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Title of the paper

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Abstract:

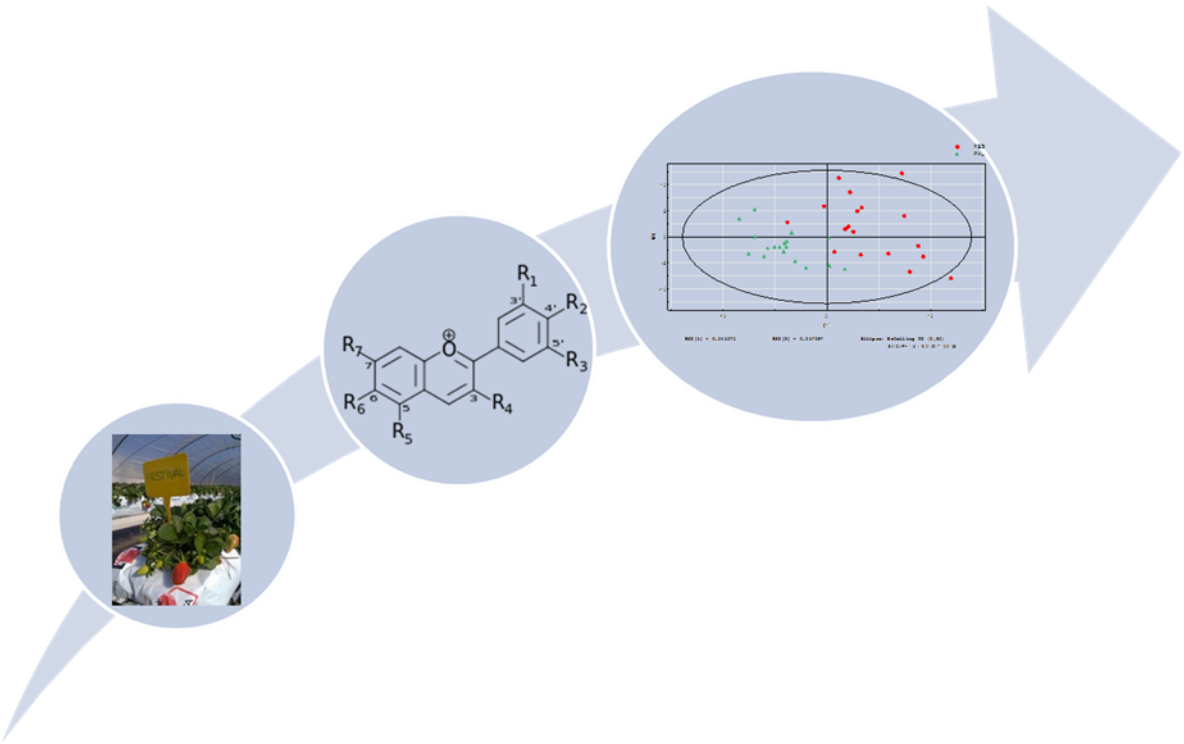
In recent years, interest in phenolic compounds has been increased due mainly to the numerous evidences of its beneficial health effects and their impact on food quality. Numerous efforts have been made in order to increase the knowledge about phenolic compounds, with an especial focus on characterization of new food sources rich in polyphenols. There has been studied the influence of different factors such as variety, pedoclimatic conditions, geographical origin, authenticity and traceability, among others on their contents, as a tool for enhancing nutritional and nutraceutical quality of plant derived foods in breeding programs.

The objective of the present study was to assess whether growth under controlled condition could be used for cultivation of strawberry to enhance bioactive polyphenols content. For this purpose, 54 samples of strawberries belonging to three varieties with different sensitivity to environmental conditions (Camarosa, Festival, Palomar) were grown in soilless system with different agronomic conditions (electrical conductivity, substrate type and coverage).

UHPLC-ESI-MS/MS analysis of polyphenolic compounds combined with chemometric methods revealed changes in compounds such as chlorogenic acid, ellagic acid, ellagic acid pentoside, ellagic acid rhamnoside, Sanguin H10, quercetin-O-glucuronide, catechin, procyanidin B2, pelargonidin-3-glucoside, cyanidin-3-glucoside and pelargonidin-3-rutinoside, which could be related to differences in organoleptic characteristics and/or beneficial health effects.

Keywords: *Phenolic compounds, strawberry, bioactivity, UPLC-ESI-MS/MS*

Graphical Abstract:



Introduction:

Polyphenolic compounds are ubiquitous in all plant organs and are, therefore, an integral part of the human diet through the consumption of edible plants and plant products, such as fresh and cooked vegetables, fresh and processed fruits, legumes, spices, and beverages such as fruit juices, tea, wine, coffee and infusions. In the last years the interest for the phenolic compounds has increased due to the evidences of their health benefits and their impact on food quality [1,2]. Numerous efforts have been made in order to increase the knowledge about phenolic compounds, with an especial focus on characterization of food sources rich in polyphenols, studying the influence of different factors [3,4] such as variety, pedoclimatic conditions, geographical origin, authenticity and traceability, among others on their contents, as a tool for increasing nutritional and nutraceutical quality of plant derived foods in breeding programs.

Materials and Methods:

The experimental design consisted of two macrotunnels (covered and uncovered) each containing three breeding lines of plants grown under different conductivities (EC = 1, 2 and 3 dS / m). Each line of breeding plants was composed of three strawberry cultivars (Palomar, Festival and Camarosa). Finally, each cultivar was grown in three different commercial substrates (coconut fiber, perlite and rockwool). The three varieties were chosen basing on their sensitivity to environmental conditions: Palomar (PAL, very sensitive), Festival (FES, sensitive) and Camarosa (CAM, resistant) [5].

Homogenized fruits (5.0 g) were extracted with 10 mL of methanol, sonicated for 15 min and then centrifuged 10 min at 10 000 rpm 4 °C. Supernatants were concentrated by means of a rotary evaporator at 40 °C and the residues were redissolved in 3 mL of 50% methanol. The concentrated extracts were filtered through 0.20 µm nylon filter prior to injection. Five microliters of this solution were injected in the UHPLC-ESI-MS/MS.

The phenolic compounds profile were obtained by means of an Agilent 1200 series ultra-performance liquid chromatography system

(Agilent, USA) coupled to a 6410 Triple Quad LC/MS system equipped with an Electrospray Ionization Source. Table 1 shows the chromatographic and MS conditions. The phenolic acids, flavonoids, ellagitannins and ellagic acid derivate were operated in the negative ion mode and the anthocyanins in the positive ion mode.

Table 1. Chromatographic and MS/MS working conditions

Chromatographic conditions		
Column	Zorbax SB-C18 (2.1 mm X 50 mm, 1.8 mm)	
Mobile phase A	0.2% water/acetic acid pH 3.10	
Mobile phase B	Acetonitrile	
Temperature	30 ºC.	
Flow rate	0.4 ml/min.	
injection volume	5 µL	
Elution conditions		
Time(min)	*% A*	*% B*
0	100	0
3	95	5
15	60	40
15.5	0	100
17	0	100
23	100	0
MS/MS conditions		
Capillary voltage	4000 V	
Gas flow rate	10 L/min	
Gas temperature	300 ºC	
Nebulizer pressure	35 psi	
Dwell time	50 ms	

Detection was performed in DMRM mode using retention times and detection windows (Delta RT) for each compound instead of a time segment. This will improve the chromatographic peaks resulting in better peak symmetry that allows reproducibility in measurement (peak areas) and accuracy of quantitation. Only the two most abundant MRM transitions were used for confirmatory analysis. The most sensitive transition was used as quantifier ion and the other one as qualifier ion.

Data were then submitted to multivariate and univariate statistical analysis in order to find significant differences between samples according to growing conditions.

Results and Discussion:

Thirty-three different compounds were identified and quantified, which are classified into different groups of phenolics: a) phenolic acids (gallic, vanillic, protocatechuic, *p*-hydroxybenzoic , chlorogenic, cinnamic, caffeic, m-coumaric, and p-coumaric); b) ellagic and ellagic derivative acid (HHDP gallohexose, ellagic acid pentoside, dimethyl ellagic pentoside, ellagic acid rhamnoside, and Sanguin H10); c) flavonols (quercetin, quercetin-3-O-glucoside, quercetin-O-glucuronide, quercetin-3-O-galactoside, kaempferol-3-O-glucoside, kaempferol acetylhexoside, isorhamnetin and quercetrin); d) flavones (luteolin and apigenin); e) flavan-3-ols (catechin, catechi tirmer, -(-) epicatechin, epicatechin gallate and procyanidins B1 and B2); e) anthocyanins (pelargonidin-3-glucoside, cyanidin-3-glucoside and pelargonidin-3-rutinoside).

To understand the interclass separation and identify potential characteristic markers for each cultivar, partial least squares discriminant analysis (PLS-DA) was applied and three binary classification models were developed. Figure 1 (A-C) shows the scores plots obtained for the three strawberry cultivars, grouped accordingly to the first two components. As it can be observed, the separation between cultivars was satisfactory in all cases and they were clearly discriminated.

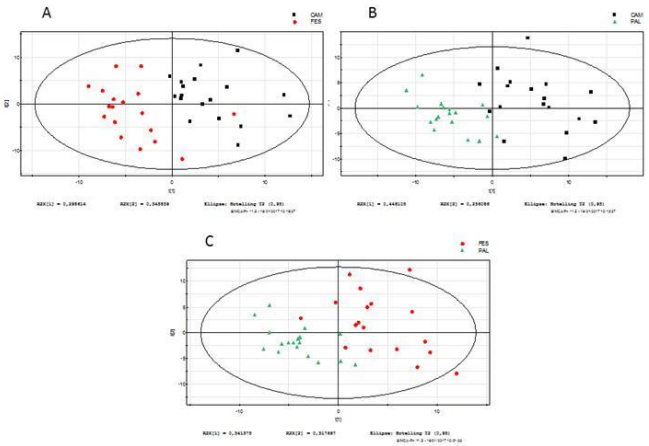


Figure 1.- Scores plots of PLS-DA models for two-class comparisons. (A) Scores plot for the comparison Festival vs. Camarosa, (B) scores plot for the comparison Palomar vs. Camarosa, (C) scores plot for the comparison Palomar vs. Festival.

Furthermore, PLS models also provide the possibility of obtaining a quantitative measure of the discriminating power of each variable by means of the variable importance for the projection (VIP) parameter. Only variables with VIP values higher than 1.0 were selected. Box-plots representing the mean values with standard deviation intervals for these selected compounds are shown in Figure 2 in order to highlight metabolomic differences found between the three cultivars.

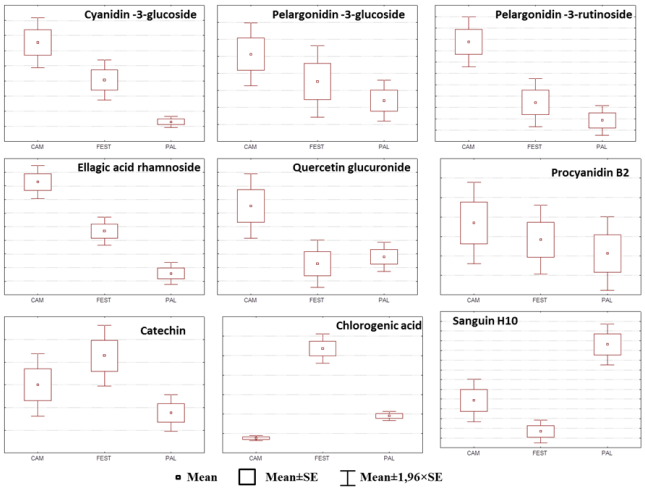


Figure 2.- Box plots with whiskers for discriminant polyphenols for differentiation of strawberry cultivars

In addition to the effect of genotype, the nutritional quality of berry fruits is also significantly affected by the environmental and agronomic conditions in which plants are cultivated. In order to evaluate the metabolic alterations associated with growing conditions of strawberries investigated in this study, PLS-DA was applied to data sets for each cultivar, considering the different agronomic conditions as categorical factors (*i.e.*macrotunnel type, electrical conductivity and substrate). Discriminant compounds are shown in Table 2 along with the percentage of change and the p-value for each comparison (covered *vs.* uncovered, EC3 *vs.* EC2 *vs.* EC1, perlite *vs.* coconut fiber,rockwool*vs.* coconut fiber, perlite *vs.*rockwool).

Table 2.-. Discriminant polyphenolic compounds associated with different growing conditions of strawberries cultivated in soilless systems.

compound	Coverage			EC			Substrate		
	Camarosa	Festival	Palomar	Camarosa	Festival	Palomar	Camarosa	Festival	Palomar
Pelargonidin-3-glucoside	37% (0.0405)	NS	NS	NS	NS	33% (0.0244)	NS	NS	NS
Pelargonidin-3-rutinoside	61% (0.0003)	69% (0.0189)	NS	NS	NS	NS	NS	NS	NS
Cyanidin-3-glucoside	43% (0.0037)	38% (0.0127)	NS	NS	NS	NS	NS	NS	NS
Ellagic acid	NS	NS	NS	NS	NS	NS	NS	NS	NS
Ellagic acid rhamnoside	NS	NS	NS	NS	NS	12% (0.0178)	NS	-12% (0.0222)*	NS
Ellagic acid pentoside	NS	NS	NS	NS	NS	NS	NS	-17% (0.0082)*	NS
Sanguin H10	NS	NS	33% (0.0153)	NS	NS	NS	NS	NS	NS
Catechin	NS	NS	NS	NS	NS	NS	NS	NS	NS
Procyanidin B2	NS	NS	NS	NS	NS	NS	NS	NS	NS
Quercetin glucuronide	NS	NS	NS	NS	NS	NS	NS	NS	NS
Chlorogenic acid	NS	NS	NS	NS	NS	NS	NS	NS	NS

* perlite *vs.* rockwool; N.S. non significant

Conclusions:

UHPLC-MS-MS technology using LC in tandem with a triple quadrupole mass spectrometer (LC/QQQ) has become the approach of choice for analysis of hundreds of target compounds in food. The application of this analytical approach and subsequent multivariate statistics such as PLS, allowed the differentiation of three strawberry cultivars characterized by different sensitivity to environmental stress (Camarosa, Festival, Palomar), and then was employed to identify changes of polyphenolic compounds associated with the use of various agronomic conditions, including different electrical conductivities of irrigation (EC = 1, 2 and 3 dS / m), macrotunnel types(covered, uncovered) and substrates (coconut fiber, perlite and rockwool).In this way, multiple discriminant compounds were identified (chlorogenic acid, ellagic acid, ellagic acid pentoside, ellagic acid rhamnoside, Sanguin H10, quercetin-O-glucuronide, catechin, procyanidin B2, pelargonidin-3-glucoside, cyanidin-3-glucoside and pelargonidin-3-rutinoside), which could be related to differences in organoleptic characteristics and/or beneficial health effects.

Conflicts of Interest: *The authors declare no conflict of interest*

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References:

1. Giampieri, F.; Tulipani, S.; Alvarez-Suarez, J.M.; Quiles, J.L.; Mezzetti, B.; Battino, M. The strawberry: Composition, nutritional quality, and impact on human health. *Nutrition* **2012**, *28*, 9-19.
2. Alvarez-Suarez, J.M.; Giamperi, F.; Tulipani, S.; Casoli, T.; Di Stefano, G.; González-Paramás, A.M.; et al. One-month strawberry-rich anthocyanin supplementation ameliorates cardiovascular risk, oxidative stress markers and platelet activation in humans. *J. Nutr. Biochem.* **2014**, *25*, 289-294.
3. Akhatou, I. and Fernández-Recamales, A. Nutritional and nutraceutical quality of strawberries in relation to harvest time and crop conditions. *J. Agric. Food Chem.* **2014a,** *62*, 5749–5760.
4. Akhatou, I. and Fernández-Recamales, A. Influence of cultivar and culture system on nutritional and organoleptic quality of strawberry. *J. Sci. Food Chem.* **2014b**, *94*, 866–875.
5. Akhatou, I.; González-Domínguez, R. and Fernández-Recamales, A. Investigation of the effect of genotype and agronomic conditions on metabolomic profiles of selected strawberry cultivars with different sensitivity to environmental stress. *Plant Physiology and Biochemistry* **2016**, *101*, 14-22