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Improving the nutritional value of kiwifruit with the application of agroindustry waste extracts

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Summary

The purpose of this study was to evaluate the effects of the application of an agro-industrial waste extract (AWE) on the quality of kiwifruit.

The AWE was obtained by extraction from apple seeds, rapeseed and rice husks and then boron (0.6 %) and zinc (1.4 %) were added. The effect of AWE as a fertilizer/biostimulant on several parameters of kiwifruit quality (weight, total soluble solids, firmness, dry matter percentage, pH, titratable acidity, antioxidant capacity, ascorbic acid) was then evaluated. The application was carried out on two cultivars of *Actinidia deliciosa*, cv. Hayward and cv. Green Light, and also in two different cultivation environments.

AWE increased the fruit weight in cv. Green Light and in cv. Hayward fruits grown in Piedmont but no increase was observed in dry matter percentage for fruits of both cultivars.

The most relevant effect of AWE was the significant increase of the ascorbic acid (AA) content in the fruits of cv. Hayward of all the tested orchards.

Fertilization with biostimulants shows a promising future in functional plant nutrition linked to an increase in food quality parameters.

Introduction

The great interest in kiwifruit cultivation and consumption is also due to its nutraceutical value, its content of ascorbic acid (AA) in particular and also its long term storage capability, which makes this valuable fruit available and exportable throughout winter time in the Northern Hemisphere.

Pre-harvest treatments and storage period greatly influence the ripening physiology of the kiwifruit, and therefore the correct management of these has a significant effect on the final quality of the product. Thus the current trend for local or export markets is to achieve an extended marketing period while maintaining high fruit quality standards (BRIGATI and DONATI, 2007).

In the global market, the quality parameters of kiwifruit, including size and shape, are essential for the product to compete effectively (HOPPING and SIMPSON, 1982; GALIMBERTI, 2002).

In the last few years, however, new parameters to define the final quality of the kiwifruit have been taken into consideration. Nowadays the fruit is considered a "functional food", due to its high antioxidant activity and significant AA content. Kiwifruit is a food able to confer health benefits and to contribute to the prevention of diseases such as cancer and heart attacks (KAUR and KAPOOR, 2001). This aspect could be important because many medical and epidemiological surveys have shown an inverse relationship between the consumption of fruits, rich in antioxidants and vitamins, and the incidence of coronary heart disease and certain cancers (RICE-EVANS and MILLER, 1996; BUB et al., 2003). In particular, AA is a strong antioxidant, along with vitamin E, beta-carotene, and many other plant-based nutrients, and blocks some of the damage caused by free radicals, which occur naturally when the human body transforms food into energy (AMES et al., 1993; BENZIE, 2000).

The broad distribution of AA throughout the plant tissues suggests this compound plays a role in a wide range of physiological phenomena. Best studied among these functions is the AA role in redox processes during photosynthesis, environment-induced oxidative stress and during wound- and pathogen-induced oxidative processes (NOCTOR and FOYER, 1998; DAVEY et al., 2000; HOREMANS et al., 2000; SMIRNOFF, 2000, FRANCESCHI and TARLYN, 2002). To satisfy commercial demand and, recently, to implement nutraceutical positive traits, currently growth regulators (auxins, gibberellins and cytokinins) are applied to kiwifruit orchards (ANTOGNOZZI et al., 1994; IWAHORI et al., 1988). In addition to growth regulators, micronutrients and amino acids are also distributed in the orchards to obtain better quality.

The development of new fertilisers/biostimulants from natural raw materials has become the focus of recent interest for fruit crops. An indispensable requirement of sustainable fruit culture is the management of the cultures with renewable resources, lessening the need for chemical products (fertilizers, growth regulators), and thus lessening their environmental burden (TENGERDY and SZAKACS, 1998).

In this study the influence on quality parameters in kiwifruits of the application of an extract from agro-industrial vegetable by-products with bioactive compounds, with added boron and zinc, is described. The substrate for agro-industrial waste extract (AWE) production was a mixture of organic waste by-products from apple seeds, rapeseed and rice husks, subjected to proteolytic and hydrolytic extraction processes. All these by-products present a high level of high value biomolecules with biological activity and many phenolic compounds – very important vegetable biochemicals involved in different physiological functions, such as fruit development (TAIZ and ZEIGER, 2006). AWE was then added with boron (0.6 %) and zinc (1.4 %): these elements, added to the agro-industrial waste extracts, help to increase fruit size: in some situations this effect is accompanied by a decrease in the dry matter content (NARDOZZA et al., 2007).

Taking into account the interaction phenomena between kiwifruit nutrition and quality, in this study an organic fertilizer with bioactive compounds was used in order to determine its influence on kiwifruit quality.

One of the main bioactive compounds present in AWE is the water-soluble phytohormonal content. It has been found that all the main phytohormones (auxins, gibberellins, cytokinins) can be extracted from vegetable organic waste by the hydrolytic process (PARRADO et al., 2007).

Apple seeds, used for the preparation of the waste extract in this study, are proven to contain high levels of growth hormones, such as gibberellins and cytokinins (LUCKWILL et al., 1969); the chemical composition of rice husks derived from waste processing depends on the type of raw material and includes proteins and amino acids (5 % albumin, 1 % globulin, 1 % prolamin, 93 % gluten) and microelements (ZEMNUKHOVA et al., 2004; JULIANO, 1985); finally, colza seeds contain a very high and varied amount of amino acids and microelements (SZCZUREK, 2010).

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Materials and methods

Agro-industrial waste-extract application

The main components of AWE are nitrogen (peptides and free amino acids) and phytohormones (auxins, gibberellins, cytokinins), as functional molecules. Some important chemical components of AWE are shown in Tab. 1 (AWE composition is indicated by the product label). As these data show, protein is an important component. The AWE was obtained from Intrachem Bio Italia.

Tab. 1: Protein, amino acids and growth regulators content of AWE: means of three samples.

Component	Concentration
Protein (g kg^{-1})	980
Free amino acids (g kg^{-1})	140
Indolacetic acid (IAA) (mg kg^{-1})	152.68
Naphtoxy acetic acid (NAA) (mg kg^{-1}) w/w)	12.51
Indolbutyric acid (IBA) (mg kg^{-1})	15.1
Gibberellic acid (mg kg^{-1})	256.3

The application was carried out on two cultivars of *Actinidia deliciosa*, cv. Hayward and cv. Green Light, grown under the same agronomical and environmental conditions, in order to better understand the interaction between the treatment and the different genotypes. The trials were located in Givoletto, Piedmont Region, North West Italy. Moreover, the same trials were repeated for the cultivar Hayward in another cultivation environment at Cisterna di Latina (Latium Region, Central Italy), under the same agronomical conditions, in order to study the effect of the different pedoclimatic conditions on the results of the application.

AWE was applied in the ratio of 1 L ha^{-1}, diluted in a water solution, twice, once two weeks before flowering and again one week after the fruit set. The produce was spread on the orchard by using a spray system for foliar application (CHAPIN et al., 1993; KIELLAND, 1994; MILLER and HSU, 1986).

The production of plots treated with AWE was compared with untreated plots. For each thesis, 25 plants in 5 sub-plots were randomly selected and 125 fruits were harvested from each plant. The fruits were harvested at conventional ripeness. Sampling was carried out randomly and the fruits were immediately stored for 3 months in normal atmosphere at 1 °C and relative humidity 95 %, simulating the usual storage conditions for these fruits. Any mould or alteration of the fruits was observed.

Reagents and standards

Potassium dihydrogen phosphate, sodium acetate, citric acid, hydrochloric acid, 2,4,6-tripyridyl-s-triazine (TPTZ) and 1,2-phenylenediamine dihydrochloride (OPDA) were purchased from Sigma Aldrich, USA, while acetic acid was purchased from Fluka Biochemika, Switzerland.

Ethylene diamine tetraacetic acid (EDTA) disodium salt was purchased from AMRESCO, USA, while sodium fluoride was purchased from Riedel-de Haen, Germany. Milli – Q water was produced by using SARTORIUS STEDIUM BIOTECH mod. ARIUM. Analytic solvent HPLC grade (methanol) was purchased from Extrasynthése (Genay, France), while dehydroascorbic acid (DHAA), ascorbic acid (AA) and cetyltrimethylammonium bromide (cetrimide) were purchased from Extrasynthése (Genay, France).

Quality parameters

Median weight/fruit, total soluble solids (TSS), firmness, dry matter percentage, pH, titratable acidity (TA), antioxidant capacity and ascorbic acid (AA) were evaluated after the storage.

Median weight (g) was evaluated by Mettler PM460 DeltaRange Electronic Balance, while firmness (kg/cm^2) was measured with a fruit pressure tester (Zenith mod. FT 327). TSS (°Brix) were measured with a digital refractometer (DBR35 Digitahi), while dry matter percentage (%) was evaluated by the difference between weighting the samples before and after 48 hours in a stove at 100 °C. TA (mmol/L) and pH were determined by titrating 10 mL of juice (rising to 100 mL final volume with Milli-Q water) with a solution of NaOH (0.2 N), using an automatic titrator (Crison Titromatic 2S).

Extraction of antioxidant compounds

For the extraction of antioxidant compounds, 10 g of fruit pulp (3 replications, each obtained by 5 fruits) were put into a 50 mL test tube and 25 mL of extraction solution (500 mL of methanol, 23.8 mL of bi-distilled water and 1.4 mL of HCl 37 %) were subsequently added to the weighed samples. After 60 minutes in the dark, the extracts were homogenized with an Ultra – Turrax (IKA-WERKE mod. T25) for about 1 min and then centrifuged for 15 min at 3,000 rpm in an ALC Centrifuge mod. PK 120. The supernatants were recovered and transferred to small glass tubes and kept frozen at -20 °C for further analysis (SLINKARD and SINGLETON, 1977).

Antioxidant capacity (FRAP assay)

Antioxidant capacity in fruit pulp was evaluated by FRAP (Ferric Reducing Antioxidant Power) assay (BENZIE and STRAIN, 1999). The method is based on the reduction of the ferric (Fe^{3+}) TPTZ (2,4,6-tripyridyl-s-triazine) complex to its ferrous form (Fe^{2+}). The FRAP reagent was prepared daily by mixing 2.5 mL of TPTZ solution and 2.5 mL of $FeCl_3 \cdot 6H_2O$ solution with 25 mL acetate buffer (0.3 M) and then warmed at 37 °C before using; 30 ml of sample (15 ml of extract sample and 15 ml of extraction buffer, dilution 1:2) was added to 90 µl of bi-distilled water and 900 µl of FRAP reagent in a 2 mL micro tube and then incubated at 37 °C for 30 minutes in a G.F.L. Shaking Water Bath mod. 1083. Absorbency at 595 nm with a UV/Vis Spectrophotometer (BECKMAN mod. DU® 530) was measured.

Standard curve was obtained using $FeSO_4 \cdot 7H_2O$ (concentration range: 100 – 1000 µmol/L) and results were expressed as mmol of Fe^{2+} equivalents/kg of fresh fruit weight.

Ascorbic Acid content

10 g of fruit pulp (3 replications of 5 fruits each) were put into a 50 mL test tube and 10 mL of extraction solution (0.1 M citric acid, 2 mM ethylene diamine tetraacetic acid (EDTA) disodium salt and 4 mM sodium fluoride in methanol – water 5:95 v/v) were then added.

The extracts were homogenized with an Ultra – Turrax (IKA-WERKE T25) for about 1 min and then centrifuged for 10 min at 4,000 rpm at room temperature in an ALC Centrifuge PK 120. The supernatants were recovered and transferred to a 15 mL test tube through filter cloth and then acidified with 4 N HCl to decrease pH solution to a value of 2.2 – 2.4 pH units (SANCHEZ et al., 2003).

Acidified samples were centrifuged for 5 min at 12,000 rpm at 4 °C with an ALC Multi Speed refrigerated centrifuge PK 121R and the supernatants were then filtered through a 0.45 µm filter (Titan 2 HPLC filter 17 mm PTFE Membrane); polyphenolic compounds were absorbed on a C_{18} cartridge for Solid Phase Extraction (Waters Corporation, Sep-Pak® C-18). Then, 250 µL of OPDA solution was added to 750 µL of extract sample for DHAA derivatization into the fluorophore 3-(1,2-dihydroxyethyl)furo[3,4-b]quinoxalina-1-one. After 37 min in the dark, the samples were analyzed by HPLC – DAD system (GONZÁLES-MOLINA et al., 2008).

An Agilent 1100 high performance liquid chromatograph, equipped with a G1311A quaternary pump, a manual injection valve and a 20 ml sample loop, coupled to an Agilent GI315D UV-Vis diode array detector, was used for the analysis. Separations of DFQ and AA were achieved on a ZORBAX Eclipse XDB – C18 column (4.6 x 150 mm, 5 mm).

The mobile phase was methanol – water (5:95, v/v) containing 5 mM cetrimide and 50 mM potassium dihydrogen phosphate. The flow rate was 0.9 mL min^{-1} (isocratic analysis) and the detector wavelengths were 348 nm for DHAA (DFQ) and 261 nm for AA detection.

AA and DHAA were identified and quantified by comparison with pattern areas from AA and DHAA; the vitamin C content was calculated by adding ascorbic acid and dehydroascorbic acid values and results were expressed as mg/100 g of fresh fruit weight (GONZÁLES-MOLINA et al., 2008).

Statistical Analysis

The results were subjected to analyses of variance (ANOVA) for means comparison by using SPSS 15.0 software and HSD Tukey multiple range tests at $p<0.05$.

Results and discussion

The storage of the fruits was successful. The results of the qualitative analysis are shown in Tab. 2. AWE significantly increased fruit weight in cv. Hayward in both locations: the median weight of the cv. Hayward fruits grown in Piedmont and in Latium increased from 96.43 g to 101.52 g and from 96.82 g to 102.97 g, respectively. However the procedure did not increase the weight of the cv. Green Light fruits, suggesting a different genotype response to the treatment. Moreover, the different effect of this product on cv. Green Light, in comparison with cv. Hayward, is probably due to the fact that at the time of the treatment cv. Hayward was at a more advanced phenological phase ("fruit walnut"), considering the ideal time to use it.

The treatment did not significantly reduce the firmness of the fruits during post-harvest. The AWE treatment did not influence dry matter percentage, TSS, TA and pH of the two cultivars.

The results of the nutritional parameters are shown in Tab. 3. The antioxidant activity of the fruits of the two cultivars was significantly affected by the application of the product only in the case of cv. Hayward, although the cv. Green Light fruits treated with AWE had slightly higher values than the control.

In Piedmont the average antioxidant capacity of the fruits increased from 13.34 mmol of Fe^{2+} equ/kg_{ffw} to 17.25 mmol of Fe^{2+} equ/kg_{ffw} and in Latium it increased from 12.33 mmol of Fe^{2+} equ/kg_{ffw} to 16.83 mmol of Fe^{2+} equ/kg_{ffw}.

The main effect of AWE was the increase in nutraceutical traits, especially the AA content. The application of phytohormones, micronutrients and amino acids from vegetal waste as nutrients is an important topic of research nowadays, and many studies show that these substances have a strong influence on many of the physiological activities of cultivated plants and plant cell cultures (TAIZ and ZEIGER, 2006; HANCOCK et al., 2008). Although the mechanisms controlling AA production and concentration are still largely mysterious, with large areas of uncertainty concerning the genetic and biochemical controls of pathway flux (HANCOCK and VIOLA, 2005), some studies indicate that the phloem is capable of synthesising AA from sugars (HANCOCK et al., 2003).

The treatment significantly increased the AA content in both cultivars and in both locations. For cv. Hayward, in Piedmont the median AA content of the fruits increased from 32.01mg/100 g_{ffw} to 41.73 mg/100 g_{ffw} and in Latium it increased from 38.10 mg/100 g_{ffw} to 45.52 mg/100 g_{ffw}. For cv. Green Light the AA content of the fruits increased from 45.05 mg/100 g_{ffw} to 51.22 mg/100 g_{ffw}. Fig. 1 shows an example of a chromatogram obtained in this analysis. Analytic peaks were easy to detect at 348 nm (DHAA) and 261 nm (AA), because there was a good peak resolution with two different retention times: 4 minutes for DHAA and 6 minutes for AA.

Although further physiological and biochemical studies should be

Tab. 2: Quality parameters in cv. Green Light and cv. Hayward: mean values in each row for the same parameter followed by different letters are significantly different as determined by Tukey's HSD test following ANOVA ($p \leq 0.05$).

Cultivar	Treatment	Fruit weight (g)	Firmness (kg/cm^2)	Dry matter content (%)	Total soluble solids (°Brix)	Titratable acidity (mol/L)	pH (pH units)
Green Light (Latium)	AWE	80.05 ± 5.3 bc	2.85 ± 0.2 a	20.73 ± 0.6 a	11.56 ± 0.4 b	0.08 ± 0.1 a	3.30 ± 0.1 a
	control	78.51 ± 3.3 c	3.24 ± 0.2 a	19.95 ± 0.6 a	13.50 ± 0.3 ab	0.07 ± 0.1 a	3.41 ± 0.1 a
Hayward (Latium)	AWE	102.97 ± 1.1 a	2.54 ± 0.1 a	21.58 ± 0.4 a	13.10 ± 0.7 ab	0.11 ± 0.1 a	3.07 ± 0.1 a
	control	96.82 ± 2.1 b	2.86 ± 0.1 a	21.55 ± 0.3 a	14.77 ± 0.1 a	0.12 ± 0.1 a	3.06 ± 0.1 a
Hayward (Piedmont)	AWE	101.52 ± 1.1 a	2.51 ± 0.1 a	20.55 ± 0.1 a	14.17 ± 0.4 a	0.11 ± 0.1 a	2.90 ± 0.1 b
	control	96.43 ± 3.0 b	2.65 ± 0.3 a	19.21 ± 0.2 a	14.00 ± 0.4 a	0.12 ± 0.1 a	2.97 ± 0.1 ab

Tab. 3: Nutraceutical parameters in cv. Green Light and cv. Hayward: mean values in each row for the same parameter followed by different letters are significantly different as determined by Tukey's HSD test following ANOVA ($p \leq 0.05$).

Cultivar	Treatments	Vitamin C (mg/100 g_{ffw})	Antioxidant Capacity (mmol of Fe^{2+} equ/kg_{ffw})
Green Light	AWE	51.22 ± 3.9 a	11.34 ± 1.1 c
	control	45.05 ± 2.4 b	11.17 ± 0.1 c
Hayward (Latium)	AWE	41.73 ± 5.4 a	17.25 ± 1.3 a
	control	32.01 ± 3.9 c	13.24 ± 2.1 b
Hayward (Piedmont)	AWE	45.52 ± 1.9 a	16.83 ± 0.9 a
	control	38.10 ± 2.9 b	12.33 ± 1.1 bc

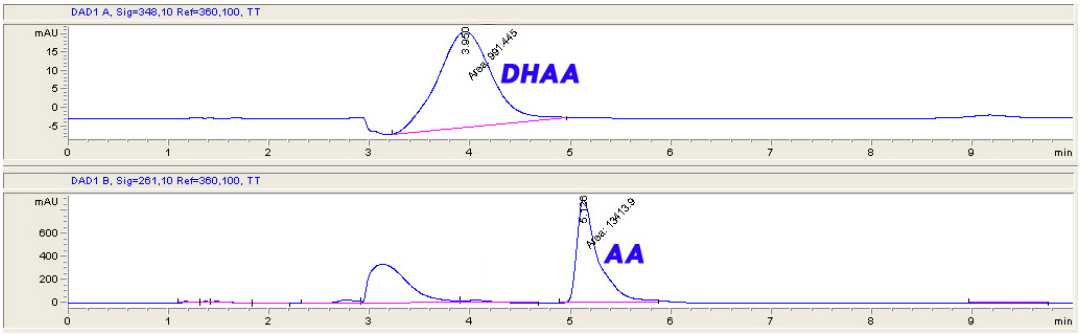

Fig. 1: HPLC separation of dehydroascorbic (DHAA) and ascorbic (AA) acid.

performed to establish the relationships between agro-industrial waste extract application and its influence on plant physiology and fruit quality, the AWE treatment in this study, in different pedoclimatic conditions and with different genotypes, was proven to have an influence on AA content and the antioxidant activity of kiwifruit production.

The raw material used for the extraction of AWE has heterogeneous characteristics and a highly variable chemical composition (PARRADO et al., 2005), and is difficult to standardize. Also the operational conditions during the formulation of the AWE, such as temperature, moisture, active biomolecular composition and concentration (as enzyme/substrate ratio) and extraction time, may influence the effectiveness of AWE and should be further analysed. The study of the extraction technology implementation and further developments on functional properties in agro industry by-products are promising research topics.

Conclusions

The use of AWE as fertilizer/biostimulant had positive effects on fruit weight, AA content and antioxidant capacity measured by FRAP.

This study confirms that fertilization with vegetal waste extracts shows a promising future in functional plant nutrition linked with an increase in food quality parameters, and further development of the technologies and study of the agronomical, physiological and biochemical relationships should be carried out to develop more sustainable strategies for future agriculture.

This is only a preliminary study, because other years of study are needed to confirm the results of this analysis, but it is clear that the application of agro-industrial waste extracts on the culture could further improve the nutritional value and the overall quality of this fruit.

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