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Application of targeted and non-targeted approaches to investigate the effect of genotype and growing conditions on the strawberry metabolome

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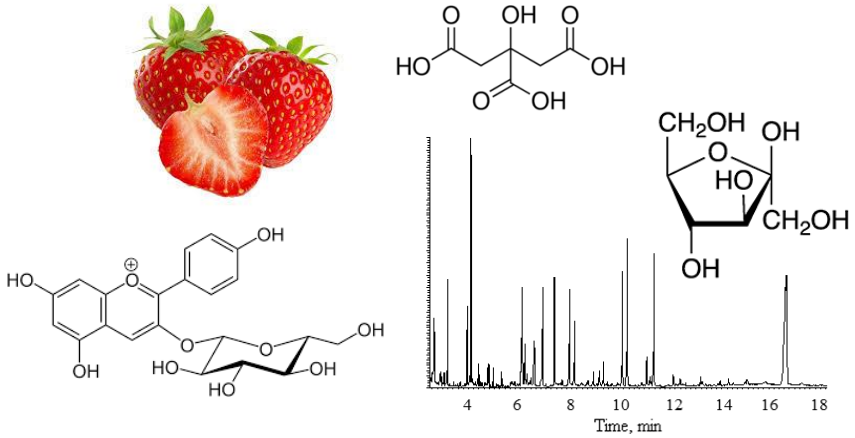

Abstract:
Strawberry is composed of numerous primary metabolites (sugars, amino acids, organic acids) and secondary metabolites (anthocyanins, flavan-3-ols, phenolic acids), which play an essential role in fruit quality, organoleptic characteristics and healthy benefits. In this context, metabolomics presents a great potential to get a deep overview of this complex chemical meshwork, which can provide valuable information on the effect of variety and agronomic conditions in the strawberry composition. We conducted a GC/MS-based non-targeted metabolomic analysis in strawberries of three varieties with different sensitivity to environmental conditions (Camarosa, Festival and Palomar), which in turn were grown in soilless systems by using various agronomic conditions (electrical conductivity, coverage and substrates). Complementarily, a targeted metabolomic approach based on UHPLC-MS/MS was also applied to identify and quantitate the main polyphenol compounds in these strawberry fruits. The most discriminant metabolites were several amino acids, sugars, organic acids, anthocyanins, ellagic acid derivatives, flavan-3-ols, chlorogenic acid and quercetin 3-O-glucuronide, which could be associated with differences in organoleptic characteristics and the biosynthesis of strawberry antioxidants.

Keywords: metabolomics; strawberry; GC-MS, polyphenols

Introduction

Primary metabolites

Sugars

Organic acids

Lipids

Amino acids

Secondary metabolites

Anthocyanins

Flavan-3-ols

Ellagitannins

Phenolic acids

- ✓ fruit quality
- ✓ organoleptic characteristics
- ✓ healthy benefits

Introduction

Un-targeted metabolomics

+

Targeted metabolomics

- ✓ Variety
- ✓ Growth conditions
- ✓ Agricultural practices
- ✓ Response to biotic and abiotic stress
- ✓ Post-harvest factors

Materials and Methods

cv. Palomar
cv. Festival
cv. Camarosa

sensitivity to environmental stress

Growing conditions

- Macrotunnel type: covered vs. uncovered
- Electrical conductivity: 1, 2 and 3 dS / m
- Soilless commercial substrates: coconut fiber, perlite and rockwool

- ✓ Extraction with methanol-water
- ✓ Derivatization and non-targeted analysis by GC-MS (primary metabolites) [1]
- ✓ Analysis by targeted LC-MS/MS (secondary metabolites) [2]

(1) Plant Physiol. Biochem. 101 (2016) 14-22
(2) J. Agric. Food Chem. 65 (2017) 9559-9567

Results and Discussion

Effect of variety on metabolomic profiles

↑ citric acid ↓ glucose, fructose

↑ alanine, norvaline, threonine, aspartate

↑ inositol

↑ anthocyanins, ellagic acid rhamnoside, quercetin 3-O-glucuronide, procyanidin B2

cv. Camarosa
(resistant cultivar)

organoleptic quality of strawberry

osmotic adjustment and protection of biochemical pathways

accumulation in osmotically challenged plants

up-regulation of the shikimate and malonate pathways

tolerance mechanisms in response to environmental stress

Results and Discussion

Effect of macrotunnel type on metabolomic profiles

covered macrotunnel → **strawberries are grown under controlled conditions, light intensity is reduced; temperature is higher; and moisture is controlled by regular irrigation**

- ↑ malic acid
- ↑ threonine ↓ norvaline

→ low sun exposure

- ↓ fructose → humid climate
- ↑ anthocyanins, ellagic acid derivatives → reduced light intensity and higher temperature

Results and Discussion

Effect of electrical conductivity on metabolomic profiles

higher electrical conductivity

- ↓ citric acid, malic acid
- ↓ alanine
- ↑ inositol
- ↑ glycerol
- ↑ anthocyanins

increase of osmoprotectants and antioxidants, decrease of essential amino acids and TCA derivatives (mainly detected in more resistant cultivars, i.e. Camarosa)

response to osmotic stress (increased salt content)

Results and Discussion

Effect of substrate on metabolomic profiles

coconut fiber

- ↑ citric acid
- ↑ glucose, fructose
- ↑ anthocyanins
- ↑ ellagic acid derivatives

increase of sugars and organic acids (organoleptic characteristics of fruits) and polyphenols (bioactive compounds)

coconut fiber is the best substrate for the production of strawberry in soilless culture

Conclusions

- ✓ Combined non-targeted and targeted metabolomics has a great potential to investigate the effect of variety and agronomic practices on strawberry composition
- ✓ cv. Camarosa (resistent cultivar) has increased levels of amino acids, inositol and polyphenols as a consequence of tolerance mechanisms in response to environmental stress
- ✓ Cultivation in covered macrotunnel induces an specific metabolic profile due to reduced light exposure, higher temperature and humidity
- ✓ Higher conductivity of irrigation leads to the increase of osmoprotectants and antioxidants, and the decrease of essential amino acids and TCA derivatives as a consequence of the response to osmotic stress
- ✓ Strawberries grown in coconut fiber have increased content of sugars, organic acids and polyphenols