
















Circular bioeconomy in strawberry cultivation: phytochemicals in fruits and leaves, and yield potential of seven genotypes

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ABSTRACT: Seven strawberry genotypes were analyzed in order to differentiate fruit production and quality. The discarded leaves (by-product) were also used to characterize their phytochemical profile with a view to potential use in the pharmaceutical and agricultural industries. The multivariate analysis gave rise to heterogeneous groups among the seven genotypes. ‘Fronteras’ and ‘Monterey’ were the most productive. ‘Camino Real’ produced fruits with the highest polyphenols concentrations. ‘Fronteras’ produced leaves with the best phytochemical profile and, together with ‘Camino Real’, the highest antioxidant activity. The genotypes differ in terms of the yield and fruit, and leaf phytochemical profiles. ‘Camino Real’ can be used to optimize and market all the organs produced (fruits and leaves) and ‘Fronteras’ to increase the fruit yield and the use of leaves as a cultivation by-product.

Key words: *Fragaria X ananassa* Duch., substrate, greenhouse, biomolecules, by-product.

INTRODUCTION

Strawberry (*Fragaria X ananassa* Duch.) consumption has increased worldwide, since they are known as rich source of fibers, vitamins, phytochemicals, and other bioactive compounds. This search for foods rich in biomolecules with health-promoting activities, together with the choice of genotypes adapted to the agroecosystems in which they are grown, reinforces the development of studies linked to the yield and phytochemical profile of strawberries (Chiomento et al. 2023a).

After establishing the planting stock with genotypes that meet the production and fruit quality requirements of the consumer market, producers have incorporated management into strawberry cultivation to help reduce production costs, such as keeping the same plants for more than one production cycle (Chiomento et al. 2023b). In the Brazilian subtropics, the management adopted to keep the same plants from one cycle to the next corresponds to renewal pruning, carried out in mid-autumn (from February to March). Among the types of pruning carried out, there is drastic pruning, which consists of removing all the leaves. Approximately 50 days after this drastic pruning, the plants begin their new reproductive cycle.



In commercial strawberry plantations, the leaves removed during pruning are mostly not used. Considering the circular bioeconomy concept (Tan and Lamers 2021), the possibility arises of using this plant organ as a cultivation by-product, since millions of tons of leaves are discarded annually (Villamil-Galindo et al. 2021). For example, the leaves could be destined for the pharmaceutical and agribusiness industries as a new source of income for producers. However, in order to understand the potential uses of the leaves and destine them for the industrial sector, it is important to characterize their phytochemical profile, since these secondary metabolites determine the effects of the new product from this plant organ transformation.

Substances already detected in strawberry leaves include caffeic acid, chlorogenic acid, cinnamic acid, ellagic acid, ferulic acid, fupenzic acid, galic acid, *p*-coumaric acid, polnolic acid, sericic acid, sinapic acid, syringic acid, vanillic acid, catechin, epicatechin, rutin, stigmasterol, and taxifolin (Cvetković et al. 2017, Akšić et al. 2019, El-Hawary et al. 2021, Chiomento et al. 2022). Despite this leaf phytochemical profile characterization, there are few studies that research, in conjunction, other end paths to plants in commercial cultivation, such as production potential and fruit quality. This holistic view can help in the choice of genotypes that will form part of the establishment of the breeding stock with a view to the circular bioeconomy of strawberry production systems.

Therefore, here we investigated whether strawberry production and the phytochemical profile of fruits and leaves differ among seven genotypes in substrate and greenhouse conditions in southern Brazil. In addition, to understand the relationship among the seven genotypes studied and the attributes analyzed, we explored the data using multivariate analysis. Our findings will allow us to strengthen links between producers, who will be able to establish their crops with genotypes rich in biomolecules and use all the plant organs as by-products for industry, depending on the purpose.

MATERIAL AND METHODS

Plant material

Bare-root daughter plants of seven strawberry genotypes from the Chilean Patagonia nursery (33°50'15.41"S; 70°40'03.06"W) were used in this study. Cultivation took place in Passo Fundo (28°15'41"S; 52°24'45"W), Rio Grande do Sul, Brazil, from May (fall) 2021 to February (summer) 2022, in a 430-m² greenhouse, installed in a northwest-southeast direction, with a semicircular roof and covered with low-density polyethylene film (150- μ m thickness and anti-ultraviolet additive).

Experimental design

Seven strawberry genotypes ('Albion', 'Aromas', 'Camino Real', 'Fronteras', 'Monterey', 'Portola', and 'San Andreas') were arranged in a randomized block design with three replications. Each experimental plot (0.50 m²) consisted of six plants. The experiment had 21 plots (7 treatments \times 3 replications) and 126 plants (21 plots \times 6 plants; $n = 126$).

Regarding flowering, we used two genotypes classified as short-day (SD) ('Camino Real' and 'Fronteras'), four genotypes of neutral day (ND) ('Albion', 'Aromas', 'Monterey', and 'San Andreas'), and one genotype classified as a summer plant ('Portola').

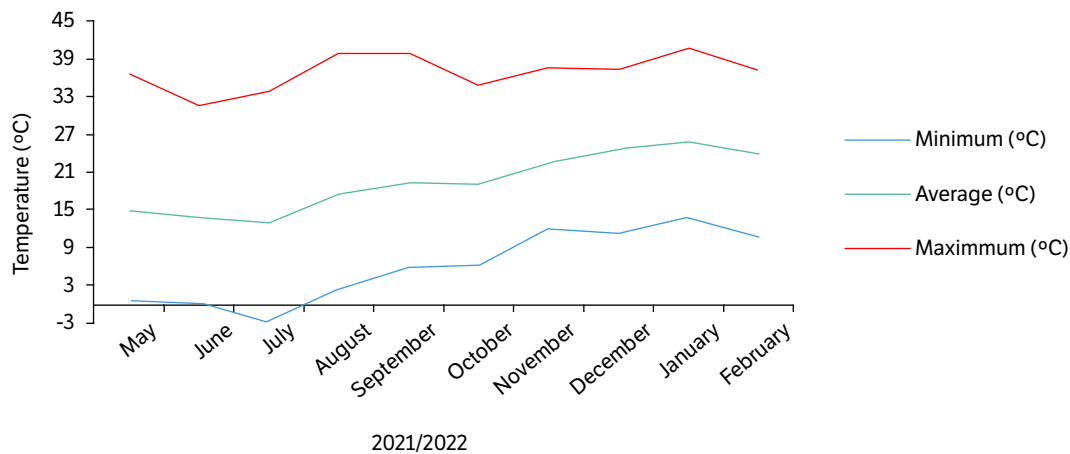
Procedures

The daughter plants were transplanted from May to July (winter) 2021 into containers (1-m long and 0.5-m wide) containing the Dallemole grow substrate, made up of pine bark, rice husk, rice ash, and class A organic compost. The daughter plants were spaced 0.17 m apart and arranged in a planting line. The substrate's physical and chemical characterization is shown in Table 1.

The irrigation used in the experiment was localized (1.41 L·h⁻¹ per dripper), with the system activated seven times a day. Nutrient solutions were supplied to the plants on a weekly basis. Along with the nutrient solution, 80 mL·L H₂O⁻¹ of Vit-Org (organo-mineral fertilizer) was added, containing humic substances, amino acids, and glycine-betaine (quaternary ammonium compound). The air temperature was monitored during the experiment using a mini-weather station installed inside the greenhouse (Fig. 1).

Table 1. Physical and chemical properties of the Dalle mole grow substrate.

| Physical properties | | | | | | |
|-----------------------|-------------------------------|------------------|------------------------------------|--------------|-----------------------|-----------|
| Density | Total porosity | Aeration space | Easily available water | Buffer water | Remaining water | |
| (kg·m ⁻³) | | | (m ³ ·m ⁻³) | | | |
| 212 | 0.885 | 0.502 | 0.144 | 0.017 | 0.222 | |
| Chemical properties | | | | | | |
| Nitrogen | P ₂ O ₅ | K ₂ O | Organic carbon | pH | Electric conductivity | C/N ratio |
| | % (m·m ⁻¹) | | | | mS·cm ⁻¹ | |
| 0.82 | 0.58 | < 0.25 | 26.10 | 7.6 | 1.05 | 33.42 |

**Figure 1.** Temperatures recorded in the cultivation environment during the experiment.

Strawberry production

Between August (winter) 2021 and January (summer) 2022, with approximately nine monthly harvests, we assessed the total number of fruits per plant (TNF, number per plant) and the total fruit production per plant (TP, g per plant), based on commercial ripeness ($\geq 85\%$ reddish visual color). The fruit was weighed, and the average fresh fruit mass (AFFM, g) was determined using the ratio between TP and TNF.

Fruit phytochemical composition

At the peak of fruit ripeness, in November (spring) 2021, 100 g of fruit from each repetition were used for analysis of total anthocyanins (TAN), total flavonoids (TFL), and total polyphenols (TPO). The fruit was analyzed in fresh mass, which is the form in which it is used for consumption.

TAN was carried out by pH differential (Giusti and Wrolstad 2001). Aliquots of extract were diluted in aqueous buffers pH 1 and 4.5, and readings were taken at 510 and 700 nm by spectrophotometry (PerkinElmer Lambda 20, Perkin Elmer). The TAN content was determined using Eq. 1.

$$A = (A_{\lambda_{\text{vis}} - \text{max}(510)} - A_{700})_{\text{pH } 1.0} - (A_{\lambda_{\text{vis}} - \text{max}(510)} - A_{700})_{\text{pH } 4.5} \quad (1)$$

Equation 2 was used to calculate the monomeric anthocyanin concentration.

$$\text{TAN} = \frac{A \times \text{MW} \times \text{DF} \times 100}{\epsilon \times l} \quad (2)$$

where: MW: molecular weight of pelargonidin-3-*O*-glucoside (433.20); DF: dilution factor; ϵ : molar absorptivity coefficient (25.660).

The results were expressed in mg of pelargonidin-3-*O*-glucoside equivalent per 100 g of fresh fruit (mg PE/100 g FF⁻¹).

TFL was carried out according to Basílio et al. (2022), and the results were expressed in mg of rutin equivalent per 100 g of fresh fruit (mg RE/100 g FF⁻¹).

TPC was determined using the Folin-Ciocalteu reagent (Singleton and Rossi 1965). Absorbance readings were taken at 760 nm, and the results were calculated using a standard curve with gallic acid and expressed in mg of gallic acid equivalent per 100 g of fresh fruit (mg GAE/100 g FF⁻¹).

Leaf phytochemical profile

In February (summer) 2022, while the plants were being pruned for the next production cycle, 100 g of fresh leaves were collected and sanitized in a 1% sodium hypochlorite solution. After soaking for 10 min, the leaves were washed with distilled water and dried on paper. The leaves were dried in an oven (40°C until constant weight), finely ground (A11 IKA mill), and stored in sealed containers in a -80°C freezer. The leaves were analyzed in dry mass, which is the form in which they can be used as a by-product.

The content of total phenols (TPH) and flavonoids (FLA) was described in the item “Fruit phytochemical composition”. In addition, antioxidant activities were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP).

The antioxidant activity measured using DPPH was carried out as described in Brand-Williams et al. (1995), and, after 30 min, the reading was carried out in a spectrophotometer (517 nm), and the percentage (%) of free radical scavenging was determined. The determination of FRAP was performed according to methods described by Benzie and Strain (1996) on samples homogenized in acidified methanol, and, after reading the absorbance at 595 nm, the results were calculated using the iron (II) sulphate (FeSO₄) standard curve and expressed in mmol of FeSO₄ per kg of dry leaves (mmol FeSO₄/kg DL⁻¹).

The polyphenol profile was carried out after extracting the samples in MeOH and filtering (Millipore 0.22- μ m filter), according to Natividade et al. (2013), with modifications. Extracts were injected (20 μ L) into a high-performance liquid chromatography (HPLC) system (UltiMate 3000RS, Dionex-Thermo Fisher Scientific, San Jose, CA, United States of America), coupled to a diode array detector and a C18 column (2.0 \times 50 mm, Luna C18 HST 2.5 μ m; Phenomenex, Torrance, CA, United States of America), with a 0.7-mL·min⁻¹ flow rate at 39°C. Phenolic compounds were identified by comparing retention times and ultraviolet spectra with commercial standards, quantified using a standard curve, measured at 280 (3-hydroxytyrosol and catechin) and 360 nm (rutin). All results were expressed in μ g of the substance per g of dry leaves (μ g/g DL⁻¹).

Data analysis

The data was subjected to analysis of variance, and the means of the treatments were compared using the Tukey's test, at a 5% probability of error ($p \leq 0.05$), with the aid of the CoStat program. The data was also subjected to principal component analysis (PCA) and hierarchical clustering (XLSTAT software, version 2020; Addinsoft, France). Both were carried out after standardizing the production and phytochemical attributes (leaves and fruit), each with a mean of 0 and variance of 1.

RESULTS AND DISCUSSION

Strawberry production

'Fronteras' and 'Albion' had higher AFFM (Table 2). Although 'Aromas' had the highest number of strawberries per plant, this genotype was not the most productive (Table 2), compared to 'Fronteras' and 'Monterey', with the highest TP.

Table 2. Fruit production of seven strawberry genotypes*.

| Genotypes | Average fresh fruit mass (g) | Total number of fruits (number per plant) | Total production (g per plant) |
|------------------------------|------------------------------|---|--------------------------------|
| 'Albion' | 10.56 ± 0.78 a | 25.33 ± 3.72 bc | 265.78 ± 24.19 ab |
| 'Aromas' | 08.26 ± 0.19 c | 36.79 ± 2.74 a | 303.73 ± 16.16 ab |
| 'Camino Real' | 10.25 ± 0.79 ab | 24.87 ± 3.75 bc | 253.68 ± 28.32 ab |
| 'Fronteras' | 11.70 ± 0.53 a | 27.16 ± 3.34 bc | 318.96 ± 49.74 a |
| 'Monterey' | 10.44 ± 0.71 ab | 31.20 ± 2.10 ab | 325.37 ± 16.39 a |
| 'Portola' | 09.86 ± 0.76 bc | 29.62 ± 3.90 abc | 288.58 ± 56.52 ab |
| 'San Andreas' | 10.33 ± 1.10 ab | 22.79 ± 1.85 c | 234.08 ± 17.34 b |
| Mean | 10.18 | 28.25 | 284.31 |
| Coefficient of variation (%) | 7.79 | 11.48 | 12.08 |

*Data was presented as mean ± standard deviation. Means followed by the same letter in the column do not differ between each other by Tukey's test ($p \leq 0.05$).

Figure 2 shows, in detail, the monthly values obtained referring to the productive performance of the seven strawberry genotypes. The productivity peaks were genotype-dependent and occurred in December ('Aromas', 'Camino Real', and 'Fronteras') and January ('Albion', 'Monterey', 'Portola', and 'San Andreas').

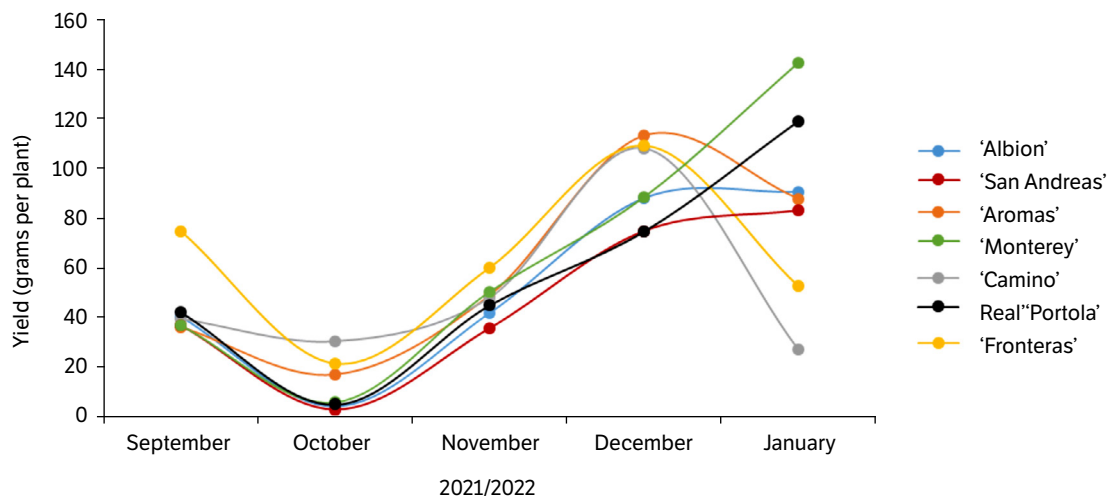
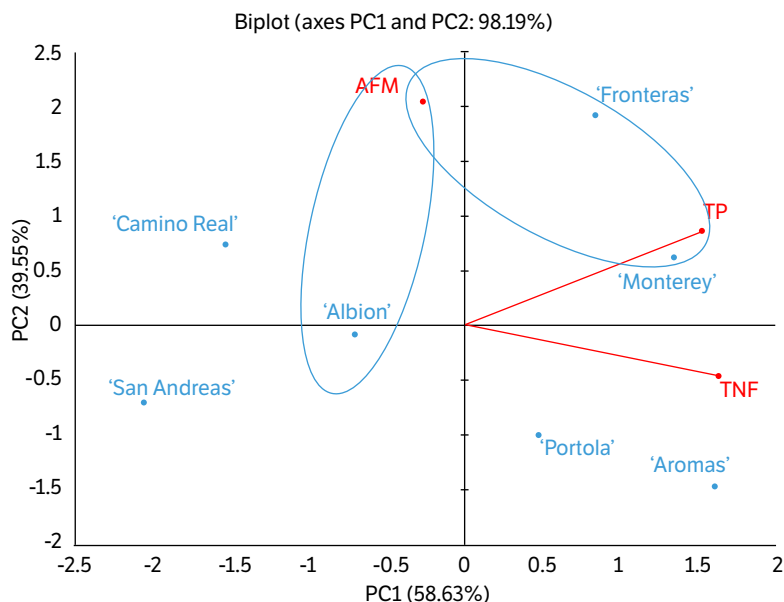


Figure 2. Fruit production per month (2021-2022) of seven strawberry genotypes in southern Brazil, grown in a greenhouse.

To establish a descriptive model for grouping samples based on yield results (AFFM, TP, and TNF), the PCA was applied to the data set. PC1 and PC2 accounted for 98.19% of the variation in the data (Fig. 3). 'Fronteras' and 'Monterey' were the most productive and were grouped together in PC1+ (Fig. 3). 'San Andreas', on the other hand, had the lowest productive potential (Table 2) and was grouped into PC1- and PC2- (Fig. 3). Although 'Albion' had the highest AFFM, it did not stand out in productivity terms, as also described by Paparozzi et al. (2018). Although this genotype showed high levels of anthocyanins,

together with ‘Portola’ and ‘San Andreas’, it had the lowest levels of total phenolic compounds and total flavonoids. These results initially showed that ‘Fronteras’ is the most suitable genotype for planting, with a view to fruit productivity and financial return.



TNF: total number of fruits; TP: total production; AFFM: average fresh fruit mass.

Figure 3. Two-dimensional projection and score resulting from cluster analysis of seven strawberry genotypes fruit production.

Fruit phytochemical composition

Although ‘Fronteras’ had good results in productivity, ‘Albion’, ‘Aromas’, and ‘Camino Real’ produced fruit with higher TAN (Table 3). ‘Camino Real’ stood out for producing higher TFL and TPO. This genotype contains 35% more TFL than ‘Portola’ (50.86 mg RE/100 g FF⁻¹), but it does not differ from ‘Fronteras’ in relation to TPO (Table 3).

Table 3. Phytochemical composition of fruits of seven strawberry genotypes*.

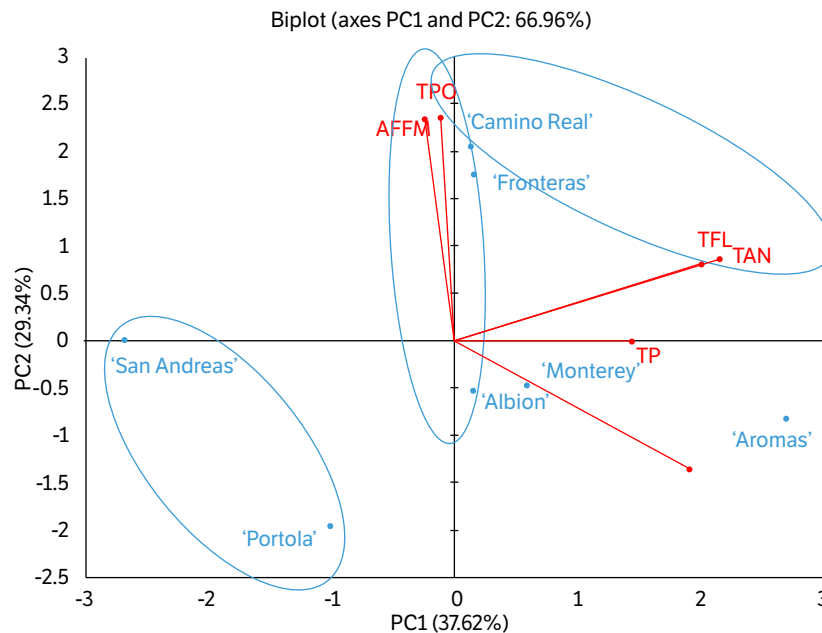
| Genotypes | Total anthocyanins (mg PE/100 g FF ⁻¹) | Total flavonoids (mg RE/100 g FF ⁻¹) | Total polyphenols (mg GAE/100 g FF ⁻¹) |
|------------------------------|--|--|--|
| ‘Albion’ | 4.57 ± 0.66 a | 71.51 ± 5.10 ab | 881.52 ± 93.29 d |
| ‘Aromas’ | 5.33 ± 0.67 a | 75.91 ± 9.26 ab | 1,089.56 ± 71.59 bc |
| ‘Camino Real’ | 5.15 ± 0.32 a | 77.67 ± 8.29 a | 1,251.29 ± 97.38 a |
| ‘Fronteras’ | 3.13 ± 0.54 bc | 52.62 ± 9.29 ab | 1,243.93 ± 48.18 a |
| ‘Monterey’ | 3.23 ± 0.12 b | 66.68 ± 5.42 ab | 1,061.49 ± 37.98 bc |
| ‘Portola’ | 3.01 ± 0.28 bc | 50.86 ± 9.07 b | 987.47 ± 42.32 cd |
| ‘San Andreas’ | 2.05 ± 0.37 c | 51.74 ± 9.97 ab | 1,147.53 ± 40.78 ab |
| Mean | 3.78 | 63.85 | 1,094.68 |
| Coefficient of variation (%) | 13.12 | 17.50 | 6.03 |

*Data was presented as mean ± standard deviation. Means followed by the same letter in the column do not differ between each other by Tukey’s test ($p \leq 0.05$).

Berries are known to be sources of polyphenols. Both ‘Camino Real’ and ‘Fronteras’ showed different results in terms of polyphenol content, including anthocyanins, which are described as having important health properties, mainly because they are able to cross the blood-brain barrier (Kalt et al. 2008), despite their low bioavailability compared to other polyphenols (Manach et al. 2005). However, the values found (Table 3) were lower than those described by Marhuenda et al. (2016) in a study of commercial strawberries. These differences found between our results and those described in the literature are mainly due to the genotypes, but other factors can influence them, such as cultivation, climate, conduction, and harvest

point. Strawberries are widely consumed not only for their taste, but also for their nutritional characteristics, including bioactive compounds with properties to scavenging free radicals by decreasing their production, which influences the action of metabolites, cell survivor, and the defense mechanism (Giampieri et al. 2015). In this way, choosing a genotype may be more interesting for consumption, as found in the results for 'Fronteras' and 'Camino Real'.

Using the yield (Table 2) and antioxidant compound data from the fruit (Table 3), a PCA was applied and explained 66.96% of the data variance (Fig. 4). 'San Andreas' and 'Portola' were grouped into PC1- and PC2-, because they had lower antioxidant compound content (Fig. 4, Table 3), as well as lower yield values (AFFM, TP, and TNF). The other genotypes were grouped into PC1+ and divided into PC2+ ('Camino Real' and 'Fronteras') and PC2- ('Monterey', 'Albion', and 'Aromas'), according to TPO content and yield performance (Fig. 4).



TNF: total number of fruits; TP: total production; AFFM: average fresh fruit mass; TAN: total anthocyanins; TFL: total flavonoids; TPO: total polyphenols.

Figure 4. Two-dimensional projection and score resulting from cluster analysis of fruit production and phytochemical composition from seven strawberry genotypes.

Leaf phytochemical profile

According to our data, after drying and grinding, these leaves of strawberry could be used in the industry (cosmetics, pharmaceuticals, food, among others) as an antioxidant source. 'Fronteras' and 'Camino Real' produced leaves with the highest levels of total phenols and antioxidant activity via DPPH (Table 4). In both genotypes, the content of total phenols and antioxidant activity measured via DPPH was close to that described by Lin et al. (2020) in strawberry leaves. Although 'Camino Real' also showed higher activity measured via FRAP, this genotype did not show the best results in relation to the (poly)phenolic profile (Table 4). The FLA content did not differ among the genotypes and ranged from 555.41 ('Aromas') to 382.60 mg RE/100 g DL⁻¹ ('Fronteras') (Table 4). Although these values were lower than those reported by El-Hawary et al. (2021), both the cultivation and the genotypes, as well as the extraction method, may have influenced them, but they are important indications of the leaf phytotherapeutic potential, as described by the authors as possible anti-hyperglycemic products.

Analysis of the phenolic profile showed the presence of higher levels of 3-hydroxytyrosol, catechin, and rutin in 'Fronteras' leaves (Table 4), compounds with free radical scavenger properties and important phytotherapeutic properties. The 3-hydroxytyrosol, a phenylethanoid, has been described to inhibit the growth of disease-inducing fungi and bacteria (Diallinas et al. 2018), and is described to occur at higher levels in olives (fruits and leaves). Although the content in

strawberry leaves is lower than that described in olive trees, the identification of this compound could be an indication of its possible use in biotechnological processes, including pharmacological ones. Furthermore, we found no reports on the detection of this phenylethanoid in strawberry plants. Thus, the content of 3-hydroxytyrosol in strawberry leaves is described here for the first time in the literature.

Table 4. Phytochemical profile of leaves of seven strawberry genotypes*.

| Genotypes | DPPH (%) | Ferric reducing antioxidant power (mM FeSO ₄ /kg DL ⁻¹) | Total phenols (mg AGE/100 g DL ⁻¹) | Flavonoids (mg RE/100 g DL ⁻¹) | 3-hydroxytyrosol (µg/g DL ⁻¹) | Catechin (µg/g DL ⁻¹) | Rutin (µg/g DL ⁻¹) |
|------------------------------|----------------|--|--|--|---|-----------------------------------|--------------------------------|
| 'Albion' | 76.21 ± 1.27 c | 164.14 ± 3.13 d | 5,743.00 ± 39.05 e | 551.52 ± 1760 ns | 708 ± 0.09 f | 97.80 ± 1.43 f | 274.29 ± 3.09 f |
| 'Aromas' | 82.55 ± 1.11 b | 175.23 ± 6.83 d | 7,271.88 ± 41.60 c | 555.41 ± 13.64 | 12.68 ± 0.31 b | 176.21 ± 5.70 b | 381.00 ± 5.73 c |
| 'Camino Real' | 93.58 ± 0.25 a | 281.49 ± 4.48 a | 10,098.90 ± 43.47 a | 484.77 ± 12.24 | 12.93 ± 0.57 b | 107.23 ± 3.75 e | 352.40 ± 1.68 d |
| 'Fronteras' | 90.86 ± 1.54 a | 260.47 ± 4.73 b | 9,943.55 ± 35.95 a | 382.60 ± 84.86 | 15.41 ± 0.02 a | 280.02 ± 2.47 a | 520.25 ± 3.35 a |
| 'Monterey' | 78.60 ± 1.79 c | 164.95 ± 0.74 d | 6,571.73 ± 73.37 d | 459.63 ± 13.50 | 10.66 ± 0.04 d | 110.39 ± 0.71 d | 402.32 ± 4.38 b |
| 'Portola' | 85.10 ± 0.38 b | 199.76 ± 9.26 c | 8,058.01 ± 20.62 b | 480.78 ± 13.82 | 8.89 ± 0.03 e | 116.11 ± 1.14 d | 337.16 ± 7.69 e |
| 'San Andreas' | 82.14 ± 0.14 b | 177.16 ± 0.97 d | 7,990.73 ± 39.05 b | 487.52 ± 15.10 | 11.84 ± 0.10 c | 149.81 ± 0.98 c | 241.13 ± 1.89 g |
| Mean | 84.15 | 203.31 | 7,953.97 | 486.03 | 923.78 | 148.22 | 358.36 |
| Coefficient of variation (%) | 1.32 | 2.66 | 1.24 | 22.29 | 2.17 | 1.93 | 1.23 |

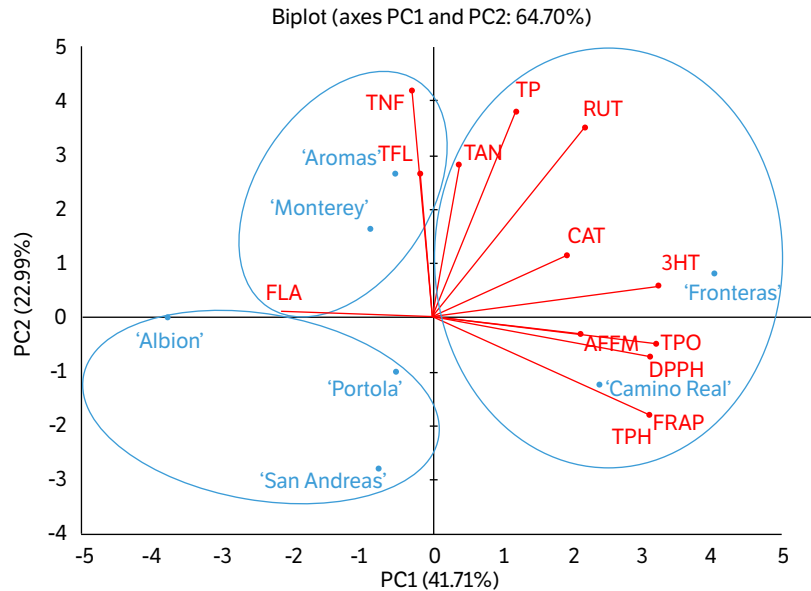
*Data was presented as mean ± standard deviation. Means followed by the same letter in the column do not differ between each other by Tukey's test ($p \leq 0.05$); ns Not significant ($p \geq 0.05$); DPPH: 2,2-diphenyl-1-picrylhydrazyl.

The flavanol catechin is also described as an antioxidant, as well as having antimicrobial, antihypertensive, antiulcer, among other effects (Musial et al. 2020). In strawberry leaves, studies have shown that this flavanol can act as a protective agent during infection by *Alternaria alternata*, acting as a fungal suppressor of induced resistance (Yamamoto et al. 2000). Thus, the use of strawberry leaves may be interesting in formulations against *A. alternata*. Although the levels of catechin found in strawberry leaves (ranging from 97.80 to 280.02 µg/g DL⁻¹, depending on the genotype) were lower than those detected by Buřičová et al. (2011) (1.5 to 4.9 mg/g DW⁻¹), catechins have several benefits due to their medicinal properties, which may be another indication of the strawberry leaf use as a by-product.

Rutin was detected in leaves and the highest content (520.25 µg/g DL⁻¹) occurred in 'Fronteras' (Table 4), and was lower than the results presented by Lin et al. (2020) (8.08 mg/g⁻¹), which can be attributed to differences among genotypes, as well as biotic and abiotic factors. The development of a product based on strawberry leaves could be interesting as it is a rutin source. This flavonoid is recommended for treatments such as varicose veins, hemorrhoids, among others, and several herbal products containing rutin have been patented (Sharma et al. 2013). Among the uses of by-products that can be generated by strawberry leaves, due to their strong antioxidant activity, we can include their application in the cosmetics industry, aiding in the stability of product preparations. However, cytotoxic studies must be carried out to determine the ideal concentration that does not cause deleterious effects (Ziemlewska et al. 2021). In addition, 'Fronteras' had the highest TP and produced fruit with the highest AFFM (Table 2). These results suggested that strawberry leaves, especially 'Fronteras', can be used as bioproducts for health applications.

The quantitative data of the secondary metabolites resulting from the HPLC analysis, as well as yield and antioxidant data were applied to PCA, and explained 64.70% of the data variance (Fig. 5). 'Fronteras' stood out from the other genotypes, followed by 'Camino Real'. Both were grouped in PC1+. 'Portola', 'Albion', and 'San Andreas' were grouped in PC1- and

PC2-, because they showed lower levels of bioactive compounds and yield indexes, both in leaves and fruits (Fig. 5). Among the genotypes, 'Fronteras' stood out mainly due to the content of flavan-3-ol (catechin), flavonol (rutin), phenylethanoid (3-hydroxytyrosol), and antioxidant activity in leaves, as well as higher TPO in fruit and better yield performance (Fig. 5). These compounds detected via HPLC are described as having potential as herbal medicines.



TNF: total number of fruits; TP: total production; AFFM: average fresh fruit mass; TAN: total anthocyanins; TFL: total flavonoids; TPO: total polyphenols; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: ferric reducing antioxidant power; TPH: total phenols; FLA: flavonoids; 3HT: 3-hydroxytyrosol; CAT: catechin; RUT: rutin.

Figure 5. Two-dimensional projection and score resulting from cluster analysis of the production and phytochemical characterization of fruits and leaves of seven strawberry genotypes.

Through the links formed by the dissimilarity cluster analysis (Fig. 6), 'Fronteras' and 'Camino Real' had fewer characteristics in common with the other genotypes, as evidenced by the amount of bioactive compounds and the production characteristics. 'Aromas' and 'Monterey' were grouped mainly by their FLA content in leaves and TFL content in fruits. 'Portola' and 'San Andreas' were grouped together due to their low production performance and antioxidant content in leaves and fruits. 'Albion' can be considered the genotype of least interest to strawberry growers, while 'Fronteras' can be recommended for its fruit, as well as its polyphenol-rich by-product (Fig. 6).

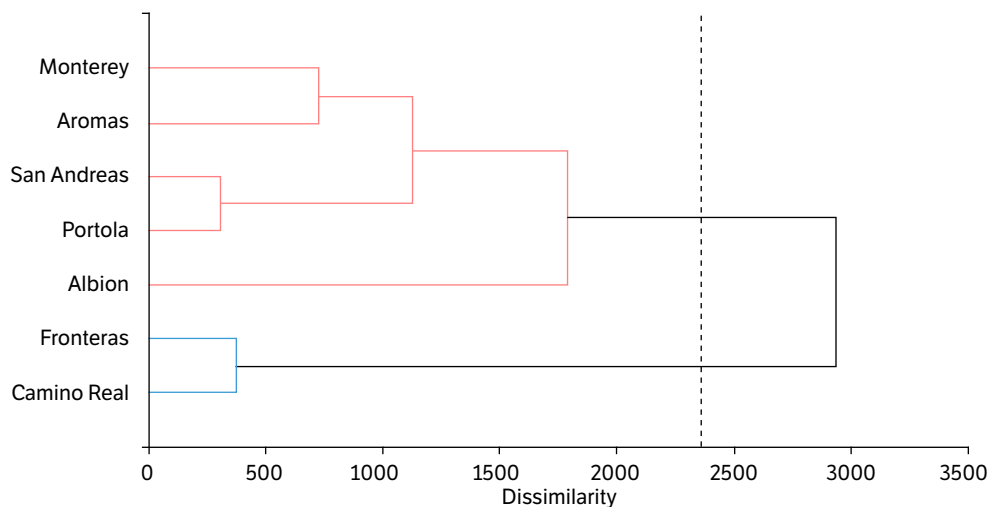


Figure 6. Dendrogram for the production and phytochemical characterization of fruits, and leaves of seven strawberry genotypes.

CONCLUSION

Strawberry genotypes grown in substrate and greenhouse in southern Brazil differ in terms of their yield potential and the phytochemical profile of their fruits and leaves. Multivariate analysis showed the formation of heterogeneous groups among the seven genotypes. 'Fronteras' and 'Monterey' are the most productive. 'Camino Real' produces fruit with the highest total polyphenol content. 'Fronteras' has leaves that are richer in phytochemicals and, together with 'Camino Real', have the highest antioxidant action. We have reported, for the first time in the literature, the presence of 3-hydroxytyrosol in strawberry leaves. 'Camino Real' can be used to optimize and market all the organs produced (fruits and leaves) and 'Fronteras' to increase fruit yield and the use of leaves as an important crop by-product to boost the strawberry production chain.

CONFLICT OF INTEREST

Nothing to declare.


AUTHORS' CONTRIBUTION


Conceptualization: Chiomento, J. L. T. and Lima, G. P. P.; **Methodology:** Chiomento, J. L. T., De Nardi, F. S., Grando, L. A., Trentin, T. S., Anzolin, J., Albrecht, G. E., Huzar-Novakowiski, J., Basílio, L. S. P., Monteiro, G. C. and Lima, G. P. P.; **Investigation:** Chiomento, J. L. T., De Nardi, F. S. and Grando, L. A.; **Writing – Original Draft:** Chiomento, J. L. T. and Lima, G. P. P.; **Writing – Review and Editing:** Chiomento, J. L. T. and Lima, G. P. P.; **Funding Acquisition:** Chiomento, J. L. T. and Lima, G. P. P.; **Supervision:** Chiomento, J. L. T.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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REFERENCES

- Akšić, M. F., Zagorac, D. D., Sredojević, M., Milivojević, J., Gašić, U., Meland, M. and Natić, M. (2019). Chemometric characterization of strawberries and blueberries according to their phenolic profile: Combined effect of cultivar and cultivation system. *Molecules*, 24, 4310. <https://doi.org/10.3390/molecules24234310>
- Basílio, L. S. P., Borges, C. V., Minatel, I. O., Vargas, P. F., Tecchio, M. A., Vianello, F. and Lima, G. P. P. (2022). New beverage based on grapes and purple-fleshed sweet potatoes: Use of non-standard tubers. *Food Bioscience*, 47, 101626. <https://doi.org/10.1016/j.fbio.2022.101626>
- Benzie, I. F. F. and Strain, J. J. (1996). The ferric reducing ability of plasma as a measure of antioxidant power: the FRAP assay. *Analytical Biochemistry*, 293, 70-76. <https://doi.org/10.1006/abio.1996.0292>
- Brand-Williams, W., Cuvelier, M. E. and Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft & Technologie*, 28, 25-30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
- Buřičová, L., Andjelkovic, M., Čermakova, A., Reblova, Z., Jurček, O., Kolehmainen, E., Verhe, R. and Kvasnička, F. (2011). Antioxidant capacity and antioxidants of strawberry, blackberry, and raspberry leaves. *Czech Journal of Food Sciences*, 29, 181-189. <https://doi.org/10.17221/300/2010-CJFS>
- Chiomento, J. L. T., De Nardi, F. S., Filippi, D., Trentin, T. S., Anzolin, A. P., Bertol, C. D., Nienow, A. A. and Calvete, E. O. (2022). Mycorrhization of strawberry plantlets potentiates the synthesis of phytochemicals during *ex vitro* acclimatization. *Acta Scientiarum. Agronomy*, 44, e55682. <https://doi.org/10.4025/actasciagron.v44i1.55682>
- Chiomento, J. L. T., De Nardi, F. S., Kujawa, S. C., Deggerone, Y. S., Fante, R., Kaspary, I. J., Dornelles, A. G., Huzar-Novakowski, J. and Trentin, T. S. (2023a). Multivariate contrasts of seven strawberry cultivars in soilless cultivation and greenhouse in southern Brazil. *Advanced Chemicobiology Research*, 2, 64-78. <https://doi.org/10.37256/acbr.2120232332>
- Chiomento, J. L. T., Silveira, D. C., Reichert Júnior, F. W., Dornelles, A. G., Trentin, T. S. and Cravero, V. P. (2023b). Prediction of the stability and yield in nine strawberry cultivars in Brazil over two cycles. *Journal of Horticultural Science and Biotechnology*, 99, 179-186. <https://doi.org/10.1080/14620316.2023.2242385>
- Cvetković, D. J., Stanojević, L. P., Stanković, M. Z., Cakić, M. D., Savić, S. R. and Miljković, M. D. (2017). Antioxidant activity of strawberry (*Fragaria x ananassa* Duch.) leaves. *Separation Science and Technology*, 52, 1039-1051. <https://doi.org/10.1080/01496395.2017.1281305>
- Diallinas, G., Rafailidou, N., Kalpaktsi, I., Komianou, A. C., Tsouvali, V., Zantza, I., Mikros, E., Skaltsounis, A. L. and Kostakis, I. K. (2018). Hydroxytyrosol (HT) analogs act as potent antifungals by direct disruption of the fungal cell membrane. *Frontiers in Microbiology*, 9, 2624. <https://doi.org/10.3389/fmicb.2018.02624>
- El-Hawary, S. S., Mohammed, R., El-Din, M. E., Hassan, H. M., Ali, Z. Y., Rateb, M. E., Naggar, E. M. B. E., Othman, E. M. and Abdelmohsen, U. R. (2021). Comparative phytochemical analysis of five Egyptian strawberry cultivars (*Fragaria X ananassa* Duch.) and antidiabetic potential of Festival and Red Merlin cultivars. *RSC Advances*, 11, 16755-16767. <https://doi.org/10.1039/D0RA10748D>
- Giampieri, F., Forbes-Hernandez, T. Y., Gasparrini, M., Alvarez-Suarez, J. M., Afrin, S., Bompadre, S., Quiles, J. L., Mezzetti, B. and Battino, M. (2015). Strawberry as a health promoter: An evidence based review. *Food and Function*, 6, 1386-1398. <https://doi.org/10.1039/c5fo00147a>
- Giusti, M. M. and Wrolstad, R. E. (2001). Anthocyanins. Characterization and measurement with UV-visible spectroscopy. In: R. E. Wrolstad (Ed.). *Current protocols in food analytical chemistry* (p. 1-13). New York: John Wiley & Sons. <https://doi.org/10.1002/0471142913>
- Kalt, W., MacKinnon, S., McDonald, J., Vinqvist, M., Craft, C. and Howell, A. (2008). Phenolics of *Vaccinium* berries and other fruit crops. *Journal of the Science of Food and Agriculture*, 88, 68-76. <https://doi.org/10.1002/jsfa.2991>
- Lin, D., Ma, Q., Zhang, Y. and Peng, Z. (2020). Phenolic compounds with antioxidant activity from strawberry leaves: A study on microwave-assisted extraction optimization. *Preparative Biochemistry & Biotechnology*, 50, 874-882. <https://doi.org/10.1080/10826068.2020.1762213>

- Manach, C., Williamson, G., Morand, C., Scalbert, A. and Rémésy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *American Journal of Clinical Nutrition*, 81, 230S-242S. <https://doi.org/10.1093/ajcn/81.1.230S>
- Marhuenda, J., Alemán, M. D., Gironés-Vilaplana, A., Pérez, A., Caravaca, G., Figueroa, F., Mulero, J. and Zafrilla, P. (2016). Phenolic composition, antioxidant activity, and *in vitro* availability of four different berries. *Journal of Chemistry*, 2016, 5194901. <https://doi.org/10.1155/2016/5194901>
- Musial, C., Kuban-Jankowska, A. and Gorska-Ponikowska, M. (2020). Beneficial properties of green tea catechins. *International Journal of Molecular Sciences*, 21, 1744. <https://doi.org/10.3390/ijms21051744>
- Natividade, M. M. P., Corrêa, L. C., Souza, S. V. C., Pereira, G. E. and Lima, L. C. O. (2013). Simultaneous analysis of 25 phenolic compounds in grape juice for HPLC: method validation and characterization of São Francisco Valley samples. *Microchemical Journal*, 110, 665-674. <https://doi.org/10.1016/j.microc.2013.08.010>
- Paparozzi, E. T., Meyer, G., Schlegel, V., Blankenship, E., Adams, S. A., Conley, M. E., Loseke, B. A. and Read, P. (2018). Strawberry cultivars vary in productivity, sugars and phytonutrient content when grown in a greenhouse during the winter. *Scientia Horticulturae*, 227, 1-9. <https://doi.org/10.1016/j.scienta.2017.07.048>
- Sharma, A., Gokulchandran, N., Sane, H., Nagrajan, A., Paranjape, A., Kulkarni, P., Shetty, A., Mishra, P., Kali, M., Biju, H. and Badhe, P. (2013). Autologous bone marrow mononuclear cell therapy for autism: An open label proof of concept study. *Stem Cells International*, 2013, 623875. <https://doi.org/10.1155/2013/623875>
- Singleton, V. L. and Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent. *American Journal of Enology and Viticulture*, 16, 144-158. <https://doi.org/10.5344/ajev.1965.16.3.144>
- Tan, E. C. D. and Lamers, P. (2021). Circular bioeconomy concepts: A perspective. *Frontiers in Sustainability*, 2, 701509. <https://doi.org/10.3389/frsus.2021.701509>
- Villamil-Galindo, E., Van de Velde, F. and Piagentini, A. (2021). Strawberry agro-industrial by-products as a source of bioactive compounds: Effect of cultivar on the phenolic profile and the antioxidant capacity. *Bioresources and Bioprocessing*, 8, 61. <https://doi.org/10.1186/s40643-021-00416-z>
- Yamamoto, A., Lucas, J. J. and Hen, R. (2000). Reversal of neuropathology and motor dysfunction in a conditional model of Huntington's disease. *Cell*, 101, 57-66. [https://doi.org/10.1016/S0092-8674\(00\)80623-6](https://doi.org/10.1016/S0092-8674(00)80623-6)
- Ziemlewska, A., Zagórska-Dziok, M. and Nizioł-Łukaszewska, Z. (2021). Assessment of cytotoxicity and antioxidant properties of berry leaves as by-products with potential application in cosmetic and pharmaceutical products. *Scientific Reports*, 11, 3240. <https://doi.org/10.1038/s41598-021-82207-2>