, basalt rock dust and animal residues were efficient in providing nutrients to maintain soybean production; thus resulting in adequate average values of grain yield.

Recent studies have shown some divergences in relation to the results obtained when using remineralizers in species of agricultural interest (AGUILERA et al., 2020). De Morais et al. (2020), when evaluating the soybean crop, after application of doses of amethyst powder, obtained an increase in the number of pods per plant, however, there was no change in grain yield, regardless of the doses applied.

For Aguilera et al. (2020), the doses of basalt rock dust (0, 1, 3 and 5 t $^{ha-1}$) did not influence the yield of three soybean cultivars, but contributed to obtain a better grain size and, thus, improve their quality.

On the other hand, Almeida Júnior et al. (2020) used doses of 0 to 24 t $^{ha-1}$ of basalt in soybean crops and obtained changes in the productive components for all agronomic characteristics tested and for yield, with an increase of 59% (dose of 24 t $^{ha-1}$ with 5,338 kg $^{ha-1}$) values above the national average (3,337 kg $^{ha-1}$, CONAB, 2023) in favor of the remineralizer.

According to Sustakowski (2020), the highest soybean yield (3,590.23 kg $^{ha-1}$) was obtained at the basalt dose of 8.4 t $^{ha-1}$; that is, an increase of 16% in relation to the productivity obtained without the application of rock dust (3,083.80 kg $^{ha-1}$).

In general, the lack of significant effects associated with the use of rocks can be linked to a number of factors such as the short cycle of the crop used as a "pilot" plant, short evaluation period, climatic conditions unfavorable to weathering and extremely sterile soils or soils with low microbial activity (SILVA et al., 2008), factors that are directly related to the remineralization capacity resulting from the use of rock dust.

In this sense, significant results can be obtained in successive crops, which is recommended when working with soil remineralizers that have a medium to long-term residual effect.

CONCLUSIONS

There was no effect of basalt rock dust and animal residues on any of the soybean yield components, but even so, expressive values were obtained, maintaining good yield results.

The components of soybean production and yield found with the application of basalt associated with the residues were the same as those found with the use of soluble chemical admixture.

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Inland water aquaculture: The role of good management practices and the use of ecotechnologies for the sustainability of the activity

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ABSTRACT

It is known that the health of fish and other aquatic organisms depend on good quality water in adequate quantity, its maintenance is a constant concern in aquaculture, as they directly affect productive performance, such as survival, growth, reproduction and susceptibility to diseases, a fact that compromises economic success. The concept of "Sustainable Aquaculture" or "Responsible Aquaculture" is increasingly discussed, which designates the desirable way of producing fish in the aquatic environment, with environmental, economic, social and political rationality. In this sense, this chapter addresses the legal aspects of aquaculture, the main impacts caused by the activity, the adoption of good management practices and the use of ecotechnologies aiming at the sustainability of the activity.

Keywords: Sustainable aquaculture, Effluent, Phosphorus, Legislation, Environment, Water quality.

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INTRODUCTION

Aquaculture (production of aquatic organisms) is a booming global economic sector due to its ability to produce healthy and nutritionally rich food, being a primary source of protein in many countries (FAO, 2020). In Brazil, the aquaculture sector stands out compared to other animal production activities, only between the years 2014 and 2023 the growth rate of the activity was 5.33% (Peixe-Br, 2024), and should be considered as a reality and no longer an activity with future growth potential, generating for Brazil an annual direct revenue of more than one billion dollars (Valenti et al., 2021).

Although it is impossible to produce fish without causing environmental changes, the current challenge for aquaculture is to develop while reducing this impact as much as possible. The development of management techniques to increase productivity without evaluating the impacts produced is not conceived, and it should be understood that environmental preservation is part of the production process (Valenti et al., 2018).

Aquaculture activities can affect the environment more or less intensely, according to the modality with which the cultivation is practiced: Extensive, Semi-intensive, Intensive and super-intensive. The environmental problems potentially associated with the creation of aquatic organisms are: Alteration of the landscape with the conversion of preserved areas for the implementation of the projects; Deterioration of water quality mainly due to leftover feed; Impacts on aquatic diversity. In this way, the measures to mitigate the problems caused by aquaculture can be divided into actions before and after the start of production.

In this sense, the concept of "Sustainable Aquaculture" or "Responsible Aquaculture" is increasingly discussed, which designates the desirable way of producing fish in the aquatic environment, with environmental, economic, social and political rationality.

This chapter is aimed at undergraduate and graduate students from different areas of knowledge, such as: environmental sciences, biological sciences, agronomic engineering, fisheries engineering, veterinary medicine, zootechnics, among other related courses, as well as for professionals in the field of aquaculture. The next items will address general aspects of the impacts caused by aquaculture activity, the adoption of good management practices and the use of ecotechnologies aiming at the sustainability of the activity.

AQUACULTURE AND SUSTAINABILITY

Sustainable aquaculture can be defined as the profitable production of aquatic organisms, maintaining a lasting harmonious interaction with ecosystems and local communities, and must be evaluated in the environmental, economic and social dimensions, which are inseparable and essential for a perennial activity (Valenti, 2008).

Aquaculture has some characteristics that make it sustainable, since the investment cost is relatively low and the productivity is high, which represents the ability to expand food production significantly, thus contributing to greater food security in the world (Siqueira, 2017). As an activity with low implementation and operational costs, as well as accessible technology, aquaculture presents itself as an alternative for generating employment and income in a competitive way in less developed regions (Siqueira, 2017). It should also be noted that in the state of São Paulo, through article 1 of State Decree No. 60,582 of June 27, 2014, the recognition of aquaculture activity as of social and economic interest (São Paulo, 2014).

Knowing that the health of fish and other aquatic organisms depend on water of good quality and in adequate quantity, its maintenance is a constant concern in fish farming, as it directly affects productive performance, such as survival, growth, reproduction and susceptibility to diseases, a fact that compromises economic success (Boyd, 1990; Mercante et al., 2020a).

In addition to the constant concern with water quality for the success of production, there is also concern about the impacts that the activity can cause on the receiving water body due to waste discharged via effluent (Mercante et al., 2020a). These impacts depend on the species cultivated, the method of cultivation, the hydrography of the region, the type of food provided, and management practices (Cao et al., 2007). In this sense, in Brazil, states have increasingly intensified the monitoring and control of water quality, a fact that has led to the readjustment of legal requirements (Mercante et al., 2020b).

LEGAL ASPECTS OF BRAZILIAN AQUACULTURE

In Brazilian legislation, the National Council for the Environment (CONAMA), a federal collegiate body of the Ministry of the Environment, provides for the classification of water bodies and environmental guidelines for their framing, as well as establishes the conditions and standards for the discharge of effluents through resolutions 357/2005 and 430/2011 and their amendments (Brasil, 2005; 2011). That is, it regulates the permissible limit of nutrient concentrations in the discharged effluent according to the framework of the water body.

Within the scope of the State of São Paulo, through State Decree 62.243 of November 1, 2016 (São Paulo, 2016), the rules and procedures for the environmental licensing of aquaculture in São Paulo are established. In particular, the aforementioned Decree was drafted with the aim of promoting actions to strengthen and encourage aquaculture in São Paulo, which has grown in recent years. Such measures allow the regularization of aquaculture activities, enabling small producers in São Paulo to leave informality and start working safely, in accordance with current legislation (Mercante et al., 2020b). Fish farmers, frog farmers, mariculturists, algae farmers and other producers of aquatic organisms in the State of São Paulo can regularize their activities through the Declaration of Conformity of Aquaculture Activity (DCAA) (São Paulo, 2016).

In this context, the technical manual prepared by Secanho et al. (2022), brings extensively and with accessible language the stages of environmental licensing of aquaculture in the state of São Paulo, with information ranging from the need, or not, of environmental licensing according to the size of production, through the different stages of the authorization process and/or regularity of the activity.

The search for sustainable production, which combines the necessary speed for an economically viable production, without compromising the due care with environmental quality, is the great challenge of the new public policies. In this sense, the directives presented above can be considered as an advance for the sustainability of the activity, however, the control of water quality, in quantity and quality, and the control of the discharge of effluents from the production systems, are still a great challenge for the producer.

In excavated systems (nurseries and tanks), the control of effluent discharge follows the standards of CONAMA resolutions 357/2005 and 430/2011 and their amendments, bringing guidelines on the water quality parameters to be met for the discharge of liquid waste from the activity. Currently, meeting these standards established by current legislation has been an obstacle to environmental regularization, especially for small and medium-sized producers, who increasingly need tools to develop a more productive activity, with the lowest possible environmental impact. In this sense, the development and popularization of the insertion of good management practices throughout the production process are crucial, as well as the development of *eco-friendly technologies* that are aimed at the environmental sustainability of the activity.

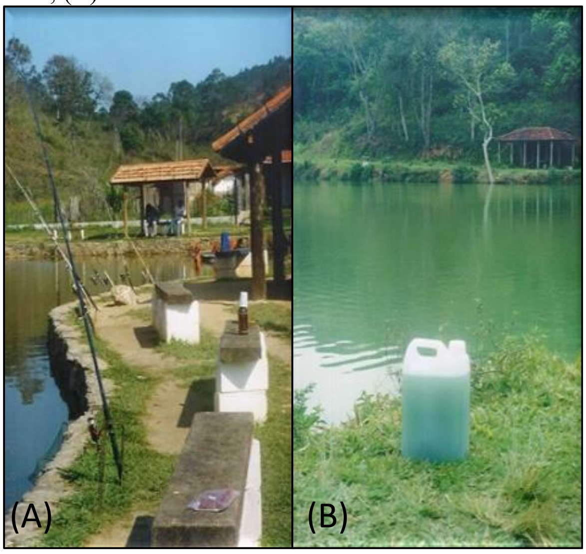
GOOD MANAGEMENT PRACTICES IN AQUACULTURE

Inadequate water management can trigger the process of artificial eutrophication, generating a chain reaction, breaking the stability of the system, since the factors act in an interconnected way, affecting the success of the enterprise (Vinatea, 1997; Sipaúba-Tavares et al., 1998). According to Boyd (1990), eutrophication in production systems occurs due to the excess of food offered to fish, which is not fully consumed, causing food leftovers and, therefore, accumulation of organic matter in the water

The economic success of this activity, according to Eler et al. (2001), depends on the good maintenance of water quality, and this quality can be influenced by several factors, but mainly by food management.

Studies carried out in fishing grounds (Figure 1) located in the metropolitan region of São Paulo indicated a high degree of water deterioration, suggesting, among other factors, that the management employed promoted an intense eutrophication process in these places (Mercante et al., 2005; Mercante et al., 2007). Also with regard to sanitary aspects, cyanotoxins (toxins produced by cyanobacteria) were found in 60% of the fish-pays, a fact related, among others, to the high concentrations of phosphorus present in the water (Honda et al., 2006), due to inadequate management.

Figure 1 – Fishing grounds located in the metropolitan region of São Paulo. (A) highlighting the fishing grounds and fishing equipment used by the visitors; (B) Flask used for water collection



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Compared to other macronutrients needed by aquatic life, phosphorus is the one that occurs in the least abundance, therefore, it is considered the limiting element to biological productivity (Wetzel, 1993). In addition, this element is one of the main causes of artificial eutrophication.

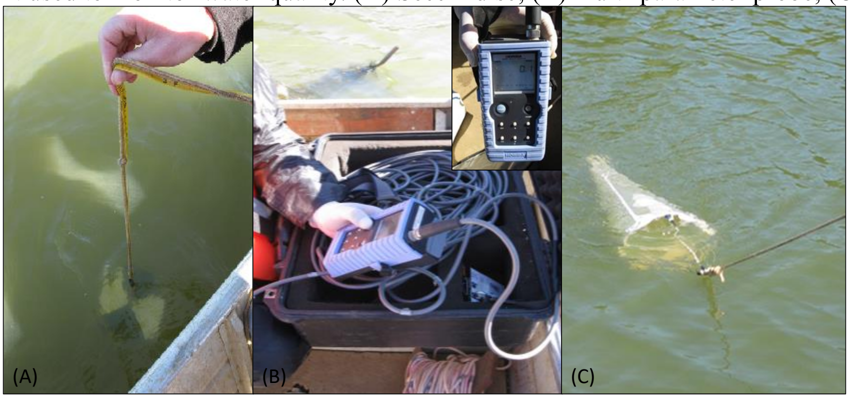
Another element of great importance for the metabolism of aquatic systems is nitrogen. This element participates in the formation of proteins, and like phosphorus, it also acts as a limiting factor in primary production (Wetzel, 1993).

The increase of nitrogen in the medium can also occur due to animal excreta, the application of ammoniac nitrogen fertilizers, such as ammonium sulfate, ammonium nitrate, and monoammonium and diamonic phosphates. When there is a high concentration of organic nitrogen in the aquatic substrate, it is released into the water in the form of ammonia, which is very toxic to aquatic organisms (Sipaúba-Tavares et al., 1998).

The assimilation of ammonia, nitrate and phosphorus by phytoplankton can lead to uncontrolled growth of this community, causing algal blooms in the environment (Paerl; Tucker, 1995).

In order to control water quality in fish farming, it is essential to monitor physical variables (e.g., temperature, turbidity, transparency of the water "secchi disk"), chemical (e.g., pH, alkalinity, hardness, dissolved oxygen, ammonia ion) and biological variables (e.g., phytoplankton and zooplankton) (Figure 2). And the understanding of the relationship between these variables, together with the application of good management practices, becomes a useful tool for environmental sustainability.

Figure 2 – Equipment used to monitor water quality. (A) Secchi disc; (B) multi-parameter probe; (C) Plankton network



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According to the general principles of the FAO Code of Conduct for Responsible Fisheries (1997), States should produce and regulate aquaculture development strategies as requirements to ensure its ecologically sustainable development, allowing the rational use of sources in their different uses. In Brazil, adhering to this global demand, the Ministry of Agriculture, Livestock and Supply – MAPA published in 2022 the "Manual of good practices in farmed fish farming" (Barcelos et al., 2022), with information that transcends from fish welfare, health, physiology, water quality, facilities to aspects of nutrition and food management. For these authors, the maintenance of water quality at levels appropriate to production is strongly and directly linked to the well-being of fish and related to "Environmental Freedom", which advocates that animals should remain in conditions free of discomfort.

Good management practices should consider several aspects that involve both fish production and the maintenance of fish in ponds for sports practices such as catch and release or fishing grounds for recreation and consumption. The implementation of good management practices aims to ensure good water quality, preserving the health and well-being of the animals, as well as controlling the impact generated in the receiving water body from the effluent discharge.

Good practices involve the elaboration of implementation projects that consider water management with monitoring and control of the flow and residence time of the water. And feeding management, which involves the effective control of nitrogen and phosphorus input from food and fertilization. In this sense, feed conversion is an important factor for pollution control, considering that only about 30% of the food offered is consumed by fish and 70% remains in the water, being either assimilated by phytoplankton or deposited at the bottom of ponds (Frasca-Scorvo et al., 2013; Moraes et al., 2016; David et al., 2017; Osti et al., 2018a). Therefore, the density of fish per production area associated with the growth phase should be considered for the practice of feeding. As a result, it is possible to drastically reduce the concentrations of phosphorus and nitrogen in the water, avoiding intense eutrophication processes, which cause both environmental and health damage to the animals.

Procedures to reduce nitrogen and phosphorus levels in the water of production systems are necessary to control eutrophication, minimizing the impact on the environment (Pereira et al., 2012; Alexander et al., 2016). Thus, the monitoring and maintenance of water quality at adequate levels for rearing is essential for productive success and can be one of the biggest obstacles to the regularization of aquaculture enterprises, so the control arising from knowledge about water quality, with the interaction between physical, chemical and biological factors, should be considered as an ally of the producer.

ECOTECHNOLOGIES AIMED AT IMPROVING WATER QUALITY

The recommended treatment techniques for aquaculture effluents are dependent on the composition and volume generated in the different production systems. The quality of the effluent generated can vary between that produced in the extensive and intensive system, depending on the characteristics of the crop. The intensive cultivation system is characterized by high stocking density and total dependence on exogenous feed, and the natural food production of the nursery is even disregarded. The amount of food should be increased according to the increasing consumption, that is, regulated according to the size of the fish, the stocking density and the temperature of the water. In this way, a large rate of water turnover is used for the removal of metabolites and food residues present in the water.

The high density of fish stocking requires a large amount of artificial food to be introduced daily to the production systems, generating a proportional amount of organic matter formed by feces and food scraps. Most of the organic matter produced in the cultivation system is in particulate form and has a short sedimentation time, being accumulated at the bottom of the system or released to the receiving water body.

In Brazil, there are few studies developed with the purpose of generating adequate and efficient technologies to reduce or improve effluent quality. Such technologies include integrated aquaculture-agriculture systems, integrated multitrophic aquaculture systems (IMTA), recirculating systems (RAS), bioflocs (BFT), natural filtration (constructed *wetlands*), sedimentation tanks, among others (Tucker; Hargreaves, 2003; Henares et al., 2020). Currently, the development and adoption of these systems aim to meet the requirements of new legislation and the pressures of environmental agencies and society itself.

Natural filtration ecotechnology (constructed *wetland* - WC) is an option in the treatment choice of liquid effluents generated by breeding ponds. Toilets are systems designed and built for the treatment of effluents in order to use natural processes in the removal of pollutants (Kivaisi, 2001). In these systems, aquatic macrophytes play an important role in the removal of nutrients by assimilation, in addition to providing substrate for the development of microorganisms that act in the mineralization of organic matter and in the absorption of nutrients (Brix, 1997; Sipauba-Tavares et al. 2015). It is also noteworthy that other processes occur in these treatment systems and contribute to the removal of nutrients from the effluent, such as sedimentation, chemical precipitation and biochemical transformations (e.g. ammoniation and denitrification) (USEPA, 2000; Braskerud, 2002).

A synthesis of the main forms of toilets developed for effluent treatment was elaborated by Salati et al. (2009). In aquaculture, toilets have been shown to be an economically interesting alternative, as they have a low cost for preparation and simple operation and maintenance, and are highly efficient in removing organic matter from aquaculture ponds (Lin et al., 2005; Henry-Silva; Camargo, 2008; Carballeira et al., 2016; Osti et al., 2018b).

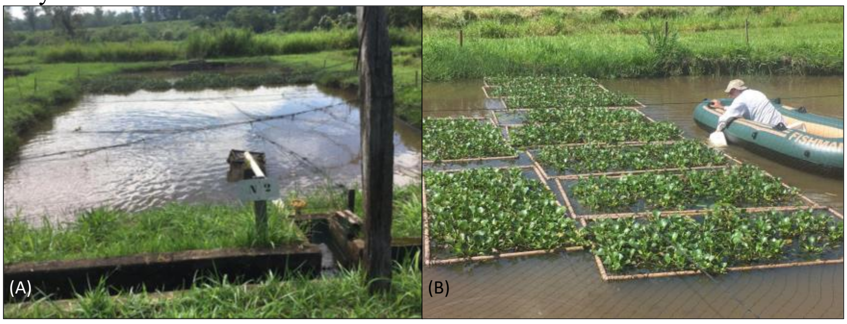
One of the great challenges of this technology is in the design of the WC, a fact that is fundamental for the planning and determination of the feasibility of using this technology (Camargo; Henares, 2014). For example, the work of Biudes (2007) concludes that the area required to treat the effluent from the breeding pond of *Macrobrachium rosenbergii* with floating aquatic macrophytes (*Eichornia crassipes*) at a nursery water renewal rate of 10% per day, corresponds to approximately 10% of the surface area of the pond. This same author shows in his work that the phase in which the macrophyte presents the best rate of nutrient absorption corresponds approximately when it is at a density between 5 and 25 kg of fresh mass/m².

The need to use at least 10% of the production area for the implementation of WCs in order to neutralize the effects of the activity on water resources can be a barrier to the economic viability of the activity, as it may be necessary to convert preserved areas or allocate the production area for the implementation of WCs.

Alternative technologies: WCs have been adopted in different regions for the purpose of pollution control, called artificial floating islands (APIs) which are designed to float on the surface of the water with floats and structured to stabilize plant roots and underground stems. Macrophytes are planted on the structures, which function similarly to natural floating mats. The use of artificial floating island systems (APIs) has been tested in the control of residues from activities such as: pig farming (Hubbard et al., 2004); in stormwater drainage systems (Headley; Tanner 2008; Lynch et al., 2015); in acid mine drainage basin sites (Gupta et al., 2020). Recently, an adaptation of this technology was devised with the aim of testing the efficiency of artificial floating islands for the treatment of tilapia effluents (Osti et al., 2020), as can be seen in Figure 3. The authors built PVC pipe structures of 2 m2 each and colonized them with free-floating macrophytes (*Eichhornia crassipes*), which occupied 10% of the tilapia production area. The results showed that the structure was efficient in the removal of nitrogen and phosphorus, reducing the load released by the effluent from the fish farming.

In common, the studies show that WC and API ecotechnologies are an economically and environmentally viable option for the removal of nutrients and metals from water and/or the retention of suspended particulate matter. However, the sizing and economic feasibility of these treatment systems are still challenges to be overcome in order to achieve the environmental sustainability of aquaculture.

Figure 3 – Tilapia fattening ponds. (A) detail of the nursery with the artificial floating island system; (B) Detail of artificial floating island systems



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CONCLUSIONS

The adoption of good management practices, ranging from soil correction, continuous monitoring of water quantity and quality, the use of good quality feed and offered in adequate quantities, among other actions that can and should be followed by the producer, aim at the sustainable development of the activity. In the last decades, aquaculture production systems with better technologies have been sought in order to reduce the impact generated on the environment, however, considering the state of the art of research developed in the State of São Paulo, we observe the scarcity of research on alternative technologies of low cost and viable to the small and medium producer aiming at the reduction or treatment of effluents and escapes from fish farming. Within the São Paulo State Aquaculture Plan, these issues were listed as a priority to comply with the current environmental legislation, recommending the use of new technologies for the treatment of effluents generated by aquaculture. It should be noted that these strategies must consider the specific conditions of each production system, where space constraints and high water flows must be contemplated. In summary, although we have developed research on this topic, it is concluded that there is a need to develop a technological package based on sustainability for the treatment of aquaculture effluent. This package should consider both good management practices and the size, cost and efficiency of the treatment.

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Contributions of genetic improvement programs for dairy livestock farming

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ABSTRACT

Genetic improvement programs have played a fundamental role in the evolution of dairy farming, promoting significant increases in productivity and milk quality. This study analyzes the main contributions of these initiatives, highlighting the techniques used, such as genetic selection and artificial insemination, and their impacts on production efficiency and animal health. Furthermore, the challenges faced by livestock farmers in implementing these programs and the future perspectives of the sector are discussed. The results indicate that genetic improvement, combined with appropriate management practices, can provide significant gains in terms of production volume, milk quality, disease resistance and animal longevity, contributing to the sustainability and competitiveness of dairy farming. This summary encapsulates the objectives, main results, and implications of the study, offering a clear and concise overview of the contributions of genetic improvement programs to dairy farming.

Keywords: Animal production, Genetic improvement, Dairy farming.

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INTRODUCTION

Genetic improvement plays an extremely important role in increasing the productivity of any animal species. However, its purpose needs to be directly related to the environmental conditions in which it is intended to use certain species. In dairy farming, this is no different. Currently in Brazil, it is common to find production systems with great imbalance between genetics and environmental conditions. There are dairy herds where the producer is able to provide optimal environmental conditions to the animals, such as management, health and nutrition, however, the low genetic capacity of these animals prevents a better response when we talk about production and profitability. The opposite is also true when we find production systems with precarious environmental conditions, but often with animals of high genetic capacity, restricting the expression of the animals' genetic potential.

Milk production in Brazil is a traditional practice. It is known that the national production represents the fifth largest producer of this product in the world and in almost all Brazilian municipalities there is at least one milk producer. However, productivity is below what would be necessary for the activity to provide sustainability, especially economic, for most dairy farmers. One way to effectively contribute to the evolution of this activity is by applying genetic improvement procedures, such as the rational use of selection and mating systems. Selection represents the choice of males and females that will be used for reproduction, while mating systems represent the strategies adopted in defining which males will be mated with which females. Through these two tools, it is possible to modify, over time, the gene frequencies of alleles of economic interest in herds and, thus, optimize the expected results, contributing to the increase of productivity and profitability of milk production systems in Brazil.

In the past, selection techniques and mating systems were practiced within herds, through empirical methods, without the use of trait information measured by more precise procedures. With the creation of the National System of Agricultural Research in Brazil in 1976, agricultural research institutions began to contribute with guidance on the use of new technologies for the sector. In the same period, there was also a great evolution in the use of new reproductive biotechniques, with the expansion of the use of Artificial Insemination (AI), Fixed Time Artificial Insemination (FTAI) and In Vitro Fertilization (IVF). In view of the above, the objective of this chapter is to demonstrate how genetic improvement is capable of contributing to the development of national dairy farming.

DEVELOPMENT

The genetic improvement programs developed in Brazil were initially coordinated by the Brazilian Agricultural Research Corporation (Embrapa), from 1975 to 1992, in several dairy farms and research centers in the Southeast of the country (FREITAS et al., 1992; LEMOS et al., 1992).

These programs involved the planning and implementation of two research projects on crossbreeding strategies between dairy breeds and on progeny testing of crossbred European−zebu bulls. The projects were part of the technical assistance program of the *Food and Agriculture Organization* (FAO), which involved the production and distribution of semen, production and distribution of females of different Holstein-Zebu genetic compositions in collaborating herds, monitoring of zootechnical records, control of milk production, processing of samples for analysis of milk fat and development of a *software* for the organization and recording of all information. The objectives of the projects were to promote and coordinate the implementation of a national selection program for milk production, to obtain experimental material for studies aimed at improving the methodology for the selection of dairy cattle and to provide an opportunity for technicians to know directly the problems encountered in the practical application of a breeding program (FREITAS et al., 1992).

Among the favorable precedents for the execution of genetic improvement projects in Brazil, the lack of genetic evaluations in national dairy bulls, used in artificial insemination, and the new norms of the Ministry of Agriculture, Livestock and Supply (MAPA), requiring progeny tests so that sires could be used, were mentioned. In addition, breeders were interested in using genetic material of proven quality. At that time, there was very little information on the productive, reproductive and economic performance of crossbred animals raised under Brazilian conditions, especially in the Southeast, Northeast and North regions of the country (HAYES et al., 2009).

The projects were conducted with great success and showed consistent results, identifying the best genetic composition of cattle for milk production, to be used in the prevailing management conditions in Central Brazil, and especially in the Southeast region. Since then, the work of genetic improvement of dairy cattle in Brazil has been expanding (HAYES et al., 2009).

In 1985, through a public-private partnership established between Embrapa Dairy Cattle, the Brazilian Association of Dairy Gir Breeders (ABCGIL) and the Brazilian Association of Zebu Breeders (ABCZ), the National Dairy Gir Improvement Program (PNMGL) was initiated, with the involvement of selector producers, collaborating dairy herds and the main state agricultural research companies. such as Minas Gerais (Epamig), Rio Grande do Norte (Emparn), Paraíba (Emepa) and the Institute of Animal Science of São Paulo (IZ), Federal Universities with research activities in agricultural sciences and artificial insemination centers. In its initial phase, the program consisted of the progeny test only for genetic evaluation of bulls for milk production. With the success achieved, new features and technologies were incorporated (MEUWISSEN et al., 2001).

Currently, the progeny test includes, in addition to the genetic evaluation of males and females for milk production, the genetic evaluation for the milk constituents (fat, protein and total solids), individual estimates of average parentage in the population, genotyping for the genes of betacasein (A2 milk), kappa-casein and beta-lactoglobulin and genetic evaluation for linear conformation and management traits (STAs) (MEUWISSEN et al., 2001).

The program has become broader, including the evaluation of young bulls to be included in the test for their fertility, conformation and temperament, also called pre-test, the program for evaluating the performance of first-calf females on pasture, called Sustainable Dairy Gyr, and the application of genomic selection techniques, which is in the implementation phase. but it has already contributed, as of 2016, to the correction of the kinship matrix and to the possibility of helping the breeder in choosing the bulls to be enrolled in the test (VANRADEN et al., 2009).

Currently, research on feed efficiency in Dairy Gyr animals is being initiated with a view to the continuous improvement of the program. The success achieved by the PNMGL was unquestionable, leading to the planning of the Guzera Breed Improvement Program for Milk (PNMGuL), initiated in 1994 along the same lines as the PNMGL, involving, in addition to the selector breeders and collaborating herds with crossbred cattle, Embrapa Dairy Cattle, the Brazilian Center for the Improvement of Guzerá (CBMG), a technical arm of the Association of Breeders of this breed, the School of Veterinary Medicine of the Federal University of Minas Gerais and state research companies. The work with the Guzera breed includes, in addition to the progeny test of bulls, an open nucleus for the selection of multiple ovulation and embryo transfer, also called Moet nucleus, and is characterized by the selection of sires of the Guzera breed of dual aptitude, meat and milk (LEMOS et al., 1992).

In the Girolando breed, the program began in 1997 and is conducted by the Brazilian Association of the breed itself, with technical coordination by Embrapa Dairy Cattle, also involving selector producers and the Agricultural Research Company of Minas Gerais (Epamig). In recent years, the Girolando breeding program has shown great evolution, with a huge increase in partnerships, an increase in the number of bulls in testing, greater participation of collaborating herds, as well as an increase in the interest of producers and the incorporation of technologies inserted in the Dairy Gyr program, with innovations, especially with a strong performance in the use of genomics in the selection work. The use of semen from proven crossbred bulls has shown a wide growth in Brazil (VARANDEN et al., 2009).

For the Sindhi breed, there was an initiative of Embrapa Dairy Cattle with the Association of Sindi breeders (ABCSindi) and the Agricultural Research Company of Paraíba (Emepa), where the PNMGL model was also followed, in 2009. At the time, the bulls were chosen to compose the first group to be included in a progeny test for milk production and constituents in this breed. Subsequently, genomic studies were carried out in which the main lineages of this breed were identified. The intention was to avoid, with the implementation of the breeding program, that there would be significant losses of genetic diversity in the breed, which has always had a small population size, and thus foster the sustainability of the selection program over future generations, and the viability of this breed as an alternative for milk production in Brazil (VARANDEN et al., 2009).

To date, the evolution of the program still comes up against the lack of herds that perform dairy controls, so that the bulls can have a sufficient number of progeny measured and, consequently, can be genetically evaluated. Pure breeds of European origin, such as Holstein, Jersey and Brown Swiss, despite not having a defined program of genetic improvement currently being carried out at the national level, benefit from the spectacular genetic evolution achieved in developed countries, as a result of the high intensity of selection practiced over time. The benefits result from the use of imported genetic material, including semen and embryos, and sometimes from the importation of animals. In addition, especially in the Holstein breed, there is structured work of the Association of Breeders of the breed providing excellent support to its Members. As a consequence, the evolution in the milk productivity of these breeds in Brazil is very expressive and consistent. In the past, there was a system of genetic evaluation of Holstein bulls, with publication of bull summaries, which was discontinued. Thus, currently, these breeds still lack a system of genetic evaluation of bulls and cows that allows the correct classification of these animals in the production conditions of Brazil (VARANDEN et al., 2009).

In this sense, there is an action started in 2012, but not yet concluded, by Embrapa Dairy Cattle with the Brazilian Association of Holstein Breeders and artificial insemination companies for Brazil's affiliation to ICAR/Interbull and for the establishment of national genomic prediction equations. Over the years, it has been observed that the application of knowledge of quantitative genetics, statistical methodologies, selection methods and mating systems has marked a new era in animal genetic improvement in Brazil, allowing substantial gains in quantitative and qualitative traits of economic importance, such as milk production. Currently, we are living in the era of genomics applied to selection work, which will certainly lead to a reduction in the generation interval, correction of pedigree errors and improvement of genetic improvement work. It is expected that the development of dairy farming will continue to occur consistently, reaching new levels in production, reproduction, and useful life of animals, bringing, as a consequence, economic return and sustainability to the national dairy activity (MEUWISSEN et al., 2001).

Genomic selection (GS) proposed by Meuwissen et al. (2001) uses high-density marker panels to predict genomic breeding values. This method is based on the assumption that part of the markers would be in disequilibrium of linkage with quantitative trait loci (QTLs), making it possible to predict genomic breeding values. The use of genomic information increases genetic gain by substantially reducing the generation interval and increasing prediction accuracy for young animals (HAYES et al., 2009). These advantages, associated with the rapid development of molecular biology techniques that enable high-density genotyping at lower and lower costs, have led to the rapid adoption of SG in the selection of the Holstein breed by several countries. In genomic selection, a reference population is genotyped with a panel of high-density SNP-like (single-base polymorphism) markers and phenotyped for the traits of interest (MEUWISSEN et al., 2001). By means of statistical procedures, these data are used to construct a prediction equation that will allow the obtaining of genomic genetic values for individuals who are genotyped, but who do not have a phenotype (candidates for selection).

Using Holstein breed data from 26 traits and a panel of approximately 50,000 markers, VanRaden et al. (2009) showed that the reliability of the predicted values was 50% for the genomic predictions and 27% for the traditional method, i.e., an average increase of 23% for the various traits. These data show the great potential of OS in the genetic improvement of dairy cattle, since the increase in the prediction accuracy of young bulls will result in greater annual genetic gain. SG is already a reality in international dairy cattle breeding and is already being incorporated into national genetic improvement programs, which will undoubtedly contribute to increasing the productivity of the dairy herd.

FINAL THOUGHTS

Milk production and productivity have shown remarkable growth in the last 30 to 40 years in Brazil. Growth in productivity has exceeded 100% and in production it is close to 500%. Productivity is still low, not exceeding 1,600 liters per cow per year considering all herds, most of which are made up of non-specialized animals and with an inappropriate management system for efficient milk production. However, when considering the productivity among the herds participating in the main genetic improvement programs in the country, as well as the productivity in the European breeds, it is clear that productivity is much higher, and it is perfectly possible to double the productivity of the national dairy herd in a very short interval, provided that producers have at their disposal policies to encourage the increase of production. through the adoption of quality-based payment measures, expansion of technical assistance with a view to the adoption of technologies in management, health, reproduction and, above all, the massification of the use of artificial insemination and other reproductive biotechniques that allow large-scale use of semen from proven bulls. Considering the information presented, as well as the future implementations of new selection procedures and crossbreeding systems, the contribution of genetic improvement programs to dairy farming is unquestionable and remarkable in Brazil.

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Peritonitis in equines – Literature review

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ABSTRACT

Peritonitis is characterized as inflammation of the peritoneum and is considered one of the most serious complications in horses. When associated with colic, they are responsible for a high mortality rate. It can present in a primary or secondary form, but diagnosing the origin of this disease is almost always very difficult. Paracentesis turns out to be the most accurate diagnostic method, being analyzed together with all the means sought for an accurate diagnosis.

Keywords: Abdomen, Inflammation, Peritoneum.

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INTRODUCTION

Peritonitis is characterized as inflammation of the mesothelium of the peritoneal cavity. It can present both acutely and chronically, septic or aseptic, and secondary to infectious processes, trauma, chemicals, parasites, visceral disease, abdominal surgery or neoplasia. Animals that have peritonitis develop a picture of colic that is secondary to another intestinal disease, and is not characterized by a primary infection. Peritonitis has a high mortality rate when associated with colic (White, 1990).

The presentation of the symptoms will depend on the intensity and extent of the peritonitis. In horses, it usually has a secondary presentation to infectious, chemical or parasitic aggressions of the peritoneum, and even to an important complication after abdominal cavity surgery (RADOSTITS et al., 2009).

It presents with a difficult diagnosis, because the clinical findings have predominant manifestations that are common to other diseases (RADOSTITS et al., 2009).

In the analysis of the peritoneal fluid, which is one of the most specific tests to confirm peritonitis, the color of the fluid will be yellowish and cloudy. Turbidity will show an increase in the number of white blood cells that can be seen depositing at the base of the tube, and the high concentration of protein causes the liquid to foam if shaken vigorously (Hillyer & Wright, 1997).

In view of the above, this paper aims to discuss peritonitis in horses, its means of confirming the diagnosis and treatment suggested in the literature.

DEVELOPMENT

Peritonitis is both an inflammation of the visceral peritoneum and can also be an inflammation of the parietal peritoneum, and is characterized as a common finding in equine colic syndrome (LUNA, 1994).

The primary form of the disease usually occurs with an interrelationship between low host immunity (HANSON, 1999). The secondary form, on the other hand, occurs through bacterial contamination of the peritoneum resulting from a hematogenous dissemination of bacteria from a primary site of infection by *Streptococcus equi* and *Rhodococcus equi*, perforation of viscera, intestinal devitalization secondary to strangulation or infarction, iatrogenic intervention, such as the application of trocaters and enterocentesis (MURRAY, 2000).

The peritoneum has very important functions for the digestive tract, such as promoting a selectively permeable barrier between the contents of the lumen and the body's tissues, facilitating the transport and digestion of food, being responsible for the absorption of the products of this digestion, and also producing hormones that regulate the activity of the digestive system. There are also cells present in this mucus-producing layer responsible for lubricating and protecting the wall of the digestive tract (JUNQUEIRA, 2004).

This differentiation between the acute or chronic form within a peritonitis setting is arbitrary. But, the authors believe it to be a useful classification, as they differ in terms of two syndromes. However, it is recognized as a difficult and complicated practice, where over the course of the disease, it can undergo changes such as going from acute to chronic or even from chronic to acute (HILLYER & WRIGHT, 1997).

The most evident clinical signs observed include moderate abdominal pain, depression, anorexia, weight loss, decreased borborygmus, diarrhea, hyperthermia, tachycardia, tachypnea, and clinical manifestation of dehydration (AUER, 2006; DABARAINER, 2006).

The diagnosis is made through the set of information obtained, based on the history and clinical symptoms, findings in the clinical examination, in addition to hematology and blood biochemistry (SEMRAD, 1992).

Among the numerous complementary diagnostic tests available, the most reliable for peritonitis is abdominal paracentesis followed by macroscopic, cytological and biochemical evaluation of the peritoneal fluid. Like any laboratory test, the aspects of the fluid cannot be interpreted in isolation, several abdominocenteses must be performed, because the initial results may be inconclusive or the clinical condition of the animal may change, especially in cases of associated colic (PARRY & BROWNLOW, 2005).

Normal peritoneal fluid is essentially plasma dialysis which, when normal, has a low volume, low cellularity, and low concentration of total proteins. Urea and glucose are substances that have a low molecular weight, diffusing easily over the mesothelium and rapidly balancing between the plasma/interstitial fluid and the peritoneal fluid. On the other hand, molecules with a higher molecular weight, such as creatinine and most enzymes, are less likely to diffuse, taking longer to balance when there is a change in their concentration or in the blood or peritoneal fluid (Parry & Brownlow, 2005).

The fluid indicative of peritonitis presents with high nucleated cell counts, high amounts of proteins, fibrins and bacteria from both intracellular and extracellular environments (MARKEL, 1988).

A treatment must be constituted for each specific cause that may be developed, a therapeutic procedure within the supposed needs presented by the animal and, if necessary, exploratory laparotomy may be indicated (RADOSTITS et al., 2009).

Because it is accompanied by a vast bacterial flora, broad-spectrum antimicrobials are needed to treat this disease. The recommended doses are higher doses to repair at high blood and tissue levels and should be maintained daily until the animal has fully recovered (RADOSTITS et al. 2009).

The most commonly used antimicrobials are Gentamicin at a dose of 2.2 to 3.3 mg/kg of body weight (BW) intravenously (IV) every eight to twelve hours; 22,000 International Units

(IU)/Kg of BW of penicillin intramuscularly (IM) every six to twelve hours. Oral administration of 15 to 25 mg metronidazole/kg BW is also recommended. Acute peritonitis, when caused by *Actinobacillus equuli*, responds rapidly to treatment with penicillin-streptomycin or ampicillin alone administered systemically. IV hydroelectrolyte therapy should be intense as it is a vital part of treatment. The use of non-steroidal anti-inflammatory drugs at a dose of 0.25 to 1.1 mg of flunixin meglumine/Kg BW IV every eight to twelve hours is indicated when peritonitis is accompanied by shock, but no information is available on the efficacy of this administration (RADOSTITS et al. 2009).

The recovery rate is considered relatively good, usually around 70% in therapy. The mortality of animals taken for surgery is about 56%, and chronic conditions do not respond well to treatment due to the time of involvement of the intestine with fibrous adhesions (RADOSTITS et al. 2009).

FINAL THOUGHTS

It is concluded from this study that secondary causes have a higher rate of causes in equine peritonitis, and that the diagnosis of the cause will most often not be detected. The safest method for confirmation is paracentesis, but it must be analyzed together with all procedures and exams done as a whole and that the treatment, when therapeutic, has a more favorable prognosis than the surgical one.

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Ozone in postharvest conservation and anthracnose control in palmer mango

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ABSTRACT

The objective of this study was to evaluate the efficiency of water-solubilized ozone, combined or not with different types of fungicides, in the control of anthracnose over 25 days of cold storage in 'Palmer' mango, as well as to evaluate the effects of this process on the postharvest quality of the fruits. 'Palmer' mango fruits harvested by hand from commercial orchards in Jaíba were used. The statistical design was completely randomized, in a 4 x 2 factorial, with the first factor being the treatments (T1: Control; T2: Ozonated water; T3: Ozonated water + 2.5 ml/L of Tebuconazole; T4: Ozonated water + 1 ml/L of thiabendazole) and the second factor two times of evaluations (0 and 25 days after storage in a closed chamber). Three replicates were used, with each replication consisting of 2 fruits. After assembling the treatments, the fruits were sent to the Postharvest Physiology laboratory of UNIMONTES, packed in polystyrene trays and stored in a cold chamber at 13°C with a relative humidity of 85% and evaluated for the incidence and severity of anthracnose, soluble solids, pH, titratable acidity, color, firmness and loss of fresh mass. Treatments 3 and 4 are efficient in the control of anthracnose in the postharvest of 'Palmer' mango fruits. The combinations of ozonated water and fungicides used did not influence the ripening of the fruits, except for the loss of fresh mass, which was lower in the treatment 3. The storage conditions contributed to the obtaining of quality fruits at the end of the 25 days of storage.

Keywords: *Mangifera indica* L., *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc, Storage, Alternative treatment.

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INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most important tropical fruits, and is highly appreciated for its characteristic flavor, aroma and color. It is a climacteric fruit that is usually harvested in the ripe green phase, has bioactive compounds such as vitamin C, β-carotene and polyphenols that contribute to the nutritional characteristics (SINGH et al., 2013). Despite its great economic importance, part of the production is lost due to problems that occur after the harvest. Brazil produced more than 1.5 million tons in 2020, in approximately 73 thousand hectares and has great growth potential for both export and the domestic market (IBGE, 2021).

One of the major causes of losses of this fruit is its susceptibility to fungal diseases, such as anthracnose, caused by the fungus *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. Anthracnose is one of the main diseases that affect fruits after harvest, and can infect a wide range of hosts from flowering, which decreases their post-harvest quality. The symptoms manifest themselves in the form of dark colored and circular-shaped punctuations on the skin of the fruits, which evolve and can go beyond the skin and reach the pulp. In the hottest months of the year, its incidence can reach 70 to 100% of the fruits in the absence of control measures, causing serious losses, as it compromises the physicochemical quality and commercialization of the product.

To curb the symptoms of this disease, the use of synthetic products is recommended. There are many fungicides recommended in preventive applications for the chemical control of this fungus, such as Thiabendazole and Tebuconazole, with application varying according to the climatic conditions of the place, stage and development of the fruits and their commercialization. This fact is especially important when the production is destined for the foreign market, since importing countries have specific legislation regarding the quality and residues of chemical products in food.

In this context, the study and development of alternative methods of postharvest disease control is of utmost importance. Among the new technologies in pest control, ozone (O3) is an alternative to maintain the quality of fruits consumed *in natura*, as it is a strong oxidant with low residual power, being an alternative disinfectant method, free of toxic residues, used for sanitizing food, such as fruits and vegetables in general (GLOWACZ et al., 2014).

In view of the above, the objective of this study was to evaluate the efficiency of water-solubilized ozone, combined or not with different types of fungicides, in the control of anthracnose over 25 days of cold storage in 'Palmer' mango, as well as to evaluate the effects of this process on the postharvest quality of the fruits.

MATERIAL AND METHODS

The fruits were harvested manually from commercial orchards in Jaíba, Minas Gerais. All fruits were at stage 2 of maturation, which is characterized by having a yellow cream pulp color. The fruits were washed with a neutral detergent at 2 mL/L, rinsed in drinking water and air-dried. The statistical design was completely randomized, in a 4 x 2 factorial, with the first factor being the treatments (T1: Control; T2: Ozonated water; T3: Ozonated water + 2.5 ml/L of Tebuconazole; T4: Ozonated water + 1 ml/L of thiabendazole) and the second factor two times of evaluations (0 and 25 days after storage in a closed chamber). Three replicates were used, with each replication consisting of 2 fruits.

Immediately after drying, all fruits, except those of the control treatment, were subjected to ozonated water bathing for about 10 minutes. The ozone gas was obtained through the ozone generator of the company Ozonfresh® and is generated by the passage of O_2 in an electrical discharge environment. The concentration used was 40g of O3/m3 of water. After the fruits were air-dried again, they were divided into batches corresponding to each treatment. Then, the doses of different fungicides were applied by immersion for 10 minutes. After assembling the treatments, the fruits were sent to the Postharvest Fruit Physiology Laboratory of the State University of Montes Claros, packed in polystyrene trays and stored in a cold chamber at 13°C with a relative humidity of 85%.

The fruits were evaluated for the incidence and severity of anthracnose. The incidence was obtained by the number of symptomatic fruits per repetition, and these values were expressed as percentages per treatment. Severity refers to the proportion of colonized tissue area, and its determination was performed with the aid of a specific diagrammatic scale for anthracnose in mangoes (CORKIDI et al., 2006), with disease severity ranging from 0 - 1% (no disease); 1 - 5% (mild disease); 6 - 9% (moderate disease); 10 - 49% (severe disease), and 50 - 100% (very severe disease) of the injured area/fruit.

The chemical characteristics evaluated were: a) soluble solids (°Brix): obtained by means of refractometry, using a digital refractometer Model N-3000E, Atago; b) pH: measured in PG1800 digital pH meter; c) titratable acidity (g Ac citric .100g of pulp-1): determined by the titration method with 0.1 M NaOH, with 1% phenolphthalein, where 10g of pulp was homogenized in 90 ml of distilled water and added three drops of phenolphthalein (1%) used as an indicator.

The analysis of the pulp color was performed using a Color Flex colorimeter model nº 45/0, with direct reflectance reading of the coordinates L* (luminosity), a* (red or green hue) and b* (yellow or blue hue), from the Hunterlab Universal Software system, measured in the median region of the fruit. From the values of a* and b*, the hue angle (°h*) and the chroma saturation index (C*) were calculated.

The firmness of the unpeeled fruits was evaluated using a penetrometer, measured in Newton (N). The loss of fresh mass (PMF) was determined by the difference between the initial mass of the fruits and the mass obtained from the same fruits in each subsequent sampling period. The results were expressed as percentage (%) of fresh mass loss.

In addition to the above-mentioned evaluations, the fruits were weighed on a precision scale to determine the percentage of mass loss, which was determined by means of the difference in mass on the day of harvest and on the 25th day of storage, using the formula: PM (%) = ((Pi-Pj)/Pi) x 100, where: PM = mass loss (%); Pi = Initial weight of the fruit (g); Pj = Mass of the fruit in the period following Mi (g). The data obtained were submitted to analysis of variance, verifying the significance of the tested factors with subsequent developments for the significant interactions. For the treatments tested, Tukey's test was performed at 5% probability, using the SISVAR software (FERREIRA, 2015).

RESULTS AND DISCUSSION

A significant interaction was observed between the treatments tested and the two evaluation periods ($p>0.05$) for the variables severity and loss of fresh mass. For the other characteristics evaluated, a difference was found only for the factor time of evaluation ($p<0.05$).

At 25 days of storage, the treatments T3 (ozonated water + 2.5 ml/L of Tebuconazole) and T4 (ozonated water + 1 ml/L of thiabendazole) had the lowest rate of disease severity, 7 and 8%, respectively (Table 1). These treatments differed from the control treatment and the treatment with only ozonated water, which had an average of more than 60% of disease severity. The efficiency of the treatments in relation to the control indicated that the treatments using Tebuconazole and Thiabendazole proved to be more efficient in controlling the severity of anthracnose.

Kechinscki et al. (2012) used ozonated water at concentrations of 2 and 4 mg/L for the postharvest treatment of papayas, combining it with hydrothermal treatment and application of carnauba wax. They concluded that the isolated use of ozonated water at these concentrations and for periods of 1 to 2 minutes was not effective in controlling anthracnose. However, they observed that the effectiveness in disinfecting the fruits was achieved only in combined treatments, such as the combination of ozonated water with hydrothermal treatment. These results corroborate the present study, since the control of anthracnose was only achieved in the combined treatments between ozonated water and fungicides.

Table 1. Severity of anthracnose in 'Palmer' mango fruits submitted to different treatments to control the disease.

Treatments	Days of Storage	
	0	25
Witness	0 bA	66 b.a.
Ozonated water	0 bA	60 b.a.
Ozonated water + 2.5 ml/L Tebuconazole	0 aA	7 aB
Ozonated water + 1 ml/L of Thiabendazole	0 aA	8 aB
CV (%)		50,9

Note: Means followed by the same letter, lowercase in the row, and uppercase in the column, do not differ from each other by Tukey's test at 5% significance

Source: authors

When analyzing the incidence of the disease, we observed that there was no significant difference between the treatments tested, only for the evaluation periods (Table 2). This is due to the generalization of this analysis, since only the presence or absence of symptoms is identified, instead of their intensity being assessed. The quantification of diseases (incidence and/or severity) is necessary both to describe the progress of the epidemic and to design management strategies that allow the rational use of available resources (SILVA; JESUS JUNIOR, 2000).

Table 2. Incidence of anthracnose in 'Palmer' mango fruits submitted to different treatments for anthracnose control.

Evaluated Characteristics	Days of Storage	
	0	25
Incidence (%)	0 b	66 to
CV (%)	43,3	

Note: Means followed by the same letter, lowercase in the line, do not differ from each other by the F test at 5% significance

Source: authors

There was an increase in the solids content during the storage period and there was no effect of the treatments under this variable. Initially, the fruits had a content of 6.88 °Brix and when they left cold storage, at 25 days, they presented an average content of 12.41 °Brix (Table 3). This increase may be related to the accumulation of reserve carbohydrates in the fruits during plant development, which undergo hydrolysis with ripening and result in the formation of soluble sugars (LIZADA, 2012).

Regarding pH, an increase of 0.21 was observed in the pH value from day 0 to day 25 (Table 3). Consequently, the storage period reduced the titratable acidity on the 25th day, which was also significant only for the storage days (Table 3). During the ripening of the mango, there is a reduction in acidity, this occurs due to the reduction in the content of citric acid, which corresponds to the most abundant organic acid in this fruit (NOGUEIRA et al., 2015). Titratable acidity can be used as a reference point for the degree of ripeness of the fruits, which is mainly attributed to the organic acids that are dissolved in the vacuoles of the cells (CHITARRA; CHITARRA, 2005).

Table 3. Chemical characteristics of 'Palmer' mango fruits submitted to different treatments in the control of anthracnose.

Evaluated Characteristics	**Days of Storage**		CV (%)
	0	25	
Soluble Solids (°Brix)	6,88 b	12,41a	11,49
Ph	3,30 b	3,51a	4,73
Titratable acidity (g ac. Citrus/100g pulp-1)	0,92 a	0,80 b	13,6

Note: Means followed by the same letter, lowercase in the line, do not differ from each other by the f-test at 5% significance

Source: authors

A decrease in luminosity and color angle (°Hue) and an increase in chromaticity (Table 4) were observed during the storage period. Changes in pulp color variables are the most evident indicators of ripening. The luminosity represents the brightness of the sample, that is, whether the yellow color of the pulp of the fruit studied is lighter or darker (CRUZ, 2010). The decrease in the values of luminosity (L*) and °Hue observed in the fruits in relation to the first day (day 0) and the last day (day 25) of storage, regardless of the technique used, indicated that there was a development from cream to yellow-orange color (Table 4).

The Hue angle represents the shade of the sample evaluated. According to the CIE L* a* b* system, graphically, the angle 0º is considered red, 90º yellow, 180º green and 270º blue (FERREIRA; SPRICIGO, 2017). In the study of mango pulp, the focus was on the variation of the intense yellow color (90°). The values of the color angle (°Hue) obtained were close to 90° until the 20th day, indicating that the color of the fruit pulp varied from cream to yellow during this period for all the preservation techniques employed.

An increase in chromaticity values was also observed during the days of storage, which characterizes an increase in the intensity of the yellow color. According to Ferreira and Spricigo (2017), as chroma increases, the saturation of colors perceived by humans also increases. This is probably due to the change in the color of the pulp, caused by the process of chlorophyll degradation and carotenoid synthesis in the fruit, as pointed out by Ebrahimi and Rastegar (2020).

Table 4. Luminosity, Chromaticity and Hue Angle of 'Palmer' mango fruit pulp submitted to different treatments to control Anthracnose.

Evaluated Characteristics	**Days of Storage**		CV (%)
	0	25	
Luminosity	84,58 a	80,83 b	1,61
Chromaticity	39,56 b	49,86 a	7,22
Hue	95,21 a	90,96 b	1,67

Note: Means followed by the same letter, lowercase in the line, do not differ from each other by the f-test at 5% significance

Source: authors

The firmness of the mangoes decreased with the storage period (Table 5). The firmness of the fruits at the time of the experiment was 90.16 N, while after 25 days of storage, this value decreased to 48.81 N, representing a decrease of about 40%. This reduction is comparatively lower than that observed by Guimarães et al. (2017), who, under refrigeration conditions at 12 ºC for 12 days, followed by three days at 25 ºC, found an average firmness below 30 N on the 15th day. These results indicate that the storage conditions adopted ensured a lower loss of firmness in the pulp of 'Palmer' mango fruits.

Table 5. Firmness of 'Palmer' mango fruits submitted to different treatments in the control of Anthracnose.

Evaluated Trait	**Days of Storage**	
	0	25
Firmness (N)	90,16 a	48,81b
CV (%)	19,53	

Note: Means followed by the same letter, lowercase in the line, do not differ from each other by the F test at 5% significance

Source: authors

For the variable fresh mass loss, it was observed that at 25 days of storage, treatment 3 (ozonated water + 2.5 ml/L of Tebuconazole) presented the lowest percentage of mass loss when compared to the other treatments (Table 3). Lima et al. (2007) found in mangoes cv. An average loss of fresh mass of 15% at the end of 12 days was observed under cold storage, a result higher than that found in the present study. According to Chitarra and Chitarra (2005), mass loss is directly linked to water reduction. The loss of water from fruits during the storage period is mainly due to transpiration (MAGUIRE et al, 2000).

Table 6. Loss of Fresh Mass of 'Palmer' mangoes submitted to different treatments in the control of Anthracnose.

Treatments	Days of Storage	
	0	25
Witness	0 bA	3,64 aA
Ozonated water	0 bA	3,62 aA
Ozonated water + 2.5 ml/L Tebuconazole	0 bA	2.94 aB
Ozonated water + 1 ml/L of Thiabendazole	0 bA	3.34 aA
CV (%)		8,22

Note: Means followed by the same letter, lowercase in the row and uppercase in the column, do not differ from each other by Tukey's test at 5% significance.

Source: authors

CONCLUSIONS

The combinations of ozonated water + 2.5 ml/L of Tebuconazole and ozonated water + 1 ml/L of Thiabendazole are efficient in the control of anthracnose in the postharvest of 'Palmer' mango fruits. The combinations of ozonated water and fungicides used did not influence the ripening of the fruits, except for the loss of fresh mass, which was lower in the use of ozonated water + 2.5 ml/L of Tebuconazole. The storage conditions contributed to the obtaining of quality fruits at the end of the 25 days of storage

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CHAPTER 7

Soil micronutrients: dynamics, availability, and fertilization management

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ABSTRACT

Micronutrients (B, Cu, Fe, Mn, Zn, and Mo) are generally required in small quantities and perform various metabolic functions in plants. In the soil, their availability can be affected by several factors, such as pH, texture, organic matter content, and the concentration of other elements. The way these factors interact can influence the absorption of micronutrients by plant roots. Once absorbed by the roots, the transport of micronutrients is affected by the way they are complexed and the available concentration in the soil solution. The deficiency of micronutrients causes various visible symptoms in plants, including chlorosis, deformation, necrosis, and reduced growth, which can be corrected with proper fertilizer management. This chapter provides updated information on the dynamics of micronutrients in soil and plants, the factors influencing their availability, and fertilization recommendations for these nutrients.

Keywords: Absorption, Chlorosis, Deficiency, Necrosis, Transport.

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INTRODUCTION

In recent decades, micronutrients have been used more routinely in fertilization practices. Of the sixteen essential nutrients for plants, seven are micronutrients: boron (B), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), molybdenum (Mo), and chlorine (Cl). Although plants generally do not require large quantities of these nutrients, the absence of any one of them in the soil can limit plant growth. This study will not address Cl.

The functions of micronutrients are divided between being constituents of prosthetic groups and activators of enzymatic reactions. Without micronutrients as activators, the enzymatic system in plants would be simply an inert mass of proteins (Gupta et al., 2008; Andrade et al., 2021).

The reasons for the inclusion of fertilizers containing micronutrients in fertilization plans can be summarized as follows:

a) Expansion of the agricultural frontier: intensive agrosilvopastoral exploitation in the Cerrado region, characterized by soil with poor chemical properties and high acidity, leading to nutrient deficiencies. Correcting acidity aims to raise the pH and make Ca and Mg bases available. Increasing the pH reduces the availability of cationic micronutrients, so their addition through fertilizer management must be anticipated.

b) Crop productivity: high crop yields lead to significant nutrient export and sometimes the depletion of reserves, especially micronutrients in soils. These deficiencies are increasingly documented in research (Resende, 2005; Abreu et al., 2007).

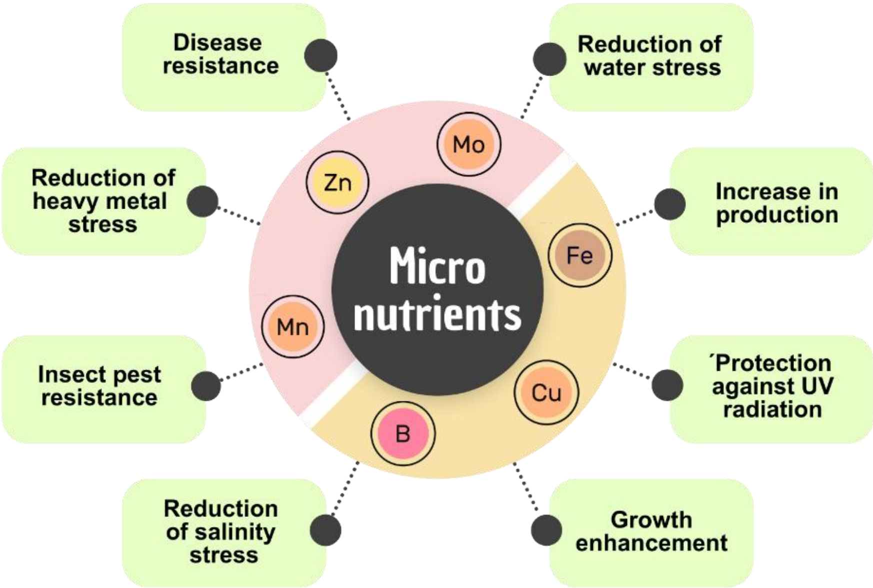
c) Fertilizer purity: current NPK fertilizer production processes remove impurities, excluding micronutrients that were indirectly supplied.

d) Nutritional specificity: certain crops have specific micronutrient requirements; for example, legumes and plants in the Brassicae family require B and Mo. Cereals show greater responses to Zn and Cu, and beet to Mn (Khaliq et al., 2019; Andrade et al., 2021).

e) Soil management: soil erosion and long-term cultivation result in the removal of micronutrients from the soils.

Figure 1 highlights the main roles of micronutrients in plants. The symptoms caused by the lack or excess of these nutrients in plants, at tissue levels, are a useful guide to establishing the deficiency or toxicity of an element. Therefore, it is important to know the distribution of micronutrients in different plant organs (Gupta et al., 2008). Some essential micronutrients and heavy metals in excess can result in toxicity to crops (Khaliq et al., 2019).

Figure 1. Plant responses to soil micronutrients under biotic and abiotic stresses.



Source: Author (2024).

DYNAMICS OF MICRONUTRIENT

Soils exhibit significant variations in micronutrient content. A comprehensive analysis shows that the abundance of these micronutrients primarily varies with the parent material (Table 1). However, even when the parent material is the same, the action of different weathering agents, such as moisture and temperature, can result in soils with distinct characteristics. Additionally, the effects of various climatic conditions, both current and past, on soil composition are evident.

Table 1. Micronutrient contents in some igneous and sedimentary rocks.

Element	Igneous rocks		Sedimentary rocks		
	Granite	Basalt	Limestone	Sandstone	Shale
	mg dm^{-3}				
Fe	27,000	86,000	3,800	9,800	47,000
Mn	400	1,500	1,100	10 - 100	850
Cu	10	100	4	30	45
Zn	40	100	20	16	95
Mo	2	1	0.4	0.2	2.6
B	15	5	35	100	100

Source: Silva et al. (2006)

The relative stability of minerals present in soils also illustrates how micronutrient levels can vary according to the weathering stage. Soils formed under more advanced weathering conditions may have a different mineralogical composition, reflecting variations in the levels of micronutrients essential for plant development.

FACTORS INVOLVED IN DYNAMICS OF MICRONUTRIENTS IN SOIL AND PLANTS

Soil texture

Soil texture influences the dynamics of micronutrients for plants, as these elements can associate with the solid phase of the soil. The energy with which micronutrients associate with the solid phase varies among them, causing differential mobility between micronutrients in the soil system (Table 2). Zn and Mn generally have very low mobility, while B is extremely mobile in the soil profile. Thus, for the first two, transport in the soil solution is essentially by diffusion. For Cu, the process of root interception assumes some importance, while for B, mass flow is the main means of transport (Rehman et al., 2018).

Table 2. Different forms of micronutrient mobility in soil.

Nutrient	Forms (%)		
	Root interception	Mass flow	Diffusion
B	3	97	0
Cu	70	20	10
Fe	50	10	40
Mn	15	5	80
Mo	5	95	0
Zn	20	20	60

Source: Camargo (2006).

The transport of micronutrients in the soil is important for aspects associated with fertilizer use and the understanding of deficiency symptoms, such as those of B and Mo, which are frequently observed in dry seasons. In the case of B, its transport to the root region depends on a water potential gradient resulting from plant transpiration, a process that decreases during dry periods (Landi et al., 2019). For Zn, initially, the establishment of a necessary concentration gradient is required, but it is not sufficient for the establishment of diffusive flow, which requires a good soil moisture content. Therefore, it is common to observe the disappearance of deficiency symptoms of these micronutrients after the first rains. However, if the rains are more intense, B deficiency may recur due to its high mobility in the soil and possible leaching, a phenomenon that does not occur with Zn (Rengel, 2015; Rehman et al., 2018).

B leaching allows us to infer about the importance of organic matter in its maintenance, as it complexes this micronutrient through radicals and organic groups like a diol group. Therefore, all soil management and conservation practices that lead to the maintenance of organic matter are beneficial for the availability of micronutrients to plants (Landi et al., 2019).

Clayey soils can adsorb part of the B, which is in the anionic form $H_2BO_3^-$ or $B(OH)_4^-$, making this nutrient unavailable for plant absorption (Chatterjee and Bandyopadhyay, 2017). In the case of Zn, soils with higher levels of iron and aluminum oxides are more affected due to their affinity for these colloids. On the other hand, Cu has a greater affinity for organic matter, being more adsorbed in organic soils (Andrade et al., 2021).

The ionic transformation of micronutrients is a relevant process, as it determines whether the nutrient will be more or less available for plant absorption (Abreu et al., 2007). Table 3 presents a summary of the absorbed forms of these micronutrients and their mobility within the plant. It is important to note that, except for B, all micronutrients are incorporated into the plant in the same form they are absorbed. Additionally, except for Mo, these micronutrients are considered immobile within the plant (Raij, 2011). Due to this immobility characteristic, the deficiency of these nutrients tends to manifest first in young leaves, where the demand for nutrients is higher during the initial stages of development.

Table 3. Forms of absorption, incorporation and mobility of micronutrients in soil.

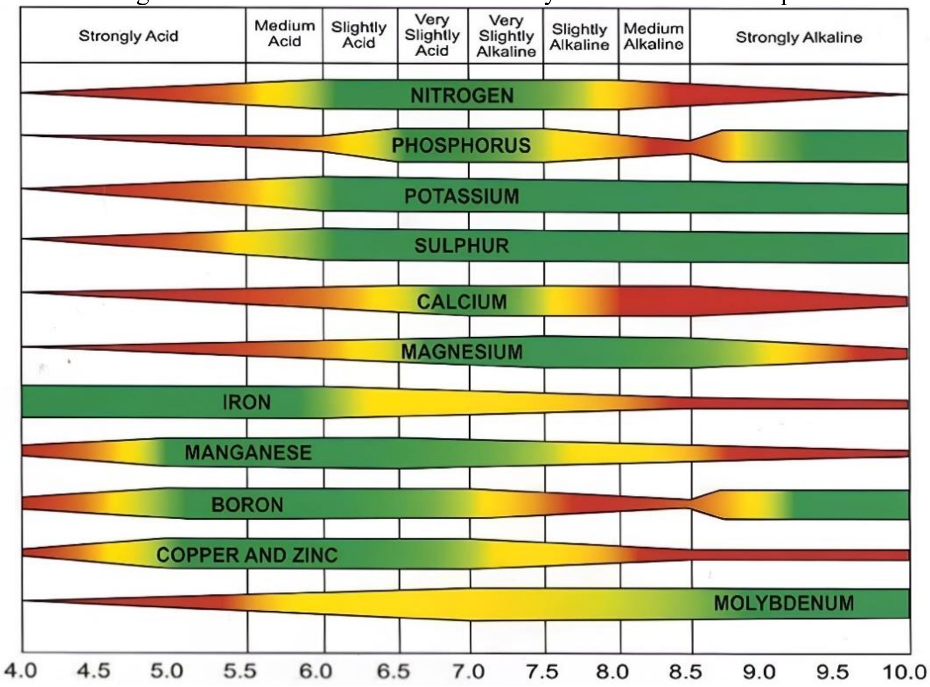
Nutrient	Absorbed form	Incorporated Form	Mobility
B	H_3BO_3	-	Immobile
Cu	Cu^{2+}	Cu^{2+}	Immobile
Fe	Fe^{2+}, Fe-Chelate	Fe^{2+}	Immobile
Mn	Mn^{2+}	Mn^{2+}	Immobile
Zn	Zn^{2+}	Zn^{2+}	Immobile
Mo	MoO_4^{2-}	MoO_4^{2-}	Mobile

Source: Dechen et al. (2018).

Soil pH

Soil pH significantly affects the availability of micronutrients due to the transformation from soluble to insoluble forms in the soil. The exception is Mo, which follows the opposite path (Figure 2). Under acidic pH conditions, some micronutrients can become sufficiently soluble to be toxic to plants. Mn, for example, can inhibit root growth in some acidic soils. Proper liming of these soils, to raise the pH close to 6.5, reduces the risk of toxicity (Mascarenhas et al., 2013).

Figure 2. Variation in nutrient availability as a function of soil pH.



Source: Agroadvance (2023).

Increases in soil pH above 6.0 induce the hydrolysis of hydrated Cu, which can lead to strong adsorption of Cu by clay and organic matter charges. Thus, the solubility of Cu^{2+} depends on the soil pH and decreases 100 times for each unit increase in pH (Fageria et al., 2002; Fonseca et al., 2010). The surface application of lime, at the correct rates in a no-tillage system, does not necessarily lead to micronutrient deficiencies (Moreira et al., 2017).

At higher pH levels, the form of H3BO3 most absorbed by the plant is minimized in solution, reducing absorption by the roots (Abreu et al., 2007; Dechen et al., 2018). The availability of Zn and Cu is affected by pH, decreasing by 100 to 1000 times for each unit increase. pH values above 6.0 strongly increase the adsorption of ions to soil colloids, affecting their availability (Abreu et al., 2007; Khaliq et al., 2019). In relation to pH, each unit increase causes a reduction in Fe availability by 1000 times and Mn by 100 times.

Unlike other micronutrients, Mo availability increases with rising pH due to the predominant form of MoO_4^{2-}, an anion absorbed by plants. Its greatest availability occurs in alkaline soils and is negatively affected by high levels of organic matter and iron and aluminum oxides, which are less impactful than the increase in pH (Chatterjee and Bandyopadhyay, 2017).

High doses of acidity correctives (over-liming) can cause drastic changes in the environment, reducing productivity due to unbalanced nutrition from micronutrient deficiencies. It is noted that, generally, considering technical, operational, and economic aspects, it is more challenging to correct the conditions resulting from over-liming than the conditions often found in acidic soils (Carneiro et al., 2018).

Soil organic matter content

The activity of microorganisms can promote changes in organic matter content and, thus, in the availability of micronutrients, either through the supply of these nutrients in response to mineralization or by decreasing their complexation (Oliosi et al., 2016). Due to the number of active sites, organic matter has a high capacity to complex micronutrients, especially cationic ones in soils with corrected acidity. Organic matter is also the main source of B, interfering with its availability to plants. Dry environments hinder the supply of B from organic matter through reduced mineralization due to decreased soil microbiological activity (Abreu et al., 2007; Dechen et al., 2018).

Oxidation-reduction potential

The availability of Fe and Mn is affected by the oxidation-reduction potential and pH of the soil. Since the forms absorbable by plants of these nutrients are Fe^{2+} and Mn^{2+}, moisture is necessary to ensure the greater presence of these ions in the form absorbable by plants (Khaliq et al., 2019). In the case of Fe, the transformation from Fe^{3+} (insoluble form) to Fe^{2+} (soluble form) occurs at an oxidation-reduction potential of -185 mV, while Mn^{4+} (insoluble form) changes to Mn^{2+} (soluble form) at an oxidation-reduction potential of 200 mV, causing Mn toxicity to occur much faster than Fe toxicity (Andrade et al., 2021).

The decrease in the oxidation-reduction potential of the environment is of great practical importance. For example, plants cultivated in lowland areas (wetlands) may suffer toxicity from Mn and Fe due to the increase in the availability of their reduced and, consequently, mobile forms (Mn^{2+} and Fe^{2+}). In the specific case of rice cultivation, this toxicity can be mitigated as rice plants possess aerenchyma, which allows the conduction of oxygen from the aerial part to the roots. Thus, under field conditions like those mentioned, the observation of a brownish-red coloration, indicating the presence of iron in the oxidized form (Fe^{3+}) on the surface of rice plant roots, is common. Additionally, there is a change from Mn^{2+} to the less active oxidized form (Mn^{4+}).

Soil use and management

Regarding the influence of soil use and management on the availability of micronutrients, in addition to the considerations already mentioned, the issue of soil compaction can be raised (Oliosi et al., 2016). There has been an increasing trend of compacted soil layers in areas with intensive agricultural exploitation. Besides aspects or factors associated with often inadequate agricultural mechanization (mainly soil preparation), the addition of inputs such as lime in high doses and repeated applications account for a large part of the compaction problems observed. In compacted layers, low oxidation-reduction potential may even occur, sufficient to cause Mn toxicity.

STUDY OF MICRONUTRIENTS

BORON

The function of B in plants is represented in the differentiation of meristematic cells. The consensus is that its main function is related to the structure of the cell wall and the substances associated with it (Chatterjee; Bandyopadhyay, 2017). The variation in plant tissues is broad, with generally higher values in dicotyledons than in monocotyledons (Gupta et al., 2008). It is present in soil solutions with a pH below 8, mainly as undissociated boric acid (H_3BO_3), the primary form taken up by roots, and it dissociates to $B(OH)_4^-$ only at higher pH values (Abreu et al., 2007; Raij, 2011).

B deficiency is a widespread nutritional disorder. Under conditions of high precipitation, B is easily leached in the form of H_3BO_3. The availability of B decreases with increasing soil pH, particularly in calcareous soils and those with high clay content (Abreu et al., 2007). Availability also decreases drastically under dry conditions, likely due to reduced B mobility through mass flow to the roots and the polymerization of boric acid (Resende, 2005; Dechen et al., 2018).

Symptoms of B deficiency in shoots are noticeable in terminal buds or the youngest leaves, which become discolored and may die. Internodes become shorter, giving plants a thick or rosette appearance (Figure 3). The deficiency is mainly found in the younger plant tissues (Dechen et al., 2018). Chlorosis can occur in mature leaves, as well as deformed leaf blades. Dropping of buds, flowers, and developing fruits are also symptoms of deficiency. With B deficiency, cells may continue to divide, but the structural components are not differentiated (Gupta et al., 2008). B is not mobile in plants, and a continuous supply is necessary at all growth points (Chatterjee; Bandyopadhyay, 2017).

Figure 3. Symptoms of B deficiency in maize plants (*Zea mays*).

Source: IPNI (2019).

COPPER

Cu is present in plants in a complexed form. Like other potentially toxic heavy metals, excess Cu binds to phytochelatins and sulfur-containing peptides (Khaliq et al., 2019). Cu in solution is present as cuprous (Cu^{+}) and cupric (Cu^{2+}). Cuprous Cu is easily oxidized to cupric Cu and, therefore, is only found in complexed forms (Abreu et al., 2007). Cu is an activator of various enzymatic systems in plants and functions in electron transport and energy capture by proteins and oxidative enzymes. It may play a role in the production of vitamin A. Deficiency interferes with protein synthesis (Raij, 2011; Dechen et al., 2018).

The native Cu supply has rarely been recognized as needing supplementation; however, in some tree plantations grown on organic soils or sands, supplementation may be required (Gupta et al., 2008). Cu can be toxic at low levels, so the need must be clearly established before replenishment (Andrade et al., 2021).

Symptoms of Cu deficiency include: i) Leaves may be chlorotic or deep blue-green with curled margins (Figure 4); ii) The bark of trees is often rough and blistered, and gum may ooze from cracks in the bark; iii) Flowering and fruiting may not develop in annual plants and may die at the seedling stage; iv) Stunted growth.

Figure 4. Symptoms of Cu deficiency in maize plants (*Zea mays*).

Source: IPNI (2019).

IRON

Fe is necessary for chlorophyll formation in plant cells. It serves as an activator for biochemical processes such as respiration, photosynthesis, and symbiotic nitrogen fixation (Gupta et al., 2008). Fe deficiency can be induced by high levels of Mn or high lime content in soils (Raij, 2011). Fe is absorbed by plants as ferrous ions (Fe^{2+}) or ferric ions (Fe^{3+}), with the latter in smaller quantities. The function of Fe in plants depends on the rapid transitions between its two oxidation states in solution (Abreu et al., 2007). Plants store Fe as ferritin, a protein that encapsulates ferric iron.

In aerobic soil conditions, Fe is largely insoluble as a constituent of oxides and hydroxides and tends to bind to organic chelates. Thus, the concentration of free Fe in soil solution is extremely low in most soils, and plants use mechanisms to mobilize Fe and make it available for root absorption (Resende, 2005; Abreu et al., 2007). Some of these mechanisms are not specific to Fe absorption. Roots expel protons and organic acids into the soil, lowering the pH of the rhizosphere, increasing Fe solubility and availability.

There are two specific mechanisms for the absorption of this nutrient (Khaliq et al., 2019). The first mechanism (characteristic of dicotyledons and non-grass monocotyledons) acidifies the rhizosphere through proton extrusion. Ferric iron is reduced to ferrous iron by reductase enzyme at the plasma membrane. The reduced iron is transported across the membrane by a specific ion transport system. The second mechanism (characteristic of maize, barley, and oats) involves the extrusion of siderophores by roots. In this case, reduction to ferrous iron does not occur (Dechen et al., 2018; Andrade et al., 2021).

Symptoms of Fe deficiency include: i) Interveinal chlorosis of young leaves (the veins remain green except in severe cases) (Figure 5); ii) Branch necrosis; iii) In severe cases, death of branches or entire plants.

Figure 5. Symptoms of Fe deficiency in maize plants (*Zea mays*).

Source: IPNI (2019).

MANGANESE

Mn acts as an enzyme activator in growth processes and aids Fe in chlorophyll formation. It is part of the system where water is split, and O_2 is released. Another protein of which Mn is an integral constituent is superoxide dismutase, common in aerobic organisms (Dechen et al., 2018). The function of this enzyme is to provide protection against free oxygen radicals formed, converting this highly toxic free radical into hydrogen peroxide (H_2O_2), which is then decomposed into water (Resende, 2005).

High Mn concentration can induce Fe deficiency. Mn is primarily absorbed as Mn^{2+}, the reduced form (Abreu et al., 2007). Tree crops can exhibit deficiencies in this nutrient; however, the requirements for this element are not commonly recognized (Raij, 2011). Some authors suggest that Mn additions can increase grain production (Khaliq et al., 2019; Andrade et al., 2021).

Symptoms of Mn deficiency include: i) Interveinal chlorosis of young leaves (gradation from pale green color with darker coloration near the veins) (Figure 6); ii) Development of gray spots (oats), interveinal white streaks (wheat), or interveinal brown spots and streaks (barley).

Figure 6. Symptoms of Mn deficiency in maize plants (*Zea mays*).

Source: IPNI (2019).

ZINC

Zn acts as a component of enzymes or as a functional, structural, or regulatory cofactor for a large number of enzymes. More than 80 Zn-containing proteins have been reported (Andrade et al., 2021). The accumulation of amino acids and amides in plants demonstrates the importance of Zn for protein synthesis. Zn is a component of RNA polymerase and constitutes ribosomes, being essential for their structural integrity. The decrease in protein content in Zn-deficient plants also results in increased RNA degradation in affected cells (Gupta et al., 2008). Unlike other metal ions such as Cu, Fe, and Mn, Zn is a divalent cation (Zn^{2+}) that does not undergo valence changes and, therefore, does not have redox activity in plants. High concentrations of divalent cations such as Ca^{2+} can inhibit Zn absorption (Abreu et al., 2007; Dechen et al., 2018).

Several experimental results indicate that P-Zn interactions exist in plants, including the inhibition of Zn translocation from roots to shoots and physiological inactivation of Zn within the shoots (Khaliq et al., 2019). P-Zn interactions in the soil occur through root infection with vesicular-arbuscular mycorrhiza, increasing the uptake rate of this nutrient. This mycorrhizal infection is decreased by an increase in P supply. Although the connection between Zn deficiency and P toxicity is not well understood, there is substantial evidence that Zn affects P metabolism in roots and increases the permeability of root cell plasma membranes to P and Cl (Raij, 2011).

Zn deficiency is widespread in plants grown in highly weathered acidic soils and is often associated with Fe deficiency (Resende, 2005). Low Zn availability in high-pH calcareous soils results primarily from Zn adsorption to clay or $CaCO_3$ (Abreu et al., 2007). Additionally, Zn uptake and translocation to the shoot are strongly inhibited by high bicarbonate concentrations.

Symptoms of Zn deficiency in plants include: i) Reduced stem length and shortened internodes (Figure 7); ii) Reduced fruit bud formation; iii) Mottled leaves with interveinal chlorosis; iv) Branch necrosis after the first year.

Figure 7. Symptoms of Zn deficiency in maize plants (*Zea mays*).

Source: IPNI (2019).

MOLYBDENUM

Although Mo is a metal, it occurs in aqueous solution primarily as the molybdate anion, MoO_4^{2-} (Abreu et al., 2007). Molybdate is relatively mobile in plants, and higher concentrations can be found in the roots than in the leaves when supplies are limited. The requirement for Mo is the lowest among minerals, except, in certain species, for nickel (Dechen et al., 2018). The functions of Mo as a nutrient for plants are related to the valence changes it undergoes as a metallic component of enzymes.

Research shows that only a few enzymes contain Mo in plants. Still, in higher plants, two Mo-containing enzymes, nitrogenase and nitrate reductase, are of vital importance in agricultural production (Gupta et al., 2008). All biological systems fixing N_2 require nitrogenase. Each nitrogenase molecule contains two Mo atoms associated with Fe. For this reason, the growth of plants that depend on N_2 fixation is stimulated by the application of Mo in deficient soils (Chatterjee and Bandyopadhyay, 2017).

Liming can increase the availability of Mo to the point of luxury consumption, which can become dangerous for ruminants, which are sensitive to excessive concentrations of Mo (Abreu et al., 2007). Plants generally have a wide range of acceptable Mo concentrations. The high concentration of Mo in seeds, although not toxic, ensures adequate seedling growth and a higher final grain yield. There is an inverse relationship between the Mo content of the seed and the yield response to added Mo fertilizer (Raij, 2011; Chatterjee and Bandyopadhyay, 2017).

Symptoms of Mo deficiency include: i) Interveinal chlorosis (Figure 8); ii) Short stature and lack of plant vigor; iii) Marginal scorching and cupping or curling of leaves.

Figure 8. Symptoms of Mo deficiency in maize plants (*Zea mays*).

Source: IPNI (2019).

AVAILABILITY OF MICRONUTRIENTS IN SOIL

Studies on the determination of total micronutrients in soils are limited. In most soils, the total micronutrient content is not related to what is potentially available to the plant (Raij, 2011). However, the total content indicates the relative abundance of B and Mo and can be useful in determining their potential for plant absorption (Resende, 2005). A summary of total micronutrients in Brazilian soils, as reported by various authors, is presented in Table 4.

Studies conducted by Chatterjee et al. (1976) in the main soils of the northern plains of India showed that available Mn and Fe were not related to their total contents. Similar relationships were reported by Kanwar and Randhawa (1974) in most soils of India. As expected, the total Fe content in the soil was high, ranging from 5.6 to 45.6 mg g^{-1}, and the total Mn content in the soil ranged from 107 to 1600 mg kg^{-1}. Compared to Fe and Mn, total Cu contents in the soil were low, ranging from 8 to 50 mg kg^{-1}. The Zn content in soils of northern India ranged from 13 to 384 mg kg^{-1} but showed no relationship with the available fraction in the soil profiles examined. In Brazilian soils, Mn contents have ranged from 20 to 3000 mg kg^{-1}, and Fe from 10 to 100 mg g^{-1}. For Cu, the contents have ranged from 10 to 80 mg kg^{-1}, while Zn does not exceed 300 mg kg^{-1} (Abreu et al., 2007; Andrade et al., 2021).

Table 4. Ranges of availability considered adequate in the interpretation of soil analysis for micronutrients according to different authors.

Literature	B	Cu	Fe	Mn	Zn
	---------------------------------- mg dm^{-3} ----------------------------------				
Raij et al. (1996)	0.21 - 0.6	0.3 - 0.8	5.0 - 12.0	1.3 - 5.0	0.6 - 1.2
Alvarez V. et al. (1999)	0.36 - 0.6	0.8 - 1.2	19.0 - 30.0	6.0 - 8.0	1.0 - 1.5
Galrão (1999)	0.3 - 0.5	0.5 - 0.8	-	2.0 - 5.0	1.1 - 1.6
Sousa and Lobato (1998)	0.5	0.5	-	5.0	1.0
RS Fertility Commission (1994)	0.1 - 0.3	0.2 - 0.5	-	-	0.2 - 0.5
Value ranges	0.1 - 0.6	0.2 - 1.2	5.0 - 30.0	1.3 - 8.0	0.2 - 1.6

Source: Resende (2005).

The B content of soils ranged from 4 to 630 mg kg^{-1} in soils of India (Kanwar and Randhawa, 1976). The total B content in some Argisols of eastern Canada ranged from 70 to 116 mg kg^{-1} (Gupta et al., 2008). In Brazilian soils, the contents ranged between 7 and 80 mg kg^{-1} (Abreu et al., 2007; Andrade et al., 2021). A study conducted on 108 soil samples showed a positive correlation between total B and hot-water soluble B, suggesting that total B can be used to some extent as an index of availability.

Of all micronutrients, total Mo is found in the smallest quantities, ranging from 0.05 to 3.2 mg kg^{-1} in soils of India (Chatterjee et al., 1976). In humid regions, the total Mo content in soils is about 2 mg kg^{-1}, and in Brazil, it has not exceeded 5 mg kg^{-1} (Abreu et al., 2007; Andrade et al., 2021). Although no real data are available showing the relationship between total and available Mo in soil, the total Mo in soil is an important reserve. Gupta et al. (2008) showed that soils containing very low amounts of Mo were not able to meet the nutrient needs of crops, even when sufficiently limed. This was not the case in soils that contained higher total Mo.

FERTILIZATION RECOMMENDATIONS

There are positive, null, and negative effects of micronutrient application, with a high degree of local specificity in terms of soil and crops. This demonstrates that broad and standardized recommendations are not the ideal strategy when aiming for the rational supply of micronutrients (Resende, 2005).

In general, to achieve good results, the management of micronutrient fertilization needs to be more refined than that adopted for macronutrients, due to the greater complexity inherent in the behavior of the former in the soil and plant. Soil analysis is the reference for sizing the application of micronutrients in areas that have never been fertilized (Abreu et al., 2007). From then on, supplementary fertilization should be confirmed through foliar analysis (Sousa, 1998).

Soil analysis has the advantage of allowing the evaluation of soil fertility and the adoption of corrective measures before planting. However, it is not advisable to manage fertilization solely based on soil analysis, which sometimes leads to dubious interpretations and recommendations. As previously mentioned, soil analysis for micronutrients is subject to interferences, and its interpretation has not yet been satisfactorily perfected (Resende, 2005; Abreu et al., 2007). In the case of Zn, for example, the critical level for maize is 1 mg dm^{-3} when the pH is around 6.0 and increases with rising pH (Galrão, 2002). The critical level of Cu is also usually higher in soils with high organic matter content. Therefore, leaf analysis of the crop is highly recommended for a more accurate diagnosis of the nutritional status of the production, which ultimately reflects the efficiency in fertilization management.

Due to the lack of detailed information for local conditions, the so-called "safety fertilization" is still widely used. For the Cerrado region, for example, safety fertilization consists of broadcasting and incorporating doses of 4.0-6.0 kg of Zn; 3.0-6.0 kg of Mn; 0.5-2.0 kg of B; 1.0-4.0 kg of Cu; and 0.2-0.4 kg of Mo per hectare every four or five years. The residual effect of this fertilization is sufficient for four or more crops, especially for Cu and Zn (Sousa, 1998; Galrão, 2002).

Although foliar fertilization is a routine practice for perennial crops when combined with the application of pesticides, for annual crops, the best method of micronutrient application is via soil. In this case, foliar fertilization is recommended when no soil application was made or if it was insufficient (Galrão, 1998). Thus, foliar fertilization should be supplementary to soil application, with its residual effect being small or nil. According to Lopes (1999), foliar sprays of Zn in maize, Mn in soybeans, and Mo in beans can yield good results compared to no application of these micronutrients. Foliar fertilization done with phytosanitary treatments is more cost-effective

(Volkweiss, 1991). Micronutrients can also be supplied via seed (mainly Co and Mo) or by soaking seedling roots in solutions containing the desired nutrients (Resende, 2005). There are a variety of mineral fertilizers available on the market that can be used as sources of micronutrients (Table 5).

Table 5. Information on different sources of micronutrients (B, Cu, Fe, Mn, Zn and Mo).

Source	Formula	Content (%)
B		
Borax	$Na_2B_4O_7 \cdot 10H_2O$	11
Boric Acid	$B(OH)_3$	17
Sodium Pentaborate	$Na_2B_{10}O_{16} \cdot 10H_2O$	18
Ulexite	$Na_2CaB_5O_9 \cdot 8H_2O$	8 - 15
Colemanite	$Ca_2B_6O_{11} \cdot 5H_2O$	10
Cu		
Copper Sulfate	$CuSO_4 \cdot 5H_2O$	25
Copper Chelate, EDTA	Na_2Cu EDTA	13
Copper Sulfate Monohydrate	$CuSO_4 \cdot H_2O$	35
Ammonium Copper Phosphate	$CuNH_4PO_4 \cdot H_2O$	32
Copper Oxide	CuO	75
Cuprous Oxide	Cu_2O	89
Fe		
FTE BR-9	-	6
FTE BR-10	-	4
FTE BR-12	-	3
BR-12 Extra	-	3
FTE BR-13	-	2
NaFe EDTA	-	5 - 14
Mn		
Manganese Sulfate	$MnSO_4 \cdot 3H_2O$	26 - 28
Manganese Chloride	$MnCl_2 \cdot 4H_2O$	25
Manganese Carbonate	$MnCO_3$	40
Manganese Dioxide	MnO_2	63
Manganese Oxide	MnO	41 - 68
Zn		
Zinc Sulfate (hydrated)	-	23 - 35
Zinc Sulfate (basic)	-	55
Zinc Oxide	-	50 - 80
Zinc Oxysulfate	-	Variable
Zinc Chloride	-	24
Zinc Nitrate	-	18

Mo		
Sodium Molybdate	$Na_2MoO_4 \bullet 2H_2O$	39
Ammonium Molybdate	$(NH_4)_6Mo_7O_{24} \bullet 4H_2O$	54
Molybdenum Trioxide	MoO_3	66

Source: IPNI (2019).

The variation in molecular formulas, the various contents, and the high quantity of micronutrients make it difficult for the farmer to decide, resulting in generalized fertilizations (Andrade et al., 2021). This is the main reason for the occurrence of deficiency symptoms in crops, although less frequently than with macronutrients.

It is not a simple task to compare results obtained from different experiments testing sources, doses, and methods of micronutrient application. Many published studies do not describe the area's history (initial availability level and previous applications of micronutrients, use of pesticides with micronutrients in their composition, etc.), the characteristics of the fertilizers, the application procedures, and other information that can greatly influence the occurrence and magnitude of responses (Raij, 2011). Thus, there are controversial results, making it difficult to draw consistent conclusions about the efficiency of micronutrient fertilization.

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Agricultural future: Proteomics as a tool in crop breeding

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ABSTRACT

Proteins are essential for genetic products and their study contributes to obtaining regulatory information. Multiomics techniques are used to improve agronomic characteristics and increase agricultural tolerance, the use of these techniques can serve as a basis to improve the genetic heritage of crops, increasing their yields and tolerance to environmental factors. Proteomic analysis makes it possible to understand all the functions, structures, and interactions of proteins present in a biological system. Through a systematic review, a search was carried out, finding 15 articles related to the proteomic technique and its application in agriculture. In proteomic analysis, one of the main advantages is the ability to study protein expression on a large scale in diverse complex biological systems and at different times and conditions. Proteomic technology has been widely used in identifying proteome changes in signaling pathways in response to stress, detecting biotic and abiotic stress markers, which can be applied in the development of genetically modified crops. These advances in plant proteomics have the potential to revolutionize agriculture by providing sustainable and adaptable solutions to address future food production challenges.

Keywords: Omics, Proteins, Breeding.

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INTRODUCTION

Proteins are essential for genetic products and their study contributes to obtaining regulatory information (Weckwerth, 2011). Through advancements, proteomics has emerged as a popular tool for further approaches in omics and systems biology (Weckwerth et al., 2014). The term proteomics was established in 1996 by the fusion of the words "protein" and "genomics", it can be described as "the efficient and/or standardized analysis of all proteins present in tissues, cells or in the subcellular compartment" (Chaturvedi et al., 2016). Proteomics is a non-targeted technology, aiming to analyze the complete proteome of the target organism, although it may also involve the targeted identification and quantification of specific proteins/peptides. The proteomic profile reveals the level of protein abundance in different types of cells and tissues in any state, as well as between samples of various combinations (Silva-Sanchez et al., 2015; Fíla et al., 2016).

One of the current obstacles related to agricultural production is associated with the need for food and the adaptation of crops for high yield and resistance, whether to pests or atmospheric elements, in order to meet global demand (Haq et al., 2023). According to data from the United Nations, it is necessary to increase agricultural production by 75% by 2050 to ensure the food supply of the entire world population (Bates et al., 2008). In addition to large-scale production, the quality of food and its ability to meet nutritional demands are also of great importance. The study of genetic diversity to discover and create new crop variations, along with understanding the genetics of simple and complex traits and efficiently introducing these variations into new cultivars, are means to achieve these goals (Haq et al., 2023; Brozynska et al., 2016). Multiomics techniques are used to improve agronomic characteristics and increase agricultural tolerance, the use of these techniques can serve as a basis for improving the genetic heritage of crops, increasing their yields and tolerance to environmental factors (Haq et al., 2023)

Proteomic analysis makes it possible to understand all the functions, structures, and interactions of proteins present in a biological system. Since defective proteins are the main causes of diseases, they can serve as useful indicators in the development of specific diagnoses and treatments for certain diseases, whether in animal or plant cells (Anjolette, 2015). Proteomics not only provides comprehensive information about proteins, but also makes it possible to obtain quantitative profiles, evaluate post-translational modifications, investigate signaling pathways, and study interactions between proteins (Kaushik et al., 2024). In recent decades, proteomic technologies have been widely employed to assimilate plant responses to various external and internal signals, contributing significantly to an understanding of plant behavior in the face of changes in environmental conditions (Kaushik et al., 2024; Weckwerth et al., 2014).

Plants are constantly exposed to unfavorable conditions, such as biotic and abiotic stresses, which include variations in the availability of water, light, nutrients, and extreme temperatures

(Bokszczanin et al., 2013). These environmental modifications play an important role in plant performance, especially during the reproductive cycle, and can reduce crop yields (Tanou et al., 2012). To cope with these environmental constraints, most plants develop defense mechanisms that involve alterations in gene expression, resulting in changes in protein translation and metabolic reprogramming. These mechanisms are essential for metabolic adaptation and plant survival under stress conditions (Tanou et al., 2012; Chaturvedi et al., 2016). Thus, in order to promote greater crop sustainability, it is essential to understand the genetic and molecular conditions of the stress response mechanisms, as well as the physiological parameters (Chaturvedi et al., 2016).

Thus, the objective of this study was to carry out a systematic literature review to better understand the use of proteomic technology in genetic improvement, its application and benefits related to agriculture, especially in relation to biotic and abiotic factors.

METHODOLOGY

Through a systematic review, 15 articles were found in a search for studies related to proteomic biotechnology and its applications. The databases used were Medline/PubMed and Scopus. The articles that were admitted were in Portuguese and English. The keywords used in the research were: "Proteomics", "Genetic improvement", "Omics", "sustainable agriculture", "molecular biology", "genome alteration", "mass spectrometry", "genetic improvement".

The inclusion criteria used for the selection of articles were: (I) articles that report the global scenario in relation to sustainable agriculture; (II) articles with studies on genetic improvement techniques; (III) articles published in national and international journals; (IV) articles with the use of proteomic technology; (V) original articles, with a time frame from January 2004 to January 2024. The following were excluded from the study: (I) articles related only to the problem of agriculture; (II) articles related to other genetic improvement techniques unrelated to omics/proteomics technology; (III) editorial articles and case studies.

An initial search was carried out based on the title of the articles, and the selected studies were evaluated according to the criteria described. The information extracted is based on the characteristics of the study, title, publication and relevant considerations.

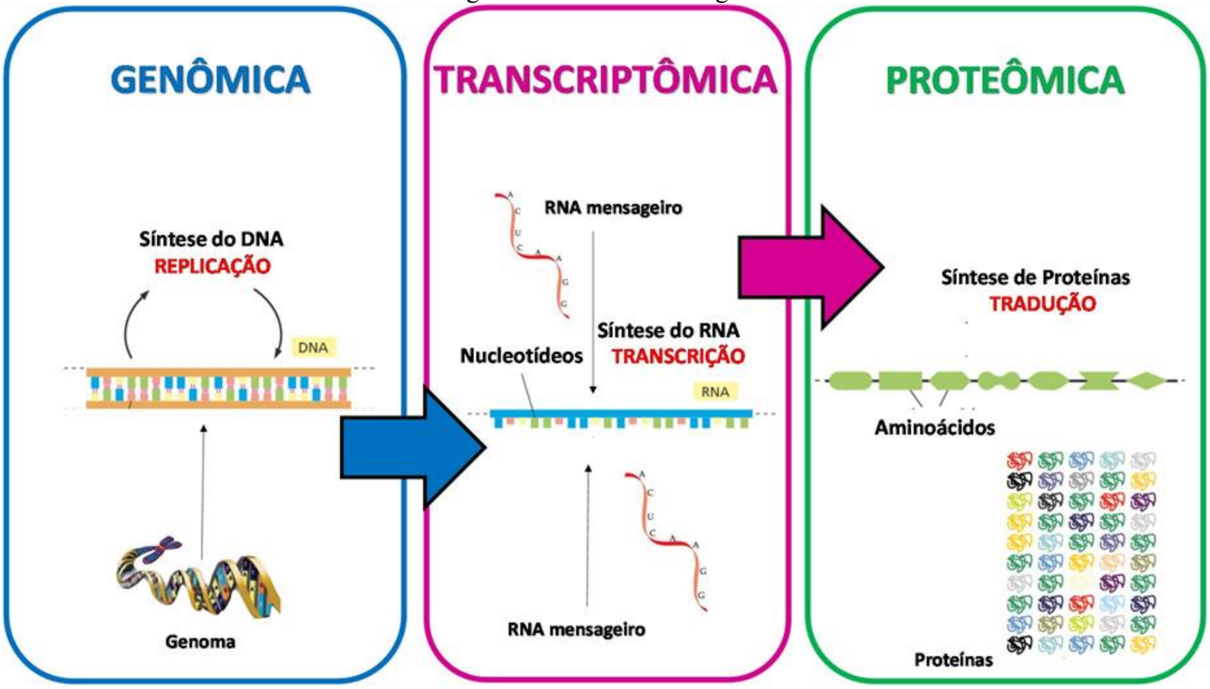
RESULT AND DISCUSSION

OMICS SCIENCES

Due to their sessile characteristic, plants develop strategies for adaptation and growth in response to changing environmental circumstances (Raza et al., 2019). At the molecular level, these tactics include changes in gene expression, from transcriptional regulation, through mRNA processing, followed by translation, protein modification, and protein renewal (Rejeb et al., 2014;

Haq et al., 2023). When exposed to controversial environmental conditions, the plant modifies its transcriptome, transcriptionally regulated genes play roles in diverse functions such as signaling, translation, transcription, metabolism, and stress response molecules (Rejeb et al., 2014). New biochemical and bioanalytical tools, such as genome sequencing, transcriptomics, proteomics, and metabolomics, are enabling more detailed analyses of these processes (Astarita & Ollero, 2015). Omics technology (figure 1) refers to the study of these processes in the genome, transcriptome, proteome, and metabolome contexts (Haq et al., 2023).

Figure 1. Omics technologies.



Overview of the fields of "omics" analyses. Genomics provides information on the complete set of genes in a complex biological system, while transcriptomics provides a complete description of the messenger RNAs in the genome. Proteomics provides information on the proteins expressed by the genome. (Anjolette, 2015)

Molecular markers are studied in genomics with the aim of discovering new patterns of variation and determining their functions in significant ecological traits (Bevan & Waugh, 2007). In crops, breeding is linked to genomic approaches to achieve advances in molecular breeding and to track elite germplasms with multi-trait assembly (Raza et al., 2019; Kole et al., 2015). Omics approaches aid in the development of crops with better yield and yield under different biotic and abiotic factors. Molecular plant breeding is an essential approach to increase crop yield and production in the presence of various factors (Raza et al., 2019).

PROTEOMICS

In recent years, studies addressing transcriptome and metabolome techniques have been conducted to identify transcriptional regulators and metabolites, providing fundamental insight into how different networks are affected and interact during the priming process (Luo et al., 2009).

However, there are limitations in estimating gene expression levels, mRNA degradation or inefficient translation, as well as in post-translational protein modifications (PTMs), protein processing and renewal, so proteomics certifies as an essential tool to bridge the gap between the transcriptome and the metabolome (Tanou et al., 2012).

The transcriptome and proteome are more complex than the genome, as distinct responses can be attributed to the same gene, and multiple proteins can be translated from the same mRNA, while the genome is a static source of information, the transcriptome and proteome are dynamic, and can vary under different conditions due to RNA processing, regulation of transcription, protein synthesis and protein modifications (Bernot, 2004). Alternative splicing, alternative promoters, or the use of polyadenylation sites can generate multiple transcripts. In addition, modifications such as glycosylation and phosphorylation can occur during or after translation, resulting in a wide variety of protein variants (Twyman, 2004; Anjolette, 2015)

Proteomic studies offer the opportunity to investigate subcellular proteomes and protein complexes, including proteins in plasma membranes, chloroplasts, mitochondria and nuclei, and, most significantly, priming-associated PTMs (Angel et al., 2012). After two-dimensional protein extract separation, the most recent advances in mass spectrometry-based proteomics (MS), such as ion mobility separations, microchip-based proteome measurements, nanoscale reversed-phase liquid chromatography, and capillary electrophoresis, have been applied as fundamental in the protein separation process (Tanou et al., 2012; Angel et al., 2012).

The variety of instruments available for proteomic analysis makes this choice complex and challenging. Currently, mass spectrometry is the main analytical method used in proteomic studies for the identification and characterization of protein compounds (Thompson; Schaeffer-Reiss; Ueffing, 2008). In proteomic analysis, one of the main advantages is the ability to study protein expression on a large scale in several complex biological systems and at different times and conditions (Barbosa et al., 2012).

The three main steps of proteomic methodologies are identification/quantification, extraction, and separation (with or without gel). Gel-free methodologies include techniques such as LC-MS, and label-based techniques such as ICAT and iTRAQ (Haq et al., 2023). Due to the complexity of the plant proteome, a single method cannot accurately assess it, so several techniques are used to improve the understanding, resolution, and comprehensiveness of the plant proteome (Altelaar et al., 2013). The choice of proteome study methodology depends on several factors, such as the availability of resources, the type of facilities and the desired applications, whether it is a global or targeted profile and the compound of interest (Anjolette, 2015). Gelless proteomics is becoming more prevalent, compared to gel-based proteomics, it has higher reproducibility and less bias (Haq et al., 2023).

GEL-FREE PROTEOMIC METHODOLOGY

Among the quantitative techniques, tag-based labeling (ICAT, iTRAQ), metabolic labeling (SILAC), and free labeling (MudPIT) stand out (Haq et al., 2023). Isotope-coded affinity labeling (ICAT) is a quantification methodology that uses a biotin affinity tag, a stable isotope ligand, and a reactive component that binds to thiol groups of proteins (cysteines) in vitro (Shiio & Aebersold, 2006). Chromatography is employed to separate the labeled triptych peptides prior to their detection by mass spectrometry (MS). ICAT is widely used to discover new proteins that influence important biological functions in specific cultivars (Barkla et al., 2013).

In the multiplex protein quantification method known as iTRAQ (isobaric labeling for relative and absolute quantification), isobaric markers are used to identify N-terminal protein and side-chain amine groups (Haq et al., 2023). This technique allows the quantification of proteins from multiple sources in a single experiment, with much higher sensitivity than ICAT. Crop researchers use iTRAQ to detect markers of biotic and abiotic stress, which can be applied in the development of genetically modified crops (Su et al., 2019).

Amino acid-stable isotope labeling in cell culture (SILAC) is the most effective metabolic labeling tool for dynamic quantitative research of the plant proteome. This technique involves in vivo labeling of cell populations cultured in medium containing N14 or N15 (Soufi & Macek, 2014). The identification of proteome changes in signaling pathways generated by PTMs in response to stress is of great value (Mastrobuoni et al., 2012).

MudPIT is a shotgun proteomic approach to analyze complex, low-abundance proteins (Zhang et al., 2010). This technology is more sensitive, it separates digested proteins using biphasic or three-phase microcapillary columns, which are then analyzed by tandem MS (Haq et al., 2023). MudPIT has been used to explore the mechanisms that regulate tiller count in rice (Lee et al., 2011).

GEL-BASED PROTEOMIC METHODOLOGIES

The gel methodology is the most popular, adaptable and recognized protein separation and quantification technique. They are more cost-effective than gel-free approaches and can be used to characterize protein isoforms and identify low-abundance proteins (Sriyam et al., 2007). Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) is critical in proteomics due to its accessibility and familiarity. The isoelectric point (pI) and molecular weight (M) are applied to differentiate proteins (Marouga et al., 2005). Proteins are classified into two groups based on their molecular weight (M) and the presence or absence of 2-mercaptoethanol. They can be visualized with dyes such as Coomassie blue, silver nitrate, or SYPRO Ruby (Rabilloud, 2013).

Difference gel electrophoresis (DIGE) was developed to overcome the limitations of 2D-PAGE, such as variations between gels and low repeatability. The DIGE technique allows the evaluation of variations in protein expression in response to biotic and abiotic stresses (Marouga et al., 2005). To reduce the co-migration interferences observed in 2D-PAGE, three-dimensional gel electrophoresis (3DGE) is used, which accurately identifies proteins and post-translational modifications (PTMs) using two distinct buffers with different ion carriers (Sriyam et al., 2007).

After digestion of the peptides, the proteins of interest are identified by mass spectrometry (MS). Computational approaches aid in the identification of proteins based on peptide mass and fragmentation (MS/MS) data (Haq et al., 2023). The process of identifying the protein by MS involves three phases: transforming molecules into gas-phase ions, separating them in an electric or magnetic field according to their mass-charge ratio (m/z), and identifying the separated ions with specific m/z values (Sriyam et al., 2007). Ionization methods include electrospray ionization (ESI), matrix-assisted laser desorption ionization (MALDI), and surface-enhanced laser desorption/ionization (SELDI) (El-Aneed et al., 2009).

MASS SPECTROMETRY

This technique works by identifying signals generated by ion transitions during fragmentation, using a mass spectrometer. Additional tools include MS tandem, linear quadrupole orbitrap trap (LTQ-Orbitrap), quadrupole trap (Q-trap), and triple quadrupole trap (Haq et al., 2023). Multiple reaction monitoring (MRM) is the process of detecting multiple changes, while selected reaction monitoring (SRM) is the process of identifying transitions in a triple quadrupole (Yocum & Chinnaiyan, 2009). On the other hand, the approaches mentioned above focus on sample accuracy. To circumvent this problem, SRM/MRM methods use isotopic labeling (Yocum & Chinnaiyan, 2009).

SRM transitions are highly specific scans that aim to detect specific analytes in complex mixtures, usually using triple-quadrupole-based mass spectrometers (Anderson & Hunter, 2006). These transitions are planned so that the first mass analysis quadrupole (Q1) is configured to transmit a narrow mass window around the desired parent ion, while the third quadrupole (Q3, the second mass analysis quadrupole) is configured to transmit a narrow mass window around the desired fragment ion (Wolf-Yadlin et al., 2007). Fragmentation occurs in the second quadrupole (Q2) via collision-induced dissociation (CID). Therefore, SRM requires the presence of two ions to produce a positive result, which makes it a highly specific detection methodology with a very low noise level, thus increasing the sensitivity of detection (Yocum & Chinnaiyan, 2009). The success of SRM transitions depends not only on the ionization efficiency of the parent ion (Q1 transmission), but also on the fragmentation efficiency of this parent ion and, consequently, on the intensity of the fragment ion (Q3 transmission) (Jenkins et al., 2006). By inserting several different SRM transitions to the same analyte or to different analytes, multiple transitions can be monitored in a single MS run. This method is known as MRM, balancing productivity and sensitivity (Yocum & Chinnaiyan, 2009).

For the identification and quantification of proteins, MRM-MS has not been as widely used due to challenges in the development of the method (Griffiths et al., 2007). Although it is theoretically possible to introduce a standard mixture of proteins into a sample at concentrations known for quantification, similar to what is done in small molecule analyses, in practice this does not guarantee robust protein quantification (Yocum & Chinnaiyan, 2009). This is because standard proteins and analyte proteins can produce different responses in the mass spectrometer due to ion suppression and variations in fragmentation (Barnidge et al., 2004). In addition, the recovery rates of the different proteins may be affected by sample preparation steps prior to MS. For this reason, isotopically labeled peptides instead of proteins are suggested for accurate protein quantification (Yocum & Chinnaiyan, 2009).

PROTEOMICS AS A TOOL IN AGRICULTURE

The secretome encompasses all secreted proteins, accounting for up to 30% of an organism's proteome, and plays critical roles in a variety of cellular processes (Skach, 2007). These proteins are essential for functions such as signal perception and transduction, stress responses, and apoptosis. The secretion process in plants is highly sophisticated and tightly regulated, and it has been observed that secreted proteins act both locally and systemically (Gupta et al., 2011). The genes responsible for encoding these proteins are less frequently present in the central genome, but are more common in mobile regions. However, the application of proteomics to analyze the secretome is limited due to several factors, namely, the secreted proteins can be expressed in low abundance; produced by specialized cell types and expressed at specific stages of development (Ahsan et al., 2007).

Chickpeas are an important legume consumed globally as a source of vegetable protein, containing 25% protein in their composition (Gupta et al., 2011). In a study with suspended culture of chickpea calluses, Gupta (2011) the secretome was characterized using classical SDS-PAGE coupled to mass spectrometry. As a result, 773 secreted proteins were identified, providing a comprehensive view of the secretome of a dicotyledon species. Regarding the identified proteins, most were related to primary and secondary metabolism (19.1%), followed by signal transduction (14.1%), proteins with different functions (11.6%) and maintenance of the redox state (9.2%) in the extracellular space. Another category included proteins involved in cellular defense (9%) and transport (8.9%) between intercellular regions. Proteins involved in protein folding (8.9%) and modification (6.7%) of proteins were also identified. Proteins whose identity was not determined were classified as unidentified (6.6%). In addition, several proteins (5.9%) involved in cell wall modifications were detected.

Proteomic analysis is very useful for the identification of proteins extracted from wheat bread grain (*Triticum aestivum* L.), in a study by Lesage et al. (2012), a comparative analysis of the proteome of two quasi-isogenic strains (NILs) was performed with the aid of two-dimensional electrophoresis (2-DE) and mass spectrometry. As a result of the analysis, during grain development, folding and stress-related proteins were more abundant in the hard strain compared to the soft line. These results suggest that protein matrix formation occurs earlier in the hard lineage, indicating an earlier stress response, possibly the unfolded protein response, compared to the soft line, leading to earlier cell death in the endosperm. In this way, new perspectives emerge on the role of purindolins in the folding machinery of storage proteins, thus affecting the development of the wheat endosperm and the formation of the protein matrix.

PROTEOMICS IN THE INVESTIGATION OF ABIOTIC AND BIOTIC MECHANISMS OF TOLERANCE

Katam et al. (2020), in a study investigated the effects of various abiotic stresses on the regulation of leaf proteins in soybean cultivars, providing valuable data on the proteomic and enzymatic responses of soybean to these stresses under field conditions. Using 2-DE protein mapping and mass spectrometry, the results revealed that simultaneous stresses cause changes in physiology, proteome and enzymatic activity, differently from what occurs in situations of individual stress. A significant degree of genetic diversity was observed in the protein abundance between the two cultivars when subjected to different types of stresses. Multiple proteins related to metabolism, heat response, and photosynthesis exhibited cross-tolerance mechanisms. This phenomenon was especially evident in cultivar R95-1705, where proteins responsive to heat, photosynthesis, metabolism and redox proteins were abundantly present in response to heat stress, as well as to combined heat and water stress in cultivar PI-471938, suggesting a relative heat tolerance in the latter.

Heat and drought are the main abiotic stressors that limit the growth and development of soybean plants, at the cellular level, plants exhibit a variety of physiological and biochemical responses to overcome these stresses (Eldakak et al., 2013). In a study, Das et al. (2016) investigated the differential expression of proteins in soybean (Glycine max L.) in response to drought and heat stress. They identified 44 proteins responsive to abiotic stress that influenced signaling cascades and molecular processes. In addition, many proteins related to photosynthesis, which were expressed in a differential manner, impacted RuBisCO regulation, electron transport, and the Calvin cycle during periods of abiotic stress. According to the results obtained, 25 proteins related to photosynthesis were downregulated under stress conditions in both soybean varieties.

Corn (Zea mays L.) It faces severe threats due to various abiotic stresses, such as drought, salinity, cold, heat, and flooding. Among these factors, drought or water deficit is the most critical, posing a significant threat to maize production worldwide (Yousaf et al., 2023). In a study on the responses to water stress in maize at the protein level, Zenda et al. (2018), employed an iTRAQ-based quantitative strategy to perform the proteomic profiling of two contrasting inbred maize strains. A comparative proteomic analysis of the leaves of these two lines and some physiological responses under water stress were performed. They analyzed 721 differentially abundant proteins (DAPs) in two maize strains, identifying both common and single proteins accumulated in response to water limitation in maize. Using an iTRAQ-based method, which resulted in a total of 721 differentially abundant proteins (DAPs), five essential sets of drought-responsive DAPs were identified, including 13 specific DAPs.

Wang et al. (2019), in proteomic analysis of the filling core proteomes of two drought-tolerant maize strains, used an iTRAQ-based strategy to identify protein expression profiles during grain development and to compare the water stress responses of the same strains (YE8112 and MO17) after a 14-day exposure to moisture deficit. As a result, a variety of molecular elements have been identified that are involved in mediating drought tolerance, There was a change in the proteome of the stressed plants compared to the control conditions. A total of 5,175 DAPs were identified in the four experimental comparisons, DAPs expressed exclusively in YE8112 were mainly involved in pathways related to "protein processing in the endoplasmic reticulum" and "tryptophan metabolism", while DAPs in MO17 were associated with "starch and sucrose metabolism" and "oxidative phosphorylation" pathways. Thus, the results revealed that YE8112 grains were comparatively more drought tolerant than MO17 grains.

Barley (Hordeum vulgare L.) stands out as one of the most salinity-tolerant crops, being an excellent model for studies on the mechanisms and inheritance of salinity tolerance, in addition to being fundamental for the development of tools that improve salt tolerance in cereals (Wang et al., 2018). In order to perform a biochemical and proteomic analysis of barley, Zhu et al. (2020), verified two pairs of quasi-isogenic strains (NILs), which are genetically almost identical, except for the target region containing QSl.TxNn.2H. According to the study, 53 and 51 differentially expressed proteins were identified in leaves and roots, respectively. QTL QSl.TxNn.2H can improve salinity tolerance by controlling the load of Na+ in the xylem, reducing the toxicity of Na+ in leaves. In addition, this QTL induces the expression of proteins related to photosynthesis, elimination of reactive oxygen species (ROS) and ATP synthase genes.

Huang et al. (2016) applied proteomics to analyze the interaction between tomato cultivars resistant to and susceptible to TYLCV infection. Proteins extracted from leaves of the resistant tomato cultivar 'Zheza-301' and the vulnerable cultivar 'Jinpeng-1', after infection by TYLCV, were confirmed by two-dimensional gel electrophoresis. A total of 86 differentially expressed proteins were identified, defined into seven groups based on their functions, which are responsible for photosynthesis, proteometabolism, carbohydrate metabolism, signal transduction, accompanying proteins, detoxification, antioxidant and amino acid metabolism. The results help identify key proteins involved in the interaction between tomatoes and TYLCV, potentially increasing resistance to the virus and providing protection against infection.

In proteomic analyses with maize infected with MCMV (Virus chlorotic stain), Dang et al. (2019), applied a comparative approach with isobaric tags for relative and absolute quantification (iTRAQ) to analyze maize infected with MCMV. A total of 972 differentially abundant proteins (DAPs) were identified, of which 661 showed increased abundance and 311 reduced abundance. Functional annotations and orientation of photosynthetic activity revealed decreased photosynthesis and significant changes in ribosomal proteins, stress responses, oxidation-reduction, and redox homeostasis. Thus, the results suggest that the combination of comparative proteomic analyses and virus-induced gene silencing can help in the identification of host proteins that modulate MCMV infection, thus contributing to guide the development of strategies.

Shah et al. (2012) used proteomic analysis of proteins released in the microenvironment of the infection sites of green and red tomato fruits with B. cinerea to identify the proteins produced by ripe green and ripe red fruits in response to infection, as well as the proteins released by B. cinerea. Thus, the analysis characterized 186 proteins in tomatoes infected with *B. cinerea*, being a viable approach both to obtain sufficient information to identify pathogen and host proteins from sites of infection, and to describe the various classes of proteins. The results provided simultaneous information on host and pathogen proteomes, identifying a significant number of several proteins involved in pathogenicity and proteins related to protection against the host oxidative stress response.

CONCLUSION

Addressing the growing challenges of agricultural production requires innovative and integrative approaches. Understanding the genetic diversity of crops and their effective introduction into new cultivars are crucial to meeting the world's growing demand for food. In this context, omics techniques, including proteomics, play a key role. Not only does proteomics provide comprehensive understanding of proteins, but it also offers the opportunity to investigate post-translational modifications, signaling pathways, and interactions between proteins, thereby contributing to a deeper understanding of plant behavior in the face of environmental changes.

Methods such as LC-MS, ICAT, and iTRAQ have been widely used to analyze the plant proteome, each bringing its own advantages and specific applications. In addition, approaches such as MudPIT have been valuable for exploring regulatory mechanisms in important cultures. By integrating these innovative methodologies, we can not only identify markers of biotic and abiotic stress, but also contribute to the development of more resilient and productive genetically modified crops. These advances in plant proteomics have the potential to revolutionize agriculture by providing sustainable and adaptable solutions to address future food production challenges.

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Elaboration of interactive didactic material for the discipline of inorganic chemistry

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ABSTRACT
Addressing the growth of higher education requires addressing dropout, which harms students and the quality of the education system. Thus, the overall objective of the project is to create innovative teaching materials to teach inorganic chemistry. The project is carried out in stages, such as: search for the menu, bibliographic research and formulation of ideas, finally, seeking feedback from students. Which confirmed the importance of investing in interactive teaching materials adapted to the needs of students.

Keywords: Chemistry, Inorganic, Material, Teaching.

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INTRODUCTION

In the Brazilian context, by focusing on reducing dropout in higher education, the project stands out as an educational approach that involves students in the learning process, recognizing that dropout should not be seen only as a definitive dropout, but as a complex phenomenon that requires exploration and discussion to guide the management of educational institutions (SENGER; DALLAGO, 2020). In the last two decades, higher education in Brazil has experienced remarkable growth in several dimensions, driven by programs such as REUNI and PROUNI, which have resulted in new public policies and investments in infrastructure (Da Silva et al., 2022). However, it is essential to address dropout as a fundamental challenge when facing the growth of higher education, whether in public or private institutions, as highlighted by Vasconcelos, Almeida and Monteiro (2009, p. 458).

> Upon entering Higher Education, students are endowed with a series of intentions and objectives that define the level and type of education and professional future they aspire to for themselves. These intentions can be translated into a greater or lesser commitment to the achievement of certain educational objectives (such as, for example, obtaining a degree) or to the achievement of these objectives in a particular educational institution – a commitment of a more institutional nature.

To combat dropout in higher education, it is crucial to implement innovative strategies and provide academic support in institutions. According to data from the MEC-INEP Higher Education Census, in 2021, the dropout rate reached 9.4% in public institutions, resulting in the loss of 165 thousand students (MEC-INEP Higher Education Census, 2022). This program seeks to create innovative and effective teaching materials for the discipline of Inorganic Chemistry, aiming to improve teaching and learning. The main focus is to provide an engaging learning experience that stimulates curiosity, critical thinking, and challenge solving, contributing to the improvement of students' performance in this area and promoting new approaches in the dissemination of knowledge.

MATERIALS AND METHODS

The main objective of this project is to create didactic resources aimed at improving the teaching and learning of the discipline of Inorganic Chemistry, also including the concepts covered in the discipline of General Chemistry I. To achieve this purpose, the project will be structured in several stages:

a) Survey of the syllabus of the discipline of Inorganic Chemistry and the contents to be addressed.
b) Conducting a bibliographic research in scientific articles related to each chapter of the content, in order to support the creation of didactic materials.
c) Elaboration of a script that describes the topics of each chapter, serving as a basis for the

production of didactic materials.

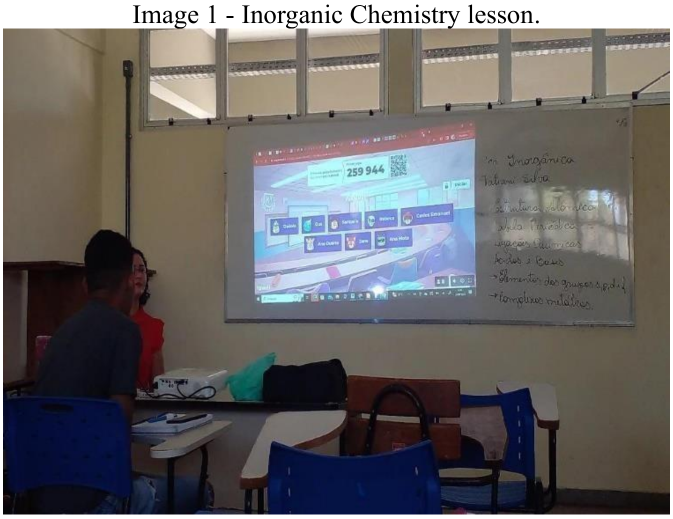
d) Precise planning, including dates and times, for the execution of each project-related activity.

e) Application of teaching materials in the classroom. And application of a questionnaire to find out the students' opinion about the resource.

RESULTS AND DISCUSSION

Alternative teaching in higher education plays a crucial role in promoting inclusive and dynamic education by adopting flexible and innovative teaching methods that adjust to students' different learning styles and interests. This creates a stimulating and engaging environment, which is essential to combat the high drop-off rate, as revealed in a survey (Christo et al., 2018, p. 163), which indicated a dropout rate of 86% among engineering students in the first periods. This focus aligned with Faria's (2001) vision of education as a continuous and comprehensive process, the project was applied in the Materials Engineering course, class of 2023, with classes on Fridays, from 2 pm to 5 pm (Image 1).

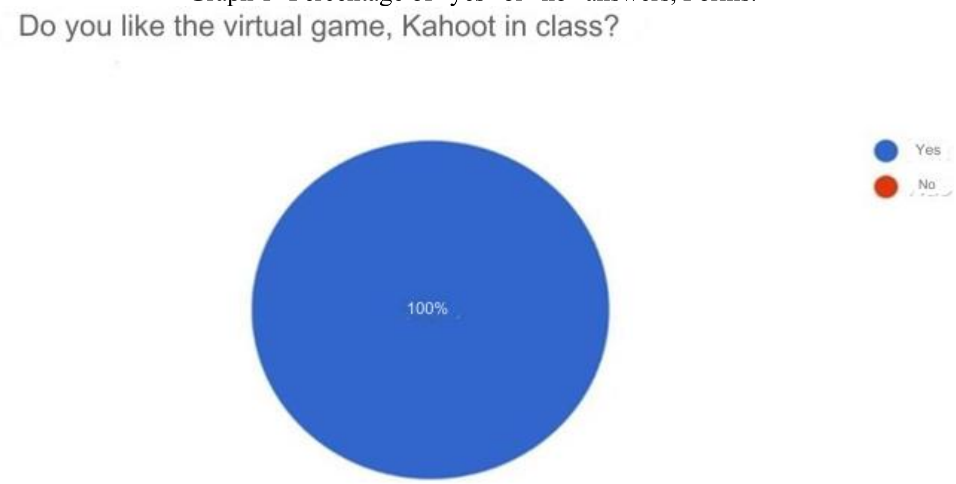
Image 1 - Inorganic Chemistry lesson.



Source: Author, 2023.

A Forms was applied to find out what the students thought about using the virtual game Kahoot in the classes of the subject. The students of the inorganic chemistry discipline, materials engineering 2023, answered the questions "Do you like the virtual game, Kahoot in class?" and "Can you learn better with this resource (Kahoot)?", where inside the classroom the students seemed excited about the game and participated. The answers of these students (Graph 1) were positive:

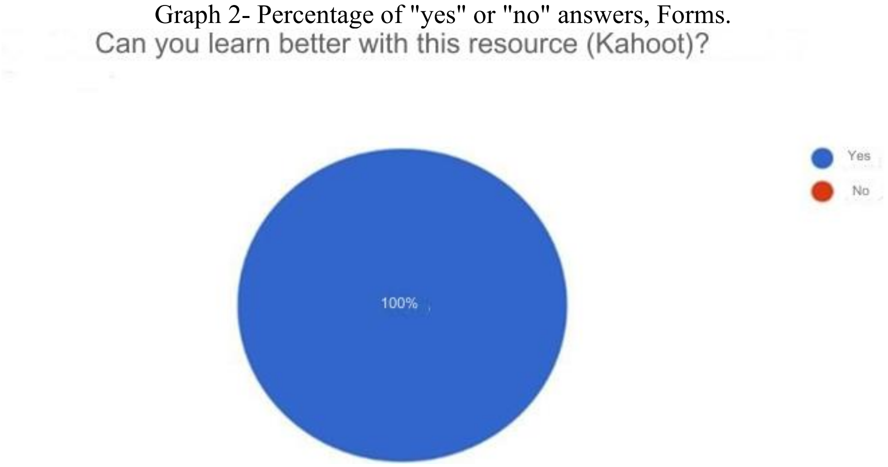
Graph 1- Percentage of "yes" or "no" answers, Forms.



Source: Author, 2023.

The students answered and 100% of the answers were "yes", stating that they liked the game. The second question (Graph 2) had the following percentage:

Graph 2- Percentage of "yes" or "no" answers, Forms.



Source: Author, 2023.

All the answers to whether the students learned better from Kahoot were positive. In addition, two students: student X1 and student X2, gave their written opinion about the resource used, organized in Table 1:

Table 1 - Students' opinion regarding the game

Student X1- "It's a way of not being in that situation where only the teacher is talking and the student listens It's more didactic and interactive."
Student X2- "A diversified teaching methodology can stimulate learning."

Source: Author, 2023.

The opinions highlight that the method is different and promising. In this way, the program should evolve within the academic scope, seeking more students, as one of the ideas against student dropout in the initial semesters. In addition to improving the quality of teaching with new methodologies.

FINAL THOUGHTS

The ongoing effects of the Covid-19 pandemic are still noticeable in the educational field, making the integration of students who come from a remote education system a critical issue to ensure their continuity at the university. The results of the program to create alternative teaching resources for the discipline of Inorganic Chemistry have been highly promising in the classroom, presenting itself as an effective solution to the challenge of student retention.

These developed materials have demonstrated positive impacts, evidenced by the improvement in students' understanding and interest in the content of the discipline, which has made the more dynamic and attractive learning process. In addition, the use of these resources has fostered greater interaction between students and knowledge, facilitating the assimilation of Inorganic Chemistry concepts. The intent is to share these materials with other educators and make them available in all future courses in the discipline as the project is completed. This not only benefits the students on our course, but also provides incentives to reduce student dropout, amplifying the positive impact on teaching.

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Conservation and postharvest quality of prata-anã banana cultivated in organic and conventional management system

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ABSTRACT

On the ecological problems and health that result by the heavy use of agrochemicals in farming, new alternatives have been demanded. This demand for organic products is growing around the world, due to the demand for healthier foods, eco-friendly and greater profitability. Thus, the work aimed to evaluate by physical, chemical and sensory methods to conservation and postharvest quality of banana ' Prata-Midget "produced in the North of Minas Gerais under conventional and organic management. The work was conducted in the laboratory of Physiology and post-harvest of the State University of Montes Claros-UNIMONTES, in exact sciences and Technology Centre on the Campus of Janaúba-MG. The fruits used were of the variety-Silver Dwarf The fruits used were of the variety-Silver and framed in the market as of Monday, being from two farms, one adopts the organic system of production and the other the traditional system the experimental design was completely randomized design (DIC) in factorial scheme 2 x 6, being the organic and conventional management and six evaluation periods (1, 3, 5, 7, 9 and 11dias after harvesting). Four replicates were used and the experimental unit was formed of four fruits. The fruits were evaluated as the coloration of the shell diameter, length, firmness, fresh pasta with and without bark, pulp/Peel, soluble solids,

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pH, titratable acidity and sensory analysis. The organic production system introduced for most physical and chemical characteristics evaluated superior results compared to the conventional system, not differing in firmness and pulp/Peel. The values of the notes for all attributes analyzed were at most 1 Note (I like extremely) and 2 (liked moderately), demonstrating that there was no satisfactory sensory acceptance on the part of assessors for both managements.

Keywords: *Musa spp.*, Storage, North of mines.

INTRODUCTION

Brazil stands out in the international market as one of the largest producers of fruits, among the fruits of greatest economic and social relevance is bananas. Due to its nutritional characteristics, it is considered a nutritious fruit, with high digestibility, wide acceptance and easy acquisition, it implies a high consumption in the various layers of society (RODRIGUES, 2009). In 2009, the world production of this fruit was over 96 million tons, with India, the Philippines and China as the largest producers. Brazilian production was more than 6.7 million tons and is the fifth largest producer in the world (AGRIANUAL, 2012). In Brazil, the crop is exploited by small, medium and large producers in an area of approximately 527 thousand hectares (IBGE, 2013), mostly managed in a conventional way, and almost all of them include the use of fertilizers, herbicides, pesticides to control the main pests and diseases and the intensive turning of the soil using agricultural machinery and implements (BORGES et al., 2004). A growing demand for food without the use of chemical inputs is mainly due to consumers' concern with food safety, due to the high levels of pesticides present in some crops, which are easily absorbed by the body, which can cause damage ranging from mild poisoning to serious health problems (RIBEIRO et al., 2013).

The adoption of alternative production systems comes as a tool to improve the quality of the fruits, introducing to the market a differentiated product, with the potential to reach new markets, especially the foreign market (LOPES, 2011). Organic agriculture is based on a holistic system, making food production compatible without the use of chemical inputs in the crop, combined with low production costs (AZADI and HO, 2010). Due to this new appeal, associated with the better remuneration achieved by organic products, many producers have migrated from the conventional to the organic production system. As the banana is a climacteric fruit, it undergoes profound biochemical transformations after harvest, having a relatively short post-harvest life and presenting marked changes during ripening. Because it is a fruit that is very sensitive to transport when ripe and does not keep for long periods, bananas should be harvested while still green. During ripening, there is an increase in the content of simple sugars, an increase in simple and organic acids, a decrease in phenolic compounds, resulting in a reduction in astringency and an increase in acidity, in addition to the release of volatile compounds, factors responsible for aroma and flavor, which are fundamental characteristics for the acceptance of the fruit (SOTO-BALLASTERO, 1992). Seen as one of the most critical phases within the production-marketing process, but the least considered, the post-harvest phase of the fruits is of paramount importance, since it defines, from the moment it is harvested to consumption, the quality and conservation capacity of the fruit (LOPES, 2011).

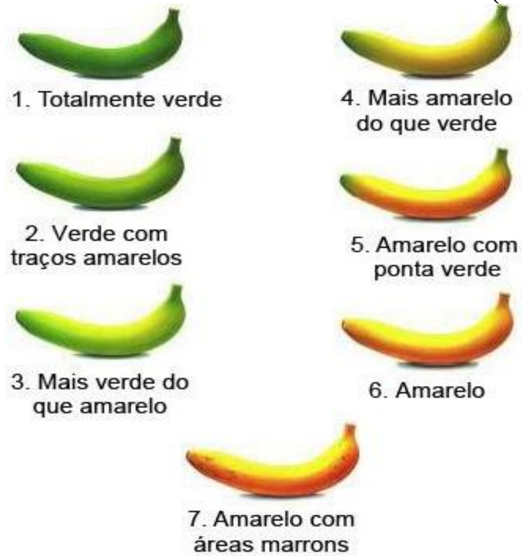
OBJECTIVE

To evaluate by physical, chemical and sensory methods the conservation and postharvest quality of the "Prata-Anã" banana produced in the North of Minas Gerais under conventional and organic management.

MATERIAL AND METHODS

The present work was carried out at the Laboratory of Postharvest Physiology, State University of Montes Claros (UNIMONTES), Department of Agricultural Sciences, Janaúba Campus, MG. The fruits used were of the Prata-Anã variety and included in the market classification as second, coming from two farms, one of organic cultivation and the other of conventional cultivation, both located in the municipality of Janaúba/MG. The characterization of the climate is semi-arid, with flat relief and silty alluvial soil. The average annual rainfall is 750 mm, concentrated from November to March. It has annual average temperature of 28 °C, insolation of 9.5h/day and relative humidity of 48%. The bunches were harvested at stage 2 of maturation (Figure 1) according to the CEAGESP Classification Standards (2006), and taken to the laboratory, where the selection of the fruits was carried out, discarding the damaged ones and those with symptoms of mechanical injuries. Subsequently, the bunches were divided into bouquets of four fruits, which were washed in running water and stored at 26°C ±1ºC and analyzed at two-day intervals

FIGURE 1 CEAGESP Classification Standards (2006)



CONVENTIONAL HANDLING

The property has its fruit production structured with a high technological level, with a production of first, second and export bananas. Management, fertilization is carried out through soil and leaf analysis, providing the plants with the necessary nutrients for their development according to their needs. These nutrients are provided in the form of chemical fertilizers, such as urea, simple superphosphate, potassium chloride, among others. When there is the occurrence of diseases and pests, alternatives are sought that are integrated in obtaining the best control, such as the use of chemical products combined with cultural control. Herbicides are used to combat weeds, since this presence of weeds delays the development of the banana plantation, decreases plant vigor, reduces the size of the bunch, hinders phytosanitary treatments, fertilization and the displacement of workers within the crop. On the property, modern techniques are used in its post-harvest infrastructure, the bunches are transported by overhead cables, taking them to the packin house, where the entire process of hygiene, classification and packaging of the products is carried out, undergoing inspections by technicians in order to ensure the quality of the product.

ORGANIC MANAGEMENT

The production of organic bananas is in an area of 9.5 hectares that was acquired in 2010, where previously there was already the planting of Prata-Anã banana conducted in a conventional way, however it was in conditions of abandonment. The irrigation system was replaced by a new one, and the area was managed, with cultural treatments and application of organic fertilizers. In management, fertilization is carried out according to the needs of the plants, through soil analysis carried out periodically. There is no involvement by any type of pest that will reduce production. Disease control is done with monthly application of mineral oil and lime application on the foot of plants that show symptoms of Panama disease (*Fusarium oxisporum* f. sp. *Together* with the organic matter that increases the amount of beneficial microorganisms and the competition between them, there will be a smaller amount of this fungus. In 2013, the producer adapted to the certification standards for pesticide-free plant products (SAT), a certification issued by the Minas Gerais Institute of Agriculture (IMA). The certification of products of plant origin Without Pesticides - SAT is offered by the IMA for the pesticide-free system at any stage. The property produces second-class bananas, since the production of first-class bananas is not satisfactory. This is due to the fact that the area was for a long time under conventional cultivation, which caused an imbalance in the soil and microfauna. This process can take a long time to reverse and, despite the organic management adopted by the producer, he has not yet been able to achieve an ecological balance in his area capable of causing an increase in the production of prime bananas.

EXPERIMENTAL DESIGN

The experiment was conducted in a completely randomized design (DIC), in a 2 x 6 factorial scheme, with organic and conventional management and six evaluation periods (1, 3, 5, 7, 9 and 11 days after harvest). Four replications were used and the experimental unit consisted of four fruits. The data of the evaluated characteristics were submitted to analysis of variance and the significance of the interactions between the tested factors was verified by means of the F test, with subsequent unfolding for the significant results. For the data of the storage periods, polynomial regression models were adjusted and submitted to analysis of variance, and the significant models with the highest coefficient of determination and that best explained the biological phenomenon were selected. The SISVAR program was used to process the data analysis (FERREIRA, 2008).

EVALUATED PARAMETERS

The physical, chemical and sensory quality of the fruits was evaluated by analyzing length, diameter, fresh fruit mass, soluble solids, titratable acidity, pH, color and firmness.

On the last day of storage, a more careful characterization of the fruits was carried out, evaluating the peel mass, pulp mass, pulp/peel ratio and sensory analysis.

Fruit length

The external curvature from the base of the peduncle insertion to the end of the fruit was measured with the aid of a caliper, and the results were expressed in centimeters.

Fruit diameter

The median region was measured with the aid of a caliper, and the results were expressed in centimeters.

Fresh fruit mass, pulp and peel

The result was performed with the aid of a semi-analytical balance and the result was expressed in grams.

PULP/PEEL RATIO

To obtain the relationship, the value of the mass of the fruit without the peel was divided by the mass of the peel and the result was expressed in pure number, with two decimal places.

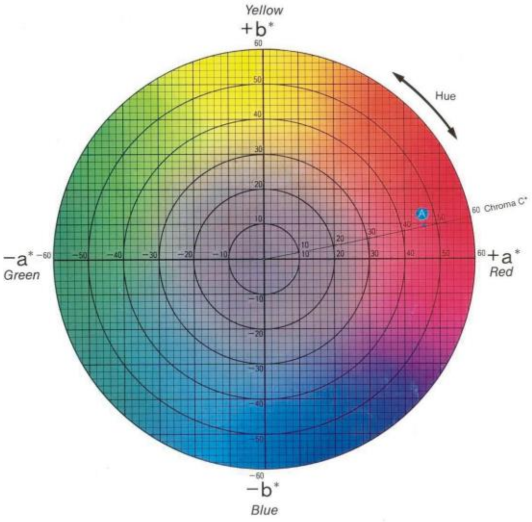
Bark staining

Color analysis was performed using a Color Flex 45/0(2200), stdzMode:45/0 colorimeter, with direct reflectance reading of the coordinates L* (luminosity), a* (red or green) and b* (yellow or blue hue), from the Hunterlab Universal Software system (Figure 2). From the values of L*, a* and b*, the hue angle (°h*) and the chroma saturation index (C*) were calculated. For each replication, an average of four measurements per fruit was used.

$$°H^* = ActG\ (A^*/B^*)\ (–1) + 90 \ □ \ \text{for negative } A^* \qquad \textbf{(A1)}$$

$$°H^* = 90 − (ActG\ (A^*/B^*)) \ □ \ \text{for } A^* \text{ positive} \qquad \textbf{(A2)}$$

$$C^* = \sqrt{}\ (a^*)^{2+(b^*)2} \qquad \textbf{(B)}$$



L, a, b Color Solid representation of the Hunterlab Universal Software system and description of hue angle (°h*) and chroma saturation index (C*).

FIRMNESS

Firmness was measured with a tabletop penetrometer (FACCHINI, model FT 011), with the results expressed in Newton (N), with a penetration depth of 2.0 mm and a tip TA 9/1000. The reading was performed on whole fruits with peel, in the median region, using four fruits for each analysis.

SOLUBLE SOLIDS

Soluble solids were determined by refractometry, using an ATAGO benchtop refractometer, model N1, with readings in the range of 0 to 95 □Brix, after extracting a sample of the pulp from the central region of each fruit. The result expressed in $^{oBrix.}$

TITRATABLE ACIDITY

The titratable acidity was determined by titrating the juice of the fruit set of each tray under agitation after extracting, crushing and homogenizing 10g of the pulp of the central region of each fruit in 90 mL of distilled water, with 0.1N NaOH, using 1% phenolphthalein as an indicator. The result was expressed in grams of malic acid per 100 g of sample.

ph

The pH determination was performed directly in the juice using a DIGIMED pH meter, model DM20, after the preparation of the samples as in the previous analyses.

SENSORY ANALYSIS

To verify the acceptance of the fruits from the different managements, sensory studies were carried out with 20 volunteer tasters. The test followed a structured 5-point scale (1- I liked it extremely, 2- I liked it moderately, 3- I didn't like it, I didn't like it or dislike it, 4- I disliked it moderately, and 5- I disliked it extremely) (Figure 3). The sensory evaluation was carried out by 20 untrained tasters, including employees, visitors and interns. The samples were presented to consumers at room temperature, in disposable plastic plates coded with repeat numbers and evaluated for pulp color, appearance, flavor, texture and overall evaluation. Sensory analyses were performed on the last day of storage, when the fruits were already ripe. The results of the sensory characteristics were evaluated and then frequency histograms were constructed with the values received by each sample.

FIGURE 3 Sensory analysis sheet to verify the acceptance of the fruits.

Nome:__________________________________

Sexo: ☐ Feminino ☐ Masculino Data: ____/____/____ Idade:______

Produto: **Banana Prata-anã**

- Você costuma consumir banana?

☐ Sempre ☐ Frequentemente ☐ Raramente ☐ Nunca

- Por favor, avalie a amostra do fruto servido e indique o quanto você gostou ou desgostou de acordo com essa escala.
    1. Gostei extremamente
    2. Gostei moderadamente
    3. Não gostei, nem desgostei
    4. Desgostei moderadamente
    5. Desgostei extremamente

	R1	R2	R3	R4
Cor				
Aparência				
Sabor				
Textura				
Avaliação global				

Comentários:______________________________

Muito Obrigada!

RESULTS AND DISCUSSION

Table 1 shows the mean length, diameter and weight of the fruits in the different managements. It was observed that there were significant differences between the managements, in the organic the fruits presented higher values, with average values found for the length were 14.68 and 13.39 cm and for the diameter were 3.47cm and 3.23cm for organic and conventional, respectively. These results are similar to those found by Lopes (2011), who found that under a temperature of ±26° the values of length and diameter were higher for the alternative culture.

However, Ribeiro *et al*. (2013), in disagreement with the results, reports that no statistical differences were detected in the characteristics of length and diameter of the Prata-Anã cultivar under organic and conventional management system and found values lower than those of the present study. According to Ribeiro (2011), the benefits found in the organic cultivation system result from the importance of organic matter, which, when applied to the soil, provides the necessary nutrients for production in a gradual manner. The improvement of the physical and biological structure of the soil is due to these nutrients, retained in the humus, as they provide greater efficiency in the ability of plants to assimilate nutrients. Donato (2003) reports that the length of the fruit is an important characteristic from the perspective of quality for classification, however, this varies with the demand of the consumer market. Research conducted by Matsuura *et al*. (2004) indicate that 87.4% of consumers prefer medium (12 to 15 cm) and large (16 to 19 cm) bananas. The advantage of the Prata-Anã banana lies in its dimensions, since they are smaller than those of the Cavendish subgroup, thus making them more practical for consumption (DAMATTO Jr., 2005).

Regarding the average mass of bananas (bouquets of four fruits), there was a significant difference, with the fruits of organic cultivation having a weight of 554.63g, a value higher than that of conventional cultivation, which was 402.45g (Table 1). The mean values observed in this study for fruit mass in organic cultivation were higher than those found by Santos *et al*. (2012) in the cultivar Prata-Anã where it found a value of 115.33g, which is higher when compared to conventional cultivation with an average value of 100.61g per fruit analyzed. Fruit mass is an important parameter for breeding work, since it cannot be considered in isolation, as it is associated with other characteristics that define fruit size, such as length and diameter, and reflect fruit quality and consequently in consumer preference (DONATO *et al*., 2006).

TABLE 1 Mean values of length (CF), diameter (DF) and fruit mass (PF) of Prata-Anã bananas cultivated under conventional and organic management (average of the evaluations of the entire storage period).

Handling	**CF (cm)**	**DF (cm)**	**MF (g)**
Conventional	13.39 b	3.23 b	402,45 b
Organic	14.68 to	3.47 to	554,63 to
CV(%)	5,35	6,12	14,55

Values followed by different letters in the columns differ statistically according to the F test(0.05).

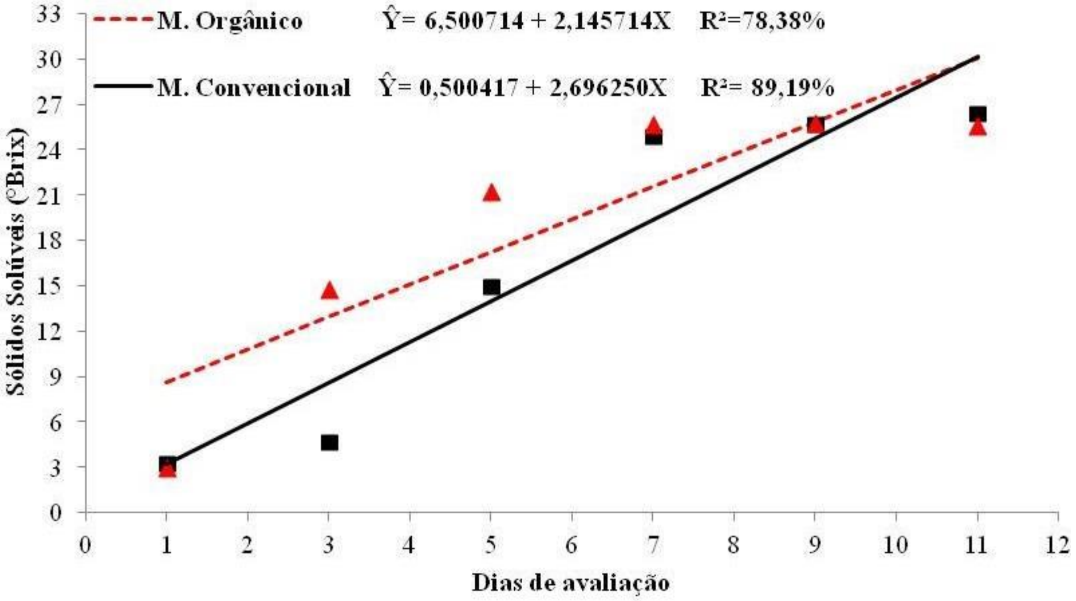
In the evaluation of the soluble solids content, a linear increase was observed throughout storage, with values ranging from 3.0 to 30 °brix, since there was a statistical difference between the two managements. At the end of storage, the organic system presented a value of 30.10 °brix and in the conventional system, 30.16 °brix (Figure 4). Soluble solids determine the amount of solids that are dissolved in the juice or pulp of fruits, being mainly made up of sugars, they are variable with the species, the cultivar, the stage of maturation and the climate.

The lower content of soluble solids may be an indication of slower ripening of the fruits, since the tendency is to increase during ripening (CASTRICINI *et al*., 2012).

In a test with several banana cultivars managed in organic and conventional systems, Ribeiro (2011) found that organic management allowed the highest average soluble solids content in relation to the conventional cultivation system in the Prata Anã cultivar, with a content of 25.20%. Pimentel *et al*. (2010) with the same cultivar obtained values of 2.13% to 20.48% in bananas planted in conventional cultivation system.

According to Ribeiro (2011), the proportion of soluble solids are of great relevance to determine the quality of the fruit, so it is an indicator of the sugar content along with acids, vitamins, amino acids and some pectins. Thus, it is an important variable both for *fresh* consumption and for the food industry.

FIGURE 4 Soluble solids of banana fruits as a function of the evaluation time in different production managements.

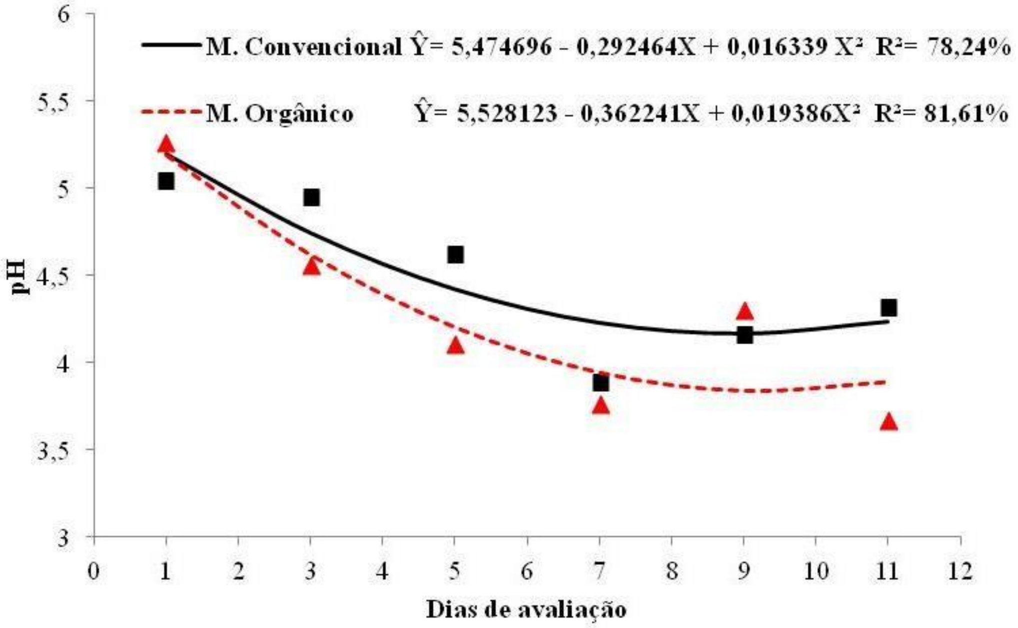


When the pH of the fruits was evaluated, a significant difference was observed between the managements, with a decline in the values, and in the conventional cultivation there were higher pH values. Figure 5 shows that there was a quadratic reduction for the pH of the fruits in both systems in the time interval evaluated. Oliveira (2010), also observed the same behavior in storage at 25° C, noting a decline in values until the 6th day of storage, with a slight increase after this period.

Nascimento Jr. *et al*. (2008) also observed a decline in pH during storage for Prata banana, where from the 10th day after harvest no changes were observed, remaining the values of 4.06.

The pH of the pulp of unripe bananas tends to fluctuate between 5.0 and 5.6, while for ripe fruit this value drops to 4.2 to 4.7 (MATSUURA and FOLEGATTI, 2001). This decrease during ripening is expected because it is associated with the accumulation of sugar and acidic constituents during fruit ripening. The fact that bananas have a predominance of malic acid, the reducing sugars, which are the precursors of organic acids, causes a decrease in pH during ripening due to their accumulation (NASCIMENTO Jr. *et al*., 2008). The small increase observed at the end of ripening can be explained by the consumption of organic acids as a respiratory substrate, causing an increase in pH (OLIVEIRA, 2010).

FIGURE 5 Variation of the pH of banana fruits as a function of the evaluation time in different managements of production.

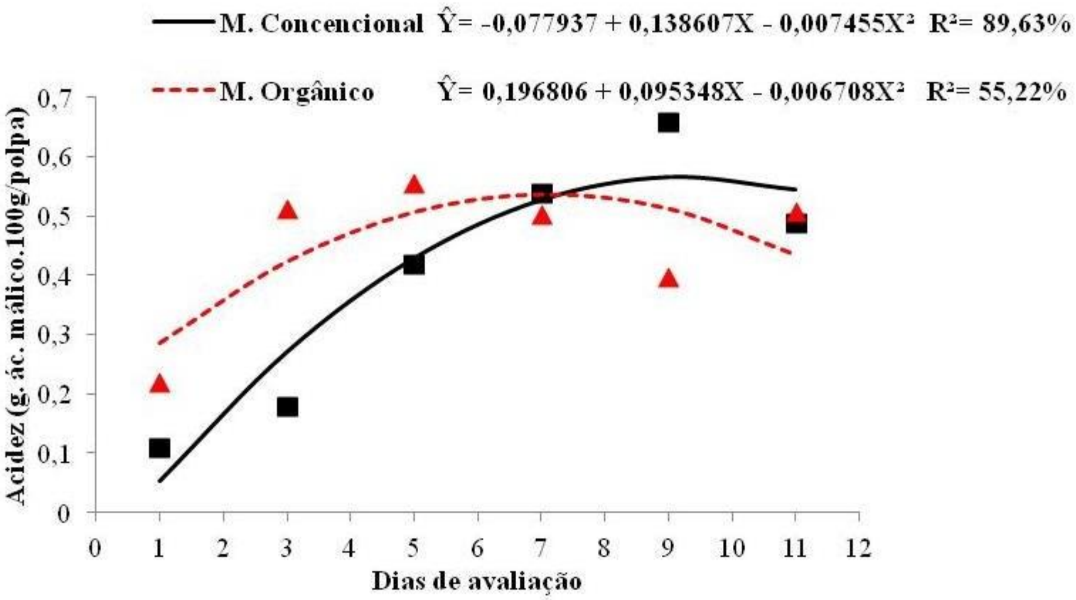


For titratable acidity, significant differences and variable behaviors were also observed within the storage period. Acidity was initially presented with 0.05 and 0.29g of malic acid/100g of pulp and at the end of the storage period it reached 0.54 and 0.43g of malic acid/100g of pulp, for conventional and organic management, respectively (Figure 6). Ribeiro (2011), working with both management systems (organic and conventional), found no statistical difference in the total titratable acidity content, and the average value of 0.21% was verified for both managements. These results differ from those found in this study, since the average levels of titratable acidity were 0.40 and 0.45g of malic acid/100g of pulp, for conventional and organic management, respectively.

On the other hand, the results of Nascimento Jr. *et al*. (2008), with Prata bananas were similar, the acidity content in the green fruit was 0.17% of malic acid per 100 g of pulp, and in the ripe fruit the value found was 0.72% of malic acid per 100 g of pulp.

According to Campos *et al*. (2003), the taste when related to sugars is favored by the increase in acidity content. Thus, with the ripening of the banana, there is an increase in the acidity content, reaching its maximum when the skin is completely yellow.

FIGURE 6 Total titratable acidity of bananas as a function of the evaluation time under different production managements.

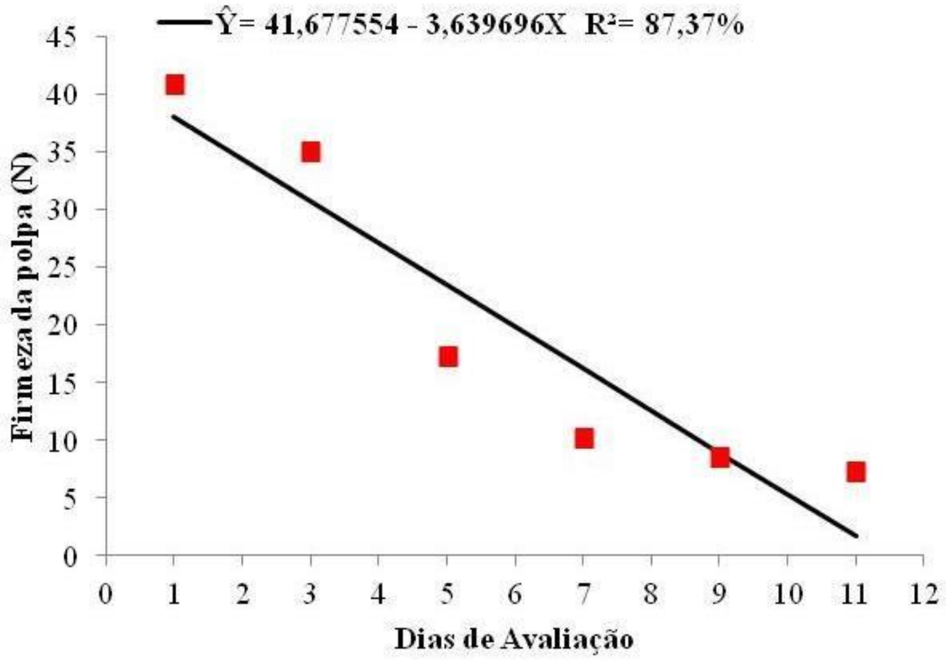


Analyzing the firmness of the fruit, no significant differences were found between the two crops, the difference that can be noticed is between the days of evaluation. During storage, the variation in firmness was very pronounced, with a decrease, where, on the day of harvest, the firmness was approximately 40.9 N and at the end of storage, 7.2 N (Figure 7). These results are consistent with those of Sarmento *et al*. (2012), when he worked with bananas from the Cavendish group in organic and conventional management systems, since he did not observe a difference in firmness for the two cultivation systems. Damatto Jr. (2008), evaluating the postharvest quality of Prata-Anã bananas fertilized with different doses of organic compost, also found no significant difference for the variable fruit firmness for the different concentrations of the compound when compared to the control.

Differently from the results obtained in this study, Ferreira (2013), on the last day of storage of Prata Anã at 25°C, found firmness values of 3.27N, which is much lower than that presented in this study. This difference is due to the ripening of the fruits, since the fruits with a firmness of 3.27N are more ripe.

According to Pereira *et al*. (2004), fruits that express low firmness are more likely to be susceptible to falling. The reduction in the firmness of the banana pulp usually occurs due to the action of the enzymes pectinmethylesterase and polygalacturonase that act on the cell wall. Differences in firmness may be related to different amounts of polysaccharides, starch and pectic substances found in banana pulps (CANO *et al*., 1997). Loss of firmness during ripening leads to lower quality and a higher incidence of mechanical damage during handling and transport (DADZIE and ORCHARD, 1997).

FIGURE 7 Variation of the firmness (N) of the banana peel Prata-Anã as a function of the time of evaluation in the different production systems.



The determination of the color of the fruits by colorimeter ascertains the differences in the color of the skin, which is spectrally close to the pattern observed by the eyes, with the advantage of being three-dimensional, eliminating evaluations of each observer (when it is done only visually) (ÁLVARES *et al*., 2003).

The characteristic L* (luminosity or brightness) of the shell ranges from 0 to 100, and that low values indicate opaque/dull bark and high values are equivalent to maximum brightness (CASTRICINI *et al*, 2012).

The value of the L* coordinate fluctuated well over the days of storage, it can be seen in Figure 8 that there is an increase in the values initially and in the last days of storage there is a decline in these values. The fruits of conventional management reached the end of 59.2, and in organic management an average value of 64.8 of fruit luminosity.

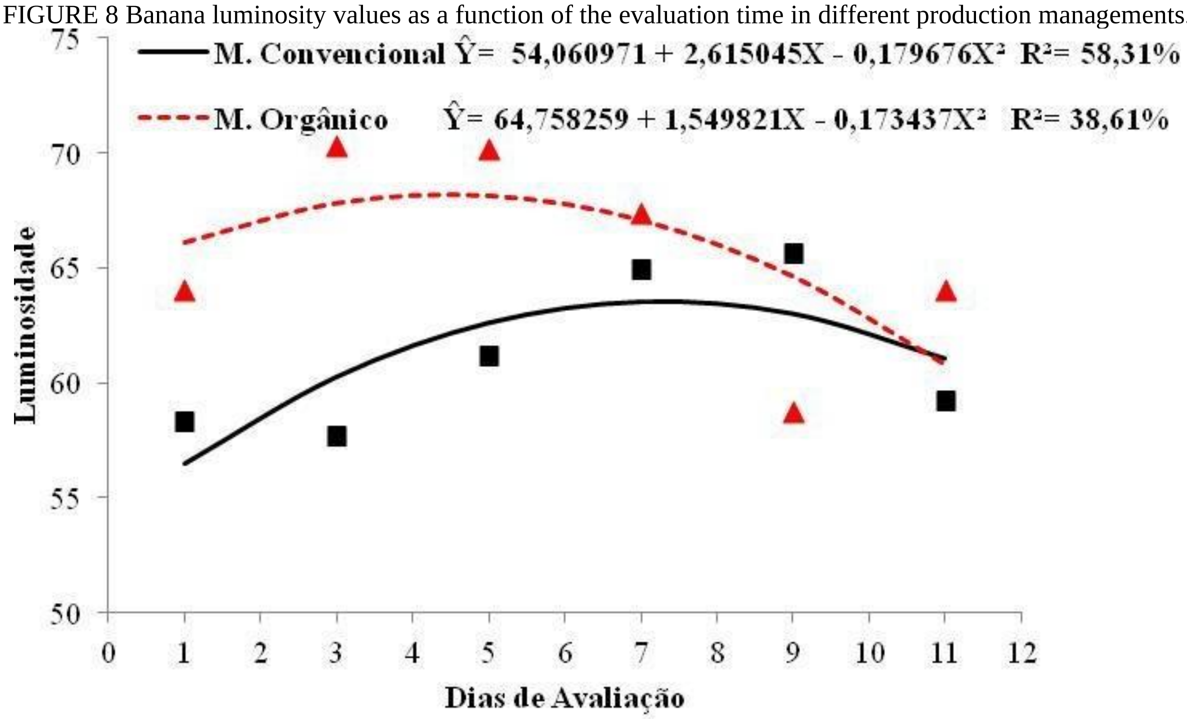
The maximum luminosity value was 63 and 68 in conventional and organic crops, respectively. Results similar to those of Pinheiro (2009), who, working with bananas cultivar Tropical, found that the values of L* at the temperature of 25ºC ranged from 61.5 to 68.1. Castricini *et al.* (2012), testing different irrigation depths with bananas cultivar BRS Platina (PA42-44) observed values ranging from 60.75 to 73.12 for peel luminosity.

The characteristic color of the banana (ripe) begins to emerge a little before the climacteric peak, due to the unmasking of pre-existing carotenoids due to the degradation of chlorophyll from the enzymatic activity of chlorophyllase. This activity evolves with increased breathing (AWAD, 1993).

Figure 9 shows the chroma (c) or chromaticity values, a parameter that expresses the intensity of the color, i.e., the saturation in terms of pigments of this color. Chroma values close to zero represent neutral colors (grays) and values close to 60 express vivid colors (MENDONÇA *et al.*, 2003). It can be seen that the behavior of color intensity was very variable in the two cultivation systems, since on the last day the values were 41.4 for the conventional system and 45.6 for the organic system.

Similar results were found by Pinheiro (2009), in which at 25ºC the chroma values for the banana cultivar Tropical increased from 41 to 46.56 at 11 days of storage.

FIGURE 8 Banana luminosity values as a function of the evaluation time in different production managements.



The color angle (hº) is a measure that has been used to express the variation of color in vegetable products, allowing the visualization of the change in the color of the fruits, from green to yellow.

The hº value in freshly harvested conventionally grown fruits was 105.87 and in organically grown fruits it was 96.86, and over the days they were stored this value only decreased linearly to 74.01 and 71.72, respectively (Figure 10).

The mean shade angle (°Hue) for the banana cultivar BRS Platina verified by Castricini *et al.* (2012) oscillated between 90.25 and 95.50, where all fruits were within the angular range of yellow color (90°).

According to the preference of consumers interviewed by Matsuura *et al.* (2004), the preferred colors for banana peels were medium yellow and dark yellow, totaling 74.6% of the preference.

FIGURE 9 Banana chroma values as a function of the evaluation time in different production managements.

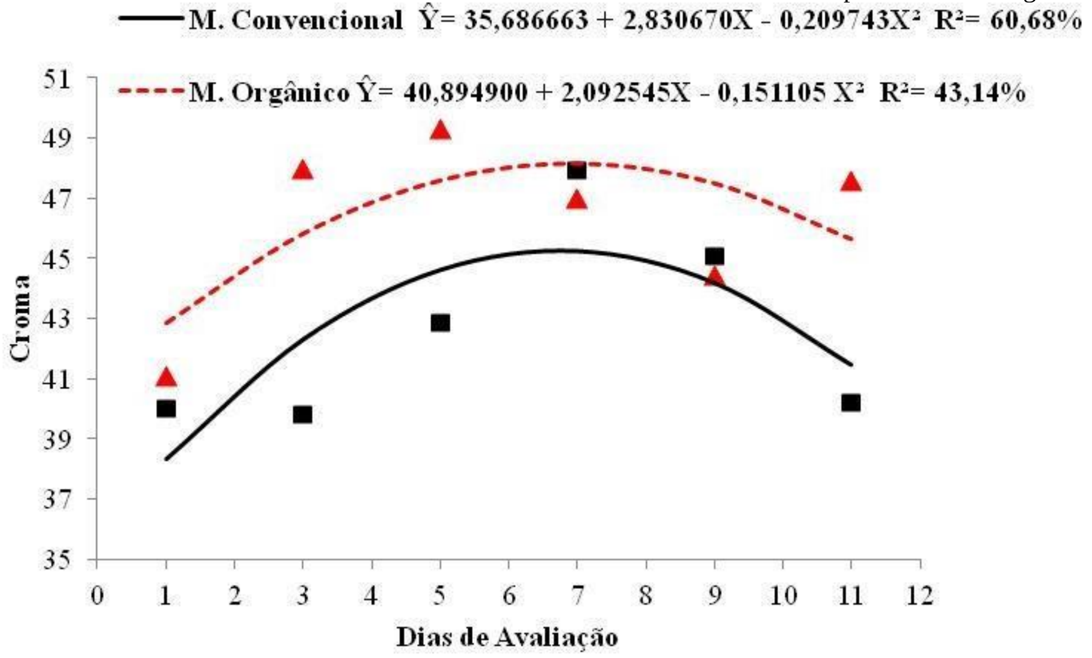
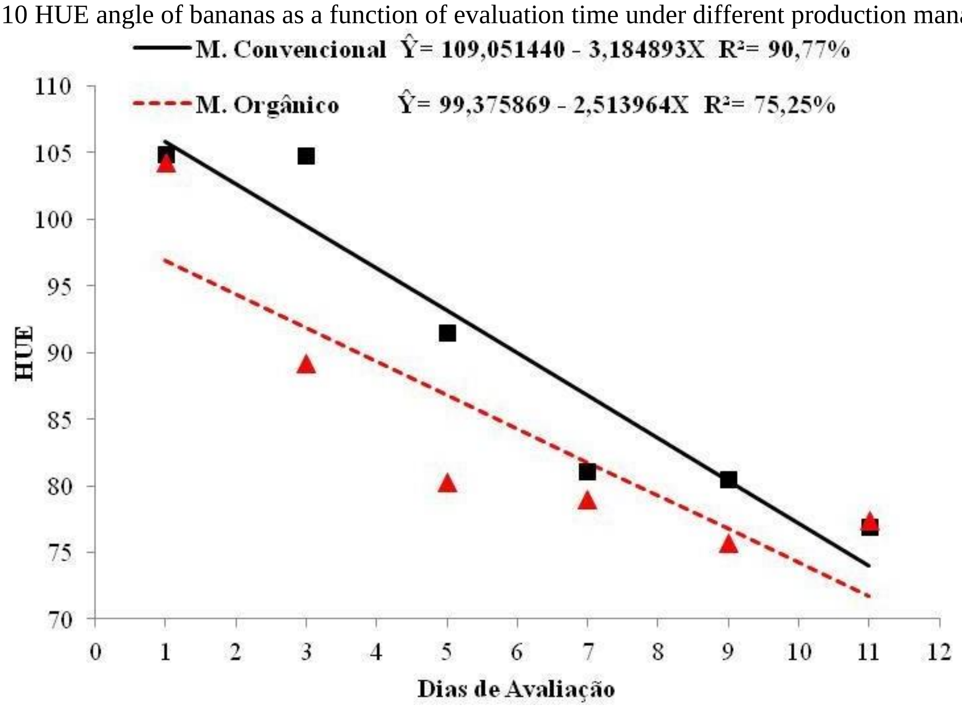


FIGURE 10 HUE angle of bananas as a function of evaluation time under different production managements.



A significant effect of the management systems was observed for the characteristics fruit weight, pulp mass, peel mass and diameter, highlighting the superiority of organic management in relation to conventional management on the last day of evaluations, except for the characteristics pulp/peel ratio and length, for which there was no significant difference between the treatments.

For the fruit mass, the difference found was quite relevant, with a difference of 38.41 grams between the crops, with emphasis on the organic management with heavier fruits (123.82g) (Table 2). Ribeiro (2011), testing some banana genotypes cultivated in conventional and organic cultivation systems in Bahia, reported distinct and superior results, of 135.08g and 90.55g, for conventional and organic, respectively. Contrary to Borges *et al.* (2011), who, evaluating the performance of banana genotypes, found a value of 86.2g for the average fruit mass of the cultivar Prata-Anã, a value much lower when compared to organic cultivation and higher when compared to conventional cultivation in the present study.

Organic cultivation also provided bananas with a higher pulp mass (88.71g) and peel mass (35.34g). Leite *et al.* (2010) evaluating the quality of Pacovan bananas sold in three different types of establishments in Rio Grande do Norte, obtained pulp mass values of 106.54, 74.98 and 94.18 grams, which are similar to those found.

Although the organic crop had the highest average fruit mass with and without peel, on the other hand, the results of the pulp/peel ratio did not give significant differences. The explanation may be based on the fact that the characterization was carried out on the last day of storage and with most of the fruits already ripe.

With ripening, the fruits start to have a higher percentage of pulp, since the peel loses more water than the pulp in this period. In addition to losing water to the pulp, the banana peel loses water to the environment through transpiration, resulting in an increase in the pulp/peel ratio during ripening (DAMATTO Jr. *et al.*, 2005). According to Matsuura and Folegatti (2001), this relationship is also known as the "ripening coefficient", which is considered a maturity index.

Similar results were reported by Calasans *et al*. (2012), who studied the characteristics of several banana genotypes and found a pulp/peel ratio of 2.44 for Prata Anã.

Damatto Jr. *et al*. (2005) found that the pulp/bark ratio was higher for the cultivar Prata-Zulu (3.43), differing from 'Prata-Anã', which presented this ratio lower (1.81). These values were found by the same author to be lower than those presented in this work.

According to Ahmad *et al*. (2001), smaller fruits have a higher pulp/peel ratio than larger fruits, as is the case of the banana cultivar Prata-Anã, which has smaller dimensions.

In the characterization of the length and average diameter of the fruits, a significant difference was found only for the diameter. For the diameter, the highest averages were obtained for organic cultivation (3.13 cm). In contradiction to Ribeiro (2011), with the cultivar Prata-Anã produced in different managements, there was no statistically significant difference for the diameter values, with the organic management resulting in 3.40 cm and the conventional 3.78 cm.

The length found for conventional cultivation was 13.03 cm and for organic cultivation was 13.88 cm, with no significant difference between managements. Ribeiro (2011), on the other hand, found totally unequal results, and there was a difference in the crops, the conventional management (16.62cm) registered a higher result than the organic one (13.96cm) and these values were much higher than those found in this study, obtaining a difference of 3.6cm larger than conventionally produced bananas.

TABLE 2 Mean values of fruit mass (MF), pulp mass (MP), peel mass (MC), pulp/peel ratio (RPC), length (CF) and diameter (DF) of Prata-Anã bananas cultivated in different managements (average of the evaluations of the last day of the storage period).

Handling	MF (g)	MP (g)	MC (g)	RPC	CF	DF
Conventional	85,41 b	61.73 b	23.49 b	2.53 to	13.03 to	2.55 b
Organic	123.82 to	88.71 to	35.34 A	2.63 to	13.88 to	3.13 to
CV (%)	15,77	14,57	17,97	6,11	5,26	6,02

Values followed by different letters in the columns differ statistically according to the F test(0.05).

The results of the sensory analyses showed a satisfactory acceptance rate for the two managements that obtained the majority of grades 1 (I liked it extremely) and 2 (I liked it moderately), which are the most convenient to receive.

In the color parameter, bananas from conventional cultivation obtained the highest amount of maximum score (1), with an acceptance percentage of (60%) compared to organic bananas, which obtained 25% of grade 1, and 60% of grade 2 (Figure 10(a)). However, there was a conventional rejection with grades 3 and 4 (15%), which are not so satisfactory to receive.

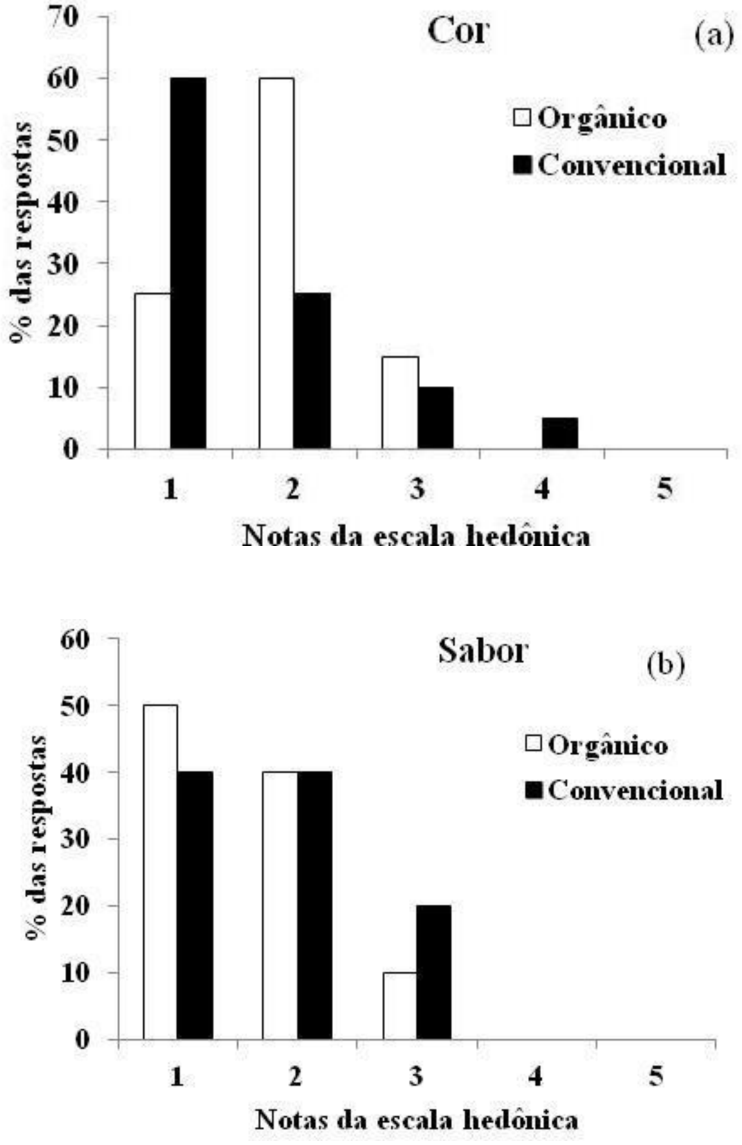
In terms of fruit flavor, the highest amount of maximum score was obtained by organic fruits with 60% of preference, with conventional fruits with 40% of grade 1 and 20% of grade 3 (neither liked nor disliked) (Figure 10 (b)). In sensory analysis, taste is one of the main attributes, as it reflects the consumer's preference for the product.

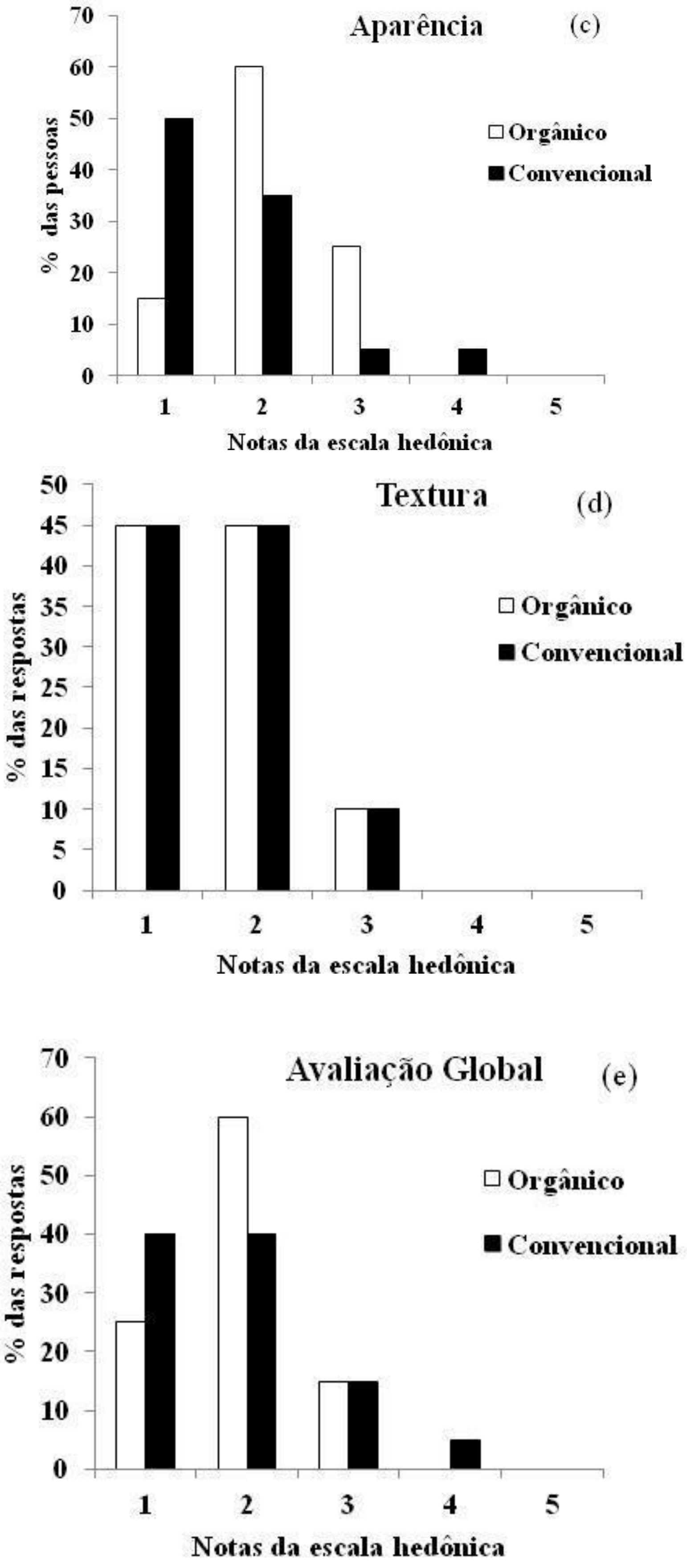
For the appearance of the fruits, 50% of the grade 1 was for the conventional management, although it still obtained 10% of the lowest scores (3 and 4), even so the organic management reached 25% of grade 3 and a small percentage of maximum grade 1 (Figure 10 (c)). These results show that the organic fruits were not apparently beautiful, but with a better flavor.

Regarding texture, organic and conventional fruits were equal, the mean scores given by the tasters were the same, obtaining 45% of grades 1 and 2 and 10% of grade 3 (Figure 10 (d)). So, in this characteristic, there was no difference between the bananas.

Finally, in the global evaluation, 40% of the maximum score 1 was verified in the fruits produced in a conventional way, leaving the organic fruits with 25% of maximum acceptance (figure 10 (e)). However, the organic ones obtained a lower percentage of low acceptance scores (grades 3 and 4) (15%), while the conventional ones obtained 20% of these grades.

Figure 10 Frequency histogram of hedonic values for color (a), taste (b), appearance (c), texture (d) and overall impression (e) of samples of Prata-Anã banana grown under organic and conventional management (1- I liked it extremely, 2- I liked it moderately, 3- I didn't like it, nor I disliked it, 4- I disliked it moderately and 5- I disliked it extremely).




Aparência
(c)
% das pessoas
Orgânico
Convencional
Notas da escala hedônica
Textura
(d)
% das respostas
Orgânico
Convencional
Notas da escala hedônica
Avaliação Global
(e)
% das respostas
Orgânico
Convencional
Notas da escala hedônica

CONCLUSION

For most of the physical and chemical characteristics evaluated, the organic production system presents superior results compared to the conventional system, with no difference in firmness and pulp/peel ratio.

The values of the scores for all the attributes analyzed were mostly scores 1 (I liked it extremely) and 2 (I liked it moderately), demonstrating that there was satisfactory sensory acceptance by the tasters for both management.

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Physical and chemical characterization of cajá-manga in Northern Minas Gerais

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ABSTRACT

A fruit native to the Polynesian islands, the cajá-manga belongs to the Anacadiaceae family. This species plays a prominent role in fruit growing in the northeast region of the country. The growing demand for its fruits has provided a larger cultivation area. Therefore, it is necessary to advance research that studies all stages of the cultivation and physiological system for the commercial production of this fruit plant. The objective of this work was to evaluate the physical and chemical characteristics of cajá-manga in the north of Minas Gerais. The experiment was carried out in a completely randomized experimental design, and consisted of ten replications and five fruits per experimental unit. The evaluations carried out were physical characteristics (length, diameter, fruit mass, pulp yield, fruit skin color, luminosity, hue angle and chromaticity), chemical characteristics (soluble solids, pH, titratable acidity and soluble solids/titratable acidity ratio).). The mean values and standard deviation of each variable were determined for the data. The cajá-manga fruit had a diameter and length of 55.16 ± 3.91 mm and 39.43 ± 3.17 mm, respectively. As for the mass, it was 54.16 ± 4.77 g, while the pulp yield was greater than 50% (79.37% ± 1.02). The fruits had pH levels (2.90 ± 0.15), soluble solids 16.04 oBrix ± 1.70, titratable

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acidity (0.90% ± 0.10). In relation to brightness (58.12 ± 2.19), hue angle (70.85 ± 1.44) and chromaticity (54.18 ± 2.48). Similar to those described in the literature for other fruits of the same botanical genus, it demonstrated that the fruit is within commercial standards.

Keywords: Fruit, Pattern, Species.

INTRODUCTION

Originally from the Polynesian islands, the mango tree (*Spondias dulcis*) belongs to the Anacardiaceae family, of the genus Spondias, as well as other fruit trees: the umbu (Spondias tuberosa), the ciriguela (*Spondias purpu*rea), the cajazeira (*Spondias mombin*) and the umbucajazeira (*Spondias* sp.) (KOHATSU, et al., 2011). This species is found in the native areas of America, Asia, Africa and Brazil. The cajá-mango is distributed in all Brazilian regions, and the Northeast and Southeast are the main regions of cultivation of this fruit, due to the edaphoclimatic conditions favorable to its development. In the north of Minas Gerais, cajá is grown mainly in backyards or in small areas. It is not a typical fruit of the Cerrado, but it adapts well and has high production in this biome, being then popularly known as a fruit of the Cerrado, from which several by-products originate. (SATURNINO, 2008). The growing demand for fruits and processed products such as cajá-mango has increased the interest of agroindustries and fruit growers for commercial exploitation, but production, for the most part, is still carried out in an extractive way (RIBEIRO et al., 2019).

The fruit has its double ellipsoid shape, when ripe it has an intense yellow color, its skin is thin and smooth, it has a fibrous pulp with a bittersweet flavor, its seed has a white color with rigid fibers, which partially extend inside the pulp. This species occurs spontaneously on the coast up to the Brazilian semi-arid region, and these fruits are well accepted by consumers in the area of occurrence during the harvest, due to the presence of compounds that provide benefits (NETO, 2018).

This fruit is composed of magnesium, potassium, zinc, copper, calcium, phosphorus and iron in large quantities, it also has vitamins such as A, B1, B6 and C, rich in fiber, it can be used for the production of juices, cocktails, liqueurs, ice cream, among others, but the highest consumption is in natura (NETO and SILVA, 2019). For fresh consumption, those that meet the quality requirements of different consumer segments are preferable, such as flavor (sugar content and acidity), good pulp yield, and good appearance, are characteristics associated with the standardization parameters of the fruit and are generally used as a criterion by consumers to evaluate the quality of the fruits (ABREU et al., 2009).

Therefore, the physical and chemical characterization of fruits is of great importance, because through this characterization it is possible to determine if the fruits produced and marketed in a given region meet the quality parameters required by consumers who consume fresh fruits and by the industry.

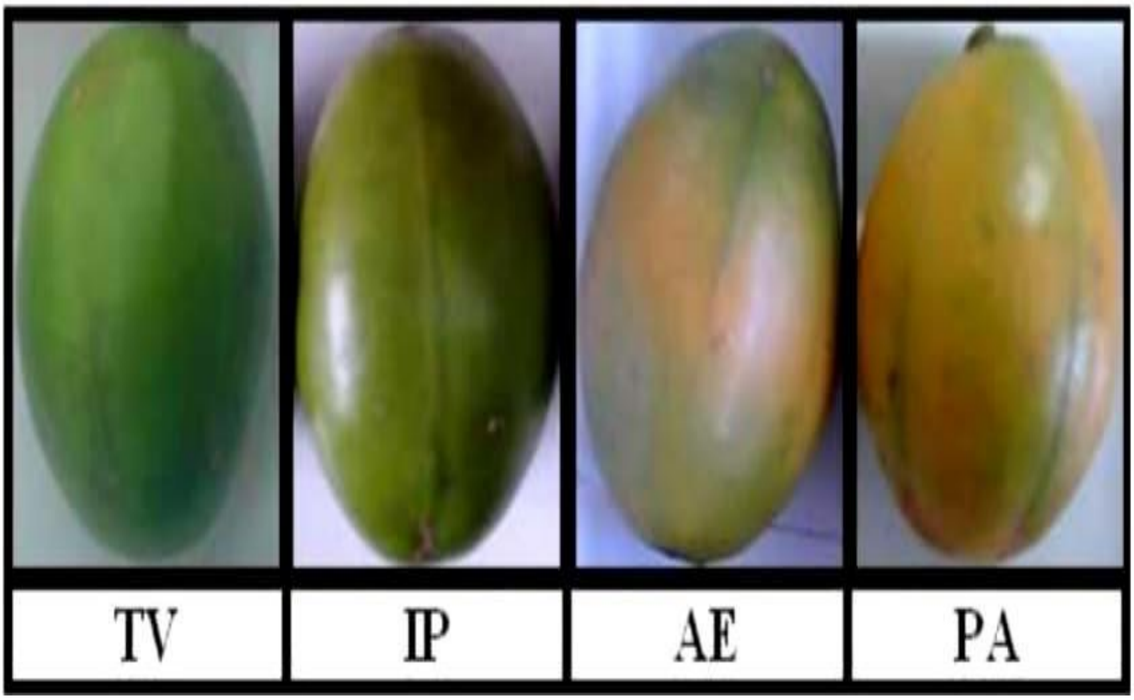
OBJECTIVE

The general objective of this work is to evaluate the physical and chemical characteristics of cajá-manga in the north of Minas Gerais.

MATERIAL AND METHODS

The study was carried out at the Laboratory of Postharvest Physiology of the State University of Montes Claros - Campus Janaúba-MG. Cajá - mango fruits from natural vegetation were used, without organized planting in the Janaúba region (15° 47'50" S and 43° 18'31" W), were harvested manually in the May 2020 harvest, at the predominant yellow maturation stage, according to the degree of skin color, as shown in figure 1. The fruits were immersed in washing tanks containing water and neutral detergent to eliminate impurities and allowed to dry in the shade. After drying the fruits, they were transferred to expanded polystyrene trays in groups of five fruits per tray.

Figure 1 Evolution of the maturation of mango tree (*Spondias dulcis) fruits*, TV - Totally Green; PI - Onset of Yellow Pigmentation; AE - Greenish Yellow and PA - Predominantly Yellow.



VARIABLES EVALUATED

Physical Characteristics

Bark color

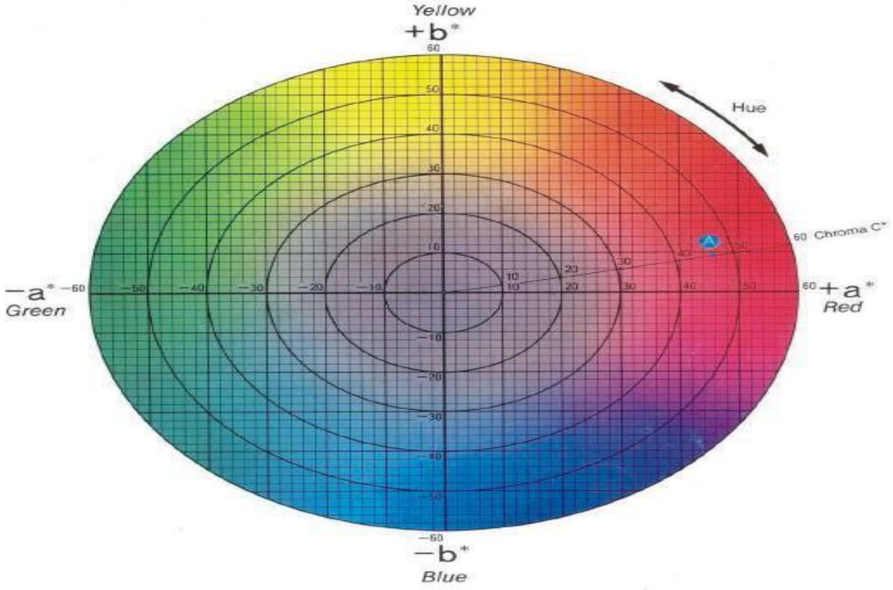
The analysis of the bark color was performed using a colorimeter Color Flex 45/0(2200), stdzMode:45/0 with direct reflectance reading of the coordinates L* (luminosity), a* (red or green hue) and b* (yellow and blue hue), from the Hunterlab Universal Software system (Figure 2), using the CIELAB scale using the 10°/D65 illuminant.

Through the values of a* and b*, the following values were obtained: Hue angle: obtained by relating through the formula: Hue = tg-1 b/a. The Hue angle is defined as starting on the + a axis and is expressed in degrees, where 0° corresponds to +a (red), 90° corresponds to +b (yellow), 180° corresponds to –a (green) and 270° corresponds to –b (blue).

Chromaticity: This value defines the intensity of the color, assuming lower values for more neutral colors (gray) and higher values for vivid colors. This variable was obtained using the formula:

$$C= (a2+b2)^{0.5}$$

Figure 2 L, a, b Color Solid representation of the Hunterlab Universal Software system and description of hue angle (ºh*) and chroma saturation index (C*).



Fresh Pasta

The fruits of each replication were weighed individually with the aid of an analytical balance and the result was expressed in grams (g).

Pulp Yield

The seeds of the fruits were removed by hand extraction and then weighed.

Fruit Dimensions

The dimensions of the fruit were estimated using a caliper, measuring the length (from the base of the peduncle to the other end) and the width (larger transverse diameter). The result was expressed in millimeters (mm).

Firmness

Firmness was measured by the penetration force required for a 4 mm diameter tip to penetrate the equatorial region of the peeled fruit at a depth of 8 mm. The results were expressed in Newton (N).

Chemical Characteristics

Soluble Solids Content

The determination of soluble solids was made after crushing the pure pulp in a mixer. An aliquot was removed for direct reading in an ATAGO benchtop refractometer, model N1. The result was expressed in °Brix, which corresponds to grams of sucrose per 100 g of solution and can generally be used as grams of soluble solids per 100 g of solution.

Titratable Acidity

The titratable acidity was determined according to the technique recommended by the AOAC (1992), titrating the juice of the fruit set from each tray under agitation after extracting, crushing and homogenizing 10g of the pulp of the central region of each fruit in 90 mL of distilled water, with 0.1N NaOH, using 1% phenolphthalein as an indicator. The result was expressed in eq. mg citric acid, 100mL-1 juice.

Soluble Solids / Titratable Acidity Ratio

The soluble solids/titratable acidity ratio was obtained by dividing the percentage of soluble solids by the titratable acidity.

ph

The pH determination was made directly in the juice using a Digital pH of the brand DIGIMED, model DM20, after the preparation of the samples as in the analysis of the titratable acidity.

Experimental Design and Statistical Analysis

The experiment was carried out in a completely randomized design, consisting of ten replications and five fruits per experimental unit. The results were submitted to analysis of variance using the statistical program SISVAR version 5.6.

RESULTS AND DISCUSSION

PHYSICAL CHARACTERISTICS

The fruits acquired in the present study obtained averages for length and width of 55.16 ± 3.90 mm and 39.43 ± 3.76 mm, respectively (Table 1). Ishak et al. (2005) described the results of length and diameter 66.80 mm and 50.20 mm respectively, which are higher than the data obtained in this study, while the results obtained by Silva et al. (2009), the fruit length was lower (41.70 mm) and the diameter result was 43.90 mm higher when compared to the values obtained in this study. According to Viana et al. (2017), there are some important characteristics for the agroindustry, such as the length and diameter of *fresh fruits* , as they directly interfere with the drying time when these fruits are destined for processing, and consequently with the cost of production due to energy demand.

The physical and physicochemical characteristics of the fruits are dependent on some factors, for example, genetic constitution, cultural management, time of harvest of the fruits and their stage of maturation (CARVALHO et al., 2013). Characterization is important for the commercialization of fruits and also for their use in the elaboration of new products (CHITARRA and CHITARRA, 2005; NASCIMENTO et al., 2014).

The fresh weight of the fruits was 54.16 g (Table 1), lower than the value observed by Neto e Silva (2019), for Brejo Paraibano-PB and João Pessoa-PB it was 99.82 and 97.47 g, respectively. According to Silva et al. (2009), for the evaluation of the weight of the cajá-mango fruits, the average weight was 28.90g, thus being lower than the result obtained in this study.

Determining the firmness of the fruits is essential to establish techniques and containers for transport and storage, in order to reduce as much as possible the damage caused by mechanical shocks, allowing greater longevity on shelves (FAGUNDES; YAMANISHI, 2001; JACOMINO et al., 2003).

As for firmness, which is a texture characteristic and corresponds to the degree of resistance of plant tissues to compression (CHITARRA and CHITARRA, 2005), the average obtained was 30.91 N (Table 2), a value close to that found by Neto and Silva (2019), which ranged from 23.83 to 50.63 N. The firmness of the pulp and fruit can be influenced by the cultivar, harvest time, cultural treatments and environmental conditions (FAGUNDES and YAMANISHI, 2001).

According to Jacomino et al. (2003), during this process the firmness can decrease from 20 to 30 times, indicating that the further the advance, the lower the firmness. This physical characteristic is correlated with the increase in enzymes responsible for structural changes in pectin, hemicellulose, and cellulose, which is the main substance that forms the primary and secondary walls of plant cells (PAULL et al., 1999; CHITARRA and CHITARRA, 2005).

According to the percentage yield of fruit pulp, the average yield was 79.37 ± 1.02% (Table

1). The value obtained in this study was higher than that found by Silva et al. (2009) and Damiani et al. (2011), in which the average yield of cajá-mango pulp was 73.58 and 61.02%, respectively. The pulp yield evaluated in this study was higher than the minimum acceptable (40%) indicated by the Ministry of Agriculture, Livestock and Supply (MAPA) and the Identity and Quality Standard (PIQ) of fruits (BRASIL, 2016).

Table 1 - Physical characteristics of mango tree (*Spondias dulcis*) *in natura*.

Evaluated Characteristics	Length (mm)	Diameter (mm)	Fresh mass (g)	Firmness (N)	Pulp yield (%)
Average/SD	55.16 ± 3.91	39.43 ± 3.17	54.16 ± 4.77	30.91 ± 3.94	79.37 ± 1.02
C.V. (%)	7.08	9.55	8.80	12.75	1.02

Mean values, standard deviations (SD) and coefficients of variation (CV) of the characteristics length (mm), diameter (mm), fresh mass (g), firmness (N) and pulp yield (%).

Table 2 shows the characteristics of the fruit peel color based on the parameters, luminosity, hue angle and chromaticity. According to Álvares et al. (2003), the determination of fruit color by colorimeter analyzes differences in skin color that spectrally approximate the pattern observed by the eyes, with the advantage of being three-dimensional, excluding evaluations of each observer (when it is done only visually).

Considering that the parameter L* (luminosity or brightness) of the shell ranges from 0 to 100, and that low values indicate opaque/dull bark and high values are equivalent to maximum brightness, this study presented an average value. An average of 58.12 ± 2.19 was observed, a value close to that found by Ribeiro et al. (2019), in the work on the greening of the cajá-manga with the use of ethylene, the fruit without the application of ethylene obtained a result 56,39, therefore, the present study obtained a mean result in terms of fruit luminosity. Chromaticity, on the other hand, demonstrates the intensity of the color, i.e., the saturation in terms of pigments according to Mendonça et al., (2003), being considered lower values for more neutral colors and higher values for vivid colors. The fruit of the present work has had the result 54.18 ± 2.48, i.e. average color saturation.

The color shade, represented by the ºhue parameter (Table 2), of the fruits was within the angular range of yellow color (90°). According to Ramos and Gomide (2007), the ºHue angle can vary from green (100º to 200º) to yellow (70º to 100º), which would simulate the ripening of the fruits.

The mean value of the Hue angle was 70.85 ± 1.44 (Table 2). According to the preference of the consumers interviewed by Matsuura et al. (2004), the preferred bark colors were medium yellow and dark yellow, totaling 74.6% of the preference. According to the CIELAB system (Figure 2), if the angle is between 0° and 90°, the higher it is, the yellower the fruit, and the smaller it is, the redder the fruit.

The yellowing or change in color of the bark of the cajá has as its main event the degradation of chlorophyll (green color), and the synthesis of other pigments is carried out at relatively low levels. During this process, the pre-existence of carotenoid pigments (yellow to orange color) is visible (SARMENTO et al., 2015). According to Castricini et al. (2015), during fruit ripening, chlorophyll degradation occurs, such as the synthesis of carotenoids, which are processes articulated by ethylene gas, and according to them, the Hue angle allows us to observe the change in pigments from green to yellow.

Table 2 - Characteristics and color of the peel of mango tree (*Spondias dulcis*) *in natura*.

Evaluated Characteristics	Luminosity	Hue Angle	Chromaticity
Average/SD	58.12 ± 2.19	70.85 ± 1.44	54.18 ± 2.48
C.V. (%)	3.94	2.04	4.57

Mean values, standard deviations (SD) and coefficients of variation (CV) of the characteristics luminosity, hue angle and chromaticity.

CHEMICAL CHARACTERISTICS

The result of the physicochemical characterization of the pulp of the mango tree was considered an acid pulp, with an average pH of 2.90 and titratable acidity of 0.9 eq. mg citric acid. 100mL-1 of juice (Table 3).

Neto and Silva (2019) observed similar values, with an average of 2.28 for pH in fruits from different microregions of the state of Paraíba. Silva et al., (2019) found values higher than those of this study (3.47) in a species of cajarana S. (*S. cytherea* Sonn), from the state of Rio Grande do Norte.

According to Benevides et al. (2008) in which they describe that in fruit processing, low pH values are ideal to favor the preservation of products, and disfavor the development of microorganisms. However, higher pH values are appreciated in the fresh fruit market (GONDIM et al., 2005).

As for soluble solids, the average was 16.04 º Brix. Neto and Silva (2019) reported lower levels of soluble solids (15.40 and 14.61) ºBrix. According to the MAPA Normative Instruction (BRASIL, 2016), which aims to establish throughout the Brazilian territory the standards of identity and quality (PIQ) of the pulp of cajá *(Spondias lutea* L.*),* a fruit pulp of the same genus as cajá-manga, the minimum content of soluble solids for commercialization is 9.0 oBrix. In this way, the fruits marketed and produced in Janaúba are a good alternative for the fresh fruit market and agribusiness.

The soluble solids/titratable acidity ratio (SS/TA) or ratio is the ratio that determines the flavor of the fruits between the soluble sugars, the higher this ratio, the sweeter the fruits will be, therefore, it indicates that these fruits are very tasty, having potential for fresh consumption. The observed ratio of this study showed a mean value of 18.03 ± 2.51ºBrix, close to that found by Silva et al. (2009), which was 15.03 ºBrix. The SS/TA ratio is a more representative index than the isolated measurement of sugars or acidity (CHITARRA and CHITARRA, 2005).

The mean values of pH, soluble solids content, and titratable acidity found in this study for the fruits of cajá-mango were higher than the minimum values (SS 9.0%, pH 2.2 and TA of 0.9g of citric acid 100g-1) established by the Identity and Quality Standard (PIQ) of the Ministry of Agriculture, Livestock and Supply (MAPA) (BRASIL, 2016), for the fruit pulp of *Spondias lutea* L, fruiting species of the same genus as the one studied in this work.

Table 3 - Chemical characteristics of mango (*Spondias dulcis*) pulp *in natura*.

Characteristics Evaluated	ph	SS	AT	RATIO
Average/SD	2.90 ± 0.15	16.04 ± 1.70	0.90 ± 0.10	18.03 ± 2.51
C.V. (%)	5.27	10.60	11.16	13.93

Mean values, standard deviations (SD), and coefficients of variation (CV) of the characteristics pH, SS: soluble solids (ºBrix), AT: titratable acidity (eq. mg citric acid/100mL of juice) and RATIO: soluble solids/titratable acidity ratio.

CONCLUSION

The results obtained in the evaluation of the physical and chemical characterization of the cajá-mango from the north of Minas Gerais, demonstrate that the fruit is within the commercial standards.

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Considerations of the Brazilian legislation on the export of animal protein

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ABSTRACT

Cattle ranching is an essential pillar of Brazilian agribusiness, playing an important role in the country's economic growth through job creation and food production. In 2023, beef production reached a record high, increasing by 900 thousand tons compared to 2022, representing 25.7% of national production and for 2024, global beef exports are projected to reach 11.90 million tons, an increase of 1% compared to 2023. In the same period, Brazil expanded its chicken meat exports by 5.6%, totaling 4.684 million tons between January and November 2023, compared to 4.436 million tons in 2022, and Brazilian pork also recorded a significant increase, with a record volume of 1.088 million tons exported, 7.3% more than in 2022. Despite these advances and Brazil's market potential, the export of animal protein faces substantial bureaucracy due to the numerous sparse regulations. However, these regulations are essential to ensure that exported products meet international quality and safety standards, protecting public health and animal health, as well as ensuring the competitiveness of Brazilian products. For success in exporting, it is imperative to follow the rules of the Ministry of Agriculture and Livestock (MAPA) and observe the Normative Instructions of other agencies, such as ANVISA, SECEX and the Federal Revenue Service. While the bureaucracy involved is complex, adhering to these regulations ensures that Brazilian products achieve the highest standards of quality and safety, promoting the competitiveness and sustainability of the industry. Therefore, understanding and complying with legal requirements are essential for the development and expansion of Brazilian animal production in the global market. This chapter aims to provide an in-depth understanding of this process, highlighting the importance of compliance with standards to ensure the success and sustainability of Brazilian animal protein exports.

Keywords: Livestock activity, Brazilian exports, Animal protein, Regulations.

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INTRODUCTION

Cattle ranching represents an important element in the Brazilian agribusiness panorama, playing a fundamental role in the country's economic growth, through job creation and food production.

Beef production was a record in 2023. In absolute terms, the volume of meat increased by 900 thousand tons compared to 2022, while exports increased by 22.8 thousand tons (CEPEA, 2024).

In 2024, global beef exports are projected to reach 11.90 million tons, with a growth of 1% compared to 2023. Brazil is expected to produce 10.84 million tons in 2024, an increase of 2.60% over the previous year and accumulating a gain of 7.81% compared to 2019, when that year production was 10.05 million tons. In addition to Brazil, China and India are expected to increase beef production in 2024.

In 2023, Brazil ended the year with an expansion of the market for poultry meat exports. Brazilian exports of chicken meat (considering all *fresh* and processed products) accumulated an increase of 5.6% in shipments made between January and November 2023. In all, 4.684 million tons were exported in 2023 *vs*. 4.436 million tons in the same period of 2022 (ABPA, 2023).

Brazilian pork also increased its share in the international market in 2023, with a record volume of *fresh* meat exported in the order of 1.088 million tons, which represents 7.3% more than 2022 and 7.19% more than the previous record, in 2021. The total exported in 2023, including *fresh* and processed pork, was 1.229 million tons (9.8% more than 2022), with revenues of US$ 2.818 billion (ABCS, 2024).

However, there is a lot of bureaucracy for the Brazilian animal protein producer to export his production to the world, in view of the number of sparse standards. Thus, for the successful export of Brazilian animal production, both live cargo and animal protein, it is necessary to follow the rules established by the Ministry of Agriculture and Livestock (MAPA) and observe the Normative Instructions, which include other bodies, such as the National Health Surveillance Agency (ANVISA), the Secretariat of Foreign Trade (SECEX) and the Federal Revenue Service of Brazil (RFB).

Because of this, the understanding of the legislation becomes fundamental, considering the number of sanitary requirements existing in the MAPA and ANVISA regulations. According to Decree No. 9,013, of 2017 (MAPA, 2022 b), any and all exports of live animals or products of animal origin are regulated by the requirements of the internal regulations and ordinances issued by MAPA, as well as any and all information on documentation and procedures necessary to export products of animal origin.

BRAZILIAN ANIMAL PRODUCTION: EXPORT

In 2022, about 27.7% of all beef exported globally originated in Brazil, totaling approximately 3.02 million tons of carcass equivalent. This year, the total production of Brazilian beef reached 10.79 million tons of carcass, and with only about 28% of this volume destined for export, these figures show not only the significant presence of Brazil in the world market of the sector, but also the breadth of the Brazilian domestic market, which absorbed an impressive 7.76 million tons of carcass (ABIEC, 2023).

In 2023, the Gross Domestic Product (GDP) of Brazilian agribusiness registered a drop of 1.37% in the third quarter, accumulating a decrease of 0.91% in the first nine months of the year (CEPEA, 2024). Brazilian beef exports, both *fresh* and processed, totaled 2.536 million tons in 2023, representing an increase of 8.15% compared to the previous year. However, total revenue decreased by 17.15%, reaching US$ 10.845 billion (ABRAFRIGO, 2024).

It is worth noting that Brazil's competitiveness in the livestock sector is mainly driven by the extension of its pastures, which encompass about 177.3 million hectares, equivalent to 20.87% of the national territory (UFG, 2022). It is notable that Brazil has the potential to play an even more prominent role in the production and export of animal protein, especially in relation to beef.

EXPORT PROVISIONS

The first step for export is to obtain the registration of the product with the Federal Inspection Service (SIF), in which the technical and sanitary standards and production processes are approved. Once these requirements are approved, in accordance with MAPA (2022a), the exporting company must prepare a qualification request with the Department of Inspection of Products of Animal Origin (DIPOA), a state agency linked to the Secretariat of Defense and Agriculture (DAS), in which it will be attached to the general list, or to the specific list of exporting companies.

The DIPOA is the body that holds the legitimacy to receive, analyze and process the application for registration of any company that wishes to sell products of animal origin to the international market. According to MAPA (2022a), Brazil currently destines its animal products to more than 150 countries.

Therefore, in order to obtain an export license, it must be ascertained whether the country is part of the general list or the specific list. For the general list, the nations that encompass it approve all laws and require nothing more than those applied in the national territory. There are situations in which certain countries require certifications regarding the slaughter process. However, this is not within the purview of SIF/DIPOA (MAPA, 2022 b).

The *Federal Drug Administration* (FDA, 2024) is the United States government agency responsible for controlling food and drugs (human and animal), dietary supplements, cosmetics, medical equipment, biological materials, and products derived from human blood. Thus, in order for a certain cargo of animal protein to arrive in the country, the containers must be certified by this sanitary control body.

For companies approved in the specific list, countries that encompass a certain economic bloc, for example, the European Union, require, in addition to Brazilian legislation, components of their local legislation or even certifications to prevent diseases that do not exist in their country. Companies registered with MAPA (MAPA, 2021), whose products are intended for animal feed, need the International Health Certificate (ISC), which will accompany the goods.

To put it simply, in the case of export to a particular nation that is part of the general list, there is a standard CSI template valid for all countries. However, if the sale is to a member of the specific list, a CSI model must be prepared to be approved by the health authorities of the importing country. In this way, this CSI will be unique for that particular approved product, in which any company in the field that wishes to optimize a sale to the foreign market of that country, will have this certificate at its disposal, regardless of the company that requested the opening of the market (SISCOMEX, 2021 b).

After confirmation of the qualification, DIPOA communicates, by means of a circular to the interested party, that the process is authorized and, subsequently, makes the certificate model available for its use on the website of the Management Information System of the Federal Inspection Service (SIGSIF). In short, the first steps to reach the international market are the type of product to be exported and the recipient country. The next step, after understanding the requirements of the recipient country, is to inform it about the interest in exporting, as well as about the quality of the product, data of the exporting company and, finally, compliance with the relevant health protocols.

INTERNATIONAL ANIMAL HEALTH CERTIFICATE

The International Animal Health Certificate (CZI) is issued by the Department of Animal Health (DSA), of the Secretariat of Agricultural Defense (SDA/MAPA) to certify animal health, after carrying out the sanitary inspection, or the tests for the diagnosis of diseases.

In this stage of certification to obtain the CZI, the animal health conditions are verified, through veterinary medical monitoring, in addition to the request for documents proving vaccination and legally required laboratory tests, the verification of the installation conditions for the development of the activity, if the animal uses appropriate inputs (such as, for example, food free of animal products and prohibited hormones and anabolic steroids) and documentary record of the activity (SICE, 2021).

ADMINISTRATIVE AND DOCUMENTARY PROCEDURES FOR EXPORT

With the definition of the type of product and the destination of the export, the administrative and documentary procedure begins.

This export cycle requires the exporting commercial company, commonly called *Trading Company* S.A., to pay attention to the institutions responsible for export supervision and, not least, the necessary documents to carry out the process. Thus, according to the provisions of Decree-Law No. 1,248/72 (BRASIL, 1972), *Trading Company* means a company, whose economic activity is organized, and duly registered with the competent body, of the Legal Regime of Exporting Commercial Companies (*Trading Companies*). Such companies are recognized in Brazil as commodity exporters, which provide for the tax treatment of the purchase of goods in the domestic market, for the specific purpose of export.

It is important to mention that the export process is not homogeneous, as each type of product requires a different bureaucratic procedure, that is, the procedure for a company to export animal protein is not the same as for exporting soybeans or coffee.

In international trade, documents play an important role, especially through contracts, which do not need to follow a pre-established form. There are cases in which an international contract is made through postal correspondence, virtual correspondence (*e-mail*) or even through facsimile communication, and only the conditions of the operation are substantial. Usually, negotiations begin with a *Letter of Intent* (LOI), which is an agreement that outlines the main aspects of a proposed contract and serves as a point of balance, of agreement between two parties (EAE, 2023).

Once the exporter meets the stipulations of the LOI, it responds with a document called Full *Corporate Offer* (FCO), issued by the seller after the conclusion of the preliminary stages of the negotiation. The final step of the export process takes place with the issuance, by the buyer, of the ICPO (*Irrevocable Corporate Purchase Order*) and BCL (*Bank Comfort Letter*) or RWA (*Ready Willing and Able*), as the POF (*Proof of Funds,* Proof of Funds), which is a document that explains the conditions of purchase and sale and bank guarantees (flexible probes) in international business (EAE, 2023).

In order for a corporate offering to be complete, it is necessary that the document describes the conditions of the sale. The buyer, when returning the draft of the signed contract, stamped and scanned by *e-mail*, will have in hand the guarantee of a legally protected and official Purchase and Sale Agreement.

In the next step, the exchange procedure begins. This documentation is, as a rule, standardized, although there are differentiations of models according to the importing country (recipient). At this point, the important thing is to be clear about the conditions of the negotiation.

The final stage refers to the consolidation of the export contract with international financial institutions. At this stage, it is necessary to pay attention to the completion of the documents referring to the exporter, in addition to the documents referring to the export contract and the documents referring to the goods.

In order for the company to export the same, the same registration is made in the REI (Registry of Exporters and Importers of SECEX), by the Ministry of Development, Industry and Foreign Trade (MDIC). It is important to note that exporters and importers are automatically enrolled in the REI when they carry out the first operation with the agency, without the need to attach any documents, which may be requested, eventually, by the Foreign Trade Department of SECEX, for routine inspection (SEBRAE, 2022).

The documents required for the Export Contract are (FIUMARO, 2022):

- Proforma Fatura;
- Letter of Credit;
- Bill or Exchange Contract.

The documents referring to the goods are:

- Export Registration;
- Credit Transaction Registration;
- Registration of Sale (VR);
- Fiscal note;
- Proof of Export (CE);
- Bill of Lading*;*
- Commercial Invoice;
- Romaneio (*Packing List*).

The other documents (only if necessary) are:

- Certificate of Origin;
- Consular Legalization;
- Transportation Insurance Certificate or Policy;
- Borderô or Delivery Letter.

MERCOSUR TREATIES AND LEGISLATION

In 1985, Argentina and Brazil signed the Iguazu Declaration, which established a bilateral commission for trade agreements. In 1988, the Treaty of Integration, Cooperation and Development was signed between the two countries, setting as a goal the establishment of a common market, to which other Latin American countries could join. Paraguay and Uruguay joined the process and the four countries became signatories to the Treaty of Asunción (1991), which established the Southern

Common Market (MERCOSUR), a commercial alliance aimed at boosting the regional economy, moving goods, people, labor and capital among themselves. In the beginning, it was agreed that the countries would have their borders open to each other, characterizing a free trade zone, where the original buyer countries would not tax or restrict their imports (NOVO, 2018).

The Treaty of Asunción, therefore, was signed on March 26, 1991, between Argentina, Brazil, Paraguay and Uruguay, with the aim of creating a common market between the agreed countries in order to form, what is popularly called, MERCOSUR (officially the Southern Common Market and, in Spanish, the Southern Common Market).

Later, in 1994, the Protocol of Ouro Preto was signed as a complement to establish that the Treaty of Asunción would be legally and internationally recognized as an organization called *Treaty for the Constitution of a Common Market* (CONGRESSO NACIONAL, 2011).

On January 1, 1995, this free trade area became a customs union, in which all signatory countries would charge a single rate, called the common external tariff, that is, they would charge the same quotas on imports.

On May 23, 2008, during an Extraordinary Meeting of Heads of State and Government held in Brasilia, the Constitutive Treaty of the Union of South American Nations (UNASUR) was signed. This treaty formalized the creation of an intergovernmental organization composed of the twelve countries of South America, with the main objective of multisectoral integration in South America.

UNASUR was conceived to promote regional cooperation in various areas, including political, economic, social, cultural and energy. The organization combined the two pre-existing regional customs unions: the Southern Common Market (MERCOSUR) and the Andean Community (CAN), thus seeking to strengthen ties between its members and present a united front on the international stage.

An important milestone for UNASUR was the creation of the position of Secretary-General, which provided the organization with clear political leadership and defined the first step towards the formation of a permanent bureaucratic body. This movement was seen as a potential precursor to replace the political bodies of MERCOSUR and CAN, aiming at greater supranational integration.

The treaty, which entered into force on March 11, 2011 after ratification by nine countries, established UNASUR's headquarters in Quito, Ecuador, and the South American Parliament in Cochabamba, Bolivia. The organization was also initially designed to achieve goals such as the elimination of socioeconomic inequalities, social inclusion, and the promotion of a South American citizenship.

However, in the following years, UNASUR faced significant challenges, including the withdrawal of several members. In 2018, six countries suspended their participations, and others followed suit in subsequent years, including Brazil in 2019, resulting in a drastic reduction in the number of active members. On 04/07/2023, Brazil returned its membership to UNASUR.

MERCOSUR has an accelerated process of economic, commercial and institutional strengthening. The States Parties have consolidated a pragmatic integration model, focused on concrete results in the short term. The meaning of the current MERCOSUR integration is to seek economic prosperity with democracy, political stability and respect for human rights and fundamental freedoms.

The Treaty of Asunción was drafted in the Spanish and Portuguese languages, both of which have equal authenticity. Paraguay was designated as depositary of the Treaty and copies were sent to the other governments. Therefore, Paraguay currently holds the *Pro Tempore Presidency* of MERCOSUR, as stipulated by the Ouro Preto Protocol, which provides for the rotation of the presidency among the States Parties in alphabetical order, lasting six months (MERCOSUR, 2024a).

MERCOSUR is an intergovernmental, open and dynamic process, where each State Party has one vote, and decisions must be made by consensus and with the presence of all members. The bloc operates through three main bodies: the Common Market Council (CMC), which is responsible for the political direction of the integration process; the Common Market Group (CMG), which is tasked with overseeing the bloc's day-to-day operations; and the MERCOSUR Trade Commission (CCM), which administers the instruments of the common commercial policy (MERCOSUR, 2024b). In addition to these bodies, there are more than 300 negotiating forums in various areas, composed of representatives from each member country, which promote initiatives for deliberation by decision-making bodies.

Over the past three decades, MERCOSUR has established permanent bodies to facilitate the implementation of its regional policies. The first of these bodies, headquartered in Montevideo, is the Commission of Permanent Representatives of MERCOSUR (CRPM). Other institutions include the MERCOSUR Secretariat (SM), the MERCOSUR Social Institute (ISM) and the Permanent Court of Review (TPR), both located in Paraguay, as well as the Institute of Public Policies for Human Rights (IPPDH) in Buenos Aires and the MERCOSUR Parliament (PARLASUR) in Uruguay (MERCOSUR, 2024 b).

In addition, solidarity financing mechanisms were implemented, such as the MERCOSUR Structural Convergence Fund (FOCEM), which offered financial assistance to more than 50 projects proposed by member countries.

REGULATORY BODIES FOR THE EXPORT OF ANIMAL PROTEIN

Ministry of Agriculture and Livestock

The Ministry of Agriculture and Livestock (MAPA) plays the main role in Brazilian exports of animal protein to the main countries of the globe. It is the body responsible for the regulation of all merchandise of agricultural origin, through the inspection and control of the international transit of products of animal origin at airports, border ports and special customs, in order to prohibit the entry of pests into the national territory that may threaten the health of the Brazilian herd. Its role has also been to ensure the entry of products of animal origin, in accordance with the standards established by internal health regulations (MAPA, 2021).

In order to consolidate an export process, the company must have a sanitary certification issued by MAPA, referring to the products that will be exported. Normative Instruction No. 36, of November 10, 2006, of MAPA regulates the Operational Procedures of International Agricultural Surveillance, with the purpose of disciplining, directing and resolving doubts regarding the principles determined by the current national legislation, in order to unify the activities developed by the Public Agents responsible for the supervision and inspection of products of animal origin in ports. airports, borders and customs posts (**MAPA, 2017).**

National Health Surveillance Agency

The National Health Surveillance Agency (ANVISA) has as its main activity, with regard to the export of animal protein, the internal control of production. Thus, its institutional objective is the sanitary control of products and services, including the environments and technologies applied in the production of animal protein, in addition to the control of the use of inputs by producers, always in accordance with international health surveillance standards (SICOMEX (b), 2021).

Its performance in the supervision and inspection of products of animal origin in ports, airports, borders and customs posts occurs at the time of importation of inputs, which are intended for the production of animal protein in the Brazilian territory.

The legal provisions that regulate and inspect the industrial and sanitary activity of products of animal origin, and that govern the inspection and industrial and sanitary inspection of products of animal origin, are Law No. 1,283, of December 18, 1950 and Law No. 7,889, of November 23, 1989.

The inspection activity is the exclusive competence of the Federal Government, in the ministerial figure of MAPA and must be in sync with the regulations of the National Health Surveillance System (SNVS). Such activity must act in the inspection of establishments (slaughterhouses and processing factories of animal products) that industrialize and process animal protein for interstate and international commercialization (export).

This activity is the property of DIPOA and SIF (MAPA, 2022 b), however, it may be delegated to the States, Federal Districts and even to the Municipalities, as long as there is recognition of the equivalence of the respective services with the MAPA, according to the provisions of the specific legislation of the Unified System for Agricultural Health Care (SUASA), in accordance with the provisions of Law No. 8,171/1991, regarding agricultural policy.

The provision of Law No. 8,171/1991 provides that only establishments able to export are slaughterhouses and factories that process products of animal origin that have the SIF, a control system of MAPA do Brasil that evaluates the quality in the production of food of animal origin (MAPA, 2022 b).

Finally, the aforementioned legal provision regulates the supervision and inspection of the activity of production and industrialization of animal protein, such as meat and its derivatives, fish and its derivatives, among others. This inspection covers, from an industrial and sanitary point of view, the *ante-mortem* and *post-mortem* inspection of animals, as well as the handling, handling, processing, industrialization, fractionation, conservation, storage, shipment and transit of any raw materials of animal origin.

Secretariat of Foreign Trade

The Secretariat of Foreign Trade (SECEX) is the competent and specific body, linked to the MDIC, and its main attribution is the regulation of export procedures and customs trade control. SECEX is also responsible for regulating, guiding, supervising, and acting in the planning and control of foreign trade activities (SISCOMEX, 2021 b).

SECEX has a single Ordinance (No. 23/2011), which centralizes in a single document all the rules issued on the administrative procedures inherent to import and export activities. It is a tax benefit that helps exporters to reduce the cost of production, which contributes to an attractive price in global trade, that is, it is a customs benefit that grants the exemption, or suspension, of taxes on products or inputs that are imported for use in the production of animal protein, to be exported in the future (SISCOMEX, 2021 b).

The exemption is levied on the following taxes: Tax on Industrialized Products (IPI), Import Tax (II), Additional to Freight for Renewal of the Merchant Marine (AFRMM) and, finally, the Tax on Operations related to the Circulation of Goods and on the Provision of Interstate and Intermunicipal Transport and Communication Services (ICMS) (SECEX, 2011).

According to the rules of the RFB, operations in the Integrated Foreign Trade System (SISCOMEX) must be carried out by the animal protein exporting company itself (slaughterhouse), provided that it is duly qualified, or through a legal representative accredited under the legal terms and conditions (RECEITA FEDERAL, 2018).

SISCOMEX's work takes place in conjunction with the other direct and indirect administration bodies that act as intervening parties in the animal protein export processes, and are accredited in the aforementioned administrative modules to act in the procedures related to their areas of competence, when the legislation determines (SISCOMEX, 2021 a).

Thus, according to Ordinance No. 23, of July 14, 2011, of the MDIC/SECEX:

> Art. 5 The qualification of the employees of the bodies involved in foreign trade operations to operate in the administrative modules of SISCOMEX will be promoted through the identification, provision of passwords and specification of the level of authorized access, observing the procedures specified in Annex I.
> Article 6 The employees of the bodies involved in foreign trade operations that are qualified to operate in SISCOMEX shall:
> I - Observe and maintain, to the fullest extent, the confidentiality of the information accessed; and
> II - Adopt the appropriate security measures, within the scope of the activities under its control, to maintain the confidentiality of information.
> Article 7 For the purpose of feeding into the SISCOMEX database, the consenting bodies shall inform the Department of Competitiveness in Foreign Trade of the legal acts that will take effect in the licensing of imports and in the registration of exports, indicating the administrative purpose, at least thirty (30) days in advance of their effectiveness, except in exceptional situations. (Text given by SECEX Ordinance No. 49, of 2013) (Revoked by art. 15 of Secex Ordinance No. 65, DOU 11/27/2020) (SECEX, 2011).

Brazilian Federal Revenue Service

The Brazilian Federal Revenue Service (RFB) is the state agency responsible for regulating the entry (import) and exit (export) of goods from the country. In this way, it has a vector role in the activity of foreign trade, aiming at the growth of activities related to exports, which, in recent years, have been breaking records in the trade balance, such as in 2019, in which the revenue accounted for more than 4 million customs declarations (RECEITA FEDERAL, 2019).

INTERNATIONAL LAW

International Legislation is a very broad science, and its objective is basically the analysis of expressions of certain legal terms, specifically contractual instruments established in international animal protein export operations.

Thus, for the constitution of the respective contracts, a terminological investigation must be carried out based on specific technical works, both North American and Brazilian, as well as texts of contracts, in what corresponds to the area of contract law. Krieger & Finatto (2004, p. 129) understand that:

> From a linguistic point of view, as we know, a terminology appears, in the first place, as a set of expressions that are linked to concepts of a thematic area or a specialty. These expressions are identified and collected and corpora terminological, that is, they are extracted from a representative textual base fixed by previously determined criteria (PICHT, 2001). Therefore, terminological recognition, we emphasize, is intrinsically related to the recognition of technical or scientific text and the identification of textual types, whether they are more or less specialized or more or less terminologically dense.

The great difficulty of international contracts, to be established between two countries, is concentrated in the language and in the different legislations, represented by the search for equivalents in the other language, of certain terminologies used by one of the contracting countries (KRIEGER & FINATTO, 2004).

FINAL THOUGHTS

In view of the above, it can be considered that animal protein export standards play a fundamental role in ensuring the quality, safety and conformity of products destined for international markets.

By adhering to these guidelines, countries have the opportunity to reinforce their reputation as reliable suppliers and foster the development of sustainable global trade. Observance of these standards not only safeguards public and animal health, but also stimulates economic growth and strengthens trade ties between nations.

In addition, it is important to note that strict compliance with standards not only ensures legal compliance, but also protects the reputation of the exporting country and strengthens its position in the international market.

It is worth mentioning that the adoption of sustainable practices throughout the production chain, from animal husbandry to transportation and processing, is essential to ensure the acceptance of products in the most demanding markets. Therefore, investment in scientific research aimed at innovative technologies, in addition to professional training, are essential to maintain competitiveness and promote the constant development of this productive sector.

It is a fact that there is a certain bureaucracy faced by producers for the export of animal protein, which can be considered a significant challenge, which can even negatively impact the efficiency and competitiveness of the sector.

Obtaining licenses and authorizations, in sanitary compliances, are obstacles that can be costly and time-consuming, since producers need to obtain documents and approvals from government authorities and regulatory agencies.

Additionally, documentation requirements for export vary between countries, which means that producers often need to familiarize themselves with the specific procedures of the destination market. This can include translating documents into different languages and hiring customs broker services, through complex customs regulations.

Another challenge would be the need to comply with different quality and food safety standards established by different importing countries, which may mean the requirement of significant investments in infrastructure, technology and training of professionals, as a way to ensure compliance with specific market requirements.

Bureaucracy can also manifest itself in the form of tariffs and trade barriers imposed by some importing countries, such as economic protectionism measures, which may include import quotas, high import tariffs, and additional certification requirements, which make it difficult for producers to export their products competitively.

Therefore, simplifying and streamlining bureaucratic processes, as well as promoting the harmonization of rules and regulations among countries, are essential measures to assist producers in maximizing their export potential and compete effectively in global markets.

Therefore, it is imperative that producers, regulatory authorities and all stakeholders maintain continuous collaboration in order to ensure compliance with these regulations and ensure the prosperity of the animal protein sector at the international level.

Finally, harmonizing standards among member countries of trading blocs can facilitate trade and reduce operating costs, promoting a more favorable environment for the sustainable growth of the animal protein industry.

TRIBUTE TO THE PASSING OF THE FIRST AUTHOR

We would like to express our deep gratitude and respect to José Eduardo Covas Fiumaro, whose contribution was essential to the realization of this book chapter. His expertise, dedication, and passion for the subject were invaluable and left an indelible mark on this work.

José Eduardo Covas Fiumaro played a key role in the development of this book chapter. Their innovative approach has significantly elevated the quality of our work. His brilliant ideas and infectious enthusiasm continue to inspire us, even after his passing.

We deeply miss him, but his legacy will live on through this book chapter. This work is dedicated to the memory of José Eduardo Covas Fiumaro, with eternal gratitude and nostalgia.

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Physical and chemical characteristics of catarina silver banana in conventional and organic cultivation

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ABSTRACT

The characterization of the fruits plays a fundamental role in the recommendation of a new cultivar, since a good variety has to present good agronomic and post-harvest characteristics. Thus, this study aimed to evaluate and compare the physical and chemical characteristics of Prata Catarina banana in conventional and organic cultivation. A completely randomized experimental design was used, consisting of two treatments, with organic and conventional cultivation methods, with fourteen replicates per treatment and four fruits per experimental unit. The work was carried out at the Laboratory of Physiology and Post-Harvest of the Center for Exact Sciences of the State University of Montes Claros (UNIMONTES), where analyses were carried out to determine the physical and chemical characteristics of the banana, such as the average fruit weight, diameter, length, fruit firmness, color, pH, soluble solids, titratable acidity and SS/TA. The organic and conventional cultivation methods did not show significant differences in terms of firmness, luminosity and chromaticity. Regarding the variable total soluble solids, the values ranged from 25.90ºBrix to 24.80ºBrix. As for the pH, differences were observed between the cultivation methods, the conventional method presented average values of 4.49, while the organic method had an average value of 4.39.It is concluded that the organic and conventional cultivation systems do not differ significantly in relation to the physical characteristics, however they present little variation in the chemical characteristics, for the attributes pH, acidity and (SS/TA).

Keywords: *Musa spp.*, Quality, Cultivar, Silver banana.

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INTRODUCTION

Banana *(Musa spp.)* It is a very important tropical fruit in the human diet and has high nutritional value, as it is a source of vitamins and minerals. In Brazil, bananas have an annual per capita consumption of 28.99 kg (FAO, 2018). More than 125 countries are dedicated to the cultivation of this fruit, with the Asian continent being the leader in production. India ranks first followed by China, Indonesia and Brazil.

According to the Brazilian Fruit Yearbook (2018), according to the latest data from the World Agricultural Production (WFP) of the Brazilian Institute of Geography and Statistics (IBGE), in 2016 alone, 6,764,324 tons of the fruit were produced, in an area of 469,711 hectares, and more than 98% of the harvest supplies the domestic market.

Commercial banana cultivars originate from two species: *Musa acuminata* (genome A) and Musa balbisiana (genome B). The nomenclature of the genome establishes the varietal groups, which group cultivars with similar characteristics, the fruits of cultivars of type AA (Gold) and AAA (Nanica) are sweeter, while the silver banana belongs to the AAB group which has as a characteristic greater acidity, there are also of the AAB type that have higher starch content, such as Terra.

The cultivars of the Silver type are the ones that most please consumers, proven by the dominance of cultivation throughout the country. Regarding the great importance of the fruit, its cultivation faces problems related to the lack of commercial cultivars. The main characteristics in the choice of cultivars to be implanted are: a) market preference; b) adaptability to the site; c) price/productivity; d) tolerance to pests and diseases; e) postage, and f) shelf life. The cultivar Catarina (SCS451), from the silver group, was registered by Epagri (Agricultural Research Company of Santa Catarina) and has a lighter skin color, longer fruit length, and higher bunch weight compared to Prata-anã, which allows a higher percentage of fruits classified as "Extra" and "First". It has a higher tolerance to "Panama Disease" compared to dwarf silver and also has high resistance to cold and winds. The main benefit of the Catarina silver banana is the excellent productivity it offers, with an average higher than the dwarf silver, which was the most productive of the variety until then.

The objective of this study is to evaluate and compare the physical and chemical characteristics of Prata Catarina banana under conventional and organic cultivation.

MATERIALS AND METHODS

The study was carried out at the Laboratory of Physiology and Post-Harvest of the Center for Exact Sciences, Department of Agricultural Sciences, State University of Montes Claros - Campus Janaúba-MG. Bananas of the Prata Catarina cultivar from commercial plantation in the municipality of Verdelândia were produced in conventional and organic methods.

Fruit harvesting should be carried out at the coolest times of the day and products should be kept protected from high temperatures, harvesting after heavy rains should be avoided, and boxes should be filled in the field. Therefore, harvesting requires some care to avoid damage and losses in the post-harvest, as some products are easily damaged, care must be redoubled so that mechanical damage does not occur that can affect the integrity and appearance of the product. The fruits had green skin with yellow traces, i.e., at maturity stage 2 according to the Von Loesecke scale (1950) (Figure 1), and were dropped and immersed in washing tanks containing water and neutral detergent to remove floral remains and eliminate latex. The analyses were performed when the fruits reached ripening stage 6 of the same scale (totally yellow skin).

The experiment was carried out in a completely randomized design, consisting of two treatments, with the cultivation methods adopted, organic and conventional, with fourteen replicates per treatment and four fruits per experimental unit.

FIGURE 1: Von Loesecke maturation scale (1950)

Fonte: VON LOESECKE (1950).

PHYSICAL ANALYSIS

For the physical analyses, samples formed by four fruits will be used.

Fruit Mass Loss

The fruits were weighed individually with the aid of an electronic scale with a precision of 0.1 g.

Fruit Dimensions

The diameter was determined using a caliper (mm) measuring the median region of the fruits, and the length was determined using a tape measure (cm) measuring the outside of the fruit, from the base of the peduncle to the other end.

Fruit firmness

Firmness was performed with a Brookfield CT3 10 KG digital texturometer. The measurements were performed in the median region of the fruit, and were determined by the penetration force, measured in Newton (N), necessary for the 4 mm diameter tip to penetrate the fruit pulp at a depth of 7 mm.

Colouring

Color analysis was performed using a Color Flex 45/0(2200) colorimeter, stdzMode:45/0 with direct reflectance reading of the coordinates L* (luminosity), a* (red or green hue) and b* (yellow or blue hue), from the Hunterlab Universal Software system, measured in the median region of the fruit. From the values of a* and b*, the hue angle (°h*) and the chroma saturation index (C*) were calculated.

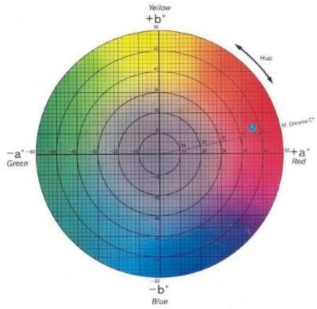
°h*= actg (a*/b*) (–1) +90 for a* negative

°h*= 90- (actg (a*/b*)) for a* positive C*= √ (a*)2 +(b*)2

The Hue angle is defined as starting at the + a axis and is expressed in degrees, where 0° corresponds to +a (red), 90° corresponds to +b (yellow), 180° corresponds to –a (green), and 270° corresponds to –b (blue).

Chromaticity: This value defines the intensity of the color, assuming lower values for more neutral colors (gray) and higher values for vivid colors.

FIGURE 2 - L, a, b Color Solid representation of the Hunterlab Universal Software system and description of Hue Angle (ºh*) and chroma saturation index (C*)



Source: Adapted by the authors, 2024.

CHEMICAL ANALYSIS

For the chemical analyses, samples composed of four crushed fruits were used.

Soluble Solids (SS)

Soluble solids were determined by refractometry, using an ATAGO optical benchtop refractometer, model N-1α, with readings in the range of 0 to 95 °Brix and the result was expressed in °Brix (IAL, 2008).

Titratable acidity (TA)

Acidity was determined by titration using 10 g of pulp diluted in 90 mL of distilled water followed by titration with 0.1 M NaOH solution, using phenolphthalein as indicator. The result was expressed in grams of malic acid per 100 g of sample (IAL, 2008).

Soluble Solids to Acidity Ratio (RATIO)

The soluble solids/titratable acidity (SS/TA) ratio was obtained by means of the ratio between the soluble solids (SS) content and the titratable acidity (TA).

ph

pH was determined using a benchtop measuring device with a glass membrane electrode calibrated with pH 4.0 and 7.0 solutions (IAL, 2008).

STATISTICAL ANALYSIS

The data were submitted to analysis of variance using the F test at 5% probability, and the means were equal.

RESULTS AND DISCUSSION

PHYSICAL CHARACTERISTICS

According to the analysis of variance of the physical characteristics (Tables 1 and 2), it was found that there is no significant difference for the variables length, diameter, firmness, chromaticity and luminosity. Significant differences were observed only for the fresh mass and HUE angle variables.

The organic and conventional cultivation methods did not show significant differences in relation to firmness, with mean values of 5.71 N and 5.62 N, respectively. The results found in this study for the two cropping systems were lower than the values found by Silva et al. (2009) in bananas at stage 7 in Terra type banana fruits, whose mean value was equal to 8.8 N.

Based on the comparison between the means of the physical characteristics (TABLE 1), through the F Test at 5% probability, it was possible to observe that for the color of the fruits, only the Hue Angle presented significant differences, the mean values were 81.93 and 84.33, which characterizes the yellow color of the fruits.

The chromaticity values did not show significant differences, the values observed were high in both cultivation systems, being 40.47 for the organic cultivation and 40.79 for the conventional cultivation, being close to vivid colors.

The luminosity values found in the conventional and organic methods were 58.88 and 58.46, respectively, and did not show differences in relation to the cultivation systems. The observed luminosity values characterize lighter colors because they are close to white.

The characteristics related to the color of the peel of the fruits such as Hue Angle, Chromaticity and Luminosity, it can be observed that there was a significant difference only for the variable Hue Angle.

TABLE 1 - Average values of physical characteristics evaluated in Prata Catararian banana Conventional and Organic system (2019)

		Physical Characteristics		
Cultivate	FIRMNESS	LUMINOSITY	HUE Angle	CHROMATICITY
Organic	5.71 to	58.46 A	84.33 to	40.47 to
Conventional	5.62 to	58.88 to	81.93 b	40.79 A
CV(%)	14,86	10,12	1,91	8,96
Means followed by the same letter do not differ statistically by the f-test at 5% probability.				

Source: AUTHORS, 2024.

Table 2 shows the length values, and the averages were similar in both cultivation methods, 19.09 in organic cultivation and 20.04 in conventional cultivation. Sarmento (2010), in studies carried out with the same cultivar, found lower values (18.4 cm). The results regarding the diameter obtained in this study were 37.14 mm in the organic system and 35.9 mm in the conventional system, when compared to the study by Sarmento (2010) showed that the values found were very close (37.3 mm).

According to Chitarra and Chitarra (2005), bananas of the Prata group are classified as Extra type when they have a minimum diameter of 28 mm and a minimum length of 15 cm, which indicates that the Prata Catarina within the two cultivation systems is included in this group.

Regarding the fresh mass variable, the organic system showed significant differences, with higher values (155.19 g) compared to the conventional system (140.30 g). For Chitarra and Chitarra (2005), size and mass are physical characteristics inherent to the species or cultivars, but they are used as quality attributes for the selection and classification of products according to the convenience of the consumer market.

TABLE 2 - Average values of physical characteristics evaluated in Prata Catararian banana Conventional and Organic system (2019)

		Physical Characteristics	
Cultivate	FRESH PASTA	LENGTH	DIAMETER
Organic	155,19 a	19,09 a	37,14 a
Conventional	140,30 b	20,04 a	35,9 a

Source: AUTHORS, 2024.

CHEMICAL CHARACTERISTICS

According to the analysis of variance of the chemical characteristics, it was found that there is a significant difference for the variables titratable acidity, pH and soluble solids/acidity ratio (RATIO). No significant differences were observed for the soluble solids variable.

Based on the comparison between the means of the chemical characteristics (TABLE 3), it was possible to observe that there were no significant differences in relation to the soluble solids variable for organic and conventional Catarina Silver, and the values ranged from 25.90ºBrix and 24.80ºBrix, respectively. Lopes (2011) studied Prata Anã bananas produced under conventional and alternative management, and found higher values, where he obtained the averages of 26.45ºBrix and 27.77ºBrix, respectively. Soluble solids indicate the amount of solids that are dissolved in the pulp and during maturation their content tends to increase due to the biosynthesis of soluble sugars or the degradation of polysaccharides (CHITARRA and CHITARRA, 2005)

Regarding the pH values, differences were observed between the conventional and organic cultivation methods, with mean values of 4.49 and 4.39, respectively. Similar values were found for the cultivar Pacovan, present in the study by Matsuura et al., (2002), which ranged from 4.30 to 4.50.

Regarding the titratable acidity variable, it was found that there was a statistical difference between the treatments (TABLE 3), the acidity values evaluated only by the conventional system were within the range suggested by some authors, which is between 0.22% and 0.65% (CHITARRA AND CHITARRA, 1994; MATSUURA et al., 2002). According to Carvalho (1989) the titratable acidity of bananas tends to increase during ripening and decreases when the fruit is very ripe, this variation occurs due to the consumption of acids during the respiratory peak characteristic of fruits in the senescence stage.

Regarding the SS/TA ratio (RATIO), significant differences can be observed between the cultivation methods (TABLE 3), Chitarra & Chitarra (2005) report that the high SS/TA ratio is very important and desirable, and is widely used for the evaluation of flavor in fruits, it is a more representative index than the isolated measurement of sugars or acidity.

TABLE 3 - Average values of the chemical characteristics evaluated in Prata Catararian banana Conventional and Organic system (2019)

Cultivate	Chemical Characteristics			
	SS	ph.	AT [1]	RATIO[2]
Organic	25.90 to	4.39 b	0.68 to	38.12 b
Conventional	24.80 a	4.49 to	0.59 b	43.05 to
CV(%)	5,99	2,44	11,44	14,19
[1] 100 g of malic acid per 100 ml of pulp			[2] Brix and acidity ratio	
Means followed by the same letter do not differ statistically by the f-test at 5% probability				

Source: AUTHORS, 2024.

CONCLUSION

The organic and conventional cropping systems do not differ significantly in relation to physical characteristics, however they present little variation for chemical characteristics, such as pH, acidity and SS/TA.

The Catarina silver cultivar has characteristics that are important to producers, such as tolerance to Panama disease and good productivity, and to consumers who have a preference for large, sweet and low-acid fruits.

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Physical and chemical characterization of banana "prata ceraíma"

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ABSTRACT

The banana tree is one of the main fruit trees of popular consumption in Brazil and is distributed throughout the national territory, being among the main crops, in planted area, volume produced and production value. Physicochemical analyses are of great importance to characterize the quality of fruits in the commercial standard, when it comes to a new variety. The objective of this study was to describe the physical and chemical characteristics of 'Prata Ceraíma' banana fruits. The fruits were harvested in the municipality of Janaúba and were taken to the Physiology and Postharvest Laboratory of the State University of Montes Claros, UNIMONTES, on the Campus of Janaúba – MG. Analyses were performed to determine the physical and chemical characteristics of the banana, such as fruit weight, diameter, length, firmness, color, pH, soluble solids, titratable acidity and soluble solids/titratable acidity ratio. The Prata Ceraíma variety characterized in this work presented important characteristics, such as soluble solids, titratable acidity and pH are within the quality standards desired in the commercialization.

Keywords: *Musa spp.*, Commercialization, Quality.

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INTRODUCTION

Brazil has been standing out in the foreign market as a major supplier of fruits. Because it has a wide variety of climates, Brazil produces fruits from temperate climates to typically tropical fruits. The production of bananas in the country is surpassed only by oranges; in volume, however, it is of great relevance in food, the country is the fourth in banana production in the world (FAO, 2018). In 2017, the Brazilian banana crop had a harvested area of 474,054 hectares, reaching a production of 6,962,134 tons of bananas, according to data from the 2017 Brazilian Fruit Yearbook. In Minas Gerais, banana cultivation has a production of around 800 thousand tons (IBGE, 2018).

The five main banana-producing municipalities in Minas Gerais are: Jaíba, Nova Porteirinha, Delfinópolis, Janaúba and Matias Cardoso, which add up to 34% of production, four of which are in the northern region of the state and one in the southern region (IBGE, 2018). According to Silva et. al. (2008), the 'Prata' banana was introduced in Brazil by the Portuguese and, for this reason, Brazilians, especially those from the Northeast and North, showed a great preference for flavor, presenting small fruits, with a sweet to mildly acidic flavor.

The cultivars considered resistant have a whole agronomic characterization, but the information regarding the physical and chemical aspects of their fruits is very incipient. The most commonly used chemical references to classify the postharvest characteristics of bananas are pH, titratable acidity, soluble solids, ratio between soluble solids and acidity or ripeness index, reducing sugars, non-reducing sugars, total sugars, pectic substances and starch content (CHITARRA, 2000). The change in the color of the epidermis in bananas is one of the most important parameters of differentiation during the ripening phases and serves as a basis for analyzing the stage of ripening of the fruit (PALMER, 1971). Thus, the objective of this study was to chemically and physically evaluate the banana 'Prata Ceraíma' in the commercial standard.

MATERIAL AND METHODS

The present study was carried out at the Laboratory of Physiology and Postharvest of the State University of Montes Claros, UNIMONTES, at the Center for Exact and Technological Sciences, Department of Agricultural Sciences, at the Campus of Janaúba – MG. The bunches of banana (*Musa* spp.) 'Prata Ceraíma' were harvested at maturity stage 2, according to the Von Loesecke (1950) maturation scale (Figure 1), obtained from a commercial farm in the municipality of Janaúba, Minas Gerais.

Figure 1 - Banana ripening scale.

Fonte: VON LOESECKE (1950).

The fruits were sent to the laboratory where the bunches were selected and divided into bouquets of four fruits. Then, the fruits were washed with water and 0.2% neutral detergent for latex coagulation and surface cleaning. Malformed, diseased or mechanically damaged fruits were selected and discarded and air-dried (Figure 2). Subsequently, the fruits were placed in expanded polystyrene trays (Figure 3). The analyses were performed when the fruits reached stage 6 on the Von Loesecke (1950) maturation scale (Figure 1), as shown in Figure 4. A treatment with fourteen replications was used, with four fruits per replication. The data were submitted to descriptive statistical analysis in order to identify the internal consistency of the samples, the mean and standard deviation.

Figure 2 - Fruits selected and discarded those that are malformed, diseased or with mechanical damage, and placed to dry in the air.

Source: Authors

Figure 3 - Fruits packed in expanded polystyrene trays.

Source: Authors

Figure 4 - Silver Ceraíma banana at stage 6 on the Von Loesecke maturation scale (1950).

Source: Authors

PHYSICAL ANALYSIS

Fruit length

The length of the fruit was evaluated (cm) with measurements using a tape measure on the external curvature of the fruit, from the base of the peduncle insertion to the end of the peduncle.

Fruit diameter

The diameter of the fruit (cm) was obtained with the aid of a digital caliper, in the median region of the fruits.

Bark staining

Color analysis was performed using a Color Flex 45/0(2200) colorimeter, stdzMode:45/0 with direct reflectance reading of the coordinates L* (luminosity), a* (red or green hue) and b* (yellow or blue hue), from the Hunterlab Universal Software system, measured in the median region of the fruit. From the values of a* and b*, the hue angle (°h*) and the chroma saturation index (C*) were calculated.

$$°h^* = \text{actg}\ (a^*/b^*)\ (-1) + 90 \text{ for } a^* \text{ negative}$$

$$°H^* = 90 - (\text{Actg}\ (A^*/B^*)) \text{ for positive } A^*$$

$$C^* = (a^*2 + b^*2)0.5$$

Average Weight

The fruit bouquets were weighed individually and the weight was averaged with the aid of an analytical scale. The result was expressed in grams (g).

Firmness of the Fruits

Firmness was performed with a Brookfield CT3 10 KG digital texturometer. The measurements will be performed in the median region of the fruit, being determined by the penetration force, measured in Newton (N), necessary for the 4 mm diameter tip to penetrate the fruit pulp at a depth of 7 mm.

CHEMICAL ANALYSIS

For the chemical analyses, samples composed of four crushed fruits were used.

Soluble solids (SS)

The determination of soluble solids was obtained by refractometry, using an ATAGO benchtop refractometer, model N-1α, with readings in the range of 0 to 95 °Brix and the result will be expressed in °Brix (IAL, 2008).

pH

The pH was determined using a bench peagameter with a glass membrane electrode calibrated with pH 4.0 and 7.0 solutions (IAL, 2008).

Titratable acidity (TA)

Titratable acidity was determined by titration using 10 g of pulp diluted in 90 mL of distilled water, followed by titration with 0.1 M NaOH solution, using phenolphthalein as indicator. The result was expressed in grams of malic acid per 100 g of sample (IAL, 2008).

Soluble Solids / Titratable Acidity Ratio

The soluble solids/titratable acidity ratio was obtained by dividing the percentage of soluble solids by the titratable acidity.

RESULTS AND DISCUSSION

PHYSICAL CHARACTERISTICS

Ceraíma silver had an average diameter of 3.16 cm and an average length of 16.14 cm. According to Chitarra and Chitarra (2005), bananas of the Silver group are classified as Extra type when they have a minimum diameter of 2.8 cm and a minimum length of 15 cm, which indicates that Ceraíma Silver fits into the Extra group, when both dimensions are considered. The evaluation of these parameters is very important for the classification, packaging and transportation of fruits and in processing operations, as they facilitate the operations of cutting, peeling or obtaining uniform products (CHITARRA and CHITARRA, 2005).

The physical aspects of diameter and length are of great importance for fruits that are intended for the processing of dehydrated products, which influences the drying process.

Prata Ceraíma, presented the value for fresh mass of 91.95g. The values of fresh fruit mass of this study were lower than those presented by Martins, *et al*. (2013), studying the postharvest quality of 'Prata Anã' and BRS Platina bananas stored under refrigeration at 15 °C, which found a fresh mass value of 'Prata Anã' banana equal to 157.25 g.

The average firmness of Ceraíma silver was equal to 8.14 N. According to CHITARRA and CHITARRA (2005), firmness is a texture characteristic and corresponds to the degree of resistance of plant tissues to compression.

The firmness found in this study was similar to the values found by Silva *et al*. (2009), in stage 7 bananas in Terra type banana fruits, whose average value was equal to 8.8 N. One of the changes observed during the ripening of tropical fruits is the reduction in firmness due to the softening caused by the progressive solubilization of protopectins into pectins or pectic acid (Proctor; Caygill, 1985).

The characteristics of the color of the peel of the fruits (Luminosity, Hue Angle and Chromaticity) are presented. The mean luminosity value found was 62.22. Being luminosity on a scale of 0 (totally black) to 100 (totally white).

According to Ribeiro (2006), in a study with 'Prata Anã' bananas, stored for 10 days at 15oC, he found average luminosity values of 50.29 to 62.05.

The variations in the color of the banana peel that occurred during fruit ripening would possibly be related to degradative processes related to the respiratory metabolism of the fruits. According to Silva *et al*. (2006), during the ripening of bananas, the degradation of the green color is intense, and the preexistence of carotenoid pigments, of yellowish to orange color, is visible.

The mean value of Hue Angle found in the peels of the fruits was 80.66. Fonseca (2013), in a study with Prata-Anã bananas, found an average Hue angle value of 81.46.

The values of Chromaticity are presented, obtaining an overall average of 46.93 in the waxy silver fruits. Rodriguez *et al*., (2015) working with banana of the Prata-anã cultivar 'Clone Gorutuba', found a value of 47.8 after refrigerated storage for 25 days at 14.5°C. Chromaticity indicates the intensity of the color, that is, it stands out in terms of the pigments of this color (MENDONÇA *et al*., 2003), assuming lower values for more neutral colors (gray) and higher values for vivid colors.

TABLE 1- Average values of physical characteristics evaluated in Banana Prata Ceraíma (2019)

CHARACTERISTICS	MEDIUM	CV (%)
Length (cm)	16,14	9,10
Diameter (cm)	3,16	3,29
Fresh mass (g)	91,95	12,34
Luminosity	62,22	8,19
Hue Angle	80,66	0,68
Chromaticity	46,93	7,22
Firmness (N)	8,14	16,34

Source: Authors

CHEMICAL CHARACTERISTICS

As for the chemical characteristics (Table 2), the soluble solids content indicates the amount of solids that are dissolved in the pulp and during maturation its content tends to increase due to the biosynthesis of soluble sugars or the degradation of polysaccharides (CHITARRA and CHITARRA, 2005). The mean SS value found was 25.61 oBrix. Lopes (2011) found similar values by studying Prata Anã bananas produced under conventional and alternative management, where they obtained the averages of 26.45oBrix and 27.77oBrix, respectively. High values of SS are desired, both for fresh consumption, as it provides better flavor, and for the industry, as it increases the yield in the preparation of products (PAIVA et al., 1997).

For the variable pH of the fruit, the mean found was 4.42. The pH of ripe banana pulp ranges from 4.2 to 4.7 (MATSUURA; FOLEGATTI, 2001).

The analysis of titratable acidity for the banana cultivar Prata ceraíma showed an average of 0.63% of malic acid per 100 g of pulp. According to the evaluated, the value is within the range suggested by several authors, which is between 0.22% and 0.65% (CHITARRA AND CHITARRA, 1994; FAGUNDES et al. 1999; CERQUEIRA, 2000 and MATSUURA et al., 2002).

The mean value between soluble solids and titratable acidity (SS/TA) ratio was 41.62. The values are included in the range analyzed by CERQUEIRA (2000), from 33.7 to 109.2, when he evaluated different banana genotypes. The SS/TA ratio is related to the taste of the fruit and is a more representative indicator than the isolated measurement of sugars or acidity, which results in the taste presented by the fruit (CHITARRA and CHITARRA, 2005).

TABLE 2- Average values of the Chemical characteristics evaluated in Banana Prata Ceraíma (2019).

CHARACTERISTICS	MEDIUM	CV (%)
SS	25,61	2,67
ph	4,42	1,50
AT[1]	0,63	13,83
SS/AT interface	41,62	15,47

Titratable acidity: 100mg of malic acid for 100mL-1 of pulp.
Source: Authors.

CONCLUSION

The Prata Ceraíma variety characterized in this work presented important characteristics, such as soluble solids, titratable acidity and pH are within the desirable quality standards in commercialization.

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Evaluation of agronomic characteristics and productivity of elephant grass (pennisetum Purpureum schum) cv. Brs Capiaçu in different dosages of phosphate fertilization

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ABSTRACT

Elephant grass BRS capiaçu is a new cultivar in the forage area, having productive potential both in the form of silage and in chopped green form, however this plant requires good availability of phosphorus in the soil to obtain this productive potential, due to the P deficit. in soils and the lack of information about it, given the need for new studies related to phosphorus and its productivity. The objective of this work was to evaluate agronomic characteristics and productivity at different levels of phosphorus (P) in elephant grass cv. BRS Capiaçu. The experimental design used was randomized blocks (DBC), with four treatments and four replications, totaling 16 experimental units. The treatments were composed of four doses of P2O5, T1=0, T2=100, T3=250 and T4=400 kg/ha. Each block contained 4 lines and 7 plants per line, with a spacing of 0.75 m between plants and 1 m between lines, totaling an area of 60 m2. The experiment was conducted in the agricultural experimentation area of the Centro Universitário Tocantinense Presidente Antônio Carlos – UNITPAC, in the municipality of Araguaína Tocantins between the months of October 2023 and June 2024.

Keywords: Elephant Grass, Phosphor, Silage.

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INTRODUCTION

The BRS Capiaçu cultivar, originally from elephant grass (Pennisetum purpureum Schum), has become one of the most widely used cultivars today, was obtained by the elephant grass improvement program conducted by Embrapa Dairy Cattle, for a higher production of biomass and nutritional value, becoming a viable animal supplementation alternative, especially in periods of food scarcity [1].

With a large dry matter production (DM), they observed yields of around 50 t/ha/year of dry matter in Minas Gerais, which placed the cultivar as the most productive among the others of the genus [1].

Elephant grass cv. BRS Capiaçu was developed with the objective of being used for the production of silage or offered chopped green in the trough. Its propagation occurs through culms and presents buds with high budding power. It is characterized by having dense clumps and erect culms, which facilitates mechanical harvesting; Leaves long, broad, green in color. The cultivar has good tolerance to water stress, but is susceptible to grassland leafhoppers.

For the supply of BRS Capiaçu forage in the form of green chopped in the trough, it is recommended that the cut be carried out when the plant reaches 2.5 to 3.0 m in height, approximately 50-70 days, in the rainy season [1]. For silage production, it is recommended when the plants reach approximately 3.5 to 4 m in height or 90 to 110 days of regrowth age [1].

In the implementation of BRS Capiaçu, three buds/m² or per row are used, with a spacing of 80 cm to 1.20 m [1]. In addition, it should be made in furrows 20 to 30 cm deep next to the fertilizer [1].

Establishment fertilization should be based on the results of soil analysis. In most tropical soils, the main limitations are related to acidity and low phosphorus contents, which must be corrected with the use of liming and fertilizers. When planting, it is recommended to apply only phosphate fertilization, distributed at the bottom of the furrows. For most soils, 120 kg/ha of P2O5 is required, which corresponds to 600 kg/ha of simple superphosphate. Potassium should be applied when the potassium content in the soil is less than 50 ppm, at a dose of 80 to 100 kg/ha of KCl[8].

This cultivar reproduces vegetatively, from the buds of the stems, with high budding power, and with approximately 30 tillers per m², it is an erect and tall plant, with thick stems, wide leaves, late flowering and good resistance to damping-off and water stress. Cultivar, which is one of the alternatives that cattle ranchers are looking for in recent times to supplement their herd in times when there is a lack of forage on the property.

Elephant grass cv. BRS Capiaçu, as well as other forages for weeding production, needs good availability of phosphorus in the soil for its growth and development. However, the soils of the Amazon region, for the most part, have low availability of phosphorus, and therefore, it is necessary to apply it so that the soil is not degraded and that production is efficient. However, there is still a lack of studies on phosphate fertilization for BRS Capiaçu elephant grass in the state of Tocantins.

SUPPORTING

Due to the drought that occurs every year, where with it comes the low supply of forage, cattle ranchers are currently looking for less expensive and more efficient alternatives for supplementing their herd.

One of the alternatives is the production of silage using elephant grass cv. BRS Capiaçu, but in order to have a quality silage and thus meet the demand of the animals, it is necessary to balance the production of biomass and the nutritional quality of the grass. Thus, the crop should be implanted at the beginning of the rainy season [1].

OBJECTIVES

General Objective

To evaluate the influence on the development of elephant grass cv. BRS Capiaçu, submitted to increasing doses of phosphate fertilization, under edaphoclimatic conditions in the municipality of Araguaína – TO.

Specific Objectives

To evaluate plant height (AP), tiller number (NP), distance between nodes (DE), stem diameter (DC), dry matter (DM) and green matter (MV) of stem and leaf, dry matter production per hectare (SMP), green matter production per hectare (PMV) and leaf:stem ratio, when subjected to increasing doses of phosphate fertilization.

THEORETICAL FRAMEWORK

ORIGIN AND CHARACTERISTICS OF ELEPHANT GRASS CV. BRS CAPIAÇU

Elephant grass belongs to the African continent, as well as the vast majority of the forages we grow in our country, more specifically from Tropical Africa, with its coordinates approximately between: Latitude 10°00'00.0"N and Longitude 20°00'00.0"E.

Its discovery was by Colonel Napier in the year 1905. With this, this cultivar spread throughout Africa and was introduced in Brazil in 1920, coming from Cuba. Today it is widespread in the five Brazilian regions.

Nowadays, this cultivar has spread in the five Brazilian regions [2]. For better germination and tillering, the use of inputs for good productivity per hectare is highlighted [1].

The BRS Capiaçu elephant grass is characterized by its tall size, broad, long and dark green leaves, yellowish green leaf sheath and stem with a thick diameter, yellowish internodes, absence of joçal (hairs), late flowering, white midrib, with a high density of tillers per m² (clump), and good resistance to damping off and water stress [6].

LIVESTOCK IN TOCANTINS

The state of Tocantins has a tradition of beef cattle raising. According to the State Secretariat of Agriculture and Livestock, since the state's inception, beef and dairy cattle breeding has grown by 95%, from about 4.2 million head of cattle in 1988 to more than 8.12 million in 2013.

In a trend scenario, the state would reach, in 2031, a total herd of 11.9 million head, producing 6.24 @/ha with a stocking of 1.05 AU/ha.

The state could slaughter about 326,000 head of males from intensive systems, representing about 25% of the total number of males slaughtered. A reduction in the slaughter age is estimated with the elimination of the 4-year category from 2027 and a production of approximately 27 million arrobas in 2031[10].

Tocantins is one of the Brazilian states with the greatest tradition in beef cattle breeding, currently having a herd of 8 million animals, distributed in all regions of the state. The Tocantins herd stands out not only for the quantity, but also for the quality of the animals and meat produced.

For 18 years, Tocantins has been internationally recognized as an area free of foot-and-mouth disease with vaccination, surpassing the mark of 99% of the herd immunized in each campaign.

In addition, the State produces the so-called "green cattle", which are pasture-fed animals, free of animal feed, which meets the preferences of the most demanding consumer markets [11].

PHOSPHATE FERTILIZATION

In Brazilian soils, forage cultivars are commonly implanted in soils of low fertility, and mainly for the availability of P, with this deficit of P in the soil, together with the non-application of P in the soil, leads to low tillering and low dry matter production [4].

The phosphorus available in the soil and the non-available phosphorus is corrected with liming to become a greater amount of P available for the plant's consumption, thus making it more feasible to increase the availability of P, which has the advantage of its dissolution by the acidity of the soil itself, being economically viable, with the reduction of expenses with the use of acids in the manufacture of water-soluble fertilizers [12].

Most Brazilian soils have a high phosphorus fixation in the soil.

The intensity of phosphorus fixation in the soil is due to issues that intervene in the availability of P in plants, one of these issues is the types of soil, such as clay, time of application, soil aeration, temperature and the level of N may interfere directly [13].

In the plant, phosphorus is absorbed in the form of an orthophosphate ion ($H_2PO_4^-$), which is either simple superphosphate or triple superphosphate.

Phosphorus is important in the formation of ATP (adenosine triphosphate), being one of the main ones for the process of photosynthesis having as a source of energy for its realization. Energy is used in the transport of assimilates, in the storage and transfer of energy, in cell division, in the enlargement of cells and in the transfer of genetic information [14].

The sources of P come from phosphate rocks, where the predominant mineral is apatite. The most common rock is fluorapatite, however, in order to make this P more soluble and available to plants, acid and temperature treatments are needed in these rocks [5].

One of the main sources of phosphorus are triple superphosphate, simple superphosphate, monoammonium phosphate (MAP), and diammonium phosphate. Triple superphosphate (41 to 46% P and 10 to 12% Ca), although it contains calcium, is a source intended to provide phosphorus. It is a relatively inexpensive source, when considering only phosphorus, simple superphosphate and acidulated phosphates contain 18 to 21% of P and 10 to 12% of S. In addition to providing the element as a nutrient, it improves the soil profile with prolonged use [15].

Phosphorus is mobile in the plant, it can move through the phloem. For the identification of deficiency symptoms, it usually occurs in the older leaves. These symptoms stand out with a yellowish or bluish green color, little brightness and even stiffness. In corn, the absence of phosphorus is manifested by the purplish color of the leaves due to the accumulation of sugars that favor the production of anthocyanin (vegetable purple pigment). Due to the symptoms on the older leaves, automatically on the first leaves of the plant after its emergence, this causes a deficiency in the growth of the plant being an irreversible problem [5].

MATERIALS AND METHODS

The experiment was carried out between November 2023 and June 2024, in the agronomy experimental field, located within the Presidente Antônio Carlos University Center – UNITAPC, in the municipality of Araguaína – TO, with the following coordinates: Latitude - 7°12'38.5" S and Longitude - 48°14'14.5" W.

Rainfall throughout the experiment was collected in the field, using a rain gauge installed between the blocks, as shown in the following table.

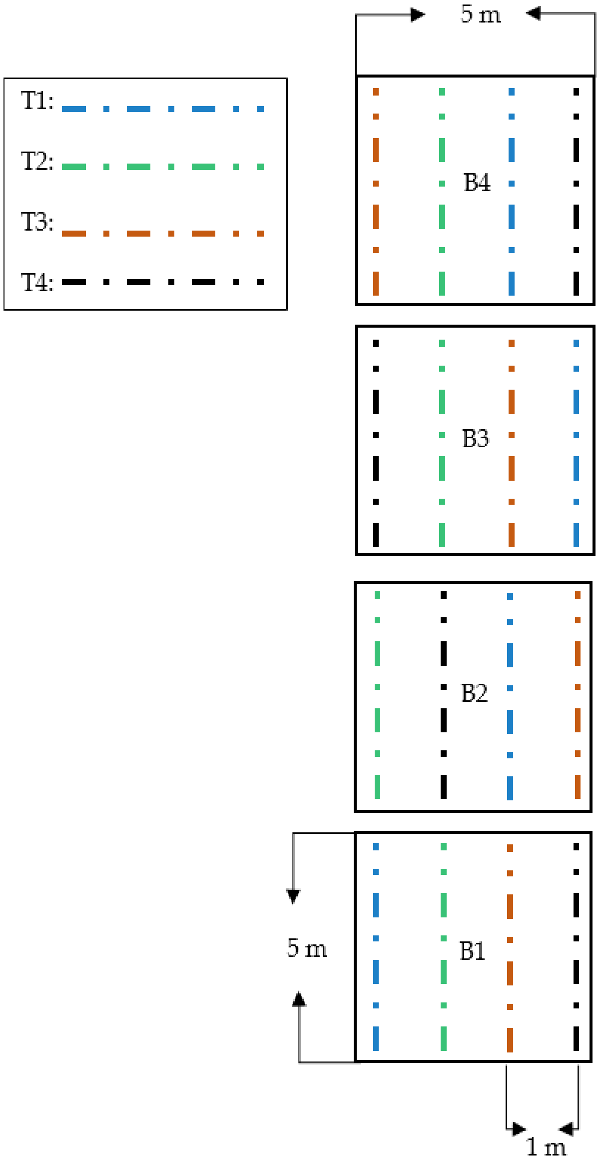
Table 1. Precipitation.

Precipitation Table	
Months	Overall (mm)
NOV	110
DEC	210
JAN	292,5
FEB	272,5
MAR	252
APR	265
MAY	5

Source: Authors, 2024.

The experimental design was in randomized blocks (Figure 1), with four treatments and four replications, totaling 16 experimental units. The treatments consisted of a control and 3 doses of P: 0, 100, 250 and 400 kg/ha of P2O5.

Figure 1. Sketch of the area.



Source: Authors, 2024.

Each block had 4 rows and 7 seedlings per row, using 28 seedlings per block, in total 112 seedlings were needed to continue the experiment.

They were prepared using propagative material from the institution itself, where they were kept in an environment protected by a 50% shade screen, on a 3x1.20 m galvanized steel countertop, along with a layer of dry grasses to maintain humidity. Irrigation was done automatically 3 times a day with an average of 5 mm/day.

Manual liming was performed according to the chemical analysis (Table 1), in order to increase the pH, and thus reduce the proportion of exchangeable $Al^{3}+$ [9]. After 60 days of liming, the planting was carried out.

Table 1. Chemical analysis of the soil in the 0-20 cm layer.

pH	**Pmeh**	**Towards**	**Ca**	**Mg**	**H+Al**	**SB**	**CTCt**	**V %**
CaCl2	**mg.dm-3**	**cmolc.dm-3**						
5,6	**3,1**	**0,08**	**1,5**	**0,8**	**1,50**	**2,38**	**3,88**	**61**

Source: Authors, 2024.

The fertilizer used was super simple, the same inserted in furrows, made in the format of rows, with a spacing of 1 m, followed by incorporation into the soil, at a depth of 20 cm.

Then, 3 buds per m² at 75 cm between plants were planted by hand with 3 buds per plant of the BRS Capiaçu elephant grass on the fertilizer, in the appropriate treatments of 0, 100, 250 and 400 kg/ha of P2O5.

When the plant reached 60 DAP, urea and KCl topdressing was carried out with application of 100 kg/ha. After 180 days of germination, when the cultivar reached the cut-off point for silage, the agronomic characteristics of BRS Capiaçu Elephant Grass were evaluated.

For data collection and sectioning, 1.5 m of the borders of each treatment line were discarded, using only the central portion. Height was measured using a measuring tape, graduated in centimeters, measuring from the base of the plant to the tip of the leaves, and counting the number of tillers.

To determine the green mass, the plant stems were cut close to the ground, mechanically, using a machete. Where leaves were separated from the stem and weighed separately.

After cutting, the material was weighed completely to determine the green matter. Then, the stems and leaves were chopped separately in a branch shredder with a particle size of 2 to 4 cm.

Of this total weight, 4 subsamples of 100 g of green matter of the stem and leaves were used, where an average was made to determine the percentage of dry matter, which was obtained by drying in a forced circulation oven at 65°C for 72 hours.

After the kiln process it was weighed on a semi-analytical balance. And after data collection, a general cut of all blocks and treatments was carried out to standardize the forage.

The data were submitted to analysis of variance, at 5% probability of error, and when a significant effect was found, regression analysis was performed. The statistical analysis software used was SISVAR 5.8.

RESULTS AND DISCUSSION

The height of the BRS Capiaçu elephant grass, in the treatments present in the study, all with the same amount of DBH, had the highest efficiency at the dose of 250 kg/ha with 3.87 m, being higher than the other doses, 100 kg/ha with 3.85 m, control with 3.81 m and 400 kg/ha with 3.72 m (Figure 2A).

The number of tillers was more efficient at dose 0 and 400 kg/ha at the dose according to the collection of 2 central m in the planting rows, with 30 tillers, being higher than the other dosages, 100 kg/ha with 26 and 250 kg/ha with 29 tillers (Figure 2B).

In the distance between nodes, the highest efficiency point was the dose of 100 kg/ha with 112.63 mm, surpassing the other dosages, where from then on the remainder hears a gradual reduction in the distance between nodes (Figure 2C).

In the stem diameter, the 100 kg/ha dose was the most efficient, with 17.45 mm in diameter, with an increase of 5.2% in relation to the 0 dose, where from 250 kg/ha, there was a reduction in the stem diameter (Figure 2D).

Figure 2. Plant height (Figure 2A), tiller number (Figure 2B), distance between nodes (Figure 2C) and stem diameter (Figure 2D) at different P2O5 dosages.

Figure 2A

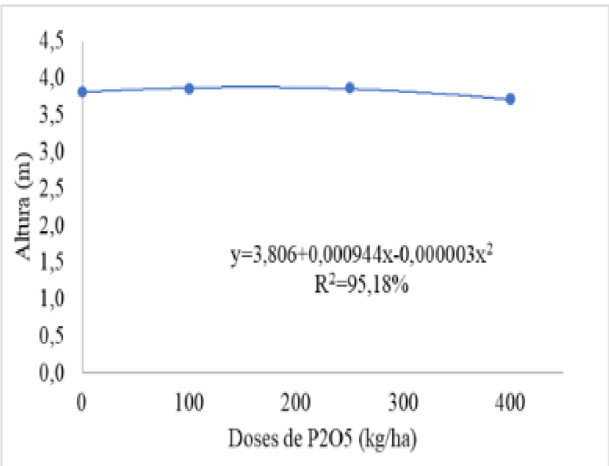


Figure 2C

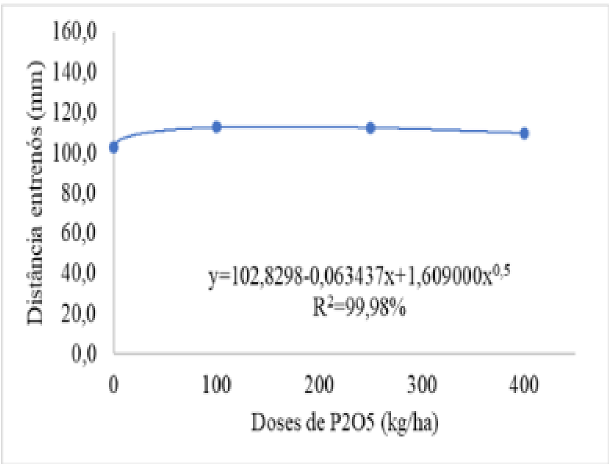


Figure 2B

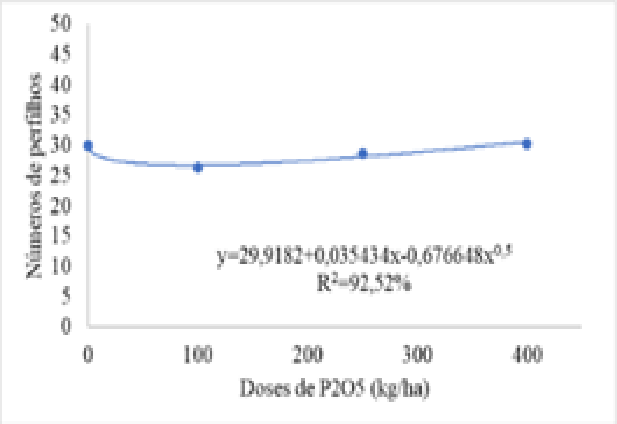
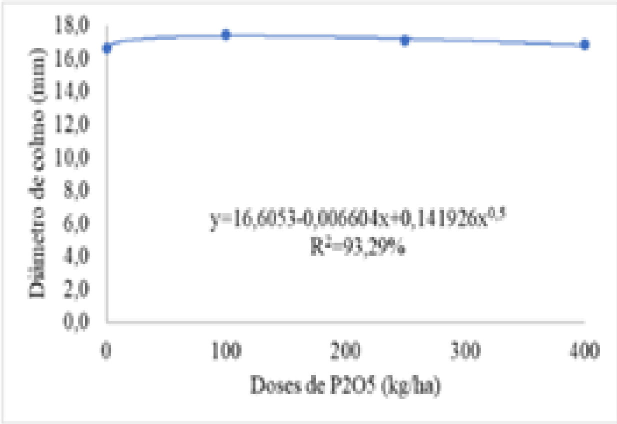


Figure 2D



Source: Authors, 2024.

The stem dry matter variable (%), had a higher result at the 100 kg/ha dose with 24.6% of average, resulting from the collection of the central 2 m in the planting rows. As a result, it exceeded all other dosages, but with a small difference from one dose to another (Figure 3A).

The variable green stem matter had its greatest influence on dose 0, with an average of 12.80 kg in the central 2 m collected in the planting rows, with a linear regression from this dose, with a negligible result (Figure 3B).

Leaf dry matter had dose 0 as the highest result, with an average of 34.5%. As a result, the other doses did not show significant differences in relation to dose 0, with a reduction in percentage (Figure 3C).

The green matter of the leaf had quadratic regression, with dose 0 being the most influential, with a considerable decrease in the dose of 100 kg/ha and an increase in the dose of 250 kg/ha, which was not significant in the result compared to dose 0 (Figure 3D).

Figure 3. Stem dry matter (Figure 3A), green stem matter (Figure 3B), leaf dry matter (Figure 3C) and green leaf matter (Figure 3D) at different P2O5 dosages.

Figure 3A

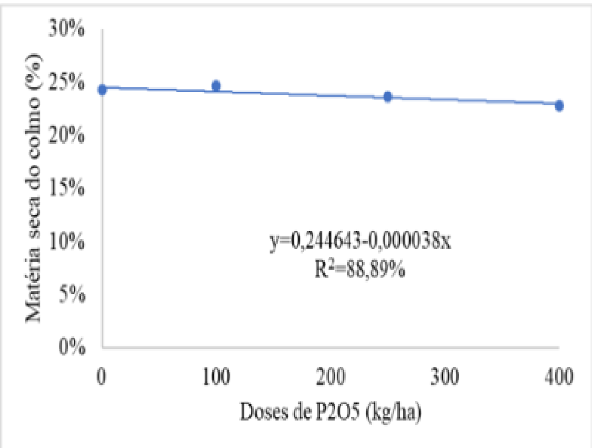


Figure 3C

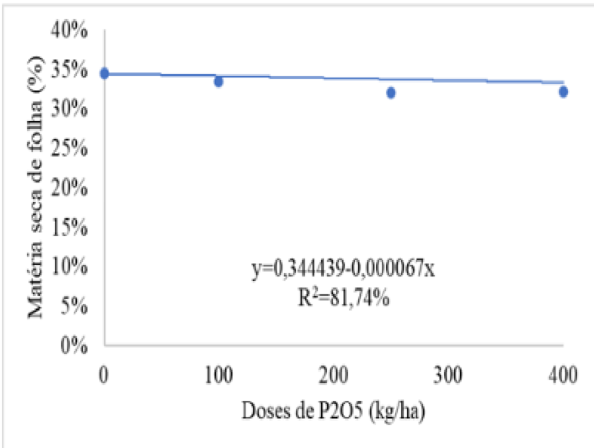


Figure 3B

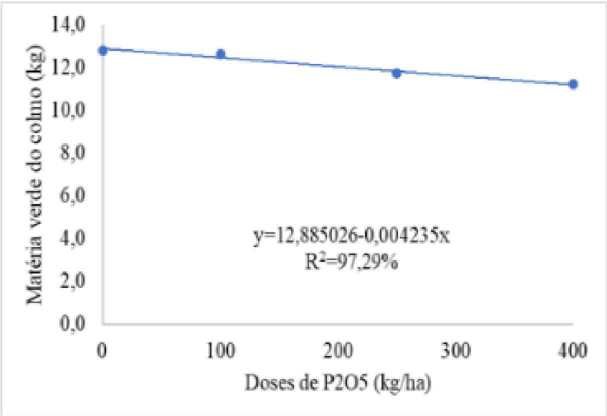
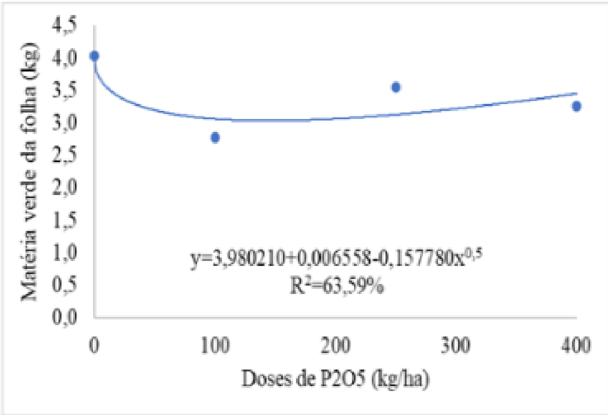


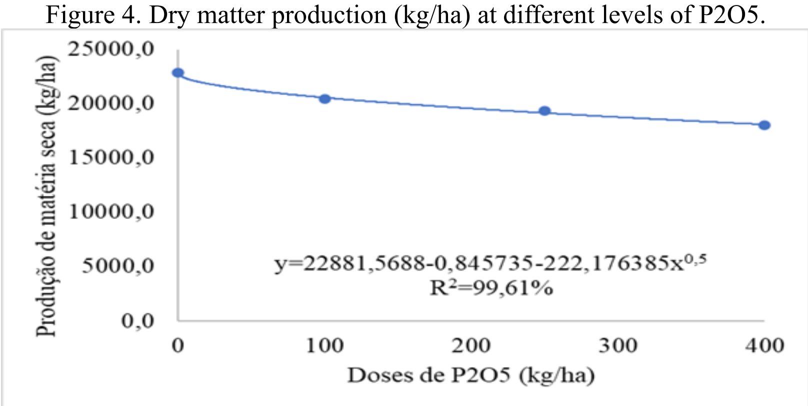
Figure 3D



Source: Authors, 2024.

Dry matter production (SMP) was adjusted to the quadratic model (Figure 4). The point where the maximum efficiency occurred was at dose 0, in which there was an estimated production of 22t/ha, an increase of 111% in relation to the second treatment, with 20t/ha. As in the PMV, we observed that the highest average production was observed in the control of the analyzed variables.

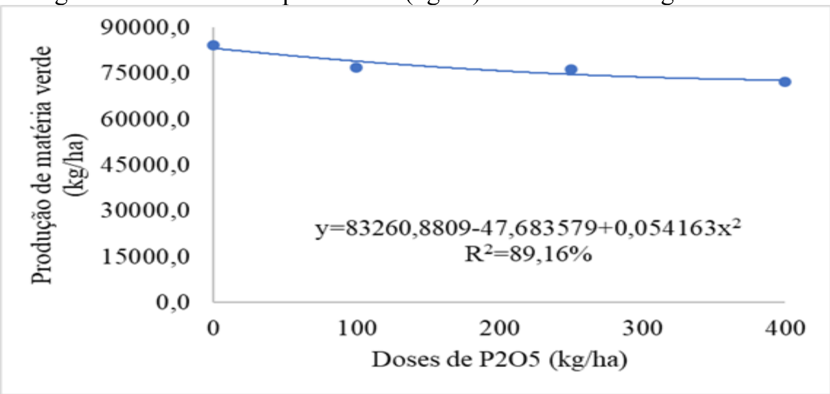
Figure 4. Dry matter production (kg/ha) at different levels of P2O5.



Source: Authors, 2024.

The production of green matter (PMV) was adjusted to the quadratic model (Figure 5). We can observe that the PMV decreases as the doses of P2O5 increase, to a certain extent where it begins to stabilize. With its maximum efficiency point at dose 0, where it obtained an average of 84 t/ha, an increase of 109% in relation to the dose of 100 kg/ha, which had an average of 77 t/ha. Consequently, the doses of P2O5 had a negligible effect on the production of green matter.

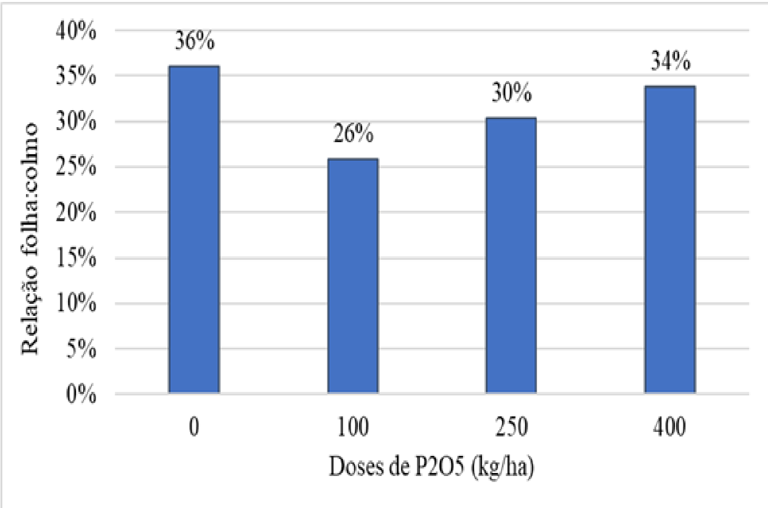
Figure 5. Green matter production (kg/ha) at different dosages of P2O5.



Source: Authors, 2024.

The leaf:stem ratio showed a better performance on average at 0, 250 and 400 kg/ha. The dose of 100 kg/ha showed a lower result than the other doses of P2O5 (Figure 6).

Figure 6. Leaf:stem ratio (%) at different dosages of P2O5.



Source: Authors, 2024.

CONCLUSION

At the end of the experiment, it was concluded that the control stood out among the other P2O5 dosages. This result may be associated with a better experimental conduction.

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