



Mini review**Mini review: fruit residues as plant biostimulants for bio-based product recovery****Andrea Ertani^{1,*}, Ornella Francioso², and Serenella Nardi¹**

¹ Dipartimento di Agronomia, Animali, Alimenti, Risorse Naturali e Ambiente, Università di Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy

² Dipartimento di Scienze Agrarie, Università di Bologna, viale Fanin 40, 40127 Bologna, Italy

* **Correspondence:** Email: andrea.ertani@unipd.it; Tel: +39 049 8272912.

Abstract: The request of natural products has augmented in the last years due to the increase in intolerance and allergy reactions showed by humans versus pesticides and certain chemical compounds used in agriculture. In response to this demand, innovative methods and new natural matrices have been exploited to obtain products able to increase plant nutrients use efficiency. In this context, agro-industrial residues contain bioactive molecules, including antioxidants and phenols, which may be used by farmers to improve crop productivity. Phenols are ubiquitous in plants and are essential components of the human diet by virtue of their antioxidant properties. They may also act as positive growth regulators by modifying the root architecture and, consequently, the uptake of macronutrients, potassium especially. In order to understand their effects on the plant metabolic pathways, agro-industrial residues were supplied to maize plants and the activity of specific enzymes was evaluated. In this review, developments and improved understanding on fruit residues on primary and secondary plants metabolism are discussed.

Keywords: phenylalanine ammonia lyase (PAL); *Zea mays*; elicitors; antioxidants; phenols

1. Overview

Fruit juices and derived products have experienced a growing consumption during the last years due to the positive effects of their ingredients on human health. Indeed, consumers are increasingly becoming aware of health problems related to food intake, therefore demanding health-promoting natural products.

Plant food processing derived by-products (agro-industrial residues) represent a disposal problem for the companies, but they are also promising sources of bioactive compounds, including

antioxidants [1,3], which may be employed in agriculture by farmers to foster crop performance [4]. Therefore, agro-industrial residues can be referred to as biostimulant compounds.

According to the European Biostimulants Industry Council [5], biostimulants are defined as products containing substances and/or microorganisms whose function when applied to plants or the rhizosphere is to stimulate natural processes, to enhance/benefit nutrient uptake, nutrient use efficiency, tolerance to abiotic stress, and crop quality. Biostimulants are of remarkable importance for sustainable agriculture as they offer an innovative solution to an increased world demand for high crop productivity under low unsustainable inputs [6]. The sale-market in Europe in particular, has been estimated to grow up to \$2 billions by 2018 [7,8]. The European Biostimulants Industry Council [9] does not recognize any direct action of biostimulants against pests; therefore, biostimulants do not fall within the regulatory framework of pesticides. Also, they must operate in plants through different mechanisms than fertilizers, regardless of the presence of nutrients in the products [9]. In this respect, the Biostimulant Coalition in North America specifies that biostimulants are not nutrients, but products that in very little amounts are able to promote plant growth and development, plant nutrient uptake and use efficiency, plant metabolic processes, or act as soil amendments to help ameliorating soil structure, function, or performance. However, because they are manufactured starting from different sources and consist of complex mixtures of active substances, the assignment of specific functions in plants to the individual components of these products is often arduous.

2. Effect on crops

In the last few years, several research has proposed the application of products derived from fruit residues in agriculture because of their content in phenols, which are particularly actives in stimulating plant metabolism [10,11]. There are a number of techniques used for the extraction of phenolic compounds from different types of vegetable residues, most of them using organic solvents (e.g. ethanol), high temperatures, and long extraction times to exhaust the materials [12,13]. However, long extraction times can cause artifacts and organic solvents may pose a risk for the environment and human health. Other extraction procedures have been recently proposed, such as ultrasound [14], pressure [15,16] and supercritical fluid extraction [17]. Controlled enzymatic hydrolysis (Figure 1) has been applied for proteins recovery from residues of animal and fish processing industries [18]. This process is advantageous in terms of environmental impact (low temperature, minimum amount of water) and because several end-products may be recovered.

In a study by Ertani et al. [10], maize (*Zea mays* L.) plants were supplied for the first time with the dry apple and blueberry residues under controlled conditions. These residues were particularly rich in phenol compounds. Plants were grown for 12 d in a nutrient solution in the absence (control) or in the presence of either cellulolytic dry apple hydrolysate (AP) or dry blueberry cool extract (BB) applied at two different doses. Plants supplied with AP and BB displayed a significant increase in root and leaf biomass and a high content in macronutrients and proteins. Conversely, sucrose and glucose concentrations drastically decreased in foliar tissues of plants treated with AP and BB (Table 1). Of particular interest was the positive impact of AP and BB on the secondary metabolism associated with the synthesis of phenolic compounds, evaluated in terms of activation of phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) gene expression. PAL catalyzes the first step of the phenylpropanoid pathway, by converting phenylalanine into *trans*-cinnamic acid and tyrosine into

p-coumaric acid. In Table 2 the content of soluble phenols in the plant tissues in relation to the treatment of plants with AP and BB is shown [10].

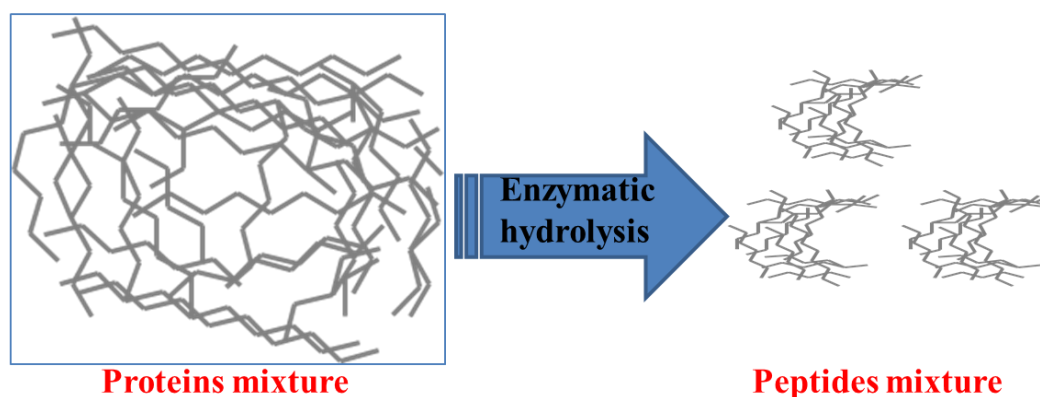


Figure 1. Enzymatic hydrolysis representation.

Table 1. Protein concentration in roots and leaves and leaf sugar concentrations of *Z. mays* plants grown in a Hoagland modified complete nutrient solution and treated for 2 d with either Apple and Blue Berry at 0.1 or 1 mL L⁻¹. Data are the means of three replicates with three plants in each (\pm SD). Values in the same column following the same letter are not statistically different at $p < 0.05$ according to Student-Newman-Keuls test.

Dosage	Proteins		Sugars		
	<i>mg g⁻¹ fresh weight</i>		Sucrose	Glucose	Fructose
	<i>s</i>	<i>es</i>	<i>mg g⁻¹ dry weight</i>		
Control	1.01 \pm 0.41 d	2.90 \pm 0.37 c	116.73 \pm 12.34 a	27.42 \pm 1.33 a	7.43 \pm 0.57 d
Apple 0.1	1.36 \pm 0.34 c	3.19 \pm 0.17 b	77.39 \pm 10.02 b	10.96 \pm 1.14 c	26.66 \pm 1.21 a
Apple 1.0	1.94 \pm 0.16 a	3.91 \pm 0.10 a	3.55 \pm 1.48 d	4.05 \pm 1.26 d	23.18 \pm 0.68 b
Blue Berry 0.1	1.85 \pm 0.10 b	3.57 \pm 0.16 b	22.47 \pm 4.42 c	22.76 \pm 1.18 b	9.79 \pm 0.61 c
Blue Berry 1.0	1.99 \pm 0.32 a	3.83 \pm 0.09 a	8.83 \pm 3.27 d	6.83 \pm 2.29 d	10.12 \pm 0.44 c

Phenols are important compounds for their ability to act as antioxidants and protectors against reactive oxygen species [19-21]. Protocatechuic, hydroxybenzoic, vanillic and *p*-coumaric acids influence several physiological effects that include improvement of plant-water relationships, stomatal function and rate of photosynthesis and respiration. These phenols also interact with several phytohormones and enzymes determining a different biosynthesis and flow of carbon into metabolites. Nevertheless, the factors regulating and controlling the quality and quantity of phenols in plant tissues still remain partially unknown.

In other study by Ertani et al. [11], vegetal extracts derived from hawthorn (*Crataegus monogyna* Jacq.) leaves, red grape (*Vitis vinifera* L.) skin material and blueberry (*Vaccinium vitis-idaea* L.) fruits were applied in maize plants in order to verify their biostimulant activity. Their content in phenols was evaluated, as well as the hormone-like activity, which was inferred via indoleacetic acid and isopentenyladenosine quantification. The biostimulant effect was demonstrated

in terms of root and leaf biomass improvement, high content in chlorophyll and sugars compared to untreated plants. Moreover, hawthorn, red grape skin and blueberry enhanced the accumulation of *p*-coumaric acid, whilst hawthorn alone increased the amounts of gallic and *p*-hydroxybenzoic acids. These evidences were likely imputable to the role of phenols in stimulating plant metabolism, and to the promotion of the auxin-mediated signal transduction pathway [22,23].

Selby and coauthors [24] further analyzed aqueous extracts from the forage grass (*Lolium perenne*) finding an elicitor of plant defense reactions in the whole plant system and in crop situations. They also showed that an elicitor from one species can be active in a completely unrelated species, thus suggesting that the biostimulant activity from *L. perenne* extracts might have a broad applicability to a wide range of crops, particularly if its mode-of-action consists in inducing plant defenses.

Table 2. Concentrations of phenols and flavonoids, selected phenolic compounds and PAL activity in leaves of *Z. mays* grown for 12 d in a modified Hoagland nutrient solution and treated for 2 d with either Apple or Blue Berry at 0.1 or 1 mL L⁻¹.

	Control	Apple 0.1	Apple 1.0	Blue Berry 0.1	Blue Berry 1.0
	(mg gallic acid g ⁻¹ fw)				
Phenols	1.02 ± 0.04 c	1.13 ± 0.05 c	1.30 ± 0.05 b	1.48 ± 0.04 a	1.52 ± 0.09 a
	(mg gallic acid g ⁻¹ fw)				
Flavonoids	0.092 ± 0.005 d	0.123 ± 0.017 c	0.111 ± 0.007 c	0.204 ± 0.010 b	0.232 ± 0.013 a
	(µg g ⁻¹ d w)				
Gallic acid	2.37 ± 0.62 c	13.73 ± 2.84 b	22.81 ± 1.47 a	14.52 ± 2.73 b	24.42 ± 1.12 a
Protocatechuic acid	12.80 ± 1.73 d	30.82 ± 3.23 c	42.38 ± 1.99 b	50.52 ± 2.85 a	49.93 ± 2.16 a
Syringic acid	n.d.	26.86 ± 2.44 a	28.32 ± 2.17 a	26.02 ± 2.81 a	25.92 ± 2.10 a
Vanillic acid	n.d.	41.27 ± 5.61 a	20.08 ± 3.72 b	n.d.	n.d.
<i>p</i> -Hydroxybenzoic	n.d.	5.89 ± 0.98 a	2.68 ± 0.99 b	6.15 ± 0.43 a	6.72 ± 0.35 a
Caffeic acid	n.d.	n.d.	2.47 ± 0.63 b	12.13 ± 1.97 a	10.86 ± 2.09 a
Chlorogenic acid	n.d.	n.d.	n.d.	n.d.	n.d.
<i>p</i> -Coumaric acid	55.01 ± 4.03 c	15.61 ± 2.28 e	149.70 ± 4.34 b	34.71 ± 2.04 d	172.46 ± 4.12 a
Ferulic acid	75.33 ± 5.62 c	83.41 ± 3.84 bc	87.18 ± 3.01 ab	84.65 ± 4.21 bc	91.32 ± 4.16 a
	(nmol cinnamic acid mg ⁻¹ protein min ⁻¹)				
PAL act	9.481 ± 0.012 c	23.845 ± 0.008	21.425 ± 0.007	23.855 ± 0.009	30.399 ± 0.017 a

3. Extraction methodology

Sánchez-Gómez et al. [25], demonstrated that grapevines treated with aqueous oak extracts via foliar application were able to modulate the phenolic and aroma composition of the grapes and the derived wines products [26,27]. One of the biggest problem in wine-producing regions is to create alternatives for processing the great amount of grape waste generated during the harvest season.

Sánchez-Gómez et al. [25], proposed the use of vine-shoot waste as biostimulant for *vitis vinifera* metabolism. The authors produced Airén waste vine-shoot aqueous extracts using four solid-liquid extraction techniques, such as conventional solid-liquid extraction (CSLE), solid-liquid dynamic extraction (SLDE-Naviglio), microwave extraction (ME), and pressurized solvent extraction (PSE). The results showed that vine-shoot waste aqueous extracts from the *V. vinifera* Airén variety obtained by CSLE and SLDE-Naviglio had the highest concentration of phenolic, volatile, and mineral valuable compounds, therefore they may have a new potential use as biostimulants or foliar fertilizers in vineyards. Their use could be implemented in the “Sustainable Viticulture” concept, where the cycle of the vine could be closed, such as using vine-shoot extracts to modulate the grape quality, return nutrient to the soil and enhance terroir effect.

In the paper of Bulgari et al. [28], the effectiveness of raw extracts from leaves or flowers of *Borago officinalis* L., were assessed in *Lactuca sativa* Longifolia. The extracts were diluted to 1 or 10 mL L⁻¹, sprayed onto lettuce plants at the middle of the growing cycle and 1 day before harvest. The leaf photosynthetic efficiency (chlorophyll *a* fluorescence and leaf gas exchanges), levels of ethylene, photosynthetic pigments, nitrate, total sugars and total phenols and flavonoids, including the activity and levels of phenylalanine ammonia lyase (PAL), were assessed. The authors reported that borage extracts increased the primary metabolism by inducing leaf pigment accumulation and the photosynthetic activity. Plant fresh weight increased upon treatments with 10 mL L⁻¹ doses, and chlorophyll *a* fluorescence data showed that flowers were able to increase the number of active reaction centers per cross section. Ethylene was three-fold lower in flower treatments. Nitrate and sugar levels did not change in response to the different treatments. Total flavonoids and phenols, as well as the total protein levels, the in vitro PAL specific activity, and the levels of PAL-like polypeptides were increased by all borage extracts, with particular regard to flowers. Flower extracts also were efficient in preventing degradation and inducing an increase in photosynthetic pigments during storage.

4. Conclusions and perspectives

The use of aqueous extracts produced from fruit juices and derived products as biostimulants in agriculture is gaining attention, even if additional studies are needed to develop new extractive techniques from these sources. Nowadays, there is a trend that encourages the use of free environmental techniques to enhance sustainability; it is the so called “green chemistry”. Such techniques are developed to reduce and/or eliminate the use of organic solvents, and the use of water as a solvent for extraction should be preferred. The resulting aqueous extracts will have the advantage of being exempt from certification based on their vegetal origin, although the concentration of bioactive compounds in such extracts may be lower than when other techniques are used, as the exhaustion of the vegetable material does not take place.

Conflict of interest

All authors declare no conflicts of interest in this paper.

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