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A Metabolomic Approach Differentiates between Conventional and Organic Ketchups

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ABSTRACT: The agronomic environments in which tomatoes are cultivated potentially affect the levels of antioxidants and other metabolites in commercial products. In this study, biochemical and metabolomic techniques were used to assess the differences between ketchups produced by organic and conventional systems. An untargeted metabolomic approach using QToF-MS was used to identify those nutrients that have the greatest impact on the overall metabolomic profile of organic ketchups as compared to conventional ones. Individual polyphenols were quantified using LC-ESI-QqQ. This multifaceted approach revealed that the agronomic environment in which tomatoes are grown induces alterations in the content of antioxidant capacity, phenolics, and other metabolites in ketchups. Organic cultivation was found to provide tomatoes and tomato-derived products with a significantly higher content of antioxidant microconstituents, whereas glutamylphenylalanine and *N*-malonyltryptophan were detected only in conventional ketchups.

KEYWORDS: ketchups, organic agriculture, conventional agriculture, metabolomics, HPLC-ESI-QTOF, LC-ESI-QqQ

INTRODUCTION

The increasing demand for organic foods can be explained mainly by consumer concerns about the quality and safety of foods and the perception that organically produced foods are healthier and safer than conventional foods. A wide range of processed organic tomato products are available including pasta sauces, tomato sauces, juices, ketchups, tinned tomatoes (whole and diced), dried tomatoes, and pastes.[1]

Fundamental differences between organic and conventional production systems, particularly in soil fertility management, may affect the nutritive composition of plants, including secondary plant metabolites.[2] Organic agriculture is an ecological production management system that promotes and enhances biodiversity, biological cycles, and soil biological activity. Organic systems rely on the activity of a diverse soil ecosystem to make nitrogen and other nutrients available to plants, whereas conventional farms utilize fertilizers containing soluble inorganic nitrogen and other nutrients, which are more directly available to plants.[3] The availability of inorganic nitrogen in particular has the potential to influence the synthesis of secondary plant metabolites, proteins, and soluble solids. It is well-known that the biosynthesis of phenolics in plants is strongly influenced by cultivar, environmental conditions, especially light, and the mode of fertilization.[4,5] Vallverdú-Queralt et al.[6] suggested that choice of cultivar is a major factor contributing to the total phenol content of tomatoes when grown under similar environmental conditions. Seven normal-sized field-grown tomatoes, grown alongside one another in Spain, showed different content of flavonols, flavanones, and phenolic and hydroxycinnamic acids. Moreover, the phenolic content of plants may be influenced by manipulating the agronomic environment in which they are grown.[7,8] The level of nitrogen influences the level of phenol.[9,10] Even if the levels of nitrogen, as well as potassium and phosphorus, were higher in organic conditions, the bioavailability of nitrogen from organic fertilization would remain lower than that achieved by synthetic fertilization; the levels of carbon-based secondary metabolites such as phenolic compounds are higher in organic plants.[9,10]

The development of tools and metabolomic markers to monitor the quality of these products is necessary. With the recent developments in plant metabolomic techniques,[11] it is possible to detect several hundred metabolites simultaneously and to compare samples reliably for differences and similarities in a semiautomated and untargeted manner.

Until now, metabolomic approaches in tomatoes have only been used to define alterations to fruit induced by mutations[12] and to investigate metabolomic changes taking place during fruit development.[13,14] To our knowledge, comprehensive biochemical studies on the effect of the agronomic environment on the phenolic content of tomato-based products have not been reported. Many studies have shown that phenol intake improves health and prevents chronic diseases.[15] We carried out a pilot study to identify the differences between organic and conventional

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ketchups on the market through a combination of MS techniques, liquid chromatography electrospray ionization time-of-flight mass spectrometry (LC-ESI-QToF-MS) and liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-QqQ), and two additional analyses, total polyphenol (TP) and hydrophilic antioxidant capacity assays.

MATERIALS AND METHODS

Standards and Reagents. All samples and standards were handled without exposure to light. Caffeic and chlorogenic acids, rutin and quercetin, Folin-Ciocalteau (F-C) reagent, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), phosphate-buffered saline (PBS), pH 7.4, (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) 97%, manganese dioxide, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma (St. Louis, MO) and naringenin, naringenin-7-*O*-glucoside, and kaempferol-3-*O*-rutinoside from Extrasynthèse (Genay, France). Ethanol and formic acid of HPLC grade were obtained from Scharlau (Barcelona, Spain), and ultrapure water (Milli-Q) was from Millipore (Bedford, MA).

Processing Conditions of Organic and Conventional Tomatoes. Conventional tomatoes receive herbicides and other pesticides as needed, whereas organic crops receive only organically approved pesticides such as sulfur and benzothiophene compounds. Conventional farms utilize fertilizers containing soluble inorganic nitrogen and other nutrients, which are more directly available to plants. Organic systems emphasize the accumulation of soil organic matter and fertility over time through the use of cover crops, manures, and composts and rely on the activity of a diverse soil ecosystem to make nitrogen (N) and other nutrients available to plants. The amount of N present in cover crops varies from year to year, but, typically, organic plots currently receive between 240 and 260 kg of N ha^{-1} per year in addition to the N fixed by the legume cover crop. Conventional tomatoes usually receive 50 kg ha^{-1} of an N−P−K starter fertilizer and 118 kg ha^{-1} of ammonium nitrate as side dressing.[16]

Processing Conditions and Ingredients of Commercial Ketchups. From a technological point of view, the main difficulty in the production of ketchups is choosing the most appropriate variety of organic or conventional tomatoes with good consistency and suitable degrees Brix to produce tomato paste between 30 and 32 °Brix. Moreover, the use of resistant and tolerant varieties can be advantageous in the management of pest control. For example, varieties such as Supreme, Gold, Fresh, and Belle are more resistant to diseases and are the most commonly used for tomato-based organic products such as ketchups.

Organic and conventional tomatoes are used to produce organic and conventional ketchups. Tomatoes are sorted, washed, and chopped, and then the tomato paste (65%, 30 and 32 °Brix) is mixed with sugar (organic ketchups) or high-fructose corn syrup (conventional ketchups), wine vinegar, salt, aroma, and trace preservatives. The mixture is pasteurized at 96 °C for 4−6 min. The entire process of ketchup manufacturing generally takes between 2 and 3 h. Finally, the ketchup is bottled.[13,17]

The manufacturing date controls are January 2010, April 2010, and September 2010. A total of 15 ketchups commercially available in Barcelona markets were analyzed on each manufacturing date: 10 conventional and 5 organic.

Extraction and Isolation of Phenolic Compounds. All ketchups (1.0 g) were weighed and homogenized with 4 mL of 80% ethanol in Milli-Q water. They were then sonicated for 5 min and centrifuged (4000 rpm at 4 °C) for 20 min. The supernatant was transferred into a flask, and the extraction was repeated. Both supernatants were combined and evaporated under nitrogen flow. Finally, the residue was reconstituted with up to 1.5 mL of Milli-Q water containing 0.1% formic acid. Samples were stored at −20 °C until analysis.

Analysis of Total Polyphenols. Solid-phase extraction (SPE) was carried out to eliminate interferences such as ascorbic acid, amino acids, and reducing sugars, which could contribute to an overestimation of the values of TP. For this procedure, Oasis MAX cartridges with 30 mg of mixed-mode anion-exchange and reversed-phase sorbent from Waters (Milford, MA) following the procedure of Vallverdú-Queralt et al.[18] were used. The eluted fractions were evaporated under nitrogen flow, and the residue was reconstituted with up to 250 μL of Milli-Q water containing 0.1% formic acid and filtered through a 13 mm, 0.45 μm PTFE filter (Waters) into an insert-amber vial for HPLC analysis.

For the TP assay, each sample was analyzed in triplicate: 20 μL of the eluted fractions was mixed with 188 μL of Milli-Q water in a thermo microtiter 96-well plate (Nunc, Roskilde, Denmark); then, 12 μL of F-C reagent and 30 μL of sodium carbonate (200 g/L) were added. The mixtures were incubated for 1 h at room temperature in the dark. After the reaction period, 50 μL of Milli-Q water was added, and the absorbance was measured at 765 nm in a UV−vis Thermo Multiskan Spectrum spectrophotometer (Vantaa, Finland). This spectrophotometer allowed the absorbance of a 96-well plate to be read in 10 s. Results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh weight (FW).[19]

Antioxidant Capacity. The antioxidant capacity in tomato juices was measured using an $ABTS^+$ radical decolorization assay and a DPPH assay following the procedure of Vallverdú-Queralt et al.[18] with minor modifications.

$ABTS^+$ Assay. One millimolar Trolox (antioxidant standard) was prepared in PBS once a week. Working standards were prepared daily by diluting 1 mM Trolox with PBS.

An $ABTS^+$ radical cation was prepared by passing a 5 mM aqueous stock solution of ABTS (in PBS) through manganese dioxide powder. Excess manganese dioxide was filtered through a 13 mm 0.45 μm filter PTFE (Waters). Before analysis, the solution was diluted in PBS, pH 7.4, to give an absorbance at 734 nm of 1.0 ± 0.1 and preincubated in ice. Then, 245 μL of $ABTS^+$ solution was added to 5 μL of Trolox or to tomato samples, and the solutions were stirred for 30 s. The absorbance was recorded continuously every 30 s with a UV−vis Thermo Multiskan Spectrum spectrophotometer for 1 h, and PBS blanks were run in each assay.

The working range for Trolox (final concentration = 0−750 μM) was based on triplicate determinations and consisted of plotting the absorbance as a percentage of the absorbance of the uninhibited radical cation (blank). The activities of the tomato samples were assessed at four different concentrations that were within the range of the dose−response curve. Each sample was analyzed in triplicate at each concentration. Results were expressed as millimoles of Trolox equivalent (TE) per 100 g of FW.

DPPH Assay. The antioxidant capacity was also studied through the evaluation of the free radical-scavenging effect on DPPH radicals. Solutions of known Trolox were used for calibration. Five microliters of tomato sample or Trolox was mixed with 250 μL of methanolic DPPH (0.025 g/L). The homogenate was shaken vigorously and kept in darkness for 30 min. Absorption of the samples was measured on a UV−vis Thermo Multiskan Spectrum spectrophotometer at 515 nm. The percentage of inhibition of the DPPH was calculated and plotted as a function of the concentration of Trolox for the standard reference data. The final DPPH values were calculated using a regression equation between the Trolox concentration and the percentage of DPPH inhibition, and results were expressed as millimoles of TE per 100 g of FW.

HPLC-ESI-QToF Analysis. The chromatography was performed on an Agilent 1200 RRLC (Agilent, Waldbronn, Germany) using a Luna C_{18} column 50 × 2.0 mm internal diameter, 5 μm (Phenomenex, Torrance, CA). The flow rate was 0.4 mL/min, and the injection volume was 5 μL. Mobile phases consisted of 0.1% formic acid in Milli-Q-water (A) and 0.1% formic acid in acetonitrile (B). Separation was carried out

over 20 min under the following conditions: 0 min, 5% B; 16 min, 40% B; 17 min, 95% B; 19 min, 95% B; 19.5 min, 5% B. The column was equilibrated for 5 min prior to each analysis. The HPLC system was coupled to a hybrid quadrupole time-of-flight QSTAR Elite (AB Sciex). The MS acquisition was performed in negative ionization using information-dependent acquisition (IDA) between m/z 90 and 1100. MS parameters were as follows: ion spray voltage, −4200 V; declustering potential, −60 V; focusing potential, −190 V; declustering potential 2, −15 V; ion release delay, 6 V; ion release width, 5 V; temperature, 400 °C with curtain gas (N_2), 50 au (arbitrary units); auxiliary gas, 50 au; and nebulizer gas (N_2), 50 au. IDA was performed using the following criteria: ions that exceed 5 counts; ion tolerance, 50 mDa; collision energy fixed at −30 V; dynamic background subtraction activated. The QToF was calibrated as recommended by the manufacturer. The sequences of injections were randomized.

The significance of the results was analyzed using MarkerView 1.2 software (Applied Biosystems, MSD SCIEX, Toronto, ON, Canada), which performs feature extraction by peak-finding for each sample and alignment using a minimum peak width of 1 mg/L with a noise threshold of 5 and a subtraction multiple factor of 1.5. Alignment used 0.04 mDa mass tolerance and 0.06 min retention time tolerance. Additionally, variables were required to be present in at least five samples. With these parameters a data set containing 1200 mass features was obtained.

LC-ESI-QqQ Analysis. To evaluate the differences between organic and conventional production systems, phenolic compounds were quantified using LC-ESI-QqQ. An API 3000 (PE Sciex, Concord, ON, Canada) triple quadrupole mass spectrometer equipped with a Turbo Ionspray source in negative-ion mode was used to obtain MS/MS data. Turbo Ionspray source settings were as follows: capillary voltage, −3500 V; nebulizer gas (N_2), 10 au (arbitrary units); curtain gas (N_2), 12 au; collision gas (N_2), 4 au; focusing potential, −200 V; entrance potential, −10 V; drying gas (N_2), heated to 400 °C and introduced at a flow rate of 8000 cm^3/min. The declustering potential and collision energy were optimized for each compound in infusion experiments: individual standard solutions (10 μg/mL) dissolved in 50:50 (v/v) mobile phase were infused at a constant flow rate of 5 μL/min using a model syringe pump (Harvard Apparatus, Holliston, MA). Full-scan data acquisition was performed scanning from m/z 100 to 800 in profile mode and using a cycle time of 2 s with a step size of 0.1 u and a pause between each scan of 2 ms. To confirm the identity of some compounds, neutral loss scan and precursor ion scan experiments were carried out. MS/MS product ions were produced by collision-activated dissociation (CAD) of selected precursor ions in the collision cell of the triple-quadrupole mass spectrometer and mass analyzed using the instrument's second analyzer. Additional experimental conditions for MS/MS included collision energy (depending on the compound), CAD gas (nitrogen) at 6 (arbitrary units), and scan range, as necessary for the precursor selected. Neutral loss scan of 162 u was carried out by scanning within the m/z range from 200 to 800 u, and precursor ion scan experiments were carried out by scanning Q1 between 300 and 800 u. In all of the experiments, both quadrupoles (Q1 and Q3) were operated at unit resolution.

For quantification purposes, data were collected in the multiple reaction monitoring (MRM) mode, tracking the transition of parent and product ions specific for each compound. In particular, we selected 15 transitions corresponding to ferulic acid-*O*-hexoside m/z 355 → 193 (CE, −20 V); ferulic acid m/z 193 → 134 (CE, −20 V); chlorogenic acid m/z 353 → 191 (CE, −20 V); cryptochlorogenic acid m/z 353 → 191 (CE, −20 V); neochlorogenic acid m/z 353 → 191 (CE, −20 V); dicaffeoylquinic acids m/z 515 → 353 (CE, −20 V); caffeic acid m/z 179 → 135 (CE, −20 V); caffeic acid-*O*-hexoside m/z 341 → 179 (CE, −20 V); quercetin m/z 301 → 151 (CE, −30 V); rutin m/z 609 → 300 (CE, −50 V); naringenin m/z 271 → 151 (CE, −30 V); naringenin-7-*O*-glucoside m/z 433 → 271 (CE, −20 V); kaempferol-3-*O*-rutinoside m/z 593 → 285 (CE, −30 V); and ethyl gallate (internal standard) m/z 197 → 169 (CE, −25 V).

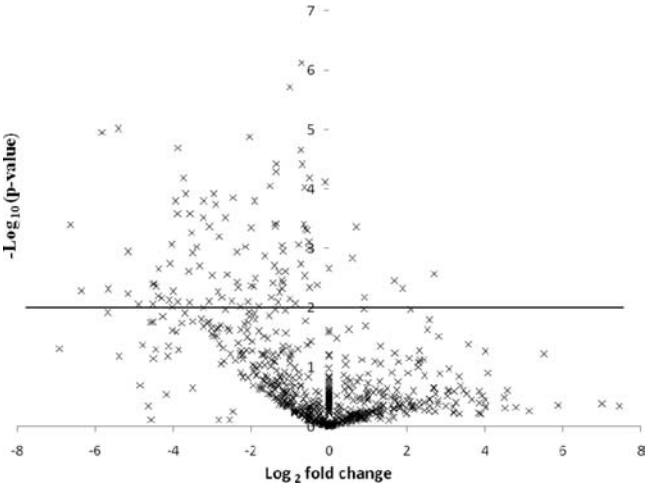

Figure 1. Volcano plot ($\log_2$ fold charge vs $-\log_{10} p$ value) representing the entire set of data (1200 features).

Quantification of phenolic compounds was performed by the internal standard method. Polyphenols were quantified with respect to their corresponding standard. When standards were not available, as in the case of caffeic-*O*-hexoside and ferulic-*O*-hexoside acids, they were quantified with respect to the corresponding hydroxycinnamic acid (caffeic and ferulic acids). The isomers of chlorogenic acid (caffeoylquinic and dicaffeoylquinic acids) were quantified with respect to the chlorogenic acid.

The liquid chromatograph was an Agilent series 1100 HPLC instrument (Agilent, Waldbronn, Germany) equipped with a quaternary pump, an autosampler, and a column oven set to 30 °C. Mobile phases consisted of 0.1% formic acid in Milli-Q water (A) and 0.1% formic acid in acetonitrile (B). The injection volume was 20 μL, and the flow rate was 0.4 mL/min. Separation was carried out for 20 min under the following conditions: 0 min, 5% B; 16 min, 40% B; 17 min, 95% B; 19 min, 95% B; 19.5 min, 5% B. The column was equilibrated for 5 min prior to each analysis.

Statistical Analysis. Mass profiles were obtained using Marker View 1.2 software and Statgraphics Plus 5.1. Analysis of variance (ANOVA) was used to remove possible mass features shared by all ketchups that could lead to a common pattern of noninformative signals. Therefore, one-way ANOVA was used to compare the effects of the different types of ketchups on metabolome modifications. Prior to each analysis, the data set was mean-centered and Pareto-scaled. We selected this scaling because it effectively increases the importance of low-concentration metabolites, albeit not to an extent that noise contributes to the model.

RESULTS AND DISCUSSION

The view that organic foods are "healthier" than those produced conventionally appears to be based on the perception that organic foods have superior sensory attributes, contain lower levels of pesticides and synthetic fertilizers, and have higher levels of nutrients and protective phytochemicals. Conversely, it has been suggested that the application of manure and reduced use of fungicides and antibiotics in organic farming could result in a greater contamination of organic foods by micro-organisms or microbial products.[20]

Untargeted Metabolomic Analysis. To establish the variations between the different organic and conventional agronomic techniques and to determine which metabolite has the greatest

Table 1. Significantly Different Compounds ($P < 0.05$) between Organic and Conventional Ketchups

compound	type	$[M - H]^-$	m/z ions	acc mass	mDa	MF	ratio mean conventional/mean organic
glutamylphenylalanine	conventional	293	164 (70), 147 (100), 103 (40)	293.1143	0.4	$C_{14}H_{18}N_2O_5$	
n-malonyl tryptophan	conventional	289	203 (40), 159 (20), 142 (40)	289.083	0.9	$C_{14}H_{14}N_2O_5$	
caffeic acid-*O*-hexoside 1	organic	341	179 (100), 135 (20)	341.0877	1.6	$C_{15}H_{18}O_9$	0.7033
caffeic acid-*O*-hexoside 2	organic	341	179 (100), 135 (20)	341.0877	0.7	$C_{15}H_{18}O_9$	0.6139
caffeic acid[a]	organic	179	135(100), 107(20)	179.0349	1.4	$C_9H_8O_4$	0.6366
neochlorogenic acid	organic	353	191 (100), 179 (80), 135 (30)	353.0877	0.7	$C_{16}H_{18}O_9$	0.3912
cryptochlorogenic acid	organic	353	191 (50) 173 (100), 135 (20)	353.0877	0.8	$C_{16}H_{18}O_9$	0.3894
chlorogenic acid[a]	organic	353	191 (100)	353.0877	1.5	$C_{16}H_{18}O_9$	0.6468
ferulic acid-*O*-hexoside	organic	355	193 (65), 178 (30), 149 (100)	355.1034	2.5	$C_{16}H_{20}O_9$	0.6240
rutin[a]	organic	609	609 (100), 300 (30)	609.1460	0.5	$C_{27}H_{30}O_{16}$	0.7101
quercetin[a]	organic	301	301 (10), 151 (100)	301.0353	0.4	$C_{15}H_{10}O_7$	0.4419
kaempferol-3-*O*-rutinoside[a]	organic	593	593 (100), 285 (60)	593.1511	0.7	$C_{27}H_{30}O_{15}$	0.6540
naringenin[a]	organic	271	151 (80), 119 (100)	271.0611	1.5	$C_{15}H_{12}O_5$	0.6790
naringenin-7-*O*-glucoside[a]	organic	433	433 (10), 271 (100)	433.1140	1.1	$C_{21}H_{22}O_{10}$	0.6503
dicaffeoylquinic acid 1	organic	515	515 (20), 353 (100), 191 (50)	515.1194	0.1	$C_{25}H_{24}O_{12}$	0.7028
dicaffeoylquinic acid 2	organic	515	515 (20), 353 (100), 191 (50)	515.1194	0.1	$C_{25}H_{24}O_{12}$	0.6065

[a] Comparison with standard.

effect on the overall metabolomic composition of ketchups, mass profiles were obtained using Marker View 1.2 software and Statgraphics Plus 5.1.

A volcano plot ($\log_2$ fold charge vs $-\log_{10} p$ value) representing the entire set of data (1200 features) is shown in Figure 1. Metabolites with statistically significant differences between organic and conventional ketchups lie above the horizontal threshold line. Then, the data set was mean-centered and Pareto-scaled after removal of all mass features showing nonsignificant p values or other mass features that were due to interferences between organic and conventional ketchup samples. A list of significantly different mass chromatographic features between organic and conventional ketchups is shown in Table 1. The elemental composition was selected according to the accurate masses and isotopic pattern (through the Formula Finder feature in Analyst QS 2.0). Then, the elemental composition obtained was sought in the *Dictionary of Natural Products* (Chapman and Hall/CRC), the MOTO database (http://appliedbioinformatics.wur.nl/moto), and CAS SciFinder. Interpretation of the observed MS/MS spectra in comparison with results found in the literature was the main tool for putative identification of metabolites.

The compounds found in significantly higher ($P < 0.05$) amounts in organic rather than conventional ketchups were caffeoylquinic and dicaffeoylquinic acids, caffeic and caffeic acid hexosides, kaempferol-3-*O*-rutinoside, ferulic-*O*-hexoside, naringenin-7-*O*-glucoside, naringenin, rutin, and quercetin, whereas glutamylphenylalanine and *N*-malonyltryptophan were detected only in conventional brands. It was possible to differentiate the isomers of caffeoylquinic, dicaffeoylquinic, and caffeic-*O*-hexoside acid in relative intensities in MS^2 spectra using LC-ESI-QToF. Table 1 shows the list of 16 compounds identified through HPLC-QToF-MS having levels that were significantly different ($P < 0.05$) between organic and conventional brands, along with retention times (rt), accurate mass (acc mass), molecular formula (MF), and millidaltons (mDa) of error between the mass found and the accurate mass of each metabolite and the MS-MS ions used for the identification.

Mass Characterization of Glutamylphenylalanine and *N*-Malonyltryptophan in Conventional Ketchups. Although mass characterization of phenolic compounds has been widely described in the literature for tomatoes and tomato-based products,[21−23] this is the first time that glutamylphenylalanine and *N*-malonyltryptophan have been found in conventional tomato ketchups.

Examination of the chromatograms in the ToF-MS mode suggested the presence of glutamylphenylalanine (m/z 293) and *N*-malonyltryptophan (m/z 289) with errors of 0.39 and 0.90 mDa, respectively. The MS^2 of m/z 293 showed ions at m/z 164, 147, and 103, which may correspond to losses of $C_5H_7NO_3^-$, NH_3, and COO, respectively. The MS^2 of m/z 289 showed ions at m/z 203, 159, and 142, which could be attributed to losses of $C_3H_2O_3^-$, COO, and NH_3, respectively. Glutamylphenylalanine is reported here for the first time, whereas *N*-malonyltryptophan is a predominant indole compound widespread in higher plants and found in leaves, fruits, and seeds of wheat and tomatoes.[22,24,25]

It is well-known that the biosynthesis of phenolic compounds in plants is strongly influenced by the cultivar[6] and mode of fertilization.[26] The level of carbon-based secondary metabolites such as phenolic compounds is usually higher in organic plants[9,10] due to their defensive role in plants under stress conditions.[27] Our results for nitrogen compounds are in line with a German review reporting a lower nitrate content in organic vegetables in nearly all cases and less protein in organic cereal grains.[28] These differences are reflected in our metabolomic analysis, which detected 14 metabolites as chemotaxonomic ketchup markers able to distinguish between varieties from different agronomic treatments. The metabolomic approach makes it possible to detect several hundred metabolites simultaneously and to reliably compare samples for differences and similarities in a semiautomated and, above all, untargeted manner.

Analysis of Total Phenolic Content and Hydrophilic Antioxidant Capacity. The F-C assay showed that organic ketchups contained significantly ($P < 0.05$) higher concentrations of TP than conventionally produced ketchups (results shown in Table 2). A similar trend was observed for hydrophilic antioxidant capacity, with the lowest being determined in conventional ketchups.

Table 2. Total Phenolic Content and Hydrophilic Antioxidant Capacity Using ABTS$^+$ and DPPH Assays Expressed as Mean ± SD of Organic and Conventional Ketchups[a]

ketchup	total phenolic content (mg GAE/ 100 g FW)	ABTS$^+$ (mmol TE/ 100 g FW)	DPPH (mmol TE/ 100 g FW)
conventional			
C_1	9.43 ± 0.10 ab	3.78 ± 0.09 a	4.95 ± 0.11 a
C_2	9.61 ± 0.12 b	3.81 ± 0.07 a	4.67 ± 0.08 b
C_3	9.03 ± 0.08 c	3.89 ± 0.10 b	4.93 ± 0.08 a
C_4	8.14 ± 0.11 d	3.45 ± 0.06 c	4.20 ± 0.09 c
C_5	9.40 ± 0.17 a	3.37 ± 0.08 c	3.94 ± 0.08 d
C_6	9.61 ± 0.13 b	3.65 ± 0.08 d	4.21 ± 0.07 c
C_7	8.62 ± 0.16 e	2.71 ± 0.08 e	3.21 ± 0.09 e
C_8	8.46 ± 0.19 e	3.24 ± 0.08 f	4.15 ± 0.10 c
C_9	7.62 ± 0.13 f	2.59 ± 0.06 g	3.46 ± 0.09 f
C_{10}	8.07 ± 0.10 d	2.65 ± 0.09 eg	3.25 ± 0.11 e
organic			
O_1	11.56 ± 0.18 g	4.25 ± 0.07 h	5.38 ± 0.12 g
O_2	12.61 ± 0.11 h	4.41 ± 0.06 i	5.50 ± 0.10 h
O_3	12.22 ± 0.15 i	4.27 ± 0.06 h	5.21 ± 0.09 i
O_4	12.37 ± 0.16 i	4.19 ± 0.07 h	5.09 ± 0.10 j
O_5	12.30 ± 0.12 i	4.53 ± 0.07 j	5.45 ± 0.09 gh

[a] Letters in the columns represent significant statistical differences ($P < 0.05$). GAE, gallic acid equivalents; TE, Trolox equivalent; FW, fresh weight; SD, standard deviation.

Data on the phenolic composition of fruits and vegetables grown either organically or conventionally remain scarce in the literature, because these compounds have only recently been considered as interesting functional microconstituents due to their role in the prevention of cancer, cardiovascular diseases, and degenerative diseases. Among papers mentioning total phenolic content, the majority describe a higher phenolic concentration in organically grown fruits or vegetables.[29] Our results are in accordance with these studies because organic ketchups showed a higher content of polyphenols and antioxidant capacity than conventional ketchups.

Quantitation of Individual Polyphenols in Conventional and Organic Ketchups. Specific polyphenol compounds were monitored in conventional and organic ketchups, with the results shown in Table 3. The main polyphenol in all ketchups was rutin, present at levels ranging between 40.88 and 77.65 μg/g FW, followed by naringenin, as reported in other studies.[6,30] Rutin and naringenin concentrations were significantly higher in the ketchups made from organically grown tomatoes. Chassy et al.[31] found significantly higher mean levels of soluble solids, flavonoids, total phenolics, and ascorbic acid in organic tomatoes than in their conventional counterparts grown in model plots over a 3-year period.

Hydroxycinnamic acids were mainly represented by ferulic acid-*O*-hexoside, the content of which ranged from 15.59−29.24 μg/g FW in conventional ketchups to 34.50−40.85 μg/g FW in organic ketchups. Caffeic acid-*O*-hexosides and caffeic acid followed a similar trend, with higher levels found in organic ketchups.

Table 3. Content of Polyphenols Expressed as Mean ± SD of Organic and Conventional Ketchups; superscript [a]

	μg/g FW			
ketchup	ferulic acid-*O*-hexoside	caffeic acid-*O*-hexoside 1	caffeic acid-*O*-hexoside 2	caffeic acid
conventional				
C_1	29.24 ± 0.72 a	3.87 ± 0.11 a	1.23 ± 0.06 a	1.41 ± 0.04 a
C_2	28.08 ± 0.40 b	3.79 ± 0.14 a	1.52 ± 0.04 b	1.34 ± 0.05 b
C_3	28.32 ± 0.87 b	3.52 ± 0.11 b	1.48 ± 0.07 b	1.33 ± 0.05 b
C_4	15.59 ± 0.36 c	2.20 ± 0.07 c	1.36 ± 0.05 c	1.18 ± 0.04 c
C_5	25.91 ± 0.58 d	3.92 ± 0.05 a	1.36 ± 0.07 c	1.90 ± 0.05 d
C_6	19.23 ± 0.64 e	2.93 ± 0.08 d	1.37 ± 0.07 c	1.24 ± 0.05 c
C_7	19.58 ± 0.72 ef	2.84 ± 0.08 d	1.03 ± 0.04 d	1.19 ± 0.05 c
C_8	22.76 ± 0.75 g	3.20 ± 0.11 e	1.36 ± 0.05 c	1.74 ± 0.05 e
C_9	26.83 ± 0.75 h	2.50 ± 0.09 f	1.15 ± 0.04 e	1.76 ± 0.06 e
C_{10}	20.14 ± 0.53 f	3.33 ± 0.10 g	1.40 ± 0.04 c	1.40 ± 0.04 a
organic				
O_1	34.84 ± 0.38 h	4.02 ± 0.09 h	2.00 ± 0.06 f	2.08 ± 0.03 f
O_2	40.85 ± 0.91 i	4.51 ± 0.13 i	2.39 ± 0.04 g	2.89 ± 0.04 g
O_3	39.62 ± 0.72 j	4.99 ± 0.11 j	2.20 ± 0.06 h	2.40 ± 0.04 h
O_4	39.04 ± 0.94 j	4.00 ± 0.10 h	1.88 ± 0.06 i	1.73 ± 0.03 i
O_5	34.50 ± 0.69 h	5.30 ± 0.06 k	2.33 ± 0.05 g	2.28 ± 0.05 j

	μg/g FW				
ketchup	neochlorogenic acid	cryptochlorogenic acid	chlorogenic acid	dicaffeoylquinic acid 1	dicaffeoylquinic acid 2
conventional					
C_1	0.56 ± 0.02 a	0.18 ± 0.01 a	10.24 ± 0.14 a	0.36 ± 0.01 a	0.24 ± 0.01 a
C_2	0.57 ± 0.01 a	0.22 ± 0.01 b	9.38 ± 0.09 b	0.50 ± 0.01 b	0.35 ± 0.01 b
C_3	0.76 ± 0.03[b,c]	0.26 ± 0.01 c	9.47 ± 0.15 bc	0.50 ± 0.02 b	0.42 ± 0.02 c

Table 3. Continued

	μg/g FW				
ketchup	neochlorogenic acid	cryptochlorogenic acid	chlorogenic acid	dicaffeoylquinic acid 1	dicaffeoylquinic acid 2
C_4	0.43 ± 0.02 d	0.14 ± 0.01 d	5.58 ± 0.10 d	0.47 ± 0.02 c	0.31 ± 0.01 d
C_5	0.73 ± 0.02 b	0.32 ± 0.01 e	9.54 ± 0.12 c	0.55 ± 0.02 d	0.44 ± 0.01 e
C_6	0.79 ± 0.03 c	0.35 ± 0.01 f	11.04 ± 0.12 e	0.48 ± 0.01 c	0.45 ± 0.01 e
C_7	0.65 ± 0.02 e	0.36 ± 0.01 f	6.49 ± 0.06 f	0.43 ± 0.01 e	0.41 ± 0.01 c
C_8	0.91 ± 0.02 f	0.29 ± 0.01 g	8.85 ± 0.12 g	0.39 ± 0.02 f	0.44 ± 0.01 e
C_9	0.88 ± 0.01 g	0.27 ± 0.01 c	7.09 ± 0.07 h	0.46 ± 0.02 c	0.34 ± 0.01 b
C_{10}	0.77 ± 0.02 c	0.32 ± 0.01 e	6.93 ± 0.08 i	0.33 ± 0.01 g	0.36 ± 0.01 b
organic					
O_1	1.57 ± 0.02 h	0.66 ± 0.01 h	13.28 ± 0.15 j	0.71 ± 0.01 h	0.71 ± 0.01 f
O_2	1.20 ± 0.02 i	0.44 ± 0.01 i	12.49 ± 0.13 k	0.74 ± 0.01 i	0.61 ± 0.02 g
O_3	2.44 ± 0.02 j	0.96 ± 0.01 j	13.53 ± 0.17 l	0.66 ± 0.01 j	0.67 ± 0.01 h
O_4	2.32 ± 0.07 k	0.57 ± 0.02 k	12.81 ± 0.13 m	0.56 ± 0.01 k	0.58 ± 0.02 i
O_5	1.48 ± 0.06 l	0.85 ± 0.02 l	13.30 ± 0.15 j	0.51 ± 0.01 b	0.53 ± 0.01 j

	μg/g FW				
ketchup	rutin	quercetin	naringenin	naringenin-7-*O*-glucoside	kaempferol-3-*O*-rutinoside
conventional					
C_1	64.00 ± 1.04 a	0.56 ± 0.02 a	44.30 ± 1.10 a	3.41 ± 0.08 a	29.37 ± 0.82 a
C_2	54.50 ± 1.59 b	0.61 ± 0.01 b	54.96 ± 1.24 b	3.34 ± 0.13 ab	29.88 ± 1.28 a
C_3	60.24 ± 1.33 c	0.67 ± 0.02 c	40.13 ± 1.25 c	3.23 ± 0.13 b	29.25 ± 0.76 ab
C_4	55.84 ± 1.24 b	0.26 ± 0.01 d	34.97 ± 1.43 d	2.22 ± 0.07 c	16.34 ± 0.52 c
C_5	49.58 ± 1.03 d	0.61 ± 0.01 b	45.66 ± 1.48 a	2.39 ± 0.09 d	29.08 ± 0.99 ab
C_6	45.28 ± 1.21 e	0.63 ± 0.02 b	37.74 ± 3.23 e	2.70 ± 0.08 e	21.31 ± 0.78 d
C_7	53.88 ± 0.98 b	0.50 ± 0.02 e	27.87 ± 1.14 f	2.25 ± 0.08 c	16.13 ± 0.64 c
C_8	49.07 ± 1.36 f	0.49 ± 0.01 e	38.55 ± 1.00 e	1.77 ± 0.07 f	28.97 ± 0.68 ab
C_9	41.96 ± 1.61 g	0.53 ± 0.01 f	29.79 ± 1.24 g	1.27 ± 0.06 g	27.48 ± 0.94 c
C_{10}	40.88 ± 1.78 g	0.39 ± 0.02 g	40.28 ± 1.58 h	1.99 ± 0.08 h	28.24 ± 0.75 bc
organic					
O_1	69.50 ± 1.26 h	0.73 ± 0.01 h	52.76 ± 1.60 i	3.69 ± 0.07 i	34.17 ± 1.02 e
O_2	75.15 ± 1.27 i	1.20 ± 0.03 i	60.81 ± 1.17 j	3.64 ± 0.07 i	35.98 ± 1.42 f
O_3	68.57 ± 1.55 h	0.80 ± 0.02 j	57.33 ± 1.88 k	4.01 ± 0.05 j	40.11 ± 1.02 g
O_4	71.90 ± 1.66 j	1.96 ± 0.05 k	67.16 ± 1.37 l	3.06 ± 0.03 k	38.65 ± 1.03 h
O_5	77.65 ± 0.88 k	1.25 ± 0.03 l	52.25 ± 1.42 m	4.49 ± 0.02 l	46.85 ± 1.28 i

[a] Letters in the columns represent significant statistical differences ($P < 0.05$). FW, fresh weight.

Chlorogenic acid isomers were also present in ketchups. Chlorogenic acid was the most abundant caffeoylquinic acid, ranging from 5.58−11.04 μg/g FW in conventional ketchups to 12.49−13.53 μg/g FW in organic ketchups. These results are in line with those reported by Caris-Veyrat et al.,[32] who retrieved significantly higher concentrations of chlorogenic acid and glycoalkaloids from organic tomatoes ($P < 0.05$) in comparison to the conventional variant. Absolute differences in the levels of dicaffeoylquinic acids were also found in the two types of ketchup, dicaffeoylquinic acid 1 being the most abundant among the majority, ranging from 0.33−0.55 μg/g FW in conventional ketchups to 0.51−0.74 μg/g FW in organic ketchups.

Flavanones were represented by naringenin and naringenin-7-*O*-glucoside, with maximum concentrations being determined in organic ketchups. Naringenin levels ranged from 27.87−54.96 μg/g FW in conventional ketchups to 52.25−67.16 μg/g FW in organic ketchups, and levels of naringenin-7-*O*-glucoside varied from 1.27−3.41 μg/g FW in conventional ketchups to 3.06−4.49 μg/g FW in organic ketchups.

Flavonols were represented by rutin followed by kaempferol-3-*O*-rutinoside and quercetin. The organic ketchups had higher levels of rutin (68.57−77.65 μg/g FW) than the conventional ones (40.88−64.00 μg/g FW). The same pattern was observed for quercetin and kaempferol-3-*O*-rutinoside.

Similarly, the concentrations of quercetin and kaempferol were much lower in the conventionally produced ketchups. Mitchell et al.[33] described results in line with our study, reporting that the mean level of the flavonoids quercetin, naringenin, and kaempferol was significantly higher ($P < 0.001$) in tomato samples from the organic cropping system than in those produced conventionally. They suggested that there is a significant difference between the two systems in the amount of flavonoids occurring in ripe fruit at harvest. Therefore, different food cultivation methods may result in differences in the content of secondary metabolites such as polyphenolic compounds. Grinder-Pedersen et al.

compared conventionally produced diets (CPD) and organically produced diets (OPD) in a human crossover intervention study (n = 16) in terms of the intake and excretion of five selected flavonoids and the effect on markers of oxidative defense. The urinary excretion of quercetin and kaempferol was higher after 22 days of intake of the OPD than of the CPD ($P < 0.05$). Therefore, the food production method affected the content of the major flavonoids, quercetin and kaempferol, in foods and also affected urinary flavonoids and markers of oxidation in humans.[34]

In another study performed to evaluate the polyphenol content of apples (Golden Delicious) grown under defined organic and conventional conditions, it was found that organically produced apples showed significantly higher concentrations of chlorogenic acid, flavonols, flavanols, and dihydrochalcones than the conventionally produced fruits.[35]

The results obtained in this study of metabolite profiling led to the distinction of features that differentiate between organic and conventional ketchups. Following chromatographic alignment and peak detection, statistical analysis was performed to identify metabolites that may serve as markers for organic and conventional ketchups. It was observed that organic cultivation can provide tomatoes and tomato-derived products with a significantly higher content of phenolic compounds, whereas glutamylphenylalanine and *N*-malonyltryptophan were detected only in conventional brands. Plant defense-related secondary metabolites are generally considered to be the most important determinant of the nutritional value of fruits and vegetables, and thus organically grown products are more health-promoting than conventional products.

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ABBREVIATIONS USED

F-C, Folin−Ciocalteu; SPE, solid-phase extraction; TP, total polyphenols; HPLC-QToF-MS, liquid chromatography coupled to a hybrid quadrupole time-of-flight mass spectrometer; LC-ESI-QqQ, liquid chromatography coupled to mass spectrometry in tandem mode; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl; PBS, phosphate-buffered saline, 5 mM; Trolox, (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid; GAE, mg of gallic acid equivalents; FW, fresh weight; SD, standard deviation; TE, Trolox equivalent; MRM, multiple reaction monitoring; ANOVA, analysis of variance.

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