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Untargeted metabolomics reveals specific withanolides and fatty acyl glycoside as tentative metabolites to differentiate organic and conventional Physalis peruviana fruits

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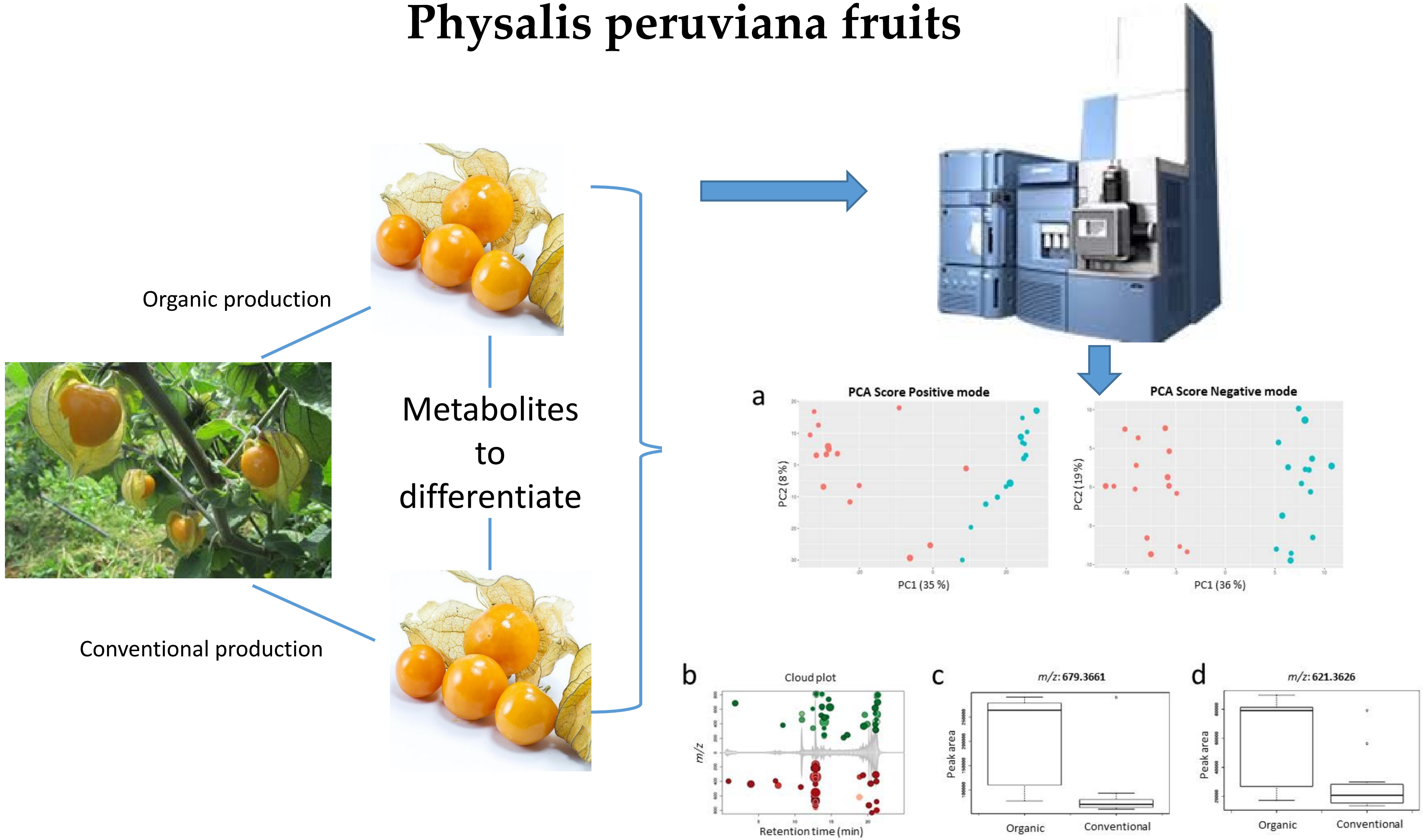
Tentative metabolites to differentiate organic and conventional Physalis peruviana fruits



Abstract:
Physalis peruvianas (goldenberry) a yellow-orange fleshed berry that attract great interest actually interest because of its chemical composition. The agronomic production systems may affect the levels of food metabolites. In previous reports organic fruits have been considered to have a higher nutritional content than conventionally grown fruits, nowadays, scientific literature is unclear and ambiguous on the accuracy of this claim. In this study, metabolomics techniques were used to assess the differences in phytochemical composition between goldenberry samples produced by organic and conventional systems. To verify that the organic samples were free of pesticides, individual pesticides were analyzed. Principal component analysis showed a clear separation of goldenberry samples from two different farming systems. Via targeted metabolomics assays, whereby carotenoids and ascorbic acid were analyzed, not statistical differences between both crops were found. Conversely, untargeted metabolomics allowed us to identify two withanolides and one fatty acyl glycoside as tentative metabolites to differentiate goldenberry fruits, recording organic fruits higher amounts of these compounds than conventional samples. Hence, untargeted metabolomics technology could be suitable to research differences on phytochemicals under different agricultural management practices and to authenticate organic products.

Keywords: Metabolomics; goldenberry; organic fruit; phytochemicals; withanolides.

Introduction

- Goldenberry, is a yellow-orange fleshed berry that attract great interest because of its chemical composition; protein, fat, carbohydrates, minerals, vitamins, fiber and high content in bioactive compounds like carotenoids, phytosterols, physalins, withanolides and polyphenols, among others, that provide health benefits and reduce risk for certain diseases, making it a fruit of great interest for future researches (Ramadan, 2011).

In previous reports organic fruits have been considered to have a higher nutritional content than conventionally grown fruits, nowadays, scientific literature is unclear and ambiguous on the accuracy of this claim (Esch, 2010). That is why it is very important to prove that the organic system is better than conventional.

May the agronomic production systems affect the levels of goldenberry metabolites?

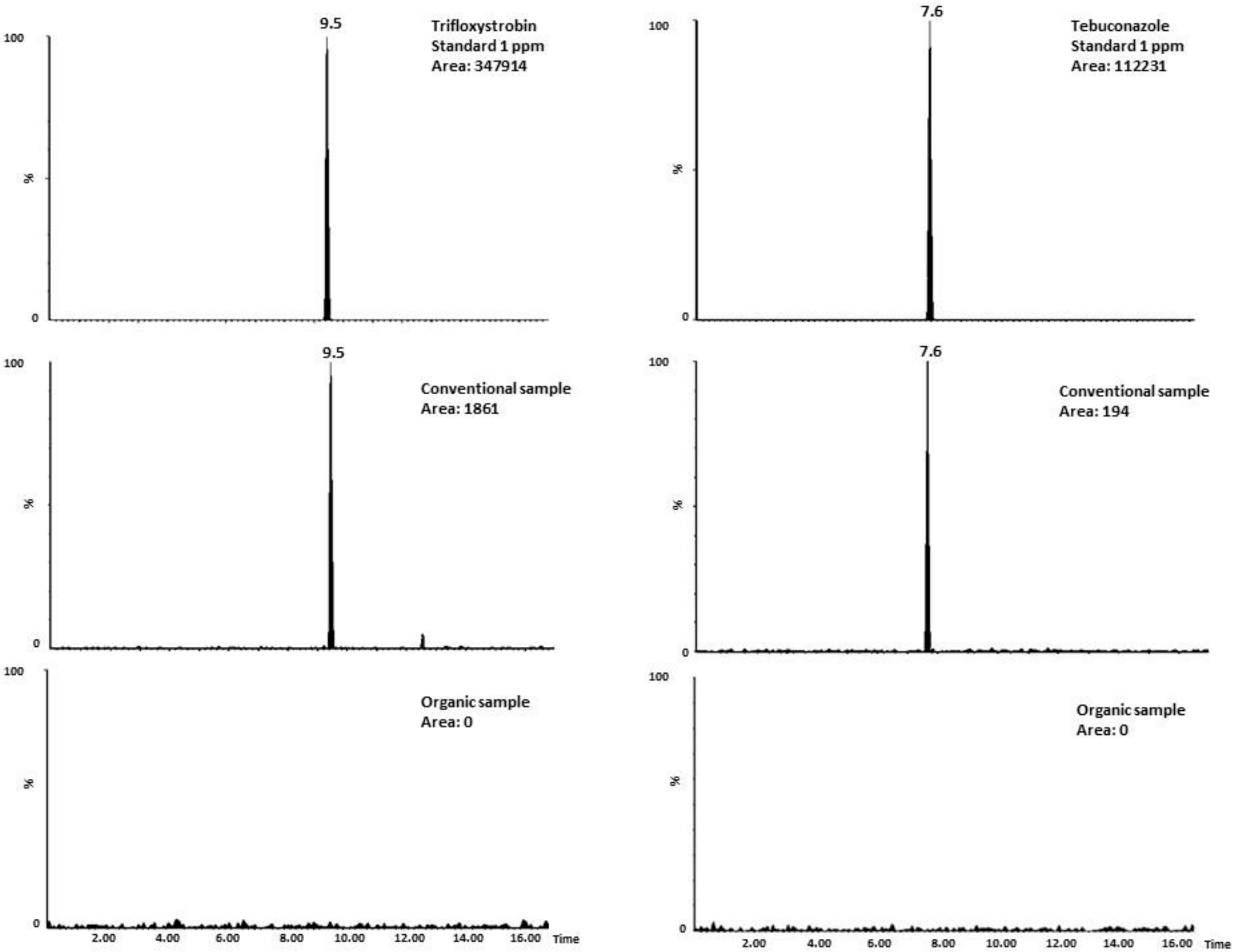
Introduction

New analytical techniques that can distinguish between both agricultural systems are being developed. For that matter, omics approaches like metabolomics, mainly untargeted metabolomics, have allowed the detection of possible differences in the chemical composition between organic and conventional production although many results are still controversial (Vallverdu-Queralt & Lamuela-Raventos, 2016).

Previous studies showed that the metabolome of organic and conventional crops was different; however, due to the limited number of available reports, it is still impossible to reach final and valid conclusions. For this reason, the main objective of this study was to explore the effect of organic and conventional growing conditions on the specific chemicals (carotenoids, ascorbic acid and pesticides) using targeted metabolomics and to research overall phytochemicals by untargeted metabolomics technique.

Results and Discussion

- **Pesticides**



Organically grown goldenberry fruit had lower pesticide residues than conventionally grown fruit. Only two out of the 25 pesticides analyzed were detected in conventional goldenberry fruits (tebuconazole, trifloxystrobin).

Chromatograms obtained by UPLC-QqQ-MS/MS from standard solution (1 ppm), conventional and organic goldenberry samples of the pesticides detected in this assay

Results and Discussion

- **Carotenoids and ascorbic acid content**

Table 1. Mean concentration of carotenoids and ascorbic acid content in goldenberry fruits produced by organic and conventional farming.

	Goldenberry fruits		
	Organic	Conventional	p-value
Carotenoids (mg 100 g^{-1} FW)			
β-carotene	0.70 ± 0.09	0.68 ± 0.09	0.76
Lutein	0.0075 ± 0.0005	0.0071 ± 0.001	0.55
Vitamins (mg 100 g^{-1} FW)			
Ascorbic acid	16.79 ± 2.40	16.51 ± 2.60	0.67

FW: fresh weight

Results are expressed as mean of three repetitions ± standard deviation.

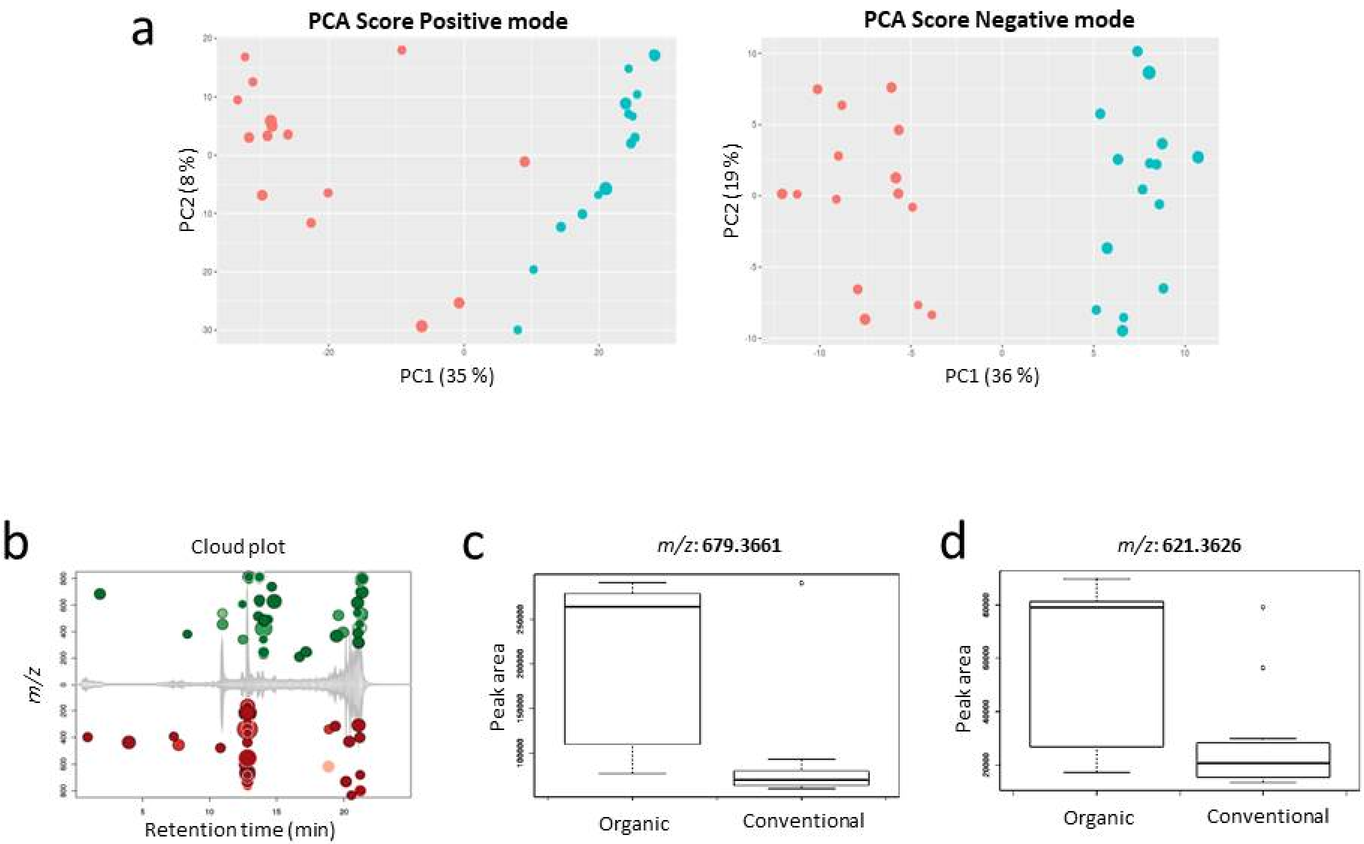
Comparing goldenberry fruits under organic and conventional farming, in this study there were no significant differences in carotenoids detected. Our results were in accordance with the previous report.

Results and Discussion

There was no evidence of the nutritional superiority of the organically grown fruits (ascorbic acid content and carotenoids content) between fruits produced by organic and conventional crops. This fact that most studies have found no differences may depend on a number of factors, such as maturity and moisture content that might have a significant effect on an apparent quality and nutritional content.

Results and Discussion

- **Untargeted metabolomics**



a. Principal Component Analysis (PCA) score plots (PC1×PC2) in positive and negative mode. The red and blue circles correspond to organic and conventional goldenberry samples, respectively. b. Cloud plot with metabolomics data visualization in positive mode. Each metabolite feature is depicted by a bubble. Up-regulated features are shown in green color and down-regulated features in red. c and d. The variation pattern of two withanolides across different farming is shown by Box-Whisker plots. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Results and Discussion

- **Metabolites identification and potential factors related**

Table 2. Metabolites tentatively identified between organic and conventional goldenberry fruits.

Putative Identification[a]	RT (min)	Molecular formula	Adducts	Theoretical Mass	Detected Mass	Mass Error (ppm)	p-value	Fold-change	Intensity O/C[b]	Id Database
Withanolides glycosides										
Physagulin D	12.67	$C_{34}H_{52}O_{10}$	$[M+H]^+$	621.3632	621.3626	0.96	0.0004	2.4	Organic	HMDB41049 FDB020922 METLIN95482
1-Acetyl-3,14,20-trihydroxywitha-5,24-dienolide 3-glucoside	12.82	$C_{36}H_{54}O_{12}$	$[M+H]^+$	679.3687	679.3661	3.83	0.0001	2.4	Organic	HMDB33572 FDB011644 METLIN89381
Fatty acyl glycoside										
Butyl (S)-3-hydroxybutyrate [arabinosyl-(1->6)-glucoside]	20.46	$C_{19}H_{34}O_{12}$	$[2M-H]^-$	907.4027	907.4002	2.75	0.001	2.1	Organic	HMDB39214 FDB018746

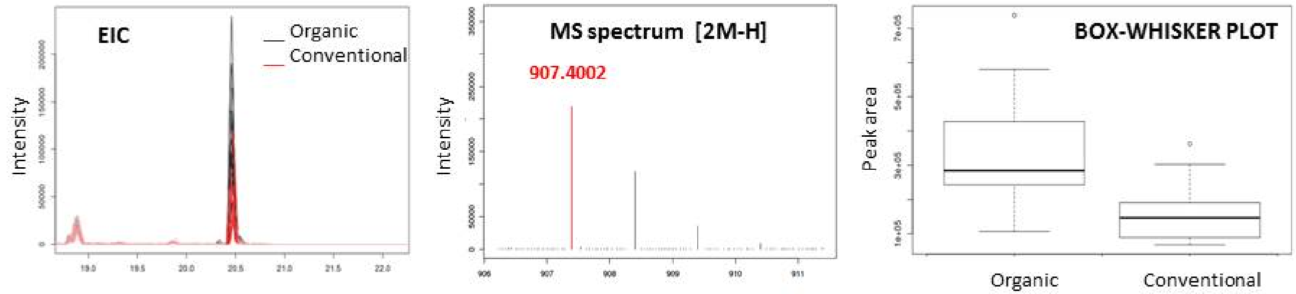
[a] The identification level for all metabolites was 2 according to Sumner et al. 2007

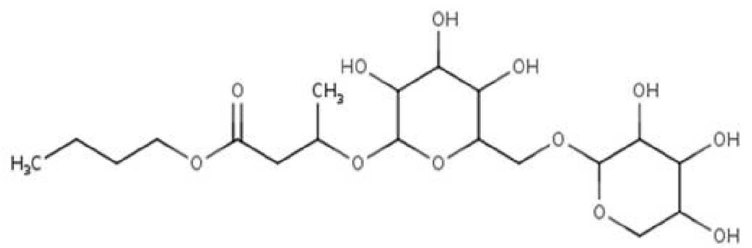
[b] Indicates in which samples the metabolites had greater intensity

O/C: Organic/Conventional

Results and Discussion

- **Metabolites identification and potential factors related**





Butyl (S)-3-hydroxybutyrate [arabinosyl-(1->6)-glucoside]

Extraction ion chromatogram (EIC), MS spectrum of m/z 907.4002 in negative mode and Box-Whisker plot for putative hydroxyester glycoconjugate [2M−H]− increased in organic goldenberry samples.

Conclusions

- The chemical composition at the metabolome level of goldenberry fruits was influenced by organic versus conventional farming practices, as it has been revealed by untargeted metabolomics technique.

- There were no significant differences in carotenoids and ascorbic acid content between both crops. This fact may be caused by several characteristics of the matrix such as variety, location, year of production, ecotype, differential ripening stage or other production related factors that might modulate bioactive compounds content.

- Untargeted metabolomics approach could potentially be suitable to explore phytochemical composition of fruits under different agricultural management practices and to authenticate organic.

Acknowledgments

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