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POLYPHENOL AND VOLATILE COMPOUNDS IN KIWIFRUIT (*ACTINIDIA DELICIOSA*) BALSAMIC VINEGAR AND DERIVATIVE PRODUCTS

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SOMMARIO
I kiwi contengono elevate quantità di antiossidanti benefici per la salute. L'aceto e nello specifico l'acido acetico in esso contenuto ha mostrato interessanti effetti metabolici ed effetti favorevoli sui fattori di rischio cardiovascolare. In questo lavoro sono stati studiati alimenti innovativi che combinano kiwi e aceto. Sono stati analizzati mediante HPLC/DAD, HPLC-MS/TOF, GC-MS e 2DGC-MS/TOF un aceto balsamico di kiwi di 3 anni (invecchiato in una botte di vetro) e di 8 anni (affinato in botti di rovere) (brevetto RM2014A000521), un giovane aceto di kiwi, una glassa a base di aceto di kiwi ed una marmellata di aceto di kiwi.

SUMMARY
Kiwifruit contains high amounts of anti-oxidants beneficial to health. Vinegar/acetic acid showed interesting metabolic effects and favorable effects on cardiovascular risk factors. In this work innovative foods combining kiwi an vinegar were studied. A kiwi balsamic vinegar 3 years old (glass barrel aged) and 8 years old (aged in oak barrels) (Patent RM2014A000521), a young kiwi vinegar, a kiwi vinegar glaze and a kiwi vinegar jam, were analysed by HPLC/DAD, HPLC-MS/TOF, GC-MS and 2DGC-MS/TOF.

INTRODUCTION

The kiwifruit is the edible berry of several *Actinidia* species. Kiwifruit contains high amounts of anti-oxidants beneficial to health. Hydroxycinnamic acids, procyanidins, and quercetin glycosides were the main polyphenol classes detected by HPLC–DAD–ESI/MS in the kiwifruit skin (Pinelli et al, 2013). Pulp raw extracts led to the isolation of caffeic acid glucosyl derivatives and coumarin glycosides in addition to the above compounds (Fiorentino et al, 2009). The main component of vinegar is acetic acid, which gives vinegar its sour taste and pungent smell. Additional components in vinegar include other organic acids amino acids, peptides, vitamins, mineral salts, and polyphenolic compounds (e.g., catechin, caffeic, ferulic acid). In recent decades, there has been increasing interest in the metabolic effects of vinegar (Petsiou et al, 2014). The majority of clinical studies have demonstrated that vinegar/acetic acid can beneficially affect glucose metabolism in healthy subjects and in patients with insulin resistance or diabetes mellitus. Vinegar has also been shown to protect from lipid accumulation in liver and skeletal muscle (Petsiou et al, 2014). There is some evidence supporting the favorable effects of vinegar on cardiovascular risk factors, such as hyperglycemia, hyperinsulinemia, hyperlipidemia, and obesity.

Innovative foods, a kiwi balsamic vinegar 3 years old (glass barrel aged) and 8 years old (aged in oak barrels) (Patent RM2014A000521) were analysed by HPLC/DAD, HPLC-MS/TOF, GC-MS and 2DGC-MS/TOF. A young kiwi vinegar and two product obtained by its processing, in particular a glaze and a jam, were also analysed.

MATERIALS AND METHODS

HPLC-DAD-MS analyses were carried out using an HP 1100L liquid chromatograph equipped with a DAD detector (Agilent Corp, Santa Clara, CA, USA). Compounds were separated by using a 150x4.6 mm i.d, 5 µm LUNA C18 column (Phenomenex, USA) (Romani et al, 2016). The HPLC system was interfaced with an Agilent TOF MS equipped with an ESI source. The TOF/MS analysis worked using full-scan mode and the mass range was set to m/z 100–1500 in both positive and negative modes (Romani et al, 2016). The acquisition and data analysis were controlled using Agilent LC-MS TOF Software (Agilent, USA).

Vinegar samples were diluted five times and put in a 20 ml vial with NaCl and analyzed by SPME-GC-MS and by SPME-GC×GC-TOF-MS. SPME-GC-MS: the profile of VOCs was determined by absorption of VOCs at 25°C (for 20-min) on a trivalent fiber Carboxen PDMS DVB 2 cm, followed by desorption at 280°C and, then, analysis by GC/MS. An Agilent 7890 a GC equipped with a 5975C MSD was used. The analyte separation was achieved with a column Agilent DB InnoWAX 50m, 0.20 µm id, 0.40 µm df. Chromatographic conditions were: initial temperature 40°C for 0,5 min, then 6°C min-1 up to 260°C. This temperature was maintained for 1 min. SPME-GC×GC-TOF-MS: the SPME conditions were the same of GC analysis. GC×GC analyses were carried out on an SRA-Agilent GC-MS 7890B, with GC 2D system, coupled to an TOF-DS Markes detector. The analyte separation was achieved with a HP-5MS UI column coupled with a InnoWAX column. A tentative compounds identification was performed by comparing Mass spectra of each peak with those reported in mass spectral databases.

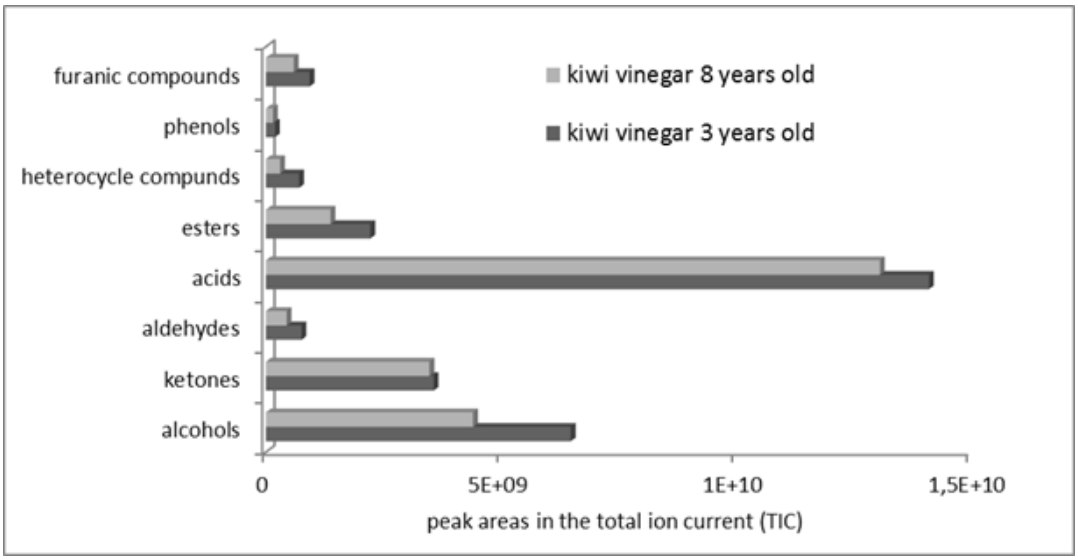
RESULTS AND DISCUSSION

HPLC/MS analysis showed the presence of gallic acid with quasi molecular ion m/z 169 $[M-H]^-$ and protocatechic acid with quasi molecular ion m m/z 153 $[M-H]^-$ and fragment ion m/z 109 $[M-44]^-$ corresponding to the loss of the $–CO_2$ group and quinic acid m/z 191 $[M-H]^-$. A peak showing a quasi molecular ion m/z 109 $[M-H]^-$ was hypnotized to be a dihydroxybenzene. Quantitative data, determined at 280 nm using gallic acid as reference compound, evidenced an higher content of secondary metabolites in the 8 years old vinegar (1146.1 mg/L) respect to the 3 years old (330.2 mg/L). Data of single compounds are shown in Table 1.

- Table 1: kiwi vinegars quantitative data, expressed as mg/L as a mean of three determination, RSD<5%.

	3 years old mg/L	8 years old mg/L
Gallic acid	2.4	182.0
Protocatechic acid	234.1	950.5
Peak m/z 110	93.7	13.6

It is worth noting that the 3 years old sample showed the higher number of volatile compounds (VOCs). In the Figure 1 identified VOCs are grouped in subclasses. In particular alcohols, ketones, aldehydes, acids, esters, heterocyclic compounds, phenols and compounds with a furanic group are present.



- Figure 1: VOCs subclasses of kiwi vinegars.

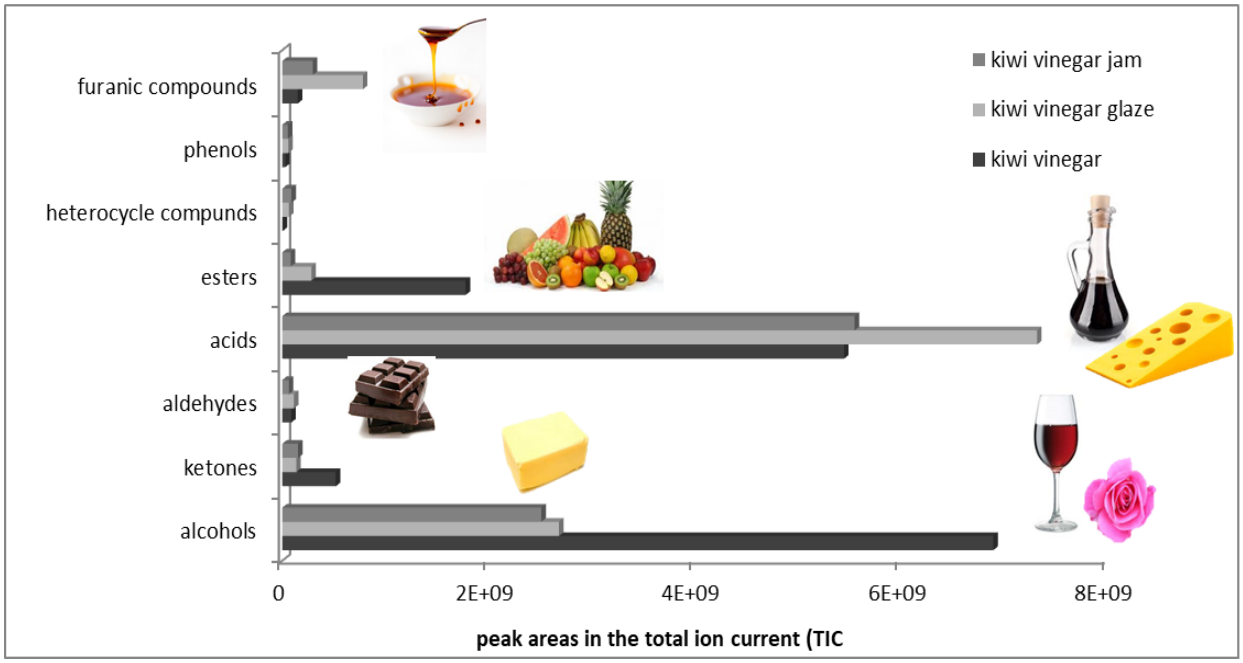
Table 1 shows the quantitative data of the compounds identified in young kiwi vinegar, glaze and jam samples. The compounds were calibrated with a gallic acid calibration curve and corrected for their respective molecular weights. The not aged vinegar showed a lower content of secondary metabolites respect to the balsamic vinegars. The glaze had a content of secondary metabolites three times higher of the jam.

- Table 2: kiwi vinegar and derivative products quantitative data, expressed as mg/L or mg/g as a mean of three determination, RSD<5%.

	Young kiwi vinegar mg/L	**Jam kiwi vinegar mg/g**	**Glaze kiwi vinegar mg/g**
Gallic acid	6.75		
unknown compound		0.31	1.85
unknown compound		0.36	1.82
Protocatechic acid	37.05	0.28	0.20
unknown compound	18.50	0.18	0.16
unknown compound		0.25	
Sum	62.30	1.37	4.04

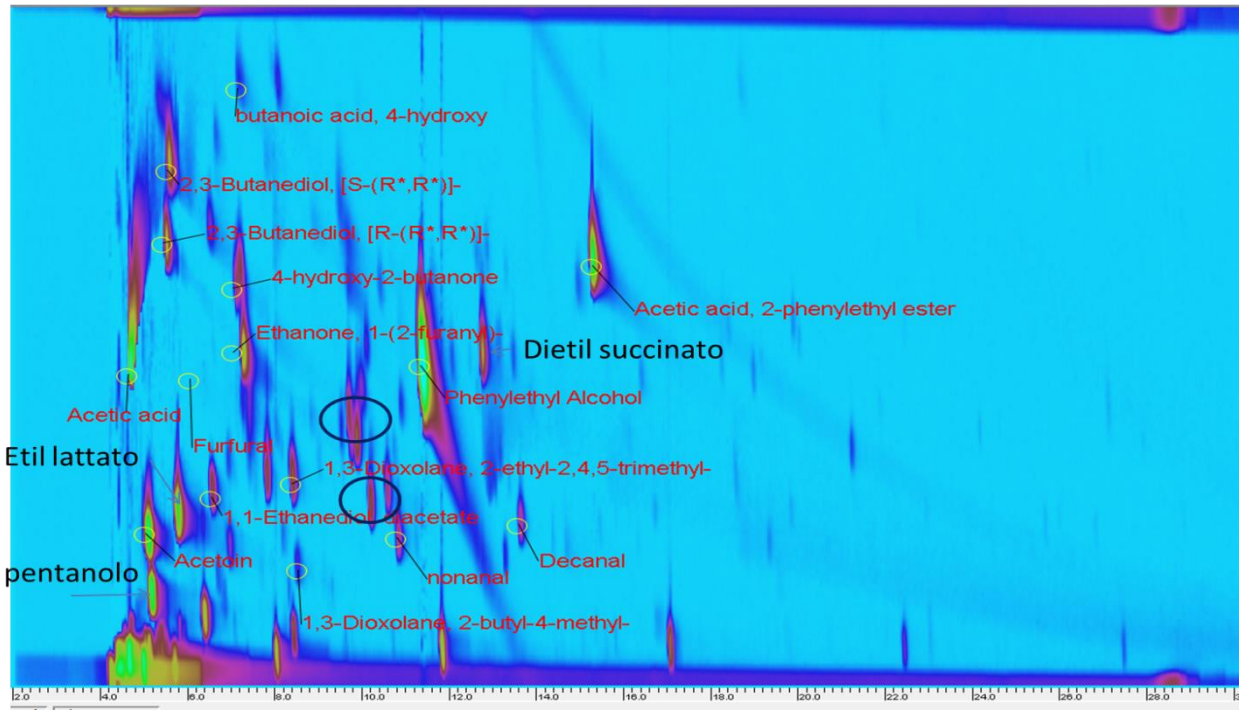
In the Figure 2 identified VOCs are grouped in subclasses with the relatives aroma descriptors. In particular alcohols, ketones, aldehydes, acids, esters, heterocyclic compounds, phenols and compounds with a furanic group are present. The furanic compounds were higher in processed samples, especially in the glaze.

Figure 2: VOCs subclasses of kiwi vinegar and derivative products with images of correspondent aroma descriptors.



HS-SPME and GC×GC-MS fingerprint analysis are ideal tools to analyze complex volatile matrices, and provide a sensitive method for the direct comparison and chemical visualization of food volatile components. HS-SPME GC×GC-TOF-MS analysis of the volatile fraction of kiwi vinegars and derivative products was submitted to advanced fingerprinting analysis of 2D chromatographic data (in Figure 3 the example of young kiwi vinegar). The use of HS-SPME-GC×GC-MS analysis permitted the creation of a comprehensive template matching fingerprinting as showed in Figure 3. This method considers, as comparative feature, each individual 2D peak together with its time coordinates, detector response and MS fragmentation pattern, and includes them in a sample template that is created by the analyst and can be used to compare plots from different samples directly and comprehensively.

Figure 3: 2D data and comprehensive template matching fingerprinting with the main identified volatile compounds of young kiwi vinegar.



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