

Extraction and Evaluation of Nutraceutical Molecules in Wastes of Fruit and Vegetables

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Abstract

Fruit and vegetables wastes not only have no commercial value, but also require expensive operations of removal and disposal, representing a significant cost for the manufacturers of plant products. However, this material may represent an important resource of commercially worth phyto-molecules endowed with various nutraceutical properties. In the present work, we have verified the possibility of using fruit and vegetable waste materials as a source of bioactive compounds. Several nutraceutical compounds of high economic interest were extracted and quantified from various waste plant materials deriving from the production phase (“first-gamma products”) or discarded during the preparation of fresh ready-to-cook or ready-to-eat packaged products (“fourth-gamma products”). Obtained results showed that, despite the precarious and uncontrolled conditions which plant wastes products are subjected, the levels of the examined bioactive compounds are present in relevant amount, also in the parts of the plant that are discarded during the preparation of the ready-to-eat/cook products.

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Introduction

The harvest and the industrial manufacture of fruit and vegetables unavoidably leads to the production of large amount of bio-waste materials, represented by fresh not packaged products such as fallen or defective fruits (*I gamma*) or discarded during the preparation of fresh ready to cook or to eat packaged products (*IV gamma*). These plant wastes are not suitable to be commercialized and in addition represent a cost, requiring expensive operations of removal and disposal. However, both wastes may theoretically represent an important resource of precious phyto-molecules endowed with various nutraceutical properties such as for example Lycopene, Phloridzin, Ursolic acid, Kaempferol, Chlorogenic acid, Arbutin, Lutein, Sulforaphane, Lactucin and Lactucopicrin.

Lycopene is a carotenoid molecule responsible for the red colour in tomato fruits. It is widely used in food industry as

additive and supplement and is the subject of a large number of studies of pharmaceutical/medical interest because of its well studied antioxidant and anticancer properties (Giovannucci et al. 1995, Stahl et al. 2003, Gann et al. 1999, Bramley et al. 2000, Tamini et al. 2009, Qu et al. 2011).

Phloridzin, one of the dihydrochalcones contained in apples, is a specific and competitive inhibitor of sodium/glucose co-transporters in intestine and kidney and for this reason it represents a potential candidate for the treatment or prevention of diabetes and obesity (Ehrenkranz et al. 2005, Najafian et al. 2012).

Ursolic acid is a triterpenoid ubiquitous in plant kingdom and its presence has been demonstrated in the cuticular wax of many fruits, including apples and pears (Belding et al. 1998, Andre et al. 2012, Li et al. 2014). It appears to increase skeletal muscle and brown fat and to possess anti-tumor and anti-inflammatory effects (Kunkel et al. 2012, Zang et al. 2014). Moreover,



it has been indicated as a potential agent against hyperglycaemia and obesity (Castro et al. 2015).

Kaempferol has been associated to a number of biological effects, inhibiting the angiogenesis process and the growth of cancer cell (Lee et al. 2010, Chen et al. 2013). In addition, it shows antioxidant and anti-inflammatory effects and inhibits *in vitro* bone resorption (Wattel et al. 2003).

Chlorogenic acid also shows anticancer properties and helps to protect from oxidative stress (Tanaka et al. 1993, Tsuchiya et al. 1996) as well as to inhibit the growth of preadipocytes (Hsu et al. 2006). Moreover, improving lipid metabolism, it has been proved to be more effective than Caffeic acid as anti-obesity agent in high fat diet induced obese mice (Cho et al. 2011). Furthermore, it contributes to enhance macrophage functions *in vivo* and *in vitro* and to influence the sleep-wake cycle (Wu et al. 2004, Shinomiya et al. 2004).

Arbutin is an important glycosylated hydroquinone compound detectable in pear fruit; it is endowed with antimicrobial properties and, due to its ability to inhibit tyrosinase, is used as a skin-lightening agent in cosmetics (Cui et al. 2005).

Lutein, a xanthophyll pigment present in the membranes of macula lutea of the primate vision apparatus, has been proven to be able to increase the macular pigment optical density, suggesting a prevention effect against damage caused by blue light as well as macular degeneration associated with aging (Landrum et al. 1997).

Sulforaphane, a naturally occurring isothiocyanate present in cruciferous vegetables, is well known to reduce risk of cancer by activation of phase II detoxification enzymes and by inducing apoptosis in cancer cells (Moreno et al., 1996). Moreover, it shows inhibitory activity against stomach cancer induced by benzo(a)pyrene and against the microbial pathogen *Helicobacter pylori* (Fahey et al. 2002).

Finally, Lactucin and Lactucopicrin, responsible of the characteristic bitter taste, largely present in *Lactuca* and *Cichorium* species, are endowed with antimalarial, anti-inflammatory and sedative properties (Bischoff et al. 2004, Wesolowska et al. 2006).

In the present work, the above cited molecules were extracted and quantified from wastes obtained after harvesting or manufacturing plant products. This material was selected among those of main interest for the companies producing fruits and vegetables (tomato, apple, pear, leek, chard, cabbage, broccoli, a variety of fruit and vegetables smoothies and a variety of chicory, radish, lettuce and endive) belonging to the AOP Unolombardia sacpa association. (tomato, apple, pear, leek, chard, cabbage, broccoli, a variety of fruit and vegetables smoothies and a variety of chicory, radish, lettuce and endive). The possibility and the feasibility to transforming bio-wastes, representing a cost for the companies that produce them, in a source of nutraceutical molecules possessing a commercial value on the market of dietary supplements or of cosmetic products ingredients was then evaluated.

Materials and Methods

Materials

All the fresh vegetable and fruit wastes were harvested and collected from farms located in Lombardia region (Italy)

and belonging to AOP Unolombardia sacpa. As concerns plant wastes of packaged products, the materials were obtained from the company "La lineaverde", Manerbio (Brescia, Italy).

Standards and reagents

All solvents and reagents were purchased from Sigma Aldrich, Germany, as well as standards of Lycopene, Ursolic acid, Phloridzin, Kaempferol, Chlorogenic acid, Arbutin, Lutein and Sulforaphane. Standards of Lactucin and Lactucopicrin were purchased from Extra synthese, France. Novozymes 50012 enzyme mixture was purchased from Novozymes, Denmark. The used water was purified by Milli-Q quality water system (Millipore Bedford, USA).

Extraction from waste material

Lycopene: Samples of tomato wastes (harvested in Alessandria, North Italy) were collected accurately separating pulp and peel. Other samples of mixed manufacturing waste of ripe tomato (pulp and peel) were harvested and collected in Collecchio. (Parma, Italy). Samples were ground with liquid nitrogen and homogenate sample (1 g) was accurately weighed and stirred for 10 minutes with 25 ml of hexane / acetone / ethanol (50:25:25, v/v/v) at 0 °C. Samples were then transferred into a flask and shaken at 300 x g in the dark for 30 minutes at 4 °C. After the addition of 5 ml of water, the upper phase was collected and concentrated by using a rotavapor. The residue was re-suspended in 1 ml of acetone, filtered and stored at -20 °C until HPLC analyses (Olives Barba 2006).

Phloridzin and Ursolic acid: Phloridzin was extracted from apple waste and Ursolic acid from apple, pear waste and from fruit smoothie preparations (mix of different fruits, table 1); Samples of apple and pear waste were accurately collected, separating pulp and peel. Both the compounds were extracted using the same method (modified) (Tsao et al. 2003). The samples were ground with liquid nitrogen, weighed (1.5 g) and placed in a test tube with 3 ml of ethanol; after they were mechanically stirred in a homogenizer for 3 minutes at 0 °C, they were placed in a ultrasonic bath for 5 minutes and left on a shaker at 300 x g, at 4 °C for 24 h. The extracts were then centrifuged and the obtained supernatant was filtered (0.45 µm) and stored at -20 °C until HPLC analysis.

Table 1. Compositions of wastes derived from fruit and vegetables smoothies.

	Percentage composition
	Ursolic acid (mg/g FW)
Mix 1	Sour black cherry 45%, pear 10%, grapes 45%
Mix 2	orange 15%, banana 17%, strawberry 40%, apple 14%, grapes 14%
Mix 3	orange 20%, banana 0.5%, strawberry 12%, apple 11%, blackberry 10%, raspberry 10%, lemon 0.5%, mulberry 10%, ribes 2%, grapes 24%
Mix 4	orange 16%, fennel 42%, banana 8%, lime 5%, lemon 0.2%, apple 20%, spinach 0.8%, grapes 8%
Mix 5	pineapple 24%, orange 11.7%, banana 0.1%, lemon 0.1%, lime 0.1%, mango 41%, apple 19%, passionfruit 4%

Arbutin: According to the method of Cui et al. (2005), with some modifications, pear waste samples were divided into three parts:

peel, pulp and core (seedless) and analyzed for the content of Arbutin. Each part was ground with liquid nitrogen and 1 gram of sample was mashed for 5 minutes with 1 ml of ethanol 95% (pre-cooled to -18 °C), containing 2% (v/v) phosphoric acid. The suspension was then transferred to a new flask, brought to 12 ml using 95% ethanol at -18 °C, placed in ultrasonic bath for 15 min and centrifuged at 3000 x g for 5 minutes. An aliquot (1 ml) of this sample was evaporated to dryness by speed vacuum. The residue was re-suspended in 1 ml of water, filtered and stored at -20 °C until HPLC analysis.

Kaempferol and Chlorogenic acid: Kaempferol and Chlorogenic acid were extracted from leek wastes, dried at 40 °C for 48 h and then ground by using a Retsch MM301 grinder. The sample (2 g) was then extracted with 20 ml of methanol by maceration at room temperature for 72 h. The extraction process was repeated until colorless residue was obtained. The samples were centrifuged at 3000 x g for 5 minutes and the recovered supernatant was then filtered. An aliquot of the sample (1 ml) was then dried by speed vacuum. The residue was re-suspended in 0.25 ml of methanol and stored at -20 °C until carrying out HPLC analysis (Faera et al. 2013).

Lutein: Lutein was extracted from wastes of leek leaves, Catalognacicory and chard. All the materials were ground using liquid nitrogen and 0.5 g of the samples were subsequently extracted with 1.5 ml of diethyl ether in a test tube in the dark for 1 h at 4 °C. After centrifugation at 3000 x g for 5 minutes, supernatant was collected and stored at -20 °C. The residue was extracted two more times in diethyl ether before recovering combined supernatants and drying them by using speed vacuum. The residue was re-suspended again in 1.5 ml of acetone, filtered and stored at -20 °C until HPLC analyses (Chang et al., 2013).

Sulforaphane extraction: Sulforaphane was extracted from cabbage and broccoli wastes; the materials were dried at 60 °C and then ground to powder. Samples were accurately weighed (150 mg) and suspended in 4 ml of 0.1 M HCl. The mixture was incubated at 45 °C for 2.5 h on a shaker in order to release Sulforaphane from the glucosinolate molecules. After that, the mixture was extracted with 20 ml of dichloromethane, mixed in a vortex for 1 minute and left at room temperature for 1 h. The organic extract was centrifuged at 3000 x g for 5 minutes in order to easily remove the residue. The clarified extract was dehydrated with anhydrous sodium sulfate, centrifuged again, and purified using an absorption column (Supelclean™ LC-Si SPE 3 ml) previously equilibrated with 3 ml of dichloromethane. After the extract was loaded onto the cartridge, the column was washed by swishing 3 ml of ethyl acetate (discarded) and the sample was collected after elution of 3 ml of methanol. An aliquot (1 ml) of the collected sample was dried and the residue resuspended in 0.5 ml of acetonitrile + 0.21 ml of water, then filtered and stored at -20 °C before HPLC analyses (Campas-Baypoli et al., 2010).

Lactucin and Lactucopicrin: Lactucin and Lactucopicrin were extracted from wastes of Catalognacicory, baby lettuce, red radish, sugarloaf and two salad mixes (Table 2) were ground by liquid nitrogen. Each sample (4 g) were homogenized with 10 ml of 0.1 M acetate buffer (containing calcium chloride 10 mM) + 0.4 ml of Novozymes 50012 hydrolytic enzymes mixture at

pH 4.5, 50 °C for 4 h. The reaction mixture was centrifuged at 4000 rpm for 5 minutes and the supernatant was recovered and mixed with 10 ml hexane. After discarding the hexane phase, samples were extracted twice in a separating funnel with 4 ml of ethyl acetate. The ethyl acetate phases were combined and dried by using a speed vacuum centrifuge. The residue was extracted with a mixture of 0.075 ml of ethyl acetate and 0.075 ml of methanol, and subsequently with a mixture of 0.025 ml of chloroform and 0.025 ml of ethanol. The two collected mixtures were combined and evaporated. The residue was solubilized in 0.050 ml of acetone, centrifuged and the supernatant was recovered and dried again. The residue obtained was re-suspended in 0.2 ml of methanol + 0.4 ml of hexane. The methanol phase was filtered and stored at -20 °C until HPLC analyses were carried out (Van Beek et al. 1990).

All the above reported extractions were made in triplicate.

Table 2. Percentage compositions of the two salad mixes (leaves).

	Percentage composition
Mix A	Baby lettuce 45%, red lettuce 25%, mizuna 10%, tatsoi 10%, red chard 10%
Mix B	curled leaves endive 34%, scarole 33%, red radish 33%

HPLC Canalysis

Unless otherwise specified, samples (20 µl) were injected into the HPLC system Kontron Instrument 420 system, equipped with a column Kinetex XB-C18, 250 mm x 4.6 mm, 5 µm. Peak identities were assessed by comparing the retention time of each peak in the analyzed specimen with that one of a standard sample. Quantitative analysis was obtained by calibration curves prepared over the expected analytical concentration range. After every run, the column was washed by methanol for 15 minutes and equilibrated with the initial mobile phase for 10 minutes before the next run. All analyses were carried out at room temperature.

Lycopene was determined as described by Olives Barbaet al. (2006). The isocratic mobile phase consisted methanol / acetonitrile (90:10, v/v) + TEA 9 µM. The flow rate was 1 ml/min. Detector wavelength was fixed at 470 nm. Under these conditions, retention time of Lycopene was found to be 24.4 min.

Phloridzin was determined as described by Tsaot et al. (2003) with modifications described below. The binary mobile phase consisted of a 2 mM sodium acetate buffer with 6% acetic acid, pH 2.55 (solvent A) and acetonitrile (solvent B); the gradient program was: 0 - 15 min, 0 - 15% B; 15 - 20 min, 15 - 30% B; 20 - 25 min, 30-50% B; 25 - 30 min, 0 - 100% B. The flow rate was 1.0 ml/min. Detector wavelength was fixed at 280 nm. Under these conditions, retention time of Phloridzin was found to be 22.6 min.

Ursolic acid was determined as described by Taralkar and Chattopadhyay (2012). The isocratic mobile phase consisted in acetonitrile/ methanol solution (80:20, v/v). The flow rate was 0.5 ml / min. Detector wavelength was fixed at 210 nm and the chromatographic run was performed at room temperature. Under these conditions, retention time of Ursolic acid was found to be 10.9 min.

Arbutin was determined as described by Cui et al. (2005), with modifications. Formic acid 0.04% was employed as mobile phase A and pure methanol as mobile phase B. The gradient procedure was 0 - 3 min, 2% B; 3 - 4 min, 2 - 25% B; 4 - 11

min, 25% B; 11 - 16 min, 25 - 50% B; 16 - 26 min, 50 - 80% B. The flow rate was 1.0 ml/min. Detector wavelength was fixed at 280 nm. Under these conditions, retention time of Arbutin was 7.0 min.

Kaempferol and Chlorogenic acid were determined as described by Zuet al. (2006). The isocratic mobile phase consisted of methanol/ water/ acetonitrile (40:15:45, v/v/v) with 1% of acetic acid. The flow rate was 1 ml / min at room temperature. Detector wavelength was fixed at 368 nm for Kaempferol and 325 nm for Chlorogenic acid. Under these conditions, retention time of Kaempferol was 7.6 min, while retention time of Chlorogenic acid was 2.2 min.

Lutein was determined as described by Tukaj et al. (2003) with some modifications described below. A methanol / 1 M ammonium acetate solution (80:20, v/v) was employed as mobile phase A and methanol/acetone (80:20, v/v) as mobile phase B. The gradient program was 100% A to 100% B in 10 min, followed by 5 min of 100% B. The flow rate was 1.0 ml/min. Detector wavelength was fixed at 450 nm. Under these conditions, retention time of Lutein was 12.3 min.

Sulforaphane was determined as described by Sivakumaret al. (2006). The HPLC system was equipped with a column C-30 Develosil RPAQEOUS-AR, 250mm x 4.6 mm, 5 µm. The isocratic mobile phase consisted of acetonitrile/water (70:30, v/v). The flow rate was 1.0 ml/min at room temperature and detector wavelength was fixed at 205 nm. Under these conditions, retention time of Sulforaphane was found to be 2.8 min.

Lactucin and Lactucopicrin were determined as described by van Beek et al. (1990). The HPLC system was equipped as described for Sulforaphane analysis. The isocratic mobile phase consisted of methanol/water (50:50, v/v) and the flow rate was 1.0 ml/min. Detector wavelength was fixed at 258 nm. Under these conditions, retention time of Lactucin was found to be 4.8 min, while about Lactucopicrin it was 23.8 min.

Results and Discussion

Reported data are presented as mg/g fresh weight (FW) or dry weight (DW), mean ± standard deviation (SD, n = 3) with the only exception of the data regarding Lycopene that are presented as mg/100 g FW.

As expected, Lycopene was concentrated mainly in the peel of ripe tomatoes. The amount of Lycopene present in mixed waste samples is slightly less than half that in the tomato peel (table 3). The results obtained do not differ much from those in the literature: values of Lycopene between 4.1 and 4.4 mg/100 g FW have been reported for two varieties of ripe tomato (Thompson et al. 2000); values of 4.8 - 14.1 mg / 100 g and 2.0 - 6.9 mg / 100 g FW have been reported, respectively, for the peel and the pulp of 12 varieties of ripe tomato (George et al. 2004).

Table 3. Lycopene content in tomato wastes.

	Lycopene mg/100 g FW
tomato mix	0.20 ± 0.002
tomato peel	4.5 ± 0.005
tomato pulp	1.6 ± 0.002

Since Phloridzin is involved in processes of plant defense against pathogenic microorganisms, its presence is more abundant in the peel than in the pulp. In the literature, the highest

concentrations of this dihydrochalcone have been reported for the leaves and bark of several species of *Malus*, followed by peel and finally pulp (Tsao et al. 2003; Escarpa et al. 1999), where it can be even undetectable in small amounts of analyzed sample. In the manuscript of Tsao, Phloridzin content in 8 varieties of apple peel in a range between 0.036 - 0.172 mg/g FW was reported. Our data, shown in table 4, are similar to these ones, except for the variety Granny in which Phloridzin levels are lower.

Table 4. Phloridzin content in wastes of the three analyzed apple cultivars.

	Peel	Pulp
Apple Golden	0.170 ± 0.005	0.101 ± 0.004
Apple Granny	0.159 ± 0.006	0.105 ± 0.002
Apple Stark	0.536 ± 0.030	0.110 ± 0.003
Pear	0.468 ± 0.005	0

	Phloridzin mg/g FW	
	Peel	Pulp
Stark	0.090 ± 0.005	0
Golden	0.080 ± 0.006	0
Granny	0.004 ± 0.001	0

The Ursolic acid values registered in apple and pear wastes are not too different from the results reported in literature for high quality fruits, i.e.: from 0.044 to 3.522 mg/g FW in apple peel (Andre et al. 2012); from 0.125 to 0.791 mg/g FW in pear peel (Lee et al. 2014). Ursolic acid is detectable in all the analyzed samples, except pear pulp. Table 5 shows that the apple Stark peel and the pear peel contain the highest values of Ursolic acid, followed by the smoothie wastes (mix6 and mix5).

Table 5. Ursolic acid content in wastes of the three apple cultivars and the pear (a), fruit and vegetable smoothies (Mix 1-6) (b).

	Ursolic acid (mg/g FW)
Mix 1	0.142 ± 0.003
Mix 2	0.207 ± 0.012
Mix 3	0.215 ± 0.016
Mix 4	0.243 ± 0.036
Mix 5	0.421 ± 0.017
Mix 6	0.424 ± 0.033

Arbutin levels in the peel and in the core were found higher compared to the pulp, (table 6), as expected on the basis of the information available in literature. The Arbutin content, while remaining not negligible, is much lower than that reported for immature pears, because it decreases rapidly during the fruit maturation process (Cui et al. 2005).

Table 6. Arbutin content in pear wastes.

	Arbutin mg/g FW
Pear peel	0.148 ± 0.016
Pear pulp	0.015 ± 0.001
Pear core	0.223 ± 0.025

Regarding Kaempferol and Chlorogenic acid, results shown in table 7 reveal that there is a relevant amount of both Kaempferol and Chlorogenic acid in the analyzed samples. According to literature, in leek leaves Kaempferol-3-O-glucoside is mainly present, while Kaempferol glycosides appear to be poorly present (0.001 mg/g DW) (Bernaert et al. 2013). However, the experimental conditions we have employed in the extraction method could promote the hydrolysis of glycosidic bonds and the release of Kaempferol during the long drying process. Indeed, the amount of Kaempferol obtained (table 7) is similar to that (0.295 mg/g DW) registered by Hertog et al. (1992) for leek leaves after extraction in hydrolytic conditions. Values of both Kaempferol and Chlorogenic acid result respectively at least 5 and 7 times lower than the amounts reported by Faeraet al. (2013) (194.5 mg/100 g and 302 mg/100 g DW respectively), which is the only article we found in literature reporting such high levels of these compounds in *Allium ampeloprasum* leaves. The review by Manach et al. (2004) reports a value of 30 mg/kg FW for Kaempferol in leek, which is not far from results obtained in this work (0.330 mg/g DW \approx 24.812 mg/kg FW, value obtained by measuring the ratio fresh weight / dry weight).

Table 7. Kaempferol and Chlorogenic acid content in leek wastes.

	Kaempferol mg/g DW	Chlorogenic acid mg/g DW
Leekleaves	0.330 \pm 0.032	0.378 \pm 0.023

The Lutein content found in leek leaves resulted much lower than that in chard and Catalognacicory (table 8), so this waste source should be regarded suitable to extract Chlorogenic acid and Kaempferol rather than Lutein. Catalognacicory seems to be a good source of Lutein (0.586 \pm 0.103 mg/g DW). The obtained values are in line with data published by Žnidarčič et al. (2011) for two *Chicoria intybus* cultivars (3.87 - 5.91 mg/100g DW), although these values are much higher than those obtained by Montefusto et al. (2015) (0.008 mg/g FW). This may be due to the difference in the analyzed experimental material: in our case, not all edible part of the Catalogna chicory was used; the white underside was discarded and only the leaves were extracted.

Table 8. Lutein content in chard, Catalogna cicory and leek wastes.

	Lutein (mg/g DW)
Chard leaves	0.297 \pm 0.050
Catalogna cicory leaves	0.586 \pm 0.103
Leek leaves	0.035 \pm 0.002

The amount of Sulforaphane present in cabbage leaves (0.100 mg/g DW \approx 0.003 mg/g FW) is in line with the data published by Liang et al. (0.001 - 0.005 mg/g FW) (Liang et al. 2006). In broccoli samples, the amount of Sulforaphane in the stems is higher than that in the florets (table 9). Although other cases of broccoli cultivars in which the stems have a quantity of Sulforaphane greater than the florets can be found (Nakagawa et al. 2006), the opposite case is more commonly present in literature. Data reported in the present work point out that, among the analyzed samples, the stems, completely unusable as food, contain the highest amount of Sulforaphane, thus representing an excellent source of this important nutraceutical molecule originated by glucosinolates.

Table 9. Sulforaphane content in cabbage and broccoli wastes.

	Sulforaphane (mg/g DW)
Cabbage leaves	0.100 \pm 0.002
Broccoli florets	0.168 \pm 0.030
Broccoli stem	0.375 \pm 0.055

Regarding the Lactucin and Lactucopicrin amount found in the waste, the mixB (see table 2) appears to be the best source for Lactucin among the analyzed samples and, together with sugarloaf, it contains also the highest amount of Lactucopicrin (table 10). These sesquiterpene lactones endowed with anti-malarial and digestive properties contribute to the bitter taste of many *Lactuca* and *Cichorium* species (Warashina et al. 2008). Indeed, in lettuce and in mix A smoothie (see table 2) samples, with almost no bitter taste, the Lactucin level is low, while Lactucopicrin is not detectable. In red radish samples, both sesquiterpene lactones are more concentrated in the leaves than in stems.

Table 10. Lactucin and Lactucopicrin contents in Catalognacicory, baby lettuce, mixA, mixB, radish and sugarloaf wastes.

	Lactucin (mg/g FW)	Lactucopicrin (mg/g FW)
Catalogna cicory leaves	0.013 \pm 0.001	0.010 \pm 0.001
Baby lettuce leaves	0.021 \pm 0.001	0
MixA	0.049 \pm 0.001	0
MixB	0.501 \pm 0.038	0.160 \pm 0.027
Red radish leaves	0.078 \pm 0.002	0.013 \pm 0.001
Red radish stems	0.050 \pm 0.002	0.004 \pm 0.0004
Sugarloaf leaves	0.046 \pm 0.003	0.200 \pm 0.002

The data obtained in this work confirm the possibility of using fruit and vegetables waste materials, representing a cost and possessing no commercial value, making their recovery disadvantageous in comparison to not waste counterparts, as a source of nutraceutical substances of high economic interest. Indeed, despite the precarious and uncontrolled conditions which the plant wastes are subjected to, the level of the examined compounds doesn't seem to be substantially reduced. Moreover, most obtained data are in line with those available in literature for counterpart not-waste materials.

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References

1. Andre, C.M., Greenwood, J.M., Walker, E.G., et al. Anti-inflammatory procyanidins and triterpenes in 109 apple varieties. (2012) *J Agric Food Chem* 60(42): 10546–10554.
[Pubmed](#) | [Crossref](#) | [Others](#)
2. Belding, R.D., Blankenship, S.M., Young, E., et al. Composition and variability of epicuticular waxes in apple cultivars. *J Am Soc Horticult Sci* 123: 348-356.
[Pubmed](#) | [Crossref](#) | [Others](#)
3. Bernaert, N., Wouters, D., De Vuyst, L., et al. Antioxidant changes of leek (*Allium ampeloprasum* var. porrum) during spontaneous fermentation of the white shaft and green leaves. (2013) *J Sci Food Agric* 93(9) 2146-2153.
[Pubmed](#) | [Crossref](#) | [Others](#)
4. Bischoff, T.A., Kelley, J.C., Karchesy, Y., et al. Antimalarial activity of Lactucin and Lactucopicrin: sesquiterpenelactones isolated from *Cichorium intybus* L. (2004) *J Ethnopharmacol* 95(2-3): 455-457.
[Pubmed](#) | [Crossref](#) | [Others](#)
5. Bramley, P. Is Lycopene beneficial to human health? (2000) *Phytochemistry* 54 (3): 233-236.
[Pubmed](#) | [Crossref](#) | [Others](#)
6. Campas-Baypoli, O.N., Sanchez-Machado, D.I., Bueno-Solano, C., et al. HPLC method validation for measurement of Sulforaphane level in broccoli by-products. (2010) *Biomed Chromatogr* 24(4): 387-392.
[Pubmed](#) | [Crossref](#) | [Others](#)
7. Castro, A.J., Frederico, M.J., Cazarolli, L.H., et al. The mechanism of action of ursolic acid as insulin secretagogue and insulinomimetic is mediated by cross-talk between calcium and kinases to regulate glucose balance. (2015) *Biochim Biophys Acta* 1850(1): 51–61.
[Pubmed](#) | [Crossref](#) | [Others](#)
8. Chang, M., Ho, S., Lee, D., Chen, C., Huang, C., Chang, J. 2013. Characterization, extraction and purification of lutein produced by an indigenous microalga *Scenedesmus obliquus* CNW-N. *Biochem Engineering Journal* 78: 24–31.
[Pubmed](#) | [Crossref](#) | [Others](#)
9. Chen, A.Y., Chen, Y.C. A review of the dietary flavonoid, Kaempferol on human health and cancer chemoprevention. (2013) *Food Chem* 138(4): 2099-2107.
[Pubmed](#) | [Crossref](#) | [Others](#)
10. Cho, K.W., Kim, Y.O., Andrade, J.E., et al. Dietary naringenin increases hepatic peroxisome proliferator-activated receptor α protein expression and decreases plasma triglyceride and adiposity in rats. (2011) *Eur J Nutr* 50(2): 81-88.
[Pubmed](#) | [Crossref](#) | [Others](#)
11. Cui, T., Nakamura, K., Ma, L., et al. Analyses of arbutin and chlorogenic acid, the major phenolic constituents in Oriental pear. (2005) *J Agric Food Chem* 53(10): 3882-3887.
[Pubmed](#) | [Crossref](#) | [Others](#)
12. Ehrenkranz, J.R., Lewis, N.G., Kahn, C.R., et al. Phlorizin: a review. (2005) *Diabetes Metab Res Rev* 21(1): 31-38.
[Pubmed](#) | [Crossref](#) | [Others](#)
13. Escarpa, A., Gonzalez, M.C. Fast separation of (poly) phenolic compounds from apples and pears by high-performance liquid chromatography with diode-array detection. (1999) *J Chromatogr A* 830(2): 301-309.
[Pubmed](#) | [Crossref](#) | [Others](#)
14. Faera, A.E., Ali, R.F.M. Proximate composition, phytochemical constituents, antioxidant activities and phenolic content of seed and leaves extract of Egyptian leek (*Allium ampeloprasum* var. kurrat). (2013) *European J Chem* 4(3).
[Pubmed](#) | [Crossref](#) | [Others](#)
15. Fahey, J.W., Haristoy, X., Dolan, P.M., et al. Sulforaphane inhibits extracellular, intracellular, and antibiotic-resistant strains of *Helicobacter pylori* and prevents benzo[a]pyrene-induced stomach tumors. (2002) *Proc Natl Acad Sci USA* 99(11): 7610-7615.
[Pubmed](#) | [Crossref](#) | [Others](#)
16. Gann, P.H., Ma, J., Giovannucci, E., et al. Lower prostate cancer risk in men with elevated plasma Lycopene levels: results of a prospective analysis. (1999) *Cancer Res* 15 (6): 1225-1230.
[Pubmed](#) | [Crossref](#) | [Others](#)
17. George, B., Kaur, C., Khurdiya, D. S., et al. Antioxidants in tomato (*Lycopersicon esculentum*) as a function of genotype. (2004) *Food Chemistry* 84: 45–53.
[Pubmed](#) | [Crossref](#) | [Others](#)
18. Giovannucci, E., Ascherio, A., Rimm, E.B., et al. Intake of carotenoids and retinol in relation to risk to prostate cancer. (1995) *J Natl Cancer Inst* 87(23):1767-1776.
[Pubmed](#) | [Crossref](#) | [Others](#)
19. Hertog, M.G.L., Hollman, P.C.H., Venema, D.P. 1992. Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *J Agric Food Chem* 40 1591-1598.
[Pubmed](#) | [Crossref](#) | [Others](#)
20. Kunkel, S.D., Elmore, C.J., Bongers, K.S., et al. Ursolic acid increases skeletal muscle and brown fat and decreases diet-induced obesity, glucose intolerance and fatty liver disease. (2012) *PLoS One* 7(6).
[Pubmed](#) | [Crossref](#) | [Others](#)
21. Landrum, J.T., Bone, R.A., Joa, H., et al. A one year study of the macular pigment: the effect of 140 days of a Lutein supplement. (1997) *Exp Eye Res* 65(1): 57-62.
[Pubmed](#) | [Crossref](#) | [Others](#)
22. Li, Y., Lu, X., Qi, H., Li, X., Xiao, X., et al. Ursolic acid induces apoptosis through mitochondrial intrinsic pathway and suppression of ERK1/2 MAPK in HeLa cells. (2014) *J Pharmacol Sci* 125(2):202–210.
[Pubmed](#) | [Crossref](#) | [Others](#)
23. Liang, H., Yuan, Q.P., Dong, H.R., Liu Y.M., et al. Determination of Sulforaphane in broccoli and cabbage by high-performance liquid chromatography. (2006) *Journal of Food Composition and Analysis* 19(5):473–476.
[Pubmed](#) | [Crossref](#) | [Others](#)
24. Manach, C., Scalbert, A., Morand, C., et al. Polyphenols: food sources and bioavailability. (2004) *Am J Clin Nutr* 79(5):727–747.
[Pubmed](#) | [Crossref](#) | [Others](#)
25. Montefusco, A., Semitaio, G., Marrese, G.P. et al. Antioxidants in Varieties of Chicory (*Cichorium intybus* L.) and Wild Poppy (*Papaver rhoeas* L.) of Southern Italy. (2015) *J Chem*.
[Pubmed](#) | [Crossref](#) | [Others](#)
26. Moreno, D.A., Carvajal, M., Lopez-Berenguer, C., et al. Chemical and biological characterization of nutraceutical compounds of broccoli. (2006) *J Pharm Biomed Anal* 41(5): 1508–1522.
[Pubmed](#) | [Crossref](#) | [Others](#)
27. Mori, H., Tanaka, T., Shima, H., et al. Inhibitory effect of Chlorogenic acid on methylazoxymethanol acetate-induced carcinogenesis in large intestine and liver of hamsters. (1986) *Cancer Lett* 30(1): 49-54.
[Pubmed](#) | [Crossref](#) | [Others](#)
28. Najafian, M., Jahromi, M.Z., Nowrozejjad, M.J., et al. Phlorizin reduces blood glucose levels and improves lipids metabolism in streptozotocin-induced diabetics rats. (2012) *Mol Biol Rep* 39(5): 5299-5306.
[Pubmed](#) | [Crossref](#) | [Others](#)
29. Nakagawa, K., Umeda, T., Higuchi, O., et al. Evaporative light-scattering analysis of Sulforaphane in broccoli samples: quality of broccoli products regarding Sulforaphane contents. (2006) *J Agric Food Chem* 54(7): 2479–2483.
[Pubmed](#) | [Crossref](#) | [Others](#)
30. Olives Barba, A.I., Camara Hurtado, M., Sanchez Mata, M.C., et al. Application of a UV–vis detection-HPLC method for a rapid determination of Lycopene and β -carotene in vegetables. 2006 *Food Chemistry* 95(2): 328–336.
[Pubmed](#) | [Crossref](#) | [Others](#)
31. Qu, M., Zhou, Z., Chen, C., et al. Lycopene protects against trimethyltin-induced neurotoxicity in primary cultured rat hippocampal neurons by inhibiting the mitochondrial apoptotic pathway. (2011) *Neurochem Int* 59(8): 1095-1113.

Pubmed | Crossref | Others

32. Sengupta, A.K., Das, B. Determination of the maximum reach envelope for Industrial workstation design, *Advances in Occupational Ergonomics and Safety*, (1999) ed. Gene C. H. Lee, IOS Press, Proceedings of the XIV Annual International Occupational Ergonomics and Safety Conference, Orlando, FL 3: 59-64.

Pubmed | Crossref | Others

33. Shinomiya, K., Omichi, J., Ohnishi, R., et al. Effect of Chlorogenic acid and its metabolites in sleep-wakefulness cycles in rats. (2004) *Eur. J Pharmacol* 504(3): 185-189.

Pubmed | Crossref | Others

34. Sivakumar, G., Bati, C.B., Perri, E., et al. Gas chromatography screening of bioactive phytosterols from mono-cultivar olive oils. (2006) *Food Chem* 95(3): 525-528.

Pubmed | Crossref | Others

35. Stahl, W., Sies, H. Antioxidant activity of carotenoids. *Molecular Aspects of Medicine*. (2003) *Mol Aspects Med* 24(6): 345-351.

Pubmed | Crossref | Others

36. Sujak, A., Gabrielska, J., Grudzinski, W., et al. Lutein and Zeaxanthin as protectors of lipid membranes against oxidative damage: The structural aspects. (1999) *Arch Biochem Biophys* 371(2): 301-307.

Pubmed | Crossref | Others

37. Tamini, R.M., Colditz, G.A., Hankinson, S.E. Circulating carotenoids mammographic density and subsequent risk of breast cancer. (2009) *Cancer Res* 69 (24): 9323-9329

Pubmed | Crossref | Others

38. Tanaka, T., Kojima, T., Kawamori, T. et al. Inhibition of 4-nitroquinoline-1-oxide-induced rat tongue carcinogenesis by the naturally occurring plant phenolic caffeic, ellagic, Chlorogenic and ferulic acids. (1993) *Carcinogenesis* 14(7): 1321-1325.

Pubmed | Crossref | Others

39. Taralkar, S.V., Chattopadhyay, S. A HPLC Method for Determination of Ursolic acid and Betulinic Acids from their Methanolic Extracts of *Vitex Negundo* Linn. (2012) *J Anal Bioanal Techniques* 3(3).

Pubmed | Crossref | Others

40. Thompson, K.A., Marshall, M.R., Sims, C.A., et al. Cultivar, maturity, and heat treatment on Lycopene content in tomatoes. (2000) *J Food Sci* 65(5): 791-795.

Pubmed | Crossref | Others

41. Tsao, R., Yang, R., Young, J.C., Zhu, H. Polyphenolic profiles in eight apple cultivars using high-performance liquid chromatography (HPLC). (2003) *J Agric Food Chem* 51(21): 6347-6353.

Pubmed | Crossref | Others

42. Tsuchiya, T., Suzuki, O., Igarashi, K. Protective effects of Chlorogenic acid on paraquat-induced oxidative stress in rats. (1996) *Biosci Biotechnol Biochem* 60(5): 765-768.

Pubmed | Crossref | Others

43. Tukaj, Z., Matusiak-Mikulin, K., Lewandowska, J., et al. Changes in the pigment patterns and the photosynthetic activity during a light-induced cell cycle of the green alga *Scenedesmus armatus*. (2003) *Plant Physiology and Biochemistry* 41(4): 337-344.

Pubmed | Crossref | Others

44. Van Beek, T.A., Maas, P., King, B.M., et al. Bitter sesquiterpene lactones from chicory roots. (1990) *J Agric Food Chem* 38: 1035-38.

Pubmed | Crossref | Others

45. Warashina, T., Miyase, T. Sesquiterpenes from the roots of *Cichorium divia*. (2008) *Chem Pharm Bull (Tokyo)* 65(10): 1445-51.

Pubmed | Crossref | Others

46. Wattel, A., Kamel, S., Menteverri, R., et al. Potent inhibitory effect of naturally occurring flavonoids quercetin and Kaempferol on in vitro osteoclastic bone reabsorption. (2003) *Biochem Pharmacol* 65(1): 34-42.

Pubmed | Crossref | Others

47. Wesolowska, A., Nikiforuk, A., Michalska, K., et al. Analgesic and sedative activities of Lactucin and some Lactucin-like guaianolides in mice. (2006) *J Ethnopharmacol* 107(2): 254-258.

Pubmed | Crossref | Others

48. Wu, H.Z., Luo, J., Yin, Y.X., et al. Effects of Chlorogenic acid, an active compound activating calcineurin, purified from *Flos Loniceræ* on macrophage. (2004) *Acta Pharmacol Sin* 25(12): 1685-1689.

Pubmed | Crossref | Others

49. Zang, L., Wu, B., Lin, Y., et al. Research progress of Ursolic acid's anti-tumor actions. (2014) *Chin J Integr Med* 20(72).

Pubmed | Crossref | Others

50. Žnidarčič, D., Ban, D., Šircelj, H. Carotenoid and chlorophyll composition of commonly consumed leafy vegetables in Mediterranean countries. (2011) *Food Chem* 129(3): 1164-1168.

Pubmed | Crossref | Others

51. Zu, Y., Li, C., Fu, Y., Zhao, C. Simultaneous determination of catechin, rutin, quercetin kaempferol and isorhamnetin in the extract of sea buckthorn (*Hippophae rhamnoides* L.) leaves by RP-HPLC with DAD. (2006) *Journal of Pharmaceutical and Biomedical Analysis* 41(3): 714-719.

Pubmed | Crossref | Others