PROYECTO

"EVALUATION OF TWO BIOSTIMULANT TREATMENTS IN PROCESSING TOMATOES CROP"

CLIENTE

PENERGETIC

Persona de contacto

Cedric Wilhelm

Responsable del Proyecto

Antonio Sánchez Sánchez

Equipo

Elena Ordiales Rey	Jesús Gil Soto
Lorena Zajara Serrano	Juan Ignacio Gutiérrez Cabanillas
Fermín Núñez Cano	Francisco Broncano Román
Angela Polo Fernández	Francisco Polo Fernández
Cecilio Polo Fernández	Andrés Polo Cuenda

Referencia CTAEX: 25/21

Referencia Expediente: 25/21

Ctra. Villafranco – Balboa Km. 1,2 – 06195 Villafranco del Guadiana (Badajoz)
Apdo. de Correos 435 – 06080 Badajoz - Tel. 0034 924 448 077 – Fax: 0034 924 241 002
ctaex@ctaex.com – www.ctaex.com

INDEX

1. INTRODUCTION

In 2020, 38,282 million kg of processing tomatoes were produced worldwide. This represents an increase of 2.4% compared to 2019, being the second year of an increase in production since the fall that had been occurring since 2015. The four main producing countries are: United States, followed by Italy, China, and Spain. World tomato production has risen sharply since 2001, from just over 25,000 million kg to the current 38,282 million kg, passing through the peak of 44,512 in 2009.

In Spain, around 3,000 million kg of processing tomatoes are produced, a quantity that has remained stable since 2014-2015. In 2020, 2,700 million kg were produced, with a 16% drop in production compared to 2019. In the 2019 campaign, 69% of Spanish tomato was produced in Extremadura, and 23% in Andalusia.

Extremadura produces 65% of Spanish tomato production. Spanish tomato production represents 7.05% of the tomato produced in the world. The Extremadura region is at the forefront of Spanish industrial tomatoes, grown in the Vegas del Guadiana and Alagón Árrago regions. In Extremadura, tomatoes are processed in 14 factories in the Vegas del Guadiana and the Alagón Árrago region. Tomato yields in Extremadura have increased from around 59 t/ha in 2001 to 91.65 t/ha in 2020. This increase has been possible thanks to the concentration of farmers in OPFH and the application of localized irrigation, fertilization, etc.

The cultivated area in Andalusia has increased substantially, from 894 ha in 2001 to 6,621 ha in the 2020 season, reaching its maximum in 2017 with 8,889 ha cultivated. Tomato production in Andalusia stands at 718 million kilos. Tomato yields have increased substantially since 2013, reaching 107.9 t/ha in 2020.

Regarding the latest developments of the 2021 campaign, heavy rains in mid-September led to the closure of the factories for 3 or 4 days in Extremadura. Another heavy episode, on Thursday 23 September, with 50-60 mm, closed the factories again. This succession of rainy spells meant that, although the crop looked promising with high yields, some tomatoes remained in the field. The brix, which was very good at the beginning, is very low in September and the production of dice will be reduced. Overall, in Spain, the forecast is still 3.1 million tons, when it should have been higher if all the fields in Extremadura could have been harvested.

2. OBJETIVES

The objective of the project consisted in the evaluation of a treatment composed of two biostimulant products: Penergetic b and Penergetic p in processing tomato crop, the main objective being the control of the most common pests in the crop and evaluation of crop development under a 20% reduction of fertilization units with respect to the CTAEX control. The influence on the development and evolution of the crop, phenology, sanitary status and finally, productivity was jointly analyzed.

3. MATERIALS AND METHODS

3.1. Trial design

The trial was developed in the experimental farm of CTAEX, in the polygon 182 and plot 1 according to the SIGPAC (6:900:0:0:182:1).

A field trial was planned, following a directed block design with elementary plots of 2520 m2. The total area of the trial was 5040 m2. Four furrows were left unplanted between the control plot and the treatments. The tomato variety on which the products were studied was H-1015, from Heinz, recommended for any transplanting season, being a variety with high Brix and good color. The plants of this variety are very rustic. Figure 1 shows the distribution of the plots in the field.

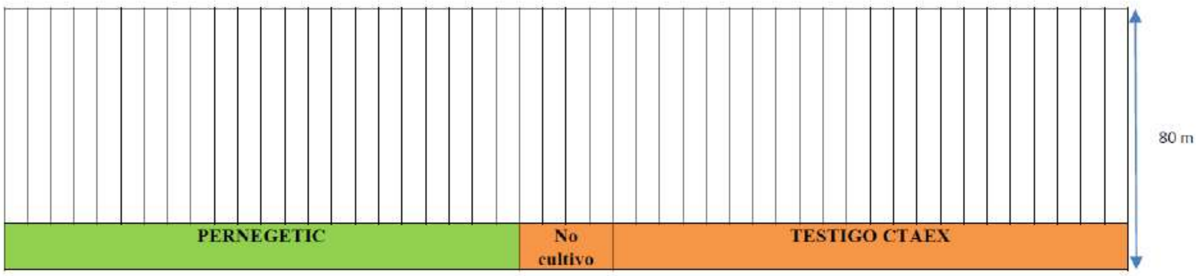

Figure 1. Distribution of the trial in the Bercial's farm.

3.2. Applied treatments

The products studied are described below:

- **Penergetic b**

Penergetic b is a product that favors the formation and regeneration of soil life, stimulating its biology and enhancing the activity of soil organisms. This stimulation favors humus formation and has a lasting impact on the maintenance of soil fertility and health.

- **Penergetic p**

Plant tonic, with stimulating effect on the immune system, providing greater resistance to stress and reinforcing the natural defenses of plants. It can also reduce susceptibility to diseases and pests. It has a positive effect on photosynthesis which influences crop growth and yield.

Two theses are considered in this project: The treatment with the products proposed by the company and a standard control with the products used in CTAEX. The following is a description of each of the treatments applied, specifying the mode and timing of application, the dosage used and the separation between treatments.

1. **Treatment with the products offered by the company**

- Application of Penergetic b in background at 2 kg/ha. In total 1 application. It will be made 20 days before transplanting.
- * Application of Penergetic p foliar at different doses and in 3 moments of application in cover crops:
    - o P-p at 0.25 kg/ha at transplanting.
    - o P-p at 0.25 Kg/ha in pre-flowering, application margin of 15 days with respect to the previous one.
    - o P-p at 0.150 kg/ha in bloom.

* In each of the foliar applications, a water volume between 100 and 500 L/ha was used.

2. **CTAEX Treatment**

No specific comparative products were used, 20% more fertilizer units were used than in the Penergetic thesis.

The applications were carried out according to the established protocol. Table 1 shows the doses and times of application.

Table 1. Dose and application moments.

PLOT	PRODUCT	APPLICATION METHOD	DATE	DOSE
PENERGETIC	PENERGETIC B	SPRAY IRRIGATION	29/04/2021	2kg/ha
	PENERGETIC P	FOLIAR	19/05/2021 04/06/2021 29/06/2021	0,25 kg/ha 0,25 kg/ha 0,150 kg/ha

Figure 2. Penergetic B and Penergetic p

Figure 3. Preparation and application of Penergetic b treatment in the background soil spraying on 29/04/2021

Figure 4. Preparation and 1st application of Penergetic p foliar treatment at the time of transplanting on 19/05/2021.

Figure 5. 2nd foliar application of Penergetic p treatment on 04/06/2021

Figure 6. 3rd foliar application of Penergetic p treatment on 29/06/2021

3.3. Climatic conditions

The climatic data constitute a fundamental source of information because they condition the development of the crop, influencing the phenological state and the incidence of plagues and diseases.

The data of maximum, average and minimum temperature, and daily precipitation, collected in table 2, have been downloaded from the web page of the Junta de Extremadura, coming from the network of weather stations distributed throughout the region, although in this case it corresponds to the *El Bercial* weather station, the closest to the test fields.

Table 2. Summary of climatic data collected in the area

Month	**Max. temperature ºC)**		**Average temperature (ºC)**	**Min. temperature (ºC)**		**Effective precipitation (mm)**
	Absolute	average		Absolute	average	
April	24.55	20.92	15.17	5.62	10.01	0.66
May	32.56	25.46	18.15	4.28	10.19	7.24
Jun	33.96	29.18	21.24	7.62	13.24	21.63
Jul	38.69	32.54	23.74	11.49	14.59	0
August	42.3	34.1	25.09	10.89	15.69	0

The temperatures recorded in the table refer to maximum temperatures (both absolute, which refers to the highest of the month, and monthly averages), averages and minimum temperatures (both absolute, which refers to the lowest of the month, and monthly averages). The daily precipitation data is the sum of the precipitations fallen throughout each month.

Figure 7 below shows the evolution of maximum, average and minimum temperatures, and rainfall over the crop cycle from transplanting on 19 May to 31 August 2021, when sampling is complete.

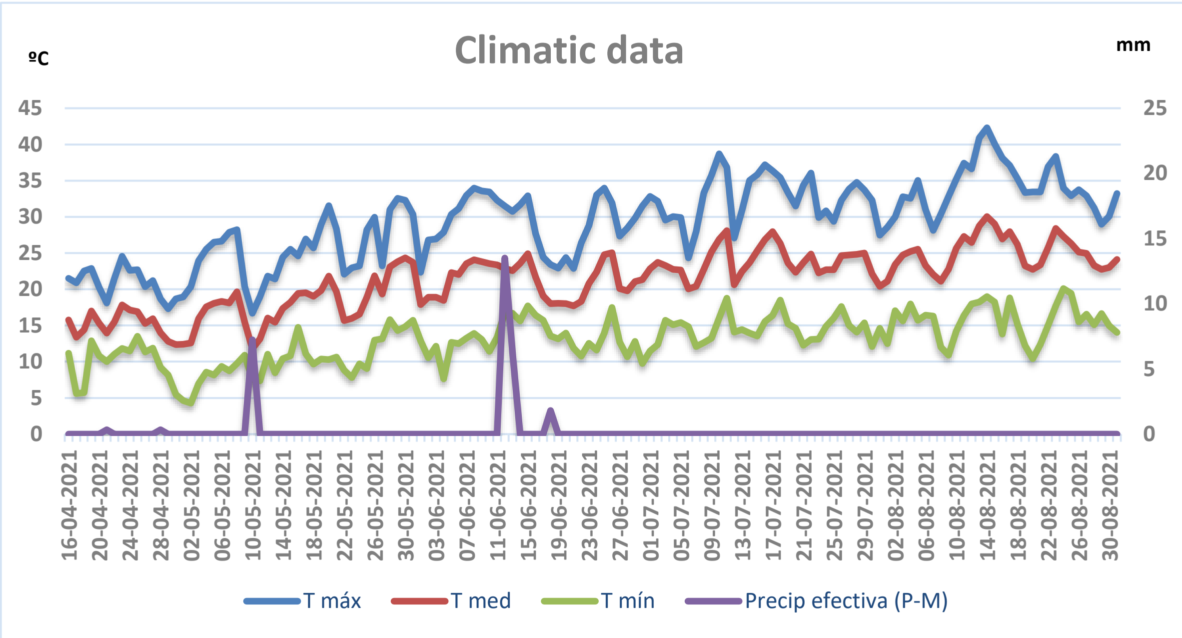

Figure 7. Evolution of temperatures and precipitation in the area

The 2021 campaign has been marked by moderately. In addition, the accumulated rainfall during the four months in which the crop was grown reached 30 mm.

3.4. Soil conditions

Before transplant, a representative soil sample was taken from the three comparative test fields, at a depth between 0 and 30 cm. The soil physicochemical analysis was carried out in CTAEX laboratories, to determine texture (% sand, clay, and silt), conductivity, pH, organic matter content, total carbonates, total nitrogen, C/N ratio, phosphorus, potassium, magnesium, calcium, and sodium.

Table 3 shows the soil texture of the trial plot.

Table 3. General soil texture of the trial plots.

Parameter	%	Type of soil
Sand (%)	88,72	**Sandy-Silt**
Silt (%)	8,00	
Clay (%)	3,28	

The soil has a sandy-silt texture, it is a very light soil with very good internal drainage and very low water and fertilizer retention capacity, being generally of very low fertility.

Table 4 shows the values of the parameters obtained from the chemical analysis of the soil.

Table 4. Results of the general chemical analysis of the soil of the test location

Element	Result	Interpretation
		VL / L / N / V / VH
Conductivity (mS/cm)	0,096	Non-saline
pH (soil/water 1/2,5)	6,98	Neutral
Organic matter (%)	0,77	Very low
Total carbonates (%)	0,34	Very low
Total nitrogen (%)	0,03	Very low
C/N ratio	12,45	Neutral
P (ppm)	37,70	Very high
K (meq /100g)	0,41	Neutral
Mg (meq /100g)	0,97	low
Ca (meq /100g)	3,70	low
Na (meq /100g)	1,08	high

The soil does not present salinity problems and the pH is adequate for the assimilation of most of the nutrients.

The nitrogen content of this soil is very low, as well as the organic matter content. The organic matter content and the C/N ratio provide information on the assimilable nitrogen that the soil will produce throughout the crop cycle. The low ratio between carbon and nitrogen content indicates an average rate of mineralization of soil organic matter, producing a normal release of nitrogen. It should be considered that the location of the trial is in an area vulnerable to nitrates, so in no case can nitrogen levels of 200 UF/ha be exceeded in the processing tomato crop.

The soil has a very high phosphorus content and neutral levels of potassium. In contrast, sodium levels are high, and magnesium and calcium levels are low.

3.5. Cultivation process

3.5.1. Transplanting

After the land preparation work, the trial was marked. Once the plots were prepared, the background fertilization was applied (29/04/2021), and on 19/05/2020 the transplanting was carried out, in a mechanized way with a FIALHO TEX DRIVER model transplanter, of 3 bodies, with a plantation density of 28,000 plants/ha (Figure 8 and 9). The trial was watered by means of drip irrigation.

Figure 8. Transplanting of the processing tomato trial on May 19

Figure 9. Newly transplanted trial

3.5.2. Irrigation

The irrigation system for this trial was drip irrigation, using P.E. drip lines with 0.3 m between drippers and a flow rate of 1.324 l/h per dripper. With these data and knowing the ETo and the kc of the crop, the irrigation dose to be contributed each day and the time that the system should be watering was calculated.

3.5.3. Fertilization

The "standard" fertilization plan designed for the trial of the processing tomato crop in the experimental farm of CTAEX consisted in providing the crop with the following fertilizing units:

- Tesis CTAEX: 159 N, 90 P, 171 K y 56 Ca
- Tesis PENERGETIC: 127 N, 72 P, 137 K y 45 Ca. 20% reduction in fertilizer units compared to the CTAEX control.

3.5.4. Weed control

To keep the trials, clean of weeds, mechanical and chemical methods were combined. The most problematic weed species in the trial plot were *Cyperus rotundus*, *Solanum sarrachoides* and *Solanum nigrum*. A first pass was made with a multiple hoe as a mechanical control measure. Pre-emergence and post-emergence herbicides were also applied, on the dates and at the doses described in table 5.

Table 5. Herbicides applied to processing tomatoes cultivation.

Date	Active substance	Dose	Application
24/04/2021	Pendimetalina 45,5%	2,0 L/ha	Pulverizer
04/06/2021	Metribuzina 70%	0,5 kg/ha	Irrigation
06/08/2021	Rimsulfuron 25%	30 g/ha	Pulverizer
06/08/2021	Cletodim 12%	0,8 L/ha	Pulverizer

3.5.5. Pest and disease control

During the crop cycle, the main pests affecting the crop were red spider mite (*Tetranychus urticae*), powdery mildew (*Erysiphe polygoni*), vasate (*Aculops lycopersici*), tomato caterpillar (*Helicoverpa* (Heliothis) *armigera*), and tuta (*Tuta absoluta*).

Table 6 shows the pesticides used in the test, as well as the doses and times of application.

Table 6. Pesticides applied to processing tomato crops

Date	Active substance	Dose	Application
21/05/2021	Deltametrina 2,5%	0,3 L/ha	Pulverizer
15/06/2021	Cimoxanilo 60%	0,3 kg/ha	Pulverizer
21/06/2021	Cimoxanilo 60%	0,3 kg/ha	Pulverizer
27/07/2021	Clorantraniliprol 35%	0,1 kg/ha	Pulverizer
03/08/2021	Azoxistrobin 25%	0,7 L/ha	Pulverizer
09/08/2021	Deltametrin 10%	0,125 L/ha	Pulverizer
09/08/2021	Abamectina 1,8%	0,15 L/ha	Pulverizer

The populations of red spider mite, vasates and heliothis were controlled with one application (except for powdery mildew, which required several more), and populations were kept low.

The following image shows plants with fungal symptomatology that had an impact on the crop.

Figure 10 shows the damage caused by powdery mildew on tomato plants.

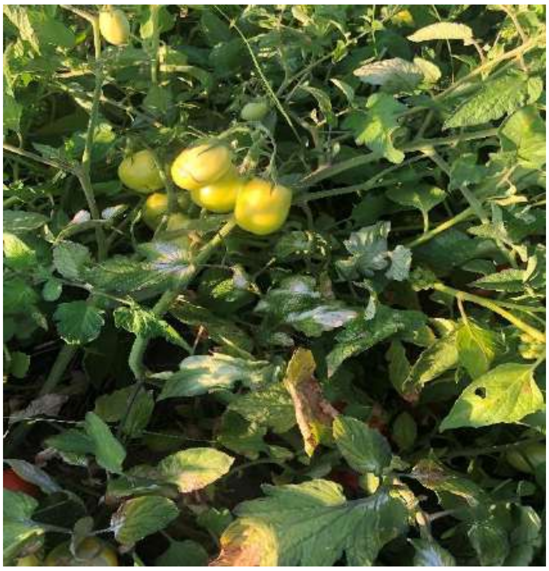

Figure 10. Powdery mildew damage to plants

In terms of diseases during the crop cycle, there were no apparent affections, apart from a not worrying incidence of powdery mildew.

3.5.6. Monitoring and control during cultivation

Durante la evolución del cultivo, se ha llevado a cabo el seguimiento del desarrollo y fenología de las plantas, del estado sanitario y de otros posibles efectos de las aplicaciones por parcela.

- **Phenology**

During the development of the trial, the evolution of different phenological stages of the plantation was monitored.

Once the first flower buds appear, phenology is collected by counting the number of buds, flowers, and fruits in the main branch of the plant, which is the one that starts from the cross, where the first fruits are found. For this, the following classification is considered:

B: Flower bud or closed flowers

F: Open flower or visible petals

G: Freshly set fruits smaller than chickpeas or petals in wilting with

unabated fruits

N: Fruit size between chickpea and walnut

V: Fruits larger than green nuts

R: Fruits larger than red nuts

The phenological state is expressed in terms of three letters, the first indicating the most delayed phenological state, the second the predominant and the third the most advanced.

- **Sanitary conditions**

Throughout the crop cycle, the crop was monitored to evaluate the possible appearance of pests and diseases. However, there was a more persistent presence of powdery mildew (Erysiphe polygoni), so several treatments had to be carried out for its control as described in the MATERIALS AND METHODS section of this report.

- **Plant coverage (%)**

This parameter was measured through Canopeo application at a time during the crop cycle, 50 and 70 days after transplanting. Eight replicates were taken for each of the two plots.

Canopeo is an automatic colour threshold image analysis tool, developed by Oklahoma State University (USA), which uses colour values in the red-green-blue (RGB) system.

Canopeo analyses and classifies all pixels in the image, and this analysis is based on the selection of pixels according to R/V ratios and the rate of excess green. The result of the analysis is a binary image in which the white pixels correspond to the pixels that meet the selection criteria (green canopy), and the black pixels correspond to the pixels that do not meet the selection criteria (non-green canopy).

- **Chlorophyll (SPAD)**

It was evaluated the chlorophyll level of leaves in three times during of the crop cycle 26, 50 and 70 days after transplanting. The chlorophyll level of the leaves was determined by means of the SPAD-502Plus (Figure 11). This instrument determines the relative amount of chlorophyll present in the plant by measuring leaf absorbance in two wavelength regions. Chlorophyll has absorption peaks in the blue (400-500 nm) and red (600-700 nm). With these two absorbances, the SPAD meter calculates a numerical value that is proportional to the amount of chlorophyll in the leaf, which is closely related to the nutritional condition of the plant.

Figure 11. Measuring Chlorophyll with SPAD-502Plus

For the measurement of these parameters, 32 samples were taken in each of the 2 test fields, to ensure a correct statistical analysis.

- **Foliar analysis**

To evaluate the nutritional status of each treatment applied, 4 foliar samples were taken from each of the two theses now of fruit development. Macro and micronutrient content was determined in the CTAEX laboratory. The analysis methodology is by ICP-MS (Inductively

Coupled Plasma Mass Spectrometry), an elemental and isotopic inorganic analysis technique capable of determining and quantifying most of the elements of the periodic table.

3.6. Harvest

Tomato samples were harvested when the plants had about 85% of red fruits. The collection of the test samples was carried out on day x of by hand, in an area of 9 m2 per repetition and with a total of 4 repetitions in each comparative trial field (thesis), being the sampling representative in terms of production and health.

Figure 12. Harvesting one of the replicates

3.7. Parameters assessed at harvest

3.7.1. Agronomical parameters

Once the samples were harvested, they were placed in plastic boxes, passed through the selection line (Figure 13), and the tomatoes were separated according to their size (< 40mm, 40-60mm, > 60mm), state of maturity (over-ripe, ripe, and green) and health status (diseased, sunburn and apical necrosis).).

Figure 13. Removal of unfit tomatoes (left) and separation by size (right)

Each group was weighed to determine the following parameters:

- <u>Gross yield (kg/ha)</u>: the yield obtained from each plot is the sum of the weights of all the groups formed in the selection line, that is, the total of the sample harvested in the field, extrapolating the result to the area of 1 hectare.

- <u>Acceptable Raw Material</u> (A.R.M.) (kg/ha and %): under this classification the mature and healthy fruits are included, expressing the result in kg/ha and in the percentage that these represent of the total (Figure 14). Tomato processing factories penalize the consignments of tomatoes that arrive at their facilities with unripe or green, ripe, over-ripe, etc., giving priority to good quality fruits, that is, the M.P.A. For this reason, it is important that the proportion of M.P.A. is as high as possible.

- <u>Average fruit weight</u> (g): the average fruit weight is determined by weighing 50 fruits from the group of Acceptable Raw Material obtained in the selection line. These 50 fruits must be representative of the sample. The average weight of the fruit is the arithmetic mean of the weight of the 50 fruits, expressed in grams.

- <u>Green fruits</u> (%): green fruits are also separated in the selection line (Figure 14) and their weight is used to determine the % of maturity of the sample. A field of industrial tomatoes is considered ready for harvest when 85% of the fruits are red.

Figure 14. Tomatoes suitable for industry (left) and immature tomatoes (right).

- <u>Over-ripe fruits</u> (%): Over-ripe fruits are those that have passed the physiological stage of maturity, losing firmness because they have been in the plant for so long (Figure 15). The fruits also reach this state due to parasite attacks, apical rot, etc. To estimate this parameter, the same procedure of manual selection in the line and subsequent weighing was followed.

- <u>Sick fruits</u> (%): this group includes all those fruits that have been attacked by plagues and/or diseases, on which bites, stains on the skin, etc. appear. (Figure 15).

Figure 15. Over-ripe fruits (left) and diseased fruits (right).

- <u>Fruits with necrosis or apical rot</u> (%): apical rot can be due to water stress, moisture imbalances, calcium deficiencies (which according to some authors it is also related to humidity), and even to genetic factors, since there are certain varieties prone to suffer from it. It manifests itself with greyish colours that turn to black and a leathery texture in the apical area of the fruit. The fruits with this physiopathy remain small, mature, and end up rotting (Figure 16).

- <u>Fruit sunburn</u> (%): Fruit sunburn (also known as sunstroke or ironing) is a problem that appears in the summer fruits of vegetables, such as tomatoes, peppers, or eggplants, due to continuous exposure to the sun, affecting the fruits that are less protected by the foliage and causing them to burn, forming discoloured spots (Figure 16).

Figura 16. Frutos con necrosis apical (izquierda) y frutos asolanados (derecha).

3.7.2. Technological parameters

Once the healthy tomatoes have been selected for each treatment, they are transferred to the CTAEX pilot plant, separated by samples, where they are processed to determine the technological parameters. Technological parameters were measured on 4 samples (replicates) of red and ripe tomatoes (2 kg) from each of the 2 test plots. Each sample was processed to obtain a juice according to the methodology used in the CTAEX. This process involved enzymatic deactivation and removal of seeds and peels, and air from the juice obtained. These parameters are sugar content (ºbrix), pH, color and viscosity.

- Sugar content (ºbrix). Brix degree expresses the number of soluble solids in tomato juice, and its measurement is made with a Belligham & Stanley Mod Rfm81 at 20º C. The values of brix degree in industrial tomatoes fluctuate between 3.6 and 6.2, although these values are extreme, being the normal interval between 4 and 5.4. Those consignments of tomatoes delivered to factories with a brix degree lower than 4 are penalized, while those with a value higher than 5.4 receive a premium. This parameter plays a decisive role in the rate of transformation of tomatoes into tomato concentrate, the destination of production. For this reason, the brix degree is a fundamental data for the transformation industries, and the geneticists, who seek to improve the varieties based on this criterion. It is a value that is determined by the variety, although it can be slightly modified by cultivation techniques such as irrigation and fertilization.

- pH: The pH is a very important factor in tomato concentrate since it influences the production process and its conservation. In industry, it is important to keep the pH below 4 to avoid microbial alterations during the production process and in its subsequent conservation. Therefore, if the batches of tomatoes delivered to industry have a pH above 4.4, citric acid is added until it is reduced to 4. This parameter is determined with a pH - meter Orion mod. 920 combined with a pH - electrode Orion mod. 8102.

- Colour: Colour is one of the most important quality parameters in tomato products. It is important that these products are as pure, intense, and dark red as possible. The compounds that give the tomato fruit its characteristic colour are mainly carotenoids, with lycopene being the one that gives the greatest red tone. The colour was determined by a Gardner colorimeter, Colorgard System 2000/05 with an optical sensor at 25ºC, obtaining the colour coordinates L* (brightness), a* (red colour) and b* (yellow colour). As for the a/b index, most of the current commercial cultivars have a/b values that usually vary between 2.1 and 2.4.
- Viscosity: In tomato processing, the concentrate used to make ketchup and certain sauces must be as consistent as possible. The consistency of a tomato juice or concentrate is a complex character, in which the viscosity of the product fundamentally intervenes. The viscosity was measured in a bostwick consistometer with a length of 50 cm. Its value is expressed in cm/30s at 25 ºC.

- Firmness: Firmness is a parameter to be considered in processing factories, like the rest of the parameters studied in this test. It is very important that the fruits have a good firmness, since they will be able to endure more time in the plant without over-ripening, nor spoiling in the vats of the trucks, since many times they must spend many hours in those vats until their processing. Obviously, fruits with little firmness are spoiled before fruits with good firmness. Fruit firmness was measured by means of TA TX TPlus texturometer, performing a compression test. 10 mm and a force of 6 g is applied by means of a 50 mm aluminium cylindrical probe. The result is expressed in grams.
- Nutritional analysis of fruit: At the same time, the macronutrient and micronutrient content of tomato fruit was determined in the laboratory. Eight samples were analysed (4 for each test plot) and approximately 2 kg of fruit were taken from each sample. The analysis methodology is by ICP-MS (Inductively Coupled Plasma Mass Spectrometry), an elemental and isotopic inorganic analysis technique capable of determining and quantifying most of the elements of the periodic table.

- Physicochemical analysis of the soil after harvest: Once the crop was harvested, a soil analysis was carried out to check the soil extractions. In this analysis, the soil was analyzed:
    - texture (% sand, clay, and silt), pH in water, electrical conductivity, organic matter, nitrogen, C/N ratio, assimilable phosphorus (Olsen), potassium, magnesium, calcium and sodium exchange, cation exchange capacity (CEC), carbonates and chlorides.

3.8. Statistical analysis

The data were analyzed using the SPSS 15.0 statistical package (SPSS Institute Inc Cary, NC). A descriptive analysis was performed to calculate the means and standard error of the mean of the measurements obtained for each parameter. The GLM (General Linear Model) procedure was used to perform a one-way analysis of variance. The level of statistical significance was defined as $P<0.05$. In cases where the effect of some of the independent variables was significant, the means were compared using Tukey's test ($P<0.05$).

4. RESULTS

4.1. Monitoring and development of the crop

4.1.1. Crop monitoring

Throughout the crop cycle it was monitored, which can be seen in the following images:

Figure 17. View of the PENERGETIC trial plot, 6 days after transplanting

Figure 18. View of the CTAEX trial plot, 6 days after transplanting

Figure 19. View of the PENERGETIC trial plot, 12 days after transplanting

Figure 20. View of the CTAEX trial plot, 12 days after transplanting

After 14 days from transplanting, the plants were at phenological stage BBB, so all three stages were flower bud stage. No petals were visible yet.

Figure 21. View of the PENERGETIC trial plot, 14 days after transplanting

Figure 22. View of the CTAEX trial plot, 14 days after transplanting

After 26 days from transplanting, the plants were at the beginning of flowering, phenological stage BBF, so the predominant stage was that of flower bud. The petals of the first flowers were beginning to dry, a symptom of a fertilized flower.

Figure 23. View of the PENERGETIC trial plot, 26 days after transplanting

Figure 24. View of the CTAEX trial plot, 26 days after transplanting

On 22 June, 34 days after transplanting, the phenological stage was BBG, the most advanced stage was fruit set. The plants were in first flowering and were more evenly matched. Differences in development between the established theses were beginning to be noticed.

Figure 25. View of the PENERGETIC trial plot, 34 days after transplanting

Figure 26. View of the CTAEX trial plot, 34 days after transplanting

On 1 July, 43 days after transplanting, the predominant phenological stage was BGV, the most advanced stage being green fruit. The plants were in full flowering and differences in development were visible to the naked eye depending on the treatment.

Figure 27. View of the PENERGETIC trial plot, 43 days after transplanting

Figure 28. View of the CTAEX trial plot, 43 days after transplanting

After 50 days from transplanting, plant development was assessed through the percentage of plant cover on the bed, measured with Canopeo. At 70 days after transplanting, this parameter was measured again.

Figure 29. Image taken from the Penergetic thesis (left), and same image processed by Canopeo (right).

Figure 30. Image taken from the CTAEX thesis (left), and same image processed by Canopeo (right).

On 12 July, 55 days after transplanting, the predominant phenological stage was BNV, the most advanced stage being green fruit. The plants were in full bloom with a high percentage of chickpea-sized fruit set.

Figure 31. View of the PENERGETIC trial plot, 55 days after transplanting

Figure 32. View of the CTAEX trial plot, 55 days after transplanting

On 14 July, 57 days after transplanting, the flowering and fruiting of the plants continued, with more and more green fruit appearing.

Figure 33. View of the PENERGETIC trial plot, 57 days after transplanting

Figure 34. View of the CTAEX trial plot, 57 days after transplanting

On 21 July, 64 days after transplanting, the predominant phenological stage was BVR, the most advanced stage being red fruit. The plants were in full growth and ripening of green fruit.

Figure 35. View of the PENERGETIC trial plot, 64 days after transplanting

Figure 36. View of the CTAEX trial plot, 64 days after transplanting

On 28 July, 71 days after transplanting, the plants were at phenological stage BVR, the most advanced stage being the red fruit stage. The plants were still flowering and continued to set fruit, the fruit cover was therefore very good, thus reducing sunburn damage.

Figure 37. View of the PENERGETIC trial plot, 71 days after transplanting

Figure 38. View of the CTAEX trial plot, 71 days after transplanting

On 6 August, 80 days after transplanting, the plants are still in the BVR phenological stage. The plants are still maturing, producing more red fruits.

Figure 39. View of the PENERGETIC trial plot, 80 days after transplanting

Figure 40. View of the CTAEX trial plot, 80 days after transplanting

On 24 August, 98 days after transplanting, the plants were in their last days in the field before harvesting.

Figure 41. View of the PENERGETIC (left) and CTAEX (right) trial plot, 98 days after transplanting.

4.1.2. Parameters evaluated during the development of the crop

During the trial, the evolution of the tomato crop in the field was studied according to the type of treatment applied, by means of two parameters: percentage of coverage of the plants on the bed and chlorophyll content.

1. **Plant coverage (%)**

This parameter was measured at two points in the crop cycle, 50 and 70 days after transplanting, taking 8 replicates for each of the 2 elementary plots of the trial.

Table 7 and figure 42 show the values of the percentage of coverage on the date previously determined.

Table 7. Coverage of plants on the bed (mean ± standard error).

Development parameter	Treatment						
	PENERGETIC			CTAEX			P
% Coverage after 50 days	68.78a	±	5.68	54.18b	±	10.46	**
% Coverage after 70 days	80.60	±	4.21	70.66	±	10.92	ns

Meaning (P): ns=no difference; *=P<0.05; **=P<0.01; ***=P<0.001.
a, b = different letters imply significant differences between treatments, P<0.05. Tukey's test.

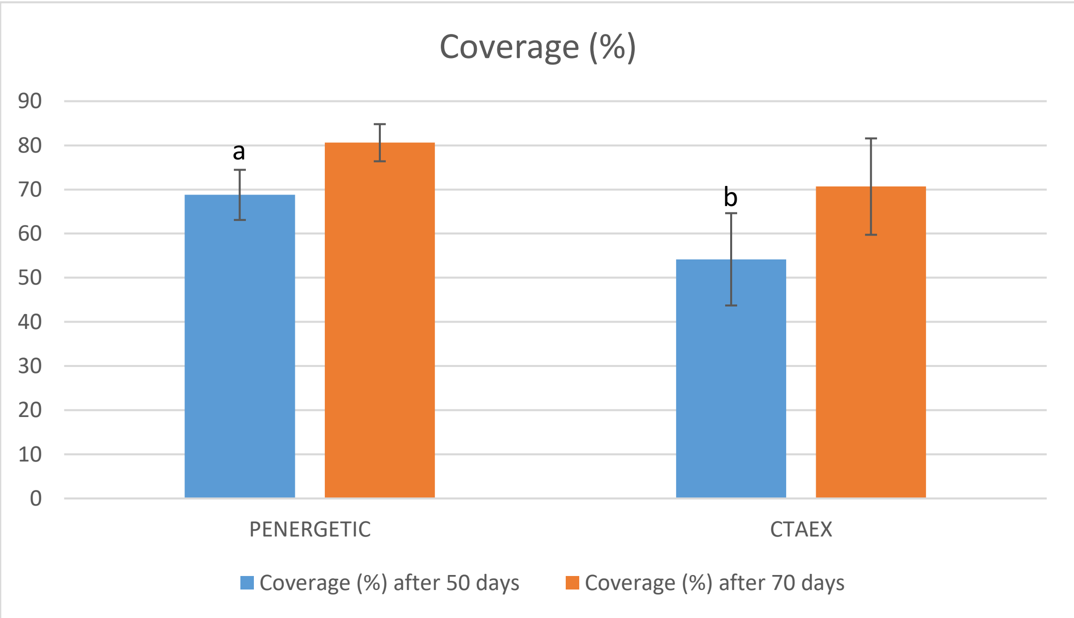

Figure 42. *Percentage plant cover at 50 and 70 days after transplanting*

At 50 days after transplanting, the coverage values of the plants on the bed ranged between 54.18 and 68.78%. The plants of the PENERGETIC thesis showed a higher percentage of cover measured with Canopeo, with significant differences compared to the CTAEX thesis (P<0.01).

At 70 days after transplanting, plant cover values on the bed ranged from 58.88 to 70.04%. No statistically significant differences (P>0.05) were found between treatments.

2. **Chlorophyll (SPAD)**

This parameter was measured at three points in the crop cycle, 26, 50 and 70 days after transplanting, taking 10 replicates for each of the two elementary plots of the trial.

Table 8 and figure 43 chlorophyll values are shown for the previously determined dates.

Table 8. Chlorophyll content (mean ± standard error).

Development parameter	Treatment						
	PENERGETIC			CTAEX			P
Chlorophyll content (SPAD) at 26 days	51.32	±	3.75	49.78	±	4.69	ns
Chlorophyll content (SPAD) at 50 days	58.40a	±	3.89	51.83b	±	1.25	**
Chlorophyll content (SPAD) at 70 days	48.79	±	0.64	47.40	±	1.7	ns

Meaning (P): ns=no difference; *=P<0.05; **=P<0.01; ***=P<0.001.
a, b = different letters imply significant differences between treatments, P<0.05. Tukey's test.

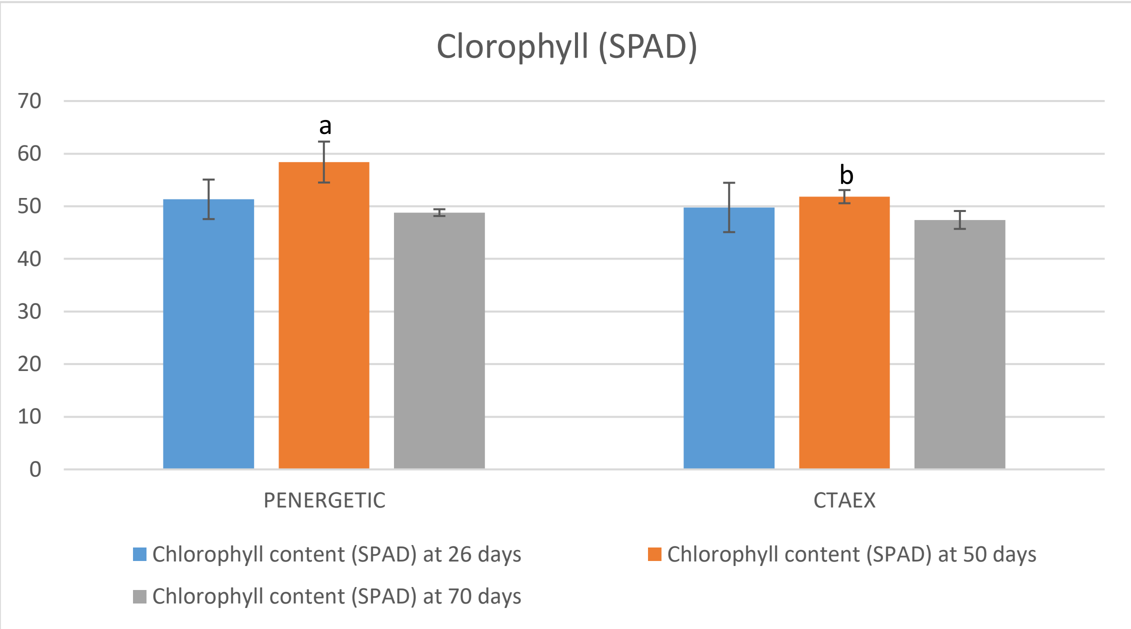

Figure 43. *Chlorophyll content (SPAD) of plants at 26, 53 and 67 days after transplanting*

At 26 days after transplanting, plant chlorophyll values ranged between 49.78 and 51.32. No statistically significant differences (P>0.05) were found between treatments.

At 50 days after transplanting, the chlorophyll values of the plants were between 51.83 and 58.40. The plants of the PENERGETIC thesis showed higher chlorophyll values and significant differences were found compared to the CTAEX thesis (P<0.01).

At 70 days after transplanting, the chlorophyll values of the plants ranged from 47.40 to 48.79. No statistically significant differences (P>0.05) were found between treatments.

3. **Leaf analysis**

The nutritional status of each treatment was evaluated by taking 4 samples of each of the two theses at the time of fruit development.

Table 9. Micro and macro elements measured in tomato leaf

Development parameter	Treatments						
	PENERGETIC			CTAEX			P
(%) N	3.06	±	0.40	3.17	±	0.34	ns
(%) P	0.50b	±	0.03	0.56a	±	0.03	*
(%) K	1.50	±	0.19	1.56	±	0.08	ns
(%) Ca	2.16	±	0.18	2.47	±	0.15	ns
(%) Mg	0.54	±	0.06	0.63	±	0.04	ns
(%) Na	0.08	±	0.02	0.09	±	0.02	ns
(ppm) Cu	14.12	±	0.77	13.97	±	1.03	ns
(ppm) Zn	14.03	±	2.62	12.73	±	0.98	ns
(ppm) B	39.87	±	1.91	41.11	±	3.01	ns
(ppm) Fe	307.66	±	84.33	277.69	±	6.66	ns
(ppm) Mn	65.74	±	8.65	97.88	±	39.11	ns

Meaning (P): ns=no difference; *=P<0.05; **=P<0.01; ***=P<0.001.
a, b = different letters imply significant differences between treatments, P<0.05. Tukey's test.

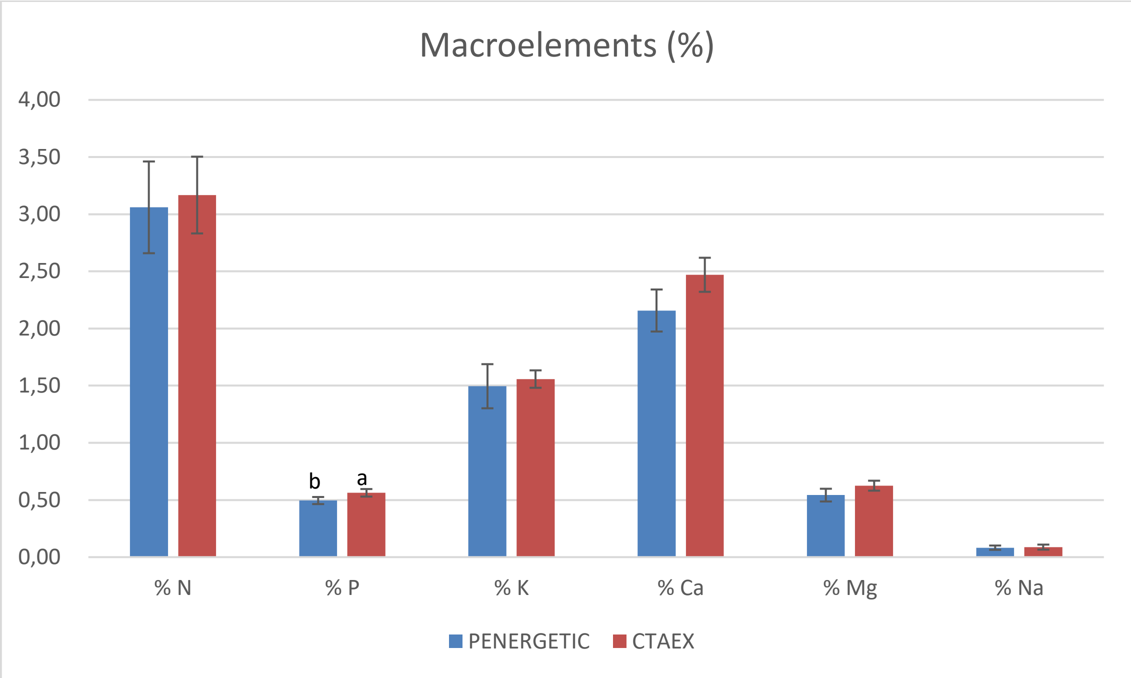

Figure 44. macro-elements analyzed in tomato leaf (%N, %P, %K, %Ca, % Mg, %Na)

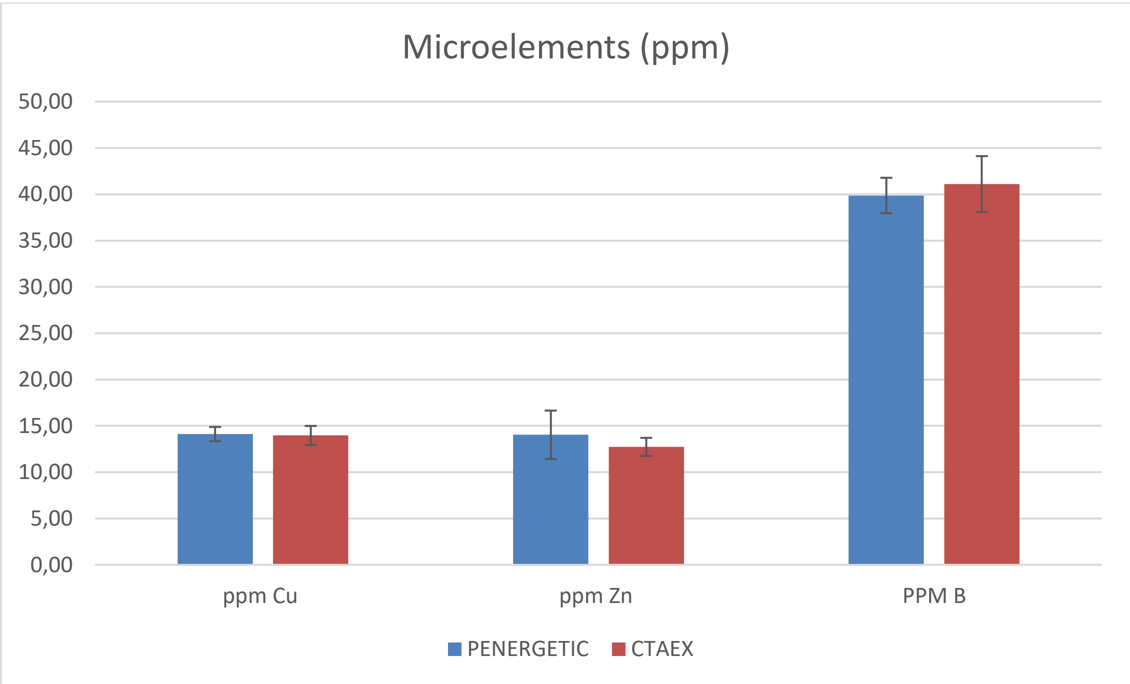

Figure 45. microelements analyzed in tomato leaf (Ppm Cu, Ppm Zn, Ppm B)

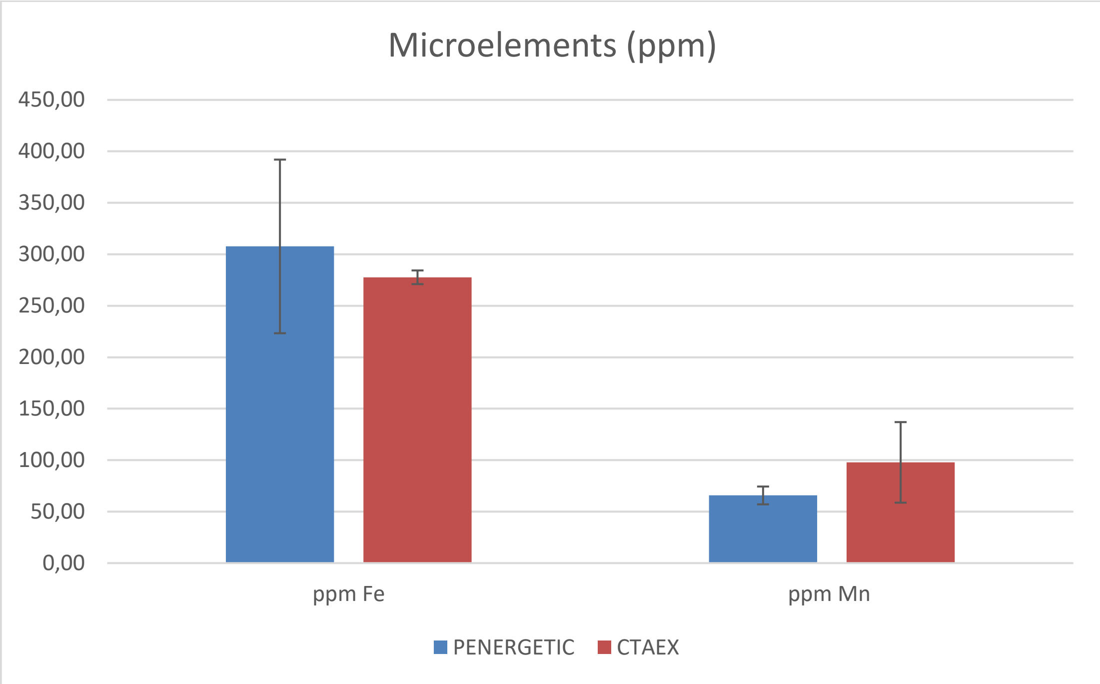

Figure 46. microelements analyzed in tomato leaf (Ppm Fe, Ppm Mn)

After 70 days after transplanting, the nutritional status of each treatment was evaluated. The plants of the CTAEX thesis showed a higher percentage of phosphorus, with significant differences compared to the PENERGETIC thesis ($P<0.05$).

4.2. Parameters evaluated after the harvest

4.2.1. Agronomic parameters

Table 10 shows the agronomic parameters evaluated from the tomatoes harvested in each thesis.

Table 10. Agronomic parameters measured in tomato cultivation (mean ± standard error).

Parámetro agronómico	Tesis						
	PENERGETIC			CTAEX			P
Gross Yield (kg/ha)	91166.67	±	8225.98	84388.89	±	13473.80	ns
A.R.M. (kg/ha)	76388.89	±	8279.83	66166.67	±	13811.64	ns
A.R.M. (%)	83.72	±	3.06	77.93	±	5.29	ns
Average fruit weight (g)	78.66a	±	2.41	72.01b	±	3.60	*
Green fruits (%)	5.88b	±	0.73	12.33a	±	2.42	*
Over-ripe fruit (%)	1.88	±	0.26	2.01	±	0.91	ns
Sick fruits (%)	2.63	±	0.55	2.98	±	0.75	ns
Sunburn fruits (%)	2.63	±	0.37	3.32	±	1.37	ns
Fruits with apical necrosis (%)	3.26	±	1.95	1.43	±	0.84	ns

Meaning (P): ns=no difference; *=P<0.05; **=P<0.01; ***=P<0.001.
a, b = different letters imply significant differences between treatments, P<0.05. Tukey's test.

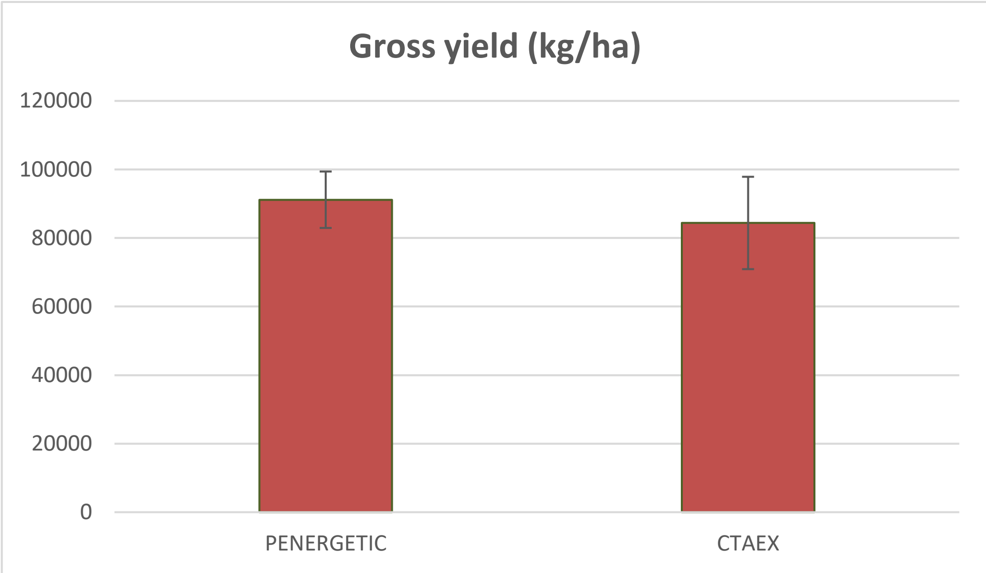

Figure 47. Gross yield (kg/ha) of the tested theses (mean and standard error)

The gross yield (kg/ha) of processing tomato crop ranged from 84,389 kg/ha to 91,167 kg/ha (figure 47). The highest value was observed for the thesis treated with PENERGETIC products, although, no significant differences were found between the two theses (P>0.05).

As mentioned above, by separating the unfit tomatoes in the selection line, the acceptable raw material (A.R.M.) is obtained. Looking exclusively at the tomatoes suitable for the industry, the PENERGETIC thesis obtained a better performance, with yields of A.R.M. of over 76,000 kg/ha. However, when the statistical study was carried out, no significant differences were found between the two treatments (P>0.05).

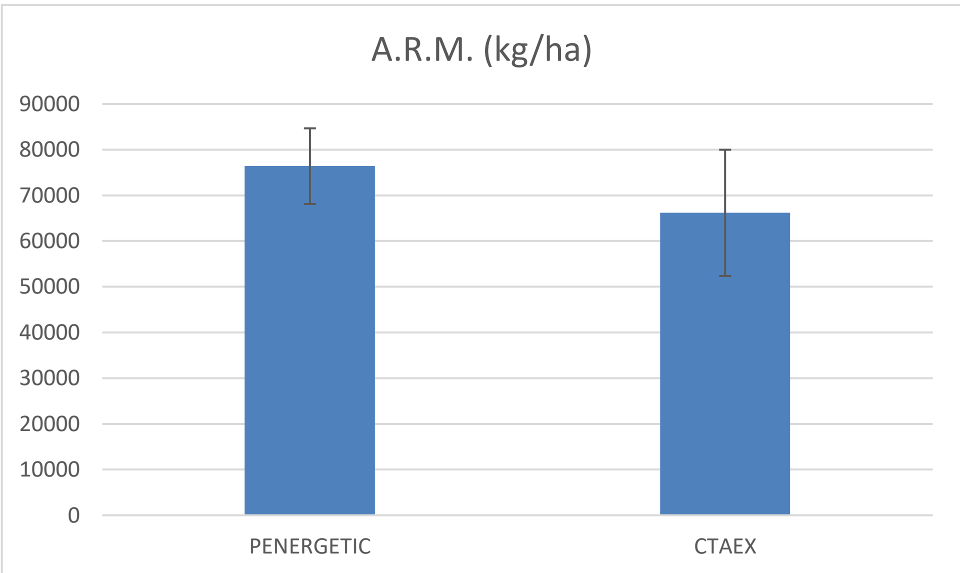

Figure 48. Acceptable raw material (kg/ha) of the tested theses (mean and standard error)

In the following figures you can see some of the tomato fruits corresponding to A.R.M. of the treatments tested.

Figure 49. Fruits of A.R.M. of the thesis PENERGETIC I

Figure 50. Fruits of A.R.M. of the thesis PENERGETIC II

Figure 51. Fruits of A.R.M. of the thesis PENERGETIC III

Figure 52. Fruits of A.R.M. of the thesis PENERGETIC IV

Figure 53. Fruits of A.R.M. of the thesis CTAEX I

Figure 54. Fruits of A.R.M. of the thesis CTAEX II

Figure 55. Fruits of A.R.M. of the thesis CTAEX III

Figure 56. Fruits of A.R.M. of the thesis CTAEX IV

When expressing the A.R.M. in percentage, the values ranged between 77.93 and 83.72 %. As was the case with the M.P.A. expressed in kg/ha, no statistically significant differences were found between the two treatments (P<0.05).

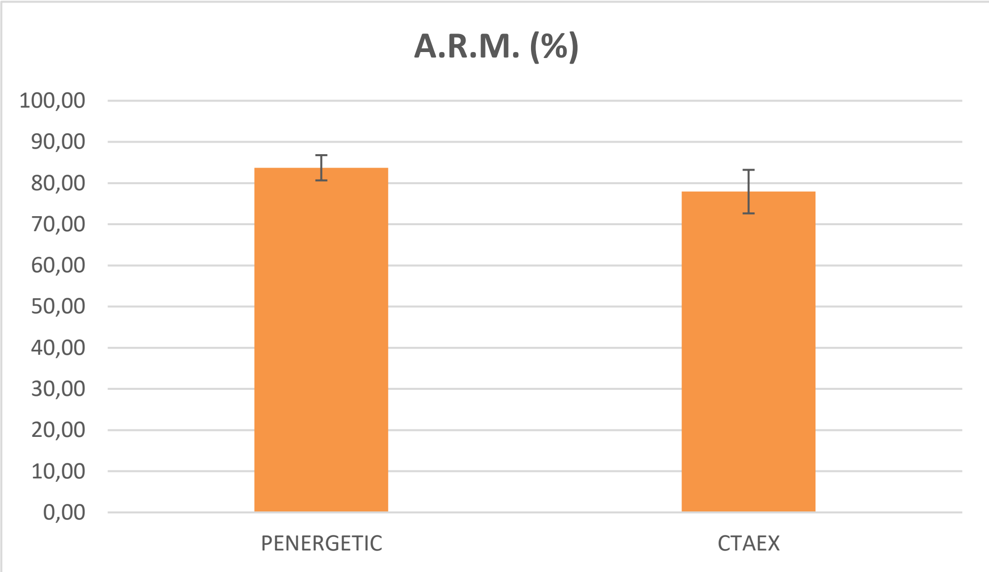

Figure 57. Acceptable raw material (%) of tested theses (mean and standard error)

The average fruit weight remained in the range 72.01-78.66 g (figure 58). In this parameter there were statistically significant differences between theses (P<0.05), being higher the average weight of the fruits in the PENERGETIC thesis.

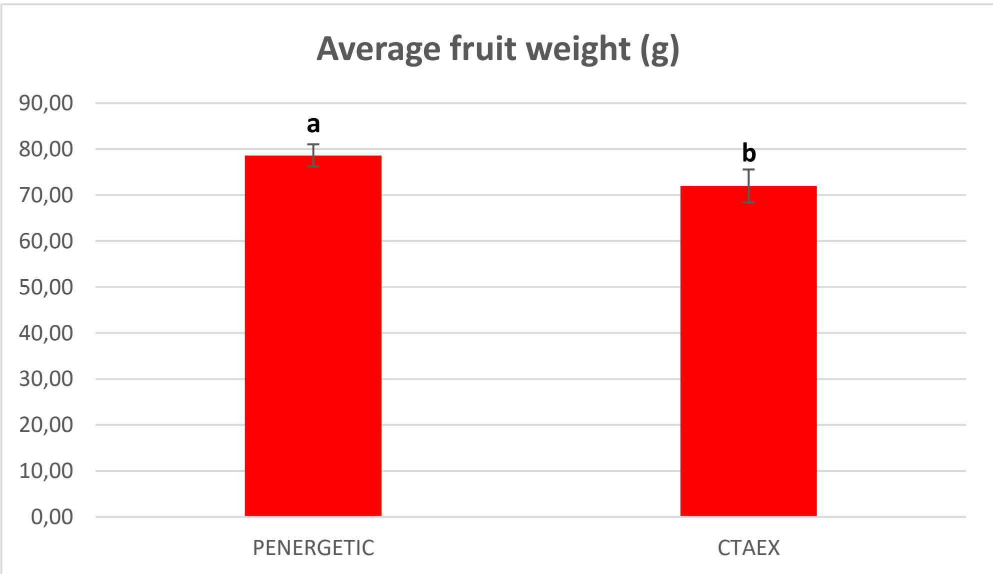

Figure 58. Average fruit weight (g) of tested theses (mean and standard error).

The percentage of green fruit was between 5.88 and 12.33%, so in general, the trial was harvested at the optimum time (figure 59). The CTAEX thesis showed the highest value of green fruit, with significant differences compared to the PENERGETIC thesis (P<0.05).

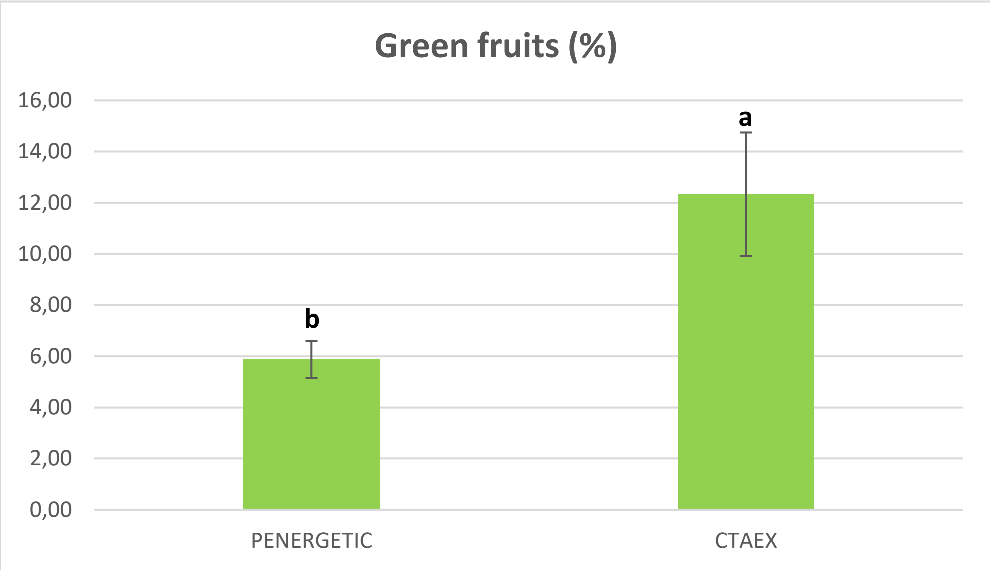

Figure 59. Percentage of green fruits of harvested tomatoes (mean and standard error)

The percentage of over-ripe fruit remained between 1.88 and 2.01% (figure 60). Little variability was observed between treatments for this parameter, so no differences were found between theses from a statistical point of view (P>0.05).

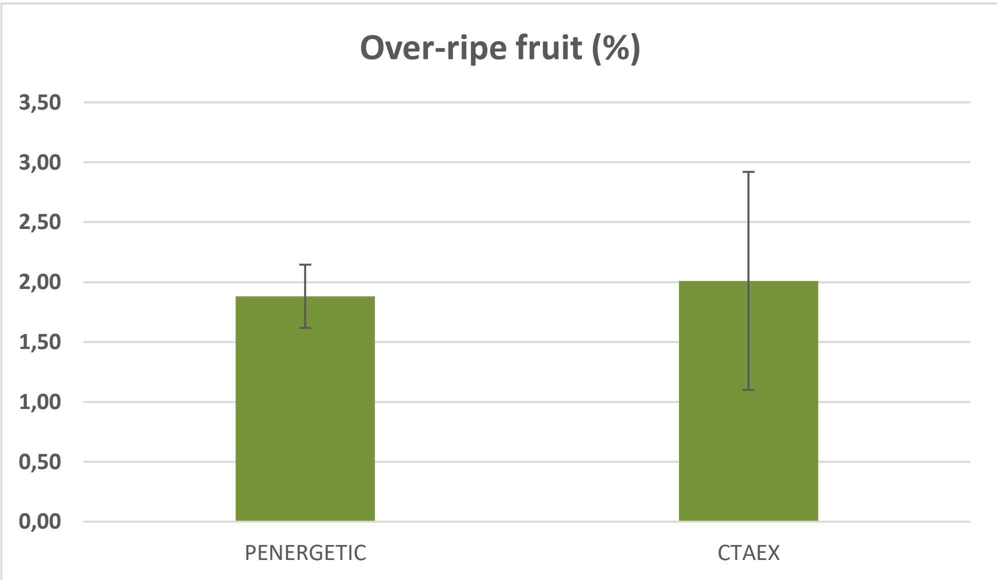

Figure 60. Over-ripe fruits (%) of the tested theses (mean and standard error).

The percentage of diseased fruits (fruits affected by diseases and pests) was between 2.63 and 2.98%. These values show the high healthiness of the crop, and as can be seen in figure 61, it was constant throughout the trial. As there was so little variability between treatments, no significant differences were found between theses (P>0.05).

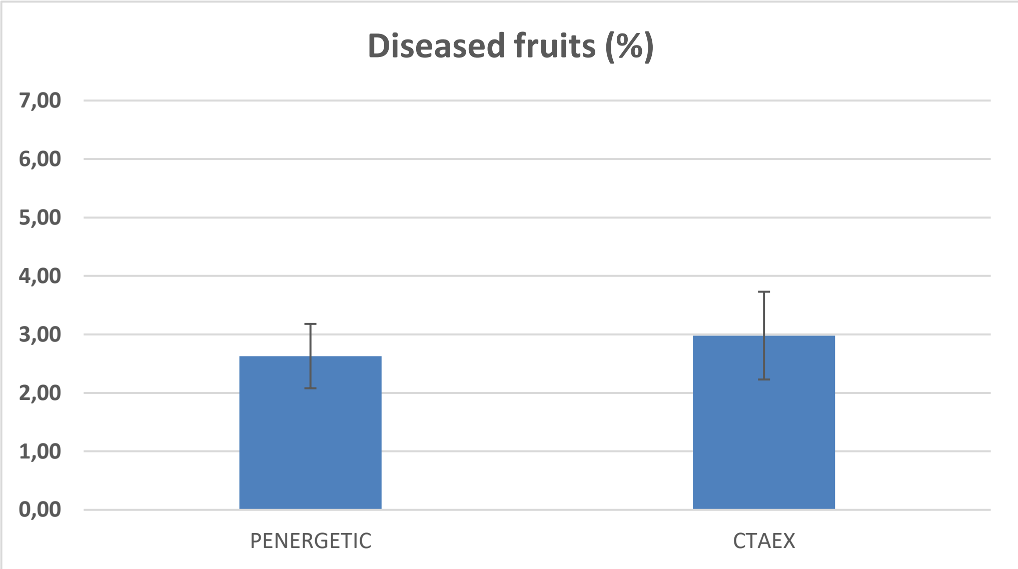

Figure 61. Diseased fruits (%) of the theses tested (mean and standard error).

The percentage of sunburn fruits varied from 2.63 to 3.32%. These values indicate the correct development of the crop and leaf area until the time of harvest since this damage occurs mainly when the tomatoes are very exposed to the sun. As with the percentage of over-ripe and sick fruit, no significant differences were found between theses (P>0.05).

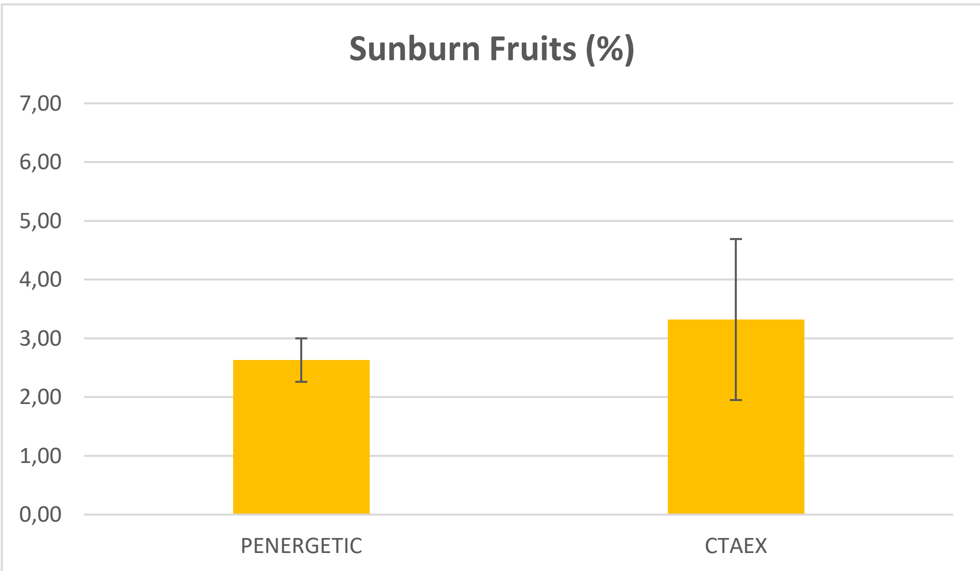

Figure 62. Percentage of fruits sunburn of the theses tested (mean and standard error)

The percentage of fruit with apical necrosis ranged from 1.43 to 3.26%. There were no statistically significant differences between treatments (P>0.05) (figure 63).

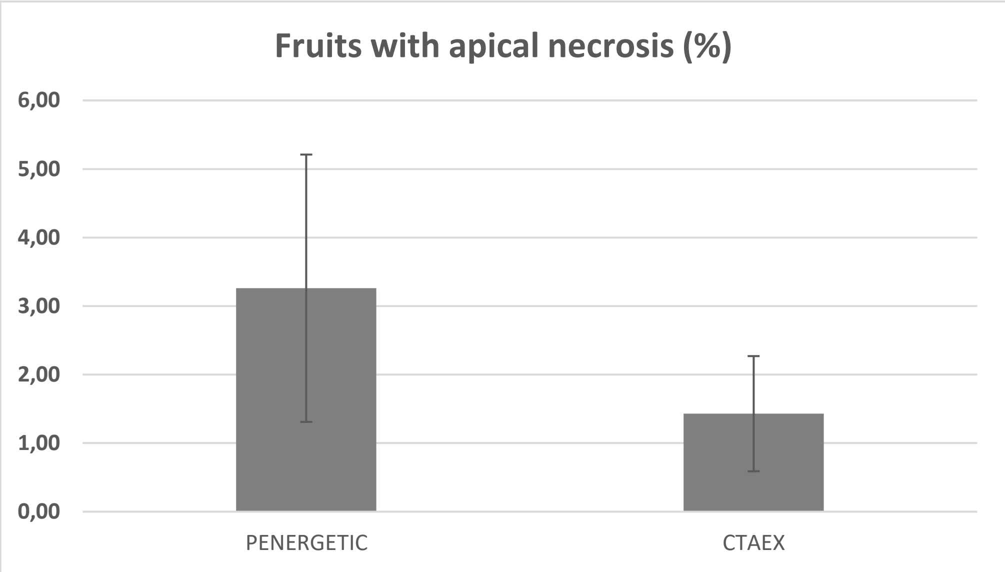

Figure 63. Fruits with apical necrosis (%) of the theses tested (mean and standard error).

4.2.2. Technological parameters

Table 11 shows the results of the technological parameters evaluated for the acceptable raw material.

Table 11. Technological parameters measured in tomato cultivation (mean ± standard error).

Technological parameter	Treatment						
	PENERGETIC			CTAEX			P
ºbrix	5.61	±	0.08	5.47	±	0.13	ns
pH	4.56	±	0.05	4.57	±	0.02	ns
Viscosity (cm/30s)	15.03	±	1.24	14.98	±	1.53	ns
Brightness (L*)	24.79	±	0.21	24.59	±	0.57	ns
Red colour (a*)	28.09	±	0.29	28.73	±	0.26	ns
Index a/b	2.05	±	0.02	2.05	±	0.01	ns
Firmness (g)	2690.69	±	323.77	2491.16	±	77.38	ns

Meaning (P): ns=no difference; *=P<0.05; **=P<0.01; ***=P<0.001.
a, b = different letters imply significant differences between treatments, P<0.05. Tukey's test.

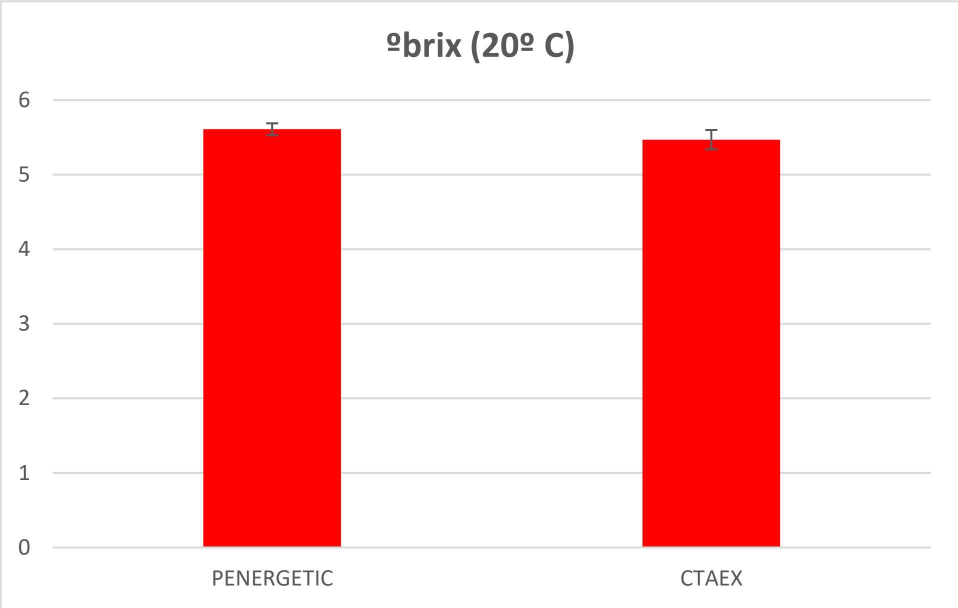

Figure 64. Brix of the tomatoes in each test field (mean and standard error).

The soluble solids content was between 5.47 and 5.61º Brix. These values are high, being above 5.4. No significant differences were found between theses (P>0.05).

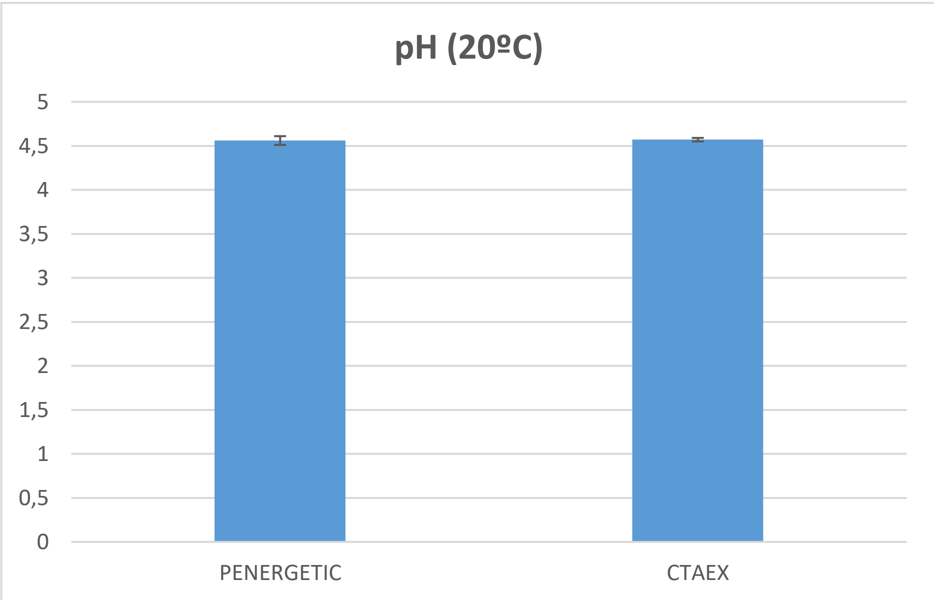

Figure 65. pH of the fruits of the theses tested (mean and standard error)

The pH of tomatoes suitable for the industry remained in the range of 4.56-4.57. In general, these values are considered high, as values below 4 are of interest. This low variability meant that no significant differences were found between theses for this parameter (P>0.05), so the products analyzed did not affect the pH of the tomatoes.

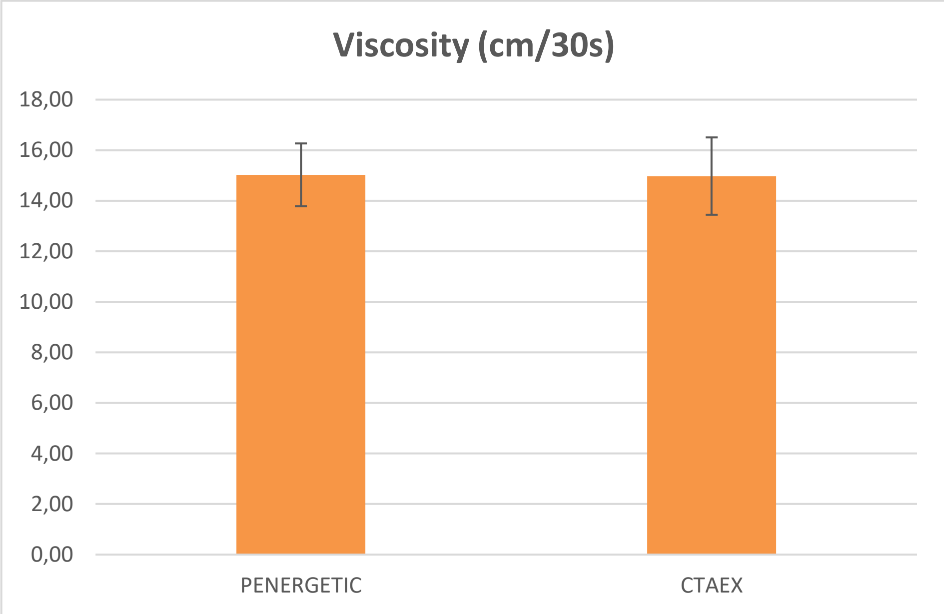

Figure 66. Viscosity (cm/30s) of the fruits of the theses tested (mean and standard error).

The viscosity (Bostwick) of the trial tomatoes ranged from 14.98 to 15.03 cm/30s. As with the previous parameters, the differences in viscosity between these were not statistically significant (P>0.05).

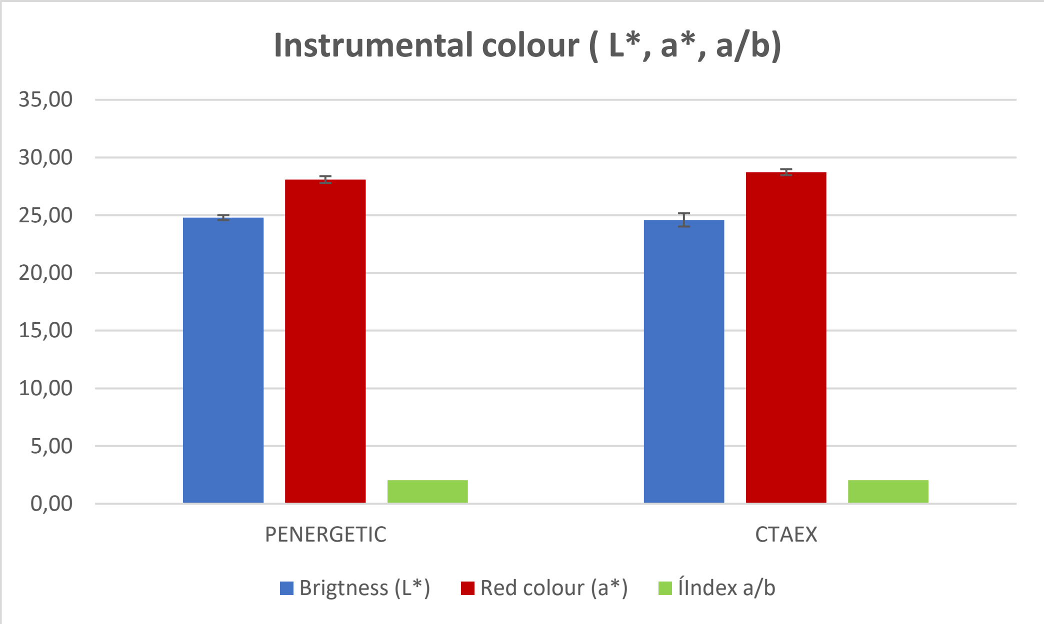

Figure 67. Colour coordinates (L, a*, a/b) in the tested theses (mean and standard error).*

As for the instrumental colour coordinates, the brightness (L*) remained between 24.59 and 24.79; the red colour (a*) between 28.09 and 28.73; and the a/b index at 2.05. In all the colour parameters, the variations between them were minimal, so, as expected, when the statistical study was carried out, there were no significant differences between treatments (P>0.05).

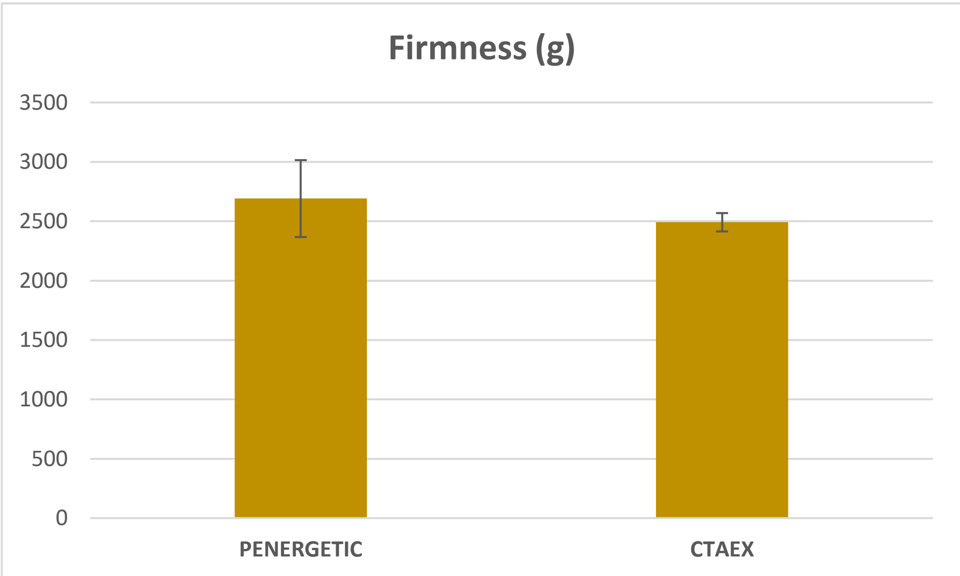

Figure 68. Firmness (g) of the fruits of each thesis tested (mean and standard error).

The firmness (g) of the tomatoes in the trial ranged from 2491.16 to 2690.69 g. As for the previous parameters, the differences in firmness between these were not statistically significant (P>0.05).

Table 12 shows the technological parameters evaluated in relation to the content of macronutrients and micronutrients in the tomato fruit.

Table 12. Micro and macro elements measured in tomato fruit.

Technological parameter	Treatment		
	PENERGETIC	CTAEX	P
(%) N	0.15 ± 0.02	0.13 ± 0.02	ns
(%) P	0.04 ± 0.01	0.04 ± 0.01	ns
(%) K	0.18 ± 0.01	0.18 ± 0.01	ns
(%) Ca	0.01 ± 0.003	0.01 ± 0.002	ns
(%) Mg	0.01 ± 0.004	0.01 ± 0.003	ns
(%) Na	0.01 ± 0.002	0.01 ± 0.002	ns
(ppm) Cu	0.44 ± 0.13	0.43 ± 0.07	ns
(ppm) Zn	2.30 ± 0.81	2.53 ± 0.73	ns
(ppm) B	0.01 ± 0.002	0.01 ± 0.003	ns
(ppm) Fe	10.29 ± 3.36	10.41 ± 2.20	ns
(ppm) Mn	0.98 ± 0.2	1.01 ± 0.12	ns

Meaning (P): ns=no difference; *=P<0.05; **=P<0.01; ***=P<0.001.
a, b = different letters imply significant differences between treatments, P<0.05. Tukey's test.

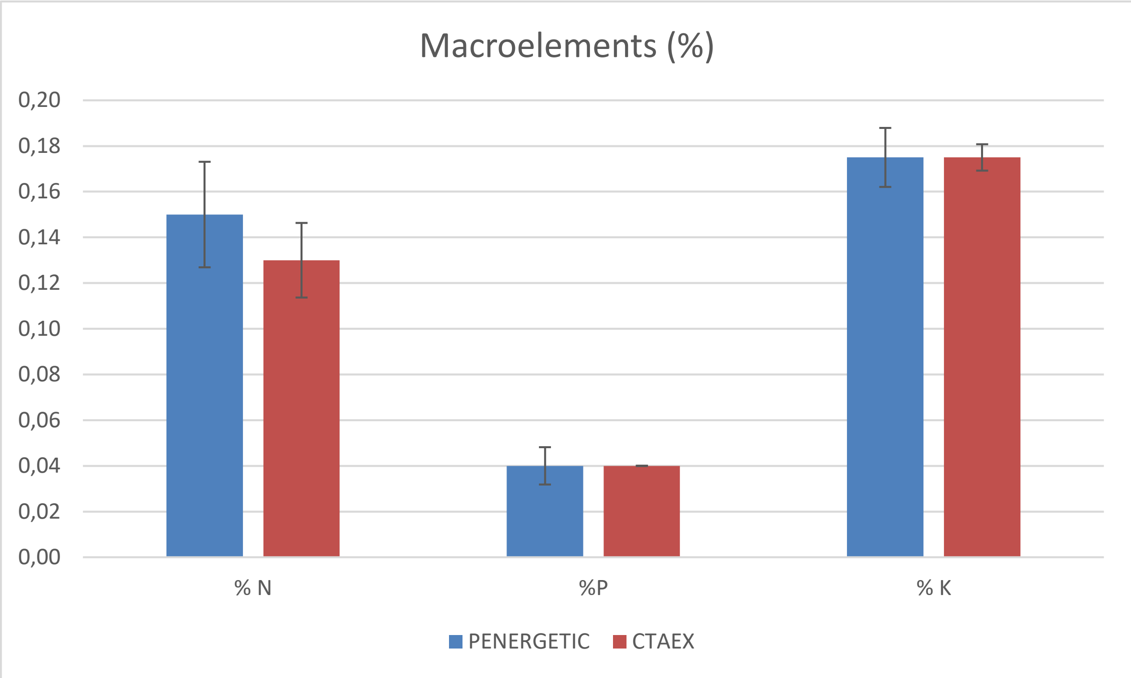

Figure 69. Macro-elements analyzed in tomato (%N, %P, %K)

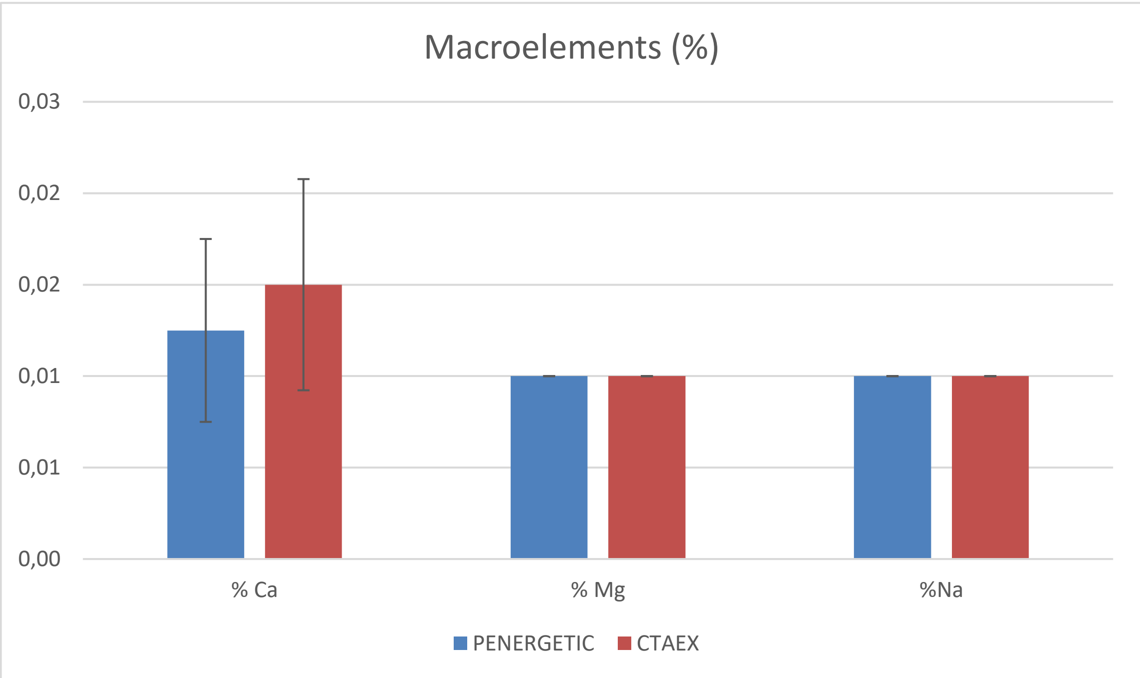

Figure 70. Macro-elements analyzed in tomato (%Ca, %Mg, %Na)

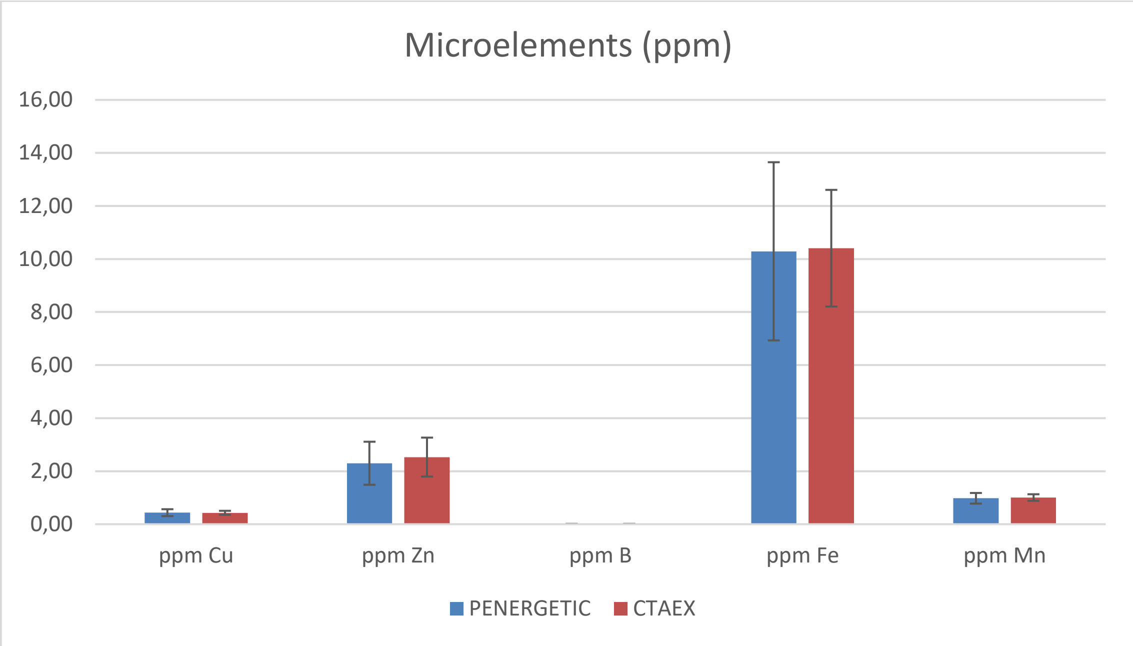

Figure 71. Microelements analyzed in tomato (ppm Fe, ppm Mn, ppm Cu, ppm Zn, ppm B)

Content of macronutrients and micronutrients in the tomato fruit of each applied treatment was evaluated, not presenting significant differences (p>0,05) for any element.

<u>Analysis of the soil after harvesting</u>

Table 13 shows the results of the physico-chemical analysis of the soil after harvesting the tomato crop.

Table 13. Result of the final soil analysis in both theses tested.

	Result	Final result PENERGETIC	Final result CTAEX	Interpretation
				VL L N V VH
Conductivity (mS/cm)	0.0956	0.0501	0.0107	Non saline/Non saline/Non saline
pH (soil/water 1/2,5)	6.98	6.23	6.73	Neutral/Acid/Neutral
Organic matter (%)	0.77	0.78	0.93	Very low/Very low/ Very low
Total carbonates (%)	0.34	0.35	0.42	Very low/Very low/ Very low
Total nitrogen (%)	0.03	0.03	0.03	Very low/Very low/ Very low
C/N ratio	12.45	10.14	14.56	Normal/Normal/Normal
P (ppm)	37.70	42.83	37.64	Very high/Very high/very high
K (meq /100g)	0.41	0.33	0.10	Normal/Normal/Low
Mg (meq /100g)	0.97	0.33	0.21	Low/Very low/Very low
Ca (meq /100g)	3.70	1.26	0.83	Low/Very low/Very low
Na (meq /100g)	1.08	0.26	0.06	High/Very low/Very low

The soil does not present salinity problems and the pH is adequate for the assimilation of most of the nutrients, being slightly more acid in the PENERGETIC thesis than in the CTAEX thesis.

The nitrogen contents of the soil in both these are very low, as well as the organic matter contents. The organic matter content and the C/N ratio provide information on the assimilable nitrogen that the soil will produce throughout the crop cycle. The ratio between carbon and nitrogen content indicates an average rate of mineralization of soil organic matter, leading to a normal release of nitrogen.

The soil has a very high phosphorus content in both theses, highlighting a higher increase of phosphorus in the PENERGETIC thesis with respect to the initial situation of the soil, normal levels of potassium in the PENERGETIC thesis and low levels in the CTAEX thesis, being the latter the one that has extracted more macroelements from the soil. Magnesium levels in both these are very low, being the extraction in the CTAEX thesis higher, the same situation occurring with Calcium. Finally, the levels of sodium in soil presented very low final levels, being the PENERGETIC thesis the one that extracted the least amount of this element in soil.

5. CONCLUSIONS

- The objective of developing the crop under a 20% reduction of the fertilization units in the PENERGETIC thesis with respect to the CTAEX control has been achieved, obtaining good results throughout the cycle and after the harvest.

- The incorporation of the PENERGETIC products in processing tomatoes had an impact on both the chlorophyll content of the leaves and the development of the plants, with significant differences between treatments, showing a higher percentage of chlorophyll and cover in the PENERGETIC thesis.

- Regarding the nutritional status at foliar level, the plants of the CTAEX thesis showed a higher percentage of phosphorus compared to the PENERGETIC thesis, these differences did not imply a deficiency in the latter, showing the good nutritional status of both theses.

- The use of PENERGETIC products made it possible to achieve a higher average fruit weight and a lower percentage of green fruit than in the CTAEX thesis. An advance in the phenological stage of ripening was observed, obtaining 85% of red fruit in less time in the PENERGETIC thesis.

- The use of PENERGETIC treatments had no impact on the rest of the agronomic parameters evaluated in the processing tomato (gross yield, acceptable raw material, percentage of over-ripe fruits, and percentage of sunburn fruits), although in comparison with the rest of the treatments, a trend in yield is observed, higher in the thesis with the PENERGETIC products. The same happens with the percentage of diseased fruits, where the PENERGETIC thesis was the one that showed a better tendency with a lower number of damaged fruits, but this would have to be corroborated in successive years.

- In relation to the technological parameters (ºbrix, Ph, Viscosity (cm/30s), Color coordinates (L*, a*, a/b) and Firmness (g)), no influence of PENERGETIC treatments was observed.

- The use of PENERGETIC treatments had no significant response on the content of macronutrients and micronutrients in the tomato fruit, although in comparison with the CTAEX thesis, a trend in nitrogen content is observed, higher in the thesis with PENERGETIC products.