Effect of biostimulants on the fruit quality of Golden Glory apple in Chihuahua, Mexico

Efecto de bioestimulantes sobre la calidad del fruto de manzana Golden Glory en Chihuahua, México

Efeito de bioestimulantes na qualidade dos frutos da Golden Glory apple em Chihuahua, México

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Abstract

The cultivation of apple (*Malus domestica* Borkh.) holds significant global importance due to their flavor and nutritional value. In Mexico, Chihuahua leads in production, yet the response of the Golden Glory variety to biostimulants remains unstudied. This study aimed to evaluate the effect of biostimulants on fruit quality in Golden Glory apple. Conducted in Namiquipa, Chihuahua, during the 2023 season, the study employed randomized experimental design with treatments (amino acids, algae, fulvic acids, and a control) spraying weekly from flowering to near harvest. Fruit characteristics were evaluated 90 days post-initial treatment. Amino acids significantly improve fruit weight (27.98 %), diameters (8.41 % polar and 9.28 % equatorial), color (178.8 %), and total soluble solids (TSS;5.72 %), while reducing malic acid content and enhancing TSS/TA ratio by 23.21 %, with no impact firmness. Fruit quality met marketing standards. Seaweed and fulvic acids treatment showed no significant improvement parameters. These results underscore amino acids 'efficacy in enhancing 'Golden Glory' apples quality.

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Resumen

El cultivo de manzano (*Malus domestica* Borkh.) tiene una gran importancia mundial debido a su sabor y valor nutricional. En México, Chihuahua lidera la producción, pero aún no se ha estudiado la respuesta de la variedad Golden Glory a bioestimulantes. Este estudio tuvo como objetivo evaluar el efecto de bioestimulantes sobre la calidad del fruto en manzana cv Golden Glory. El estudio se realizó en Namiquipa, Chihuahua, durante la temporada 2023, mediante un diseño experimental completamente al azar con tratamientos (aminoácidos, algas, ácidos fúlvicos y un control) asperjados semanalmente desde floración hasta cerca de la cosecha. Las características del fruto se evaluaron 90 días después del tratamiento inicial. Los aminoácidos mejoraron significativamente el peso de fruto (27,98 %), el diámetro (8,41 % polar y 9,28 % ecuatorial), el color (178,8 %) y los sólidos solubles totales (SST; 5,72 %), redujeron el contenido de ácido málico y mejoraron la relación SST/AT un 23,21 %, sin afectar la firmeza. La calidad del fruto cumplió con los estándares de comercialización. Los tratamientos con algas marinas y ácidos fúlvicos no mostraron mejoras significativas en la mayoría de los parámetros evaluados. Estos hallazgos destacan la eficacia de los aminoácidos para mejorar la calidad de manzanas ‘Golden Glory’.

Palabras clave: aminoácidos, algas marinas, ácidos fúlvicos, (*Malus domestica* Borkh.).

Resumo

O cultivo da maçã (*Malus domestica* Borkh.) possui significativa importância mundial devido ao seu sabor e valor nutricional. No México, o Chihuahua lidera a produção, mas a resposta da variedade Golden Glory aos bioestimulantes permanece não estudada. Este estudo teve como objetivo avaliar o efeito de bioestimulantes na qualidade dos frutos da maçã Golden Glory. Conduzido em Namiquipa, Chihuahua, durante a temporada de 2023, o estudo empregou um desenho experimental aleatório com tratamentos (aminoácidos, algas, ácidos fúlvicos e um controle) pulverizados semanalmente desde a floração até perto da colheita. As características dos frutos foram avaliadas 90 dias após o tratamento inicial. Os aminoácidos melhoram significativamente o peso dos frutos (27,98 %), diâmetros (8,41 % polares e 9,28 % equatoriais), cor (178,8 %) e sólidos solúveis totais (SST; 5,72 %), ao mesmo tempo que reduzem o teor de ácido málico e melhoram a relação SST/AT em 23,21 %, sem firmeza de impacto. A qualidade dos frutos atendeu aos padrões de comercialização. O tratamento com algas marinhas e ácidos fúlvicos não apresentou parâmetros de melhoria significativos. Estes resultados sublinham a eficácia dos aminoácidos na melhoria da qualidade das maçãs ‘Golden Glory’.

Palavras-chave: aminoácidos, algas marinhas, ácidos fúlvicos, (*Malus domestica* Borkh.).

Introduction

Biostimulants are substances such as humic and fulvic acids, protein hydrolysates, botanical extracts from algae, chitosan, and other biopolymers, as well as microorganisms, which, when applied in small quantities to plants, enhance nutrient efficiency, abiotic stress tolerance, and crop quality, regardless of their nutrient content (Garza-Alonso *et al*., 2022). These effects manifest through morphological, physiological, biochemical, epigenomic, proteomic, and transcriptomic changes (González-Morales *et al*., 2021). Additionally, biostimulants modulate plant metabolism by stimulating the production of plant hormones and growth regulators (Mannino *et al*., 2020).

Biostimulants have gained importance in agriculture due to their ability to improve mineral uptake, potentially reducing the need for chemical fertilizers, decreasing environmental damage, and supporting sustainable agriculture (Kapłan *et al*., 2023). Due to their hormonal, mineral, and phytoregulatory content, they can also mitigate abiotic stress in plants (de Araújo *et al*., 2021) and improve crop quality characteristics (Afonso *et al*., 2022; Almutairi *et al*., 2023).

Biostimulants have been introduced in apple cultivation to regulate physiological processes and improve fruit growth and quality (Kapłan *et al*., 2023). Quality attributes are crucial in fruits as they determine commercial value, market type, and consumer acceptance (Fenili *et al*., 2018). Previous studies in apple have found significant increases in various fruit quality parameters such as weight, length, color, and diameter, as well as an increase in yield with the use of biostimulants (Ayub *et al*., 2019; Kiczorowski, 2019, Di-Vaio *et al*., 2021).

The apple (*Malus domestica* Borkh) is the fourth most consumed fresh fruit worldwide due to its taste and nutritional value (Costa *et al.,* 2022). Mexico is among the top 20 apple-producing countries (SADER, 2023). 2022 Mexico produced 808,906.03 tons of apples, with Chihuahua contributing 85 % of the 31,682 hectares cultivated (SADER, 2023). The Golden Glory variety, notable for its adaptation to Chihuahua’s climate and its fruit’s quality, remains understudied in terms of its response to biostimulants (Molina-Corral *et al.,* 2021).

However, although the benefits of biostimulants on apple fruit quality are known, responses to these products can vary significantly depending on the plant variety due to differences in the physiology and genetics of each variety (Makhaye *et al.,* 2021). Studies have shown that different varieties can respond differently to the application of biostimulants, suggesting that the intrinsic characteristics of each variety influence the effectiveness of these products (Świerczyński *et al*., 2021). Therefore, the objective of the present study is to evaluate the effect of three types of biostimulants: amino acids, seaweed, and fulvic acids on the quality characteristics of the Golden Glory apple cultivar.

Materials and methods

Experimental area

The study was carried out during the 2023 apple tree production cycle in the “Loya” orchard, Namiquipa, Chihuahua (29°6’47”N,107°26’ 7” W;1,828 m asl). The apple trees (*Malus domestica* Borkh), Golden Glory variety grafted on M-11 rootstock, were ten years old, 4 meters tall, planted 2 x 4 m apart, in very friable soils, the characteristics of which are shown in the table 1.

The crop was irrigated using a drip system with irrigation sheets were set at 225 cm distributed in 15 irrigations, edaphic fertilization of 138N-45P-40K and foliar fertilization 110Ca—20Mg, and agricultural practices (mechanical weeds control, pests, and diseases management) as recommended by the Regional Agricultural Union of Fruit Growers of the state of Chihuahua (UNIFRUT, 2023). Daily monitoring of minimum and maximum relative humidity and average temperature in the orchard was also performed (weather station AddWave®, ADCO Telemetry, Kempten Germany) (figure 1).

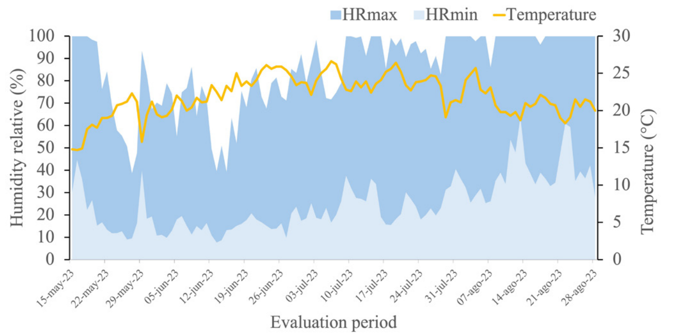

Figure 1. Prevailing climatic conditions during the development of the trial.

The trial was established under a completely randomized experimental design, testing biostimulants based on amino acids (Basfoliar Amino SL®): Free amino acids (glutamic 28.2 %, aspartic 8.8 %, lysine 6.3 %, leucine 6 %, glycine 5.9 %, alanine 5.4 %, arginine 5.2 %, valine 4.3 %, proline 4 %, threonine 3.8 %, isoleucine 3.6 %, histidine 3.4 %, phenylalanine 3.4 %, serine 3.2 %, tyrosine 2.6 %, methionine 2 %, taurine 0.9 %, tryptophan 0.7 %, hydroxyproline 0.5 %), organic matter 19.60 %, total nitrogen 3.1 % w.v^{-1}, nitrogen 3.1 % w.v^{-1} (organic/protein N), P_2O_5 3.5 % w.v^{-1}, K_2O 0.4 % w.v^{-1}, CaO 0.3 % w.v^{-1}, Mg 0.2 % w.v^{-1}, B 7ppm, Zn 6ppm, choline chloride (B9) 100 ppm, niacin (B3) 50 ppm, pantothenic acid (B5) 3 ppm, riboflavin (B2) 2 ppm, vitamin (B12) 122 mg.kg^{-1}), seaweed (Stimulus Maxx®): Seaweed extracts (*Ascophyllum nodosum*) 20.37 % w.v^{-1} total nitrogen 3.77 % w.v^{-1}, urea nitrogen 3.77 % w.v^{-1}, P_2O_5 1.73 % w.v^{-1}, K_2O 4.81 % w.v^{-1}, B 153 ppm, Cu EDTA 40.4 ppm, Fe EDTA 326 ppm, Mn 297 ppm, Mo 8.93 ppm, Zn EDTA 138 ppm), and fulvic acids (K-tionic®): Carbon from total humic extract 100 g.L^{-1} (Carbon from humic acids 10 %, carbon from fulvic acids 90 %), total nitrogen 6 g.L^{-1}, urea nitrogen 6 g.L^{-1}, K_2O 38 g.L^{-1}, and water as a control. Each treatment had ten repetitions (one tree per experimental unit), applied at 10 mL.L^{-1} of water weekly from full flowering to one week before harvest. Treatments were sprayed using a precision turbine agricultural sprinkler (Swissmex®, model 840001, MNX) at 6:00 a.m.

Table 1. Physicochemical properties of the experiment soil.

Parameters	Depth (cm)
	0-60
Sand	56
Silt	19
Clay	25
Textura name	Flat/ sandy crumb
pH	7.03
EC (mmhos.cm^{-1})	0.90
Organic matter	0.86
N-NO_3 (kg.ha^{-1})	108.75
P (kg.ha^{-1})	25.70
K (mg.kg^{-1})	562.70
Ca (mg.kg^{-1})	2262.50
Mg (mg.kg^{-1})	337.50
Fe (mg.kg^{-1})	0.48
Mn (mg.kg^{-1})	1.20
Zn (mg.kg^{-1})	3.38
Cu (mg.kg^{-1})	0.78

Parameters evaluated

The fruits were collected at maturity, determined by sampling untreated trees to assess average commercial size (mid-August). Ten fruits were randomly selected from the middle part of the tree.

Physical characteristics of the fruit

The weight was determined using a digital scale (OHAUS, model E01140, New Jersey, USA), and equatorial and polar diameters were measured with a vernier (Starret ®, EC799A-6/150, Massachusetts, USA). The fruit shape index (FSI) was calculated using the following formula (Jemrić *et al*., 2013).

$$FSI = \frac{Ecuatorial\ diameter}{Polar\ diameter}$$

Fruit firmness (lb.in^{-2}) was determined with a manual penetrometer (Bishop®, FT 327, Alfonsine, Italy) with a punch diameter of 11.3 mm, measuring at the equator of the fruits (Magness and Taylor, 1925). Fruit color was determined with the scale proposed by Hernández *et al*. (1999): 1) green epidermis; 2) rough green epidermis with rough lenticels; 3) waxy green epidermis; 4) epidermis with transition to yellow color; 5) whitish yellow epidermis (yellowish) and 6) yellow epidermis with a tendency towards orange.

The result was expressed as a percentage using the following formula:

$$Color\ (\%) = \left[\frac{\frac{C_1 + C_2}{2}}{6}\right] * 100$$

Where: C1= equatorial zone color (observation 1), C2= equatorial zone color (observation 2), 6= number of categories of the color scale.

Chemical characteristics of the fruit

For the evaluation of total soluble solids (TSS), extraction juice was from each fruit, and a drop was placed in the cell of a refractometer (ATAGO®, Pallete Model PR-32α 0- 32 %, USA) calibrated with distilled water. The results were expressed in degrees Brix (°Brix).

For titratable acidity (TA): 10 mL of apple juice was placed in a flask, and three drops of 1 % phenolphthalein were added. The titration was carried out with 0.1 N sodium hydroxide until pink. The result was expressed as a percentage of malic acid using the equation (Association of Official Agricultural Chemists [AOAC], 1990).

$$TA\ (\%\ malic\ acid) = \left[Volume_{\ NaOH\ (ml)} x Normality_{NaOH\ (\frac{meq}{ml})} x \frac{0.067 \frac{g}{meq}}{Juice\ volume_{\ (ml)}}\right] x100$$

The TSS/TA ratio was calculated by dividing the total soluble solids by the malic acid content (De Bruyn *et al*., 1971).

Statistical analysis

The physical and chemical variables of apple fruits were evaluated for normality using Shapiro-Wilk tests and for homoscedasticity using Levine's test. ANOVA and Tukey's test ($p<0.05$) or Kruskal-Wallis/Conover-Iman test (Color, TSS, TA, TSS/TA) ($p<0.05$) were conducted. Principal component analysis (PCA) was performed after Bartlett's test (χ^2 907, $p<0.01$) to assess treatment on fruit quality. Data were processed using the Info Stat (Info Stat 2021v. Grupo Info Stat, Argentina) and JAMOVI 2.5.2.0.

Results and discussion

The application of biostimulants had a notable impact on fruit quality parameters in apple cv Golden Glory, mainly using amino acids. Amino acids treatment significantly increased fruit weight by

27.98 % compared to the control, with an average weight of 167.27 ± 0.03 g (figure 2a). This increase is higher than Arabloo et al. (2017) and Kiczorowski et al. (2019) for other apple cultivars reported.

In contrast, seaweed and fulvic acids did not show significant differences compared to the control, with averages of 147.91 ± 0.03 and 141.53 ± 0.03 $g.fruit^{-1}$, respectively.

The enhanced fruit weight whit amino acids may be attributed to their role in preventing protein degradation and participating in the biosynthesis of non-protein nitrogenous compounds (Bulgari *et al*., 2019; Francesca *et al*., 2020), leading to the accumulation of soluble compounds and increased fruit weight (Puglisi *et al.*, 2020). Amino acids can act as signaling molecules, promoting the synthesis of phytohormones like auxins and cytokinins, which are crucial for cell division and growth (Francesca *et al*., 2020; Kuchay *et al*., 2021).

Regarding fruit dimensions, the equatorial and polar diameters increased by 8.41 % and 9.28 %, respectively, with amino acid treatment, averaging 71.9 ± 0.96 mm and 66.1 ± 0.88 mm (figure 2b). Seaweed and fulvic acids treatments did not show significant differences in these parameters. The spherical shape of the fruits, indicated by a consistent shape index of 0.91, was maintained across all treatments, which is representative of this variety (figure 2c). This improvement in fruit size aligns with findings by Gonçalves *et al*. (2020) and Khan *et al*. (2012), who reported size increases in cherry and grapes with amino acids and seaweed. Amino acids enhance size by improving nitrogen assimilation and promoting cell division through phytohormones signaling (Francesca *et al*., 2020; Kuchay *et al*., 2021).

Fruit firmness did not show significant differences between treatments, averaging 11.0 $lb.in^{-2}$ ± 1.10 per fruit (figure 2d). Although there were no statistical differences between treatments, fruit firmness remained within the range indicated by NMX-FF-061-SCFI-2023, which is >10 lb for green apple varieties. Firmness is a critical quality parameter for determining harvest timing, packaging, transportation, and storage conditions (Wang *et al*., 2023). These results are consistent with Lobo *et al*. (2019) and Di-Vaio *et al.* (2021), who also found no significant effects on firmness with similar treatments.

Color intensity increased significantly with amino acids and seaweed treatments, showing an increase of 178.8 %, with an average value of 84.37 % ± 6.3 (figure 2e). Fulvic acids did not show significant differences compared to the control. The enhanced color may result from increased pigment production, such as carotenoids, which are influenced by amino acid and seaweed extract (Puglisi *et al*., 2020; Fernández-Cancelo *et al*., 2021). Similar effects were reported by Basak (2008) in apples and Cozzolino *et al*. (2021) in tomatoes.

Total soluble solids (TSS) content was highest in the amino acids treatment, increasing by 5.72 % compared to the control, reaching 13.85 °Brix (figure 3a). Fulvic acids also increased TSS, although to a lesser extent (4.58 %), while seaweed treatment showed not significant difference.

SST is crucial for fruit palatability and maturity influencing consumer preference (Yang *et al*., 2020). The increase in TSS may be due to the enhancement of photosynthetic rates and upregulation of carbohydrate metabolism genes, which increases sugar content in fruits (Shehata and Abdelgawad, 2019; Malik *et al*., 2022; Liava *et al*., 2023).

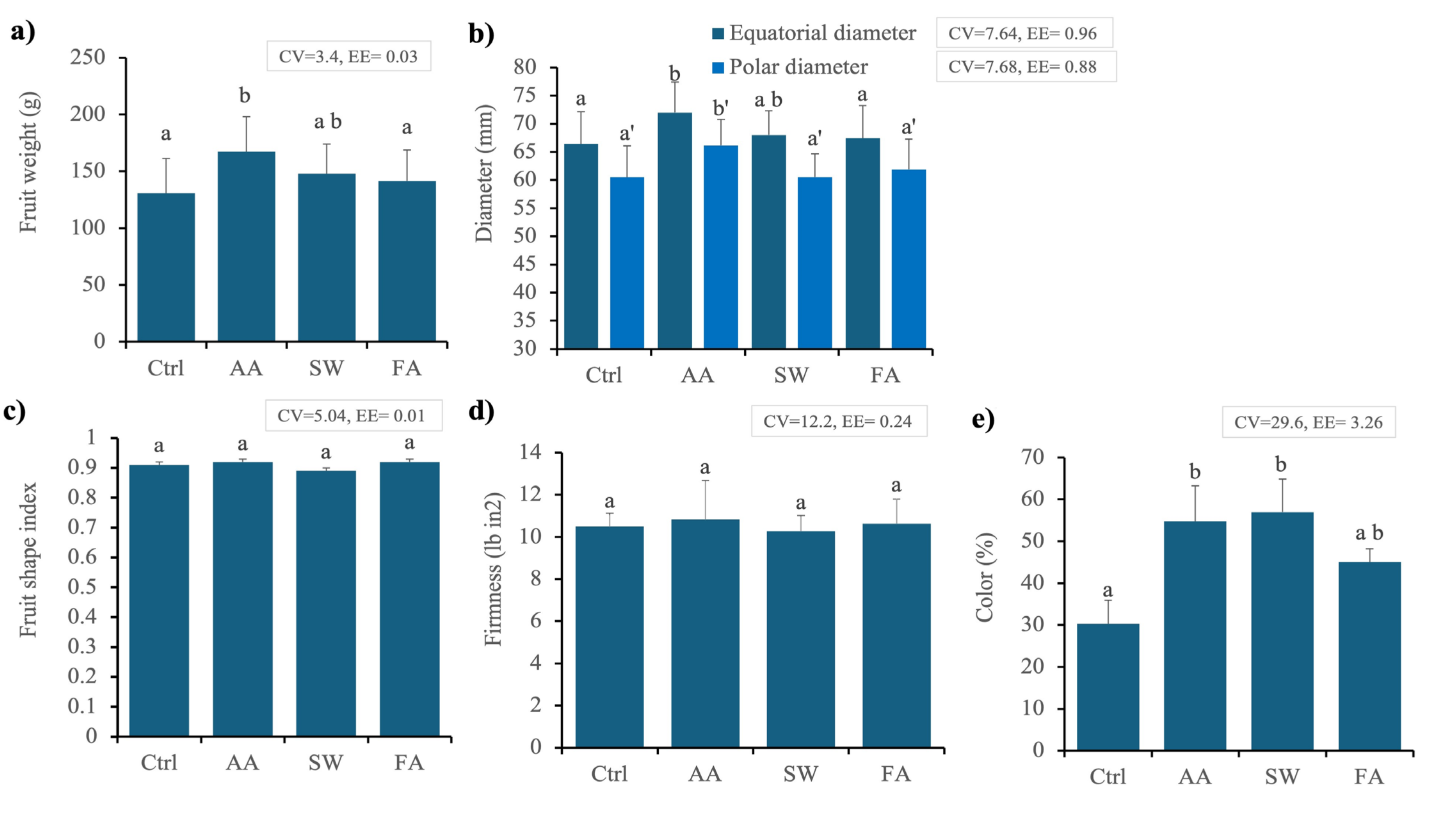

Figure 2. Physical properties of cv Golden Glory apples treated with biostimulants for 90 days during fruit development. Ctrl=control, AA= amino acids, SW=seaweed, FA= fulvic acid. Bars with same letters show no significant differences ($p<0.05$, Tukey test or Kruskal-Wallis Conover-Iman[1]).

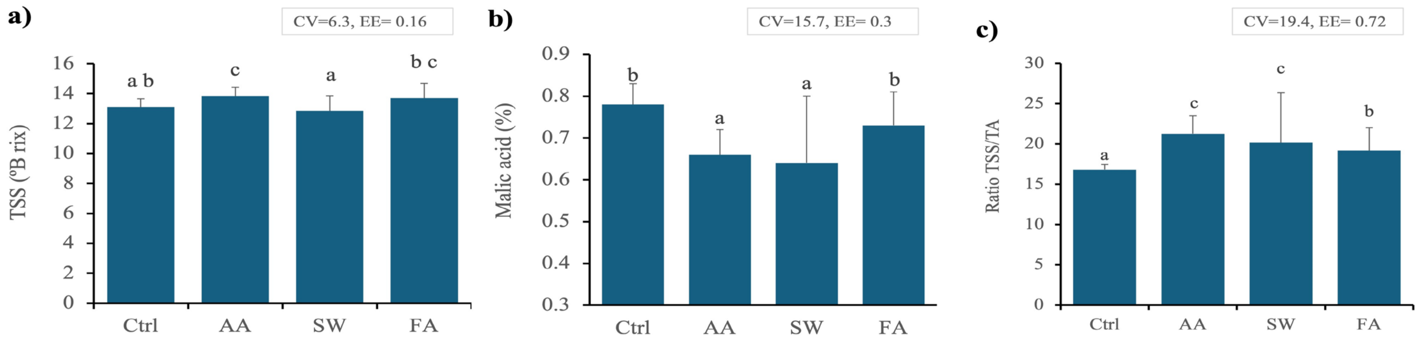

Figure 3. Chemical properties of cv Golden Glory apples treated with biostimulants for 90 days. Ctrl=control, AA= amino acid, SW=seaweed, FA= fulvic acid. Bars with same letters show no significant differences (p<0.05, Kruskal-Wallis Conover-Iman test).

The malic acid content or titratable acidity (TA) decreased significantly with amino acid and seaweed treatments, reducing by up to 16.66 %, with an average of 0.65 % malic acid (figure 3b), but values remained within the NMX-F-045-1982 for apples (30-60 g of malic acid per 100 g). The decreased aligns with findings by Di-Vaio *et al*. (2021) and Yao *et al*. (2023), who reported reductions in TA with similar treatments. The decrease in TA may be due to the enhanced metabolism and utilization of organic acids for energy and growth, influenced by biostimulants (Soppelsa *et al*., 2018).

The SST/TA ratio improved significantly with amino acid and seaweed treatments, by 23.21%, reaching 20.70 ± 4.21 (figure 3c). Fulvic acids treatment also significantly improved the TSS/TA ratio by 14.22 % compared to the control, reaching 19.19 ± 2.81. This ratio is critical for sensory quality consumer acceptance, with the optimal range being 15-38 for apples (Huang et al., 2018). The improvement in the TSS/TA ratio aligns with findings of Mosa et al. (2023).

Principal component analysis (PCA) highlighted the effects of biostimulants on the analyzed parameters. The first two components (PCs) explained 89.9 % of the variance, with PC1 at 65.5 % and PC2 at 24.4 % (figure 4).

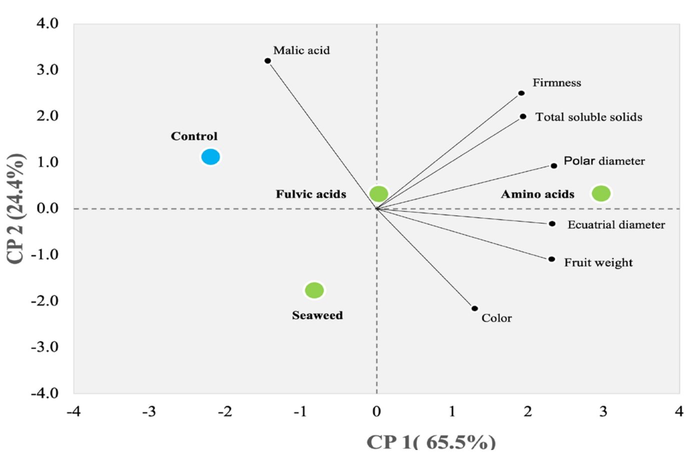

Figure 4. Analysis of principal components of characteristics of cv Golden Glory apples fruits treated with biostimulants for 90 days during fruit development.

Amino acids treatment was positively associated with firmness, TSS, polar diameter, equatorial diameter, weight, and color, showing a significant and favorable influence. Seaweed treatment and the control had different effects, located in opposite quadrants, suggesting different impacts on the variables studied. Fulvic acids did not show a strong correlation with any specific variable, indicating a neutral effect. Together, these results highlight the effectiveness of amino acids in improving multiple quality parameters, while the rest of the treatments have varied effects.

The results of this study reveal that the use of biostimulants based on amino acids, seaweed, and fulvic acids promotes to a greater extent the quality characteristics of the Golden Glory apple, serving as a tool for decision-making in the management of apple orchards.

Conclusions

The effect of biostimulants on fruit quality in apple cv Golden Glory showed differences, mainly with amino acids. The amino acids treatment improves various fruit quality parameters, such weight, polar and equatorial diameters, color, and total soluble solids, compared to untreated trees. They also reduced malic acid content and improved the TSS/TA ratio without negatively affecting the firmness of the fruit. In contrast, seaweed and fulvic acids treatments did not significantly improve most of the evaluated parameters. These findings highlight the effectiveness of amino acids as biostimulants in enhancing the quality and commercial value of Golden Glory apples.

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