COMPOSITION AND HEALTH-PROMOTING PROPERTIES OF KIWIFRUIT AND ANNONA

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Summary

Since time untold, tropical and subtropical fruits have been fundamental dietary components for indigenous populations of the warm climate areas. In many cases – sometimes correctly, other times out of superstition – for these populations the connection between consumption of specific fruits and health benefit has been a part of both folklore and traditional medicine. Nowadays, science has confirmed that several of those links between food and health were real. Modern clinical and biological studies, aided by quickly improving analytical techniques, are providing researchers with a seemingly endless mass of information on the reasons behind the beneficial effects of fruit consumption. In this context, after digging deeply into the composition of the best known and most diffused fruits and vegetables, researchers are shifting the focus of their attention toward tropical and exotic fruits, that is, plants that grow wild in limited areas or that are still cultivated only on a local basis.

In this chapter, an overview on the benefits that may be gained from the consumption of the fruits from two genera, *Actinidia* and *Annona*, and on the chemicals responsible for such effects, the composition and properties of these fruits are presented. The former, kiwifruit, has crossed the borders of the geographical area from which originates (i.e., South-Eastern Asia) and has become available to consumers worldwide, whereas for the latter, annona, most species are still little-known in many countries. Both fruits however share a common feature: they are extremely rich in several classes of bioactive compounds, and emphasis will be placed on some of the most recent studies both from the analytical and from the clinical point of view.

1. Introduction

It has been common knowledge for decades that a healthy and balanced diet is strictly related to a lower risk of insurgence of a wide number of different diseases. Nonetheless, in recent years globalization is determining a progressive alteration of traditional diets towards Western standards, and, as a result, several chronic affections typical of Western countries – cardiovascular pathologies, cancer, diabetes, and obesity disorders, to name the most representative examples – are steadily increasing in frequency, even in those areas where they have been so far less common. In addition, other health problems often appear as a consequence of dietary deficiencies deriving from the lack of single nutrients or even entire groups of macronutrients.

In this context, it is nowadays universally accepted that fruits and vegetables are fundamental components of a balanced diet. The World Health Organization (WHO) suggested that intake of no less than 400g/day of fruits and vegetables may provide a sufficient level of protection against the non-transmissible chronic diseases mentioned above, as well as preventing conditions related to micronutrient deficiencies. This is the obvious consequence of fruits and vegetables being extraordinary sources of bioactive compounds, ranging from macronutrients such as carbohydrates, proteins or fats, to micronutrients such as vitamins, minerals and phenolic compounds.

The WHO recommendation makes evident that there is still room for improvement in terms of dietary practices given that, for different cultural and/or geographic reasons, in many countries the current average consumption of fruit and vegetables is considerably below the suggested level, regardless of the ubiquitous availability of fresh and derived products.

The market, however, is dominated by a relatively limited variety of products, which are mainly those well adapted to specific regions and for which breeding, agricultural and cost-benefit transportation methods have been optimized over the years to ensure their presence on the benches of any market around the world (a place where oranges, tomatoes or soya, to name a few, are not available is nowadays hardly conceivable). These 'common' products have been the subjects of countless studies, both from the analytical and pharmacological points of view. Their composition is well known, the variability of the nutraceutical content depending on species, subspecies and variety has been elucidated, and even the modification of their chemical composition resulting on environment, climate, soil and external stress has been investigated. They have been hybridized and genetically engineered, selected for their taste, appeal to consumers and richness in bioactive compounds. Their whole fruits, extracts of main components have been tested *in vitro* for their activity against many healthy or tumor cell lines, and they have been used in many epidemiological studies to reveal solid connections between their regular consumption and the insurgence of a variety of diseases.

Conversely, there is still an enormous pool of fruits and vegetables that have been scarcely studied. In particular, those referred to as 'tropical' and/or 'exotic' fruits, which are in many cases endemic to specific ecoregions, are often neglected outside the geographic areas where they grow wild or are cultivated on a small scale. Leaving aside some generic information that can be obtained by folklore or traditional medicine, in many cases limited or no data on the potential benefits that could be gained from their consumption are available to the scientific community and consumers alike. In this light, investigation on the composition of these less studied fruits may bring forth a cascade of highly desirable consequences. Knowledge on their chemical composition, their biological and toxicological properties, their nutritional values and pharmacological activity may provide consumers with a wider choice of fresh health-beneficial products, as well as possibly providing researchers with new lead compounds to be developed for potential medical applications. In addition, an increased demand of (so far) underexploited tropical fruits may help in both protecting the biodiversity, and in boosting local farming economy in emerging countries, given that in most cases tropical and exotic fruits are cultivated in African, South American and Asian tropical areas, where they are actually essential dietary components for those populations resident in warm climate latitudes.

The present chapter will focus mainly on two fruits, that is, kiwifruit and annona. Where available, the composition of the various parts of the plant or of the fruit will be reviewed. Composition data will be discussed in a combined fashion, drawn simultaneously from different studies (i.e. carried out with different techniques, and on fruit samples with different origin and history), with the aim to provide the reader more with a comprehensive view of the qualitative profile rather than specific quantitative data on each component. On top of that, this chapter will try to cover in a compendious fashion the biological properties, nutritional features and health-promoting activity of kiwifruit and annona.

2. Kiwifruit

2.1. Facts and Figures about Kiwifruit

Kiwifruit (often shortened to 'kiwi') is a generic name that covers species belonging to the genus *Actinidia*, namely *Actinidia deliciosa* and *Actinidia chinensis*. These species are native to Southern China, although other commercially less relevant *Actinidia* species have been found to originate from India, Japan and South-Eastern Siberia. The kiwifruit, long classified as *(Actinidia chinensis* Planch.), was originally placed in the family Dilleniaceae, but it was later allotted its separate family, Actinidiaceae. In 1986 China's leading authority on this fruit renamed the stiff-haired form (which includes the kiwifruit) *A. deliciosa* (A. Chevalier) C.F. Liang et A.R. Ferguson var. *deliciosa,* retaining *A. chinensis* for the smooth-skinned form.

Originally named 'yang tao', it was introduced in New Zealand in the early 20th century, where it was renamed 'Chinese gooseberry' by the local growers. In the late fifties its name was changed once again, becoming 'kiwi' (after the typical New Zealand bird, which is brown and furry just like the fruit) for commercial reasons. New Zealand has been the leading world producer for several decades. Since the nineties however, Italy, where kiwi was introduced in the early seventies, has replaced New Zealand as the top producer, with a market volume of about 450,000 tons per year (2009 data from the Food and Agriculture Organization of the United Nations). China, Italy, New Zealand, Chile and Greece are the biggest producers, accounting collectively for 87% of the world kiwifruit production. Presently, there are no less than 30 different varieties cultivated in different part of the world. However, most of the production derives from the 'Hayward' cultivar of *A. deliciosa* (predominant in Italy, New Zealand), which has green flesh, an acidic taste and green aroma, and from the 'Hort16A' variety of *A. chinensis*, which is the main cultivar employed in China, and is yellow-fleshed, sweeter and with an intense tropical flavor. ‘Hort16A’ has been available on the market only for a decade, whereas ‘Hayward’ has been intensively produced since the 1960s. ‘Hort16A’ is also more productive than ‘Hayward’.

Figure 1a. Kiwifuit

The kiwifruit is borne on a vigorous, woody, twining vine or climbing shrub reaching about 9 m, with dark-green deciduous oval leaves about 10 cm long, and 5- to 6-petalled buff yellow flowers, 2.5 to 5 cm broad. The oval fruit is up 6 to 10 cm long, with a brown skin densely covered with short, stiff brown hairs. It has a soft textured, bright green or golden flesh, with rows of minute black, edible seeds, unnoticeable in eating. It is generally cropped in autumn, and its market availability spans from November to April (as far as the northern hemisphere crop is concerned).

2.2. Kiwifruit Composition

The aroma of kiwifruit is the result of a complex mixture of a large number of volatile compounds and, as for most food products, it is one of the key factors for consumer acceptance, along with sweetness and acidity. The volatile profile is a very subtle balance of components, which can be strongly influence by several factors such as ripening, storage and post-harvest treatments.

The volatile components of the kiwifruit bouquet have been investigated mostly by gas chromatography-mass spectrometry (GC-MS), although more recent investigations have relied on gas chromatography-olfactometry (GC-O), gas chromatography-mass spectroscopy-olfactometry (GC/MS-O) and multidimensional gas chromatography-olfactometry (GC/GC-O). Volatile isolation, on the other hand, has been carried out by applying a number of methodologies, even though different techniques are likely to yield quite different results. In some cases, methods based on solubility (i.e., solvent extraction or solid phase micro-extraction) may result in underestimation of the least soluble compounds and of the most volatile low molecular weight compounds. Conversely, volatility-based methods, such as headspace and distillation, may result in an underestimation of high molecular weight less volatile compounds. Dynamic headspace, on the other hand, may generate volatile profiles which are intermediate between those obtained by the isolation protocols mentioned above.

Being the most diffused cultivar, *A. deliciosa* var. 'Hayward' has been the subject of most investigation, whereas the other cultivars have been less studied. To date, more than 90 individual components of the volatile fraction of kiwifruit have been described in the literature. Esters, such as ethyl butanoate, methyl butanoate and methyl benzoate, and six-carbon aldehydes and alcohols, i.e., (*Z*) and (*E*)-hexenal, hexanal, (*Z*)- and (*E*)-3-hexenol have been shown to be the main components of the aroma. On top of those, a range of sulfur-containing compounds methyl (methylthio)acetate and ethyl (methylthio)acetate, dimethyl disulfide, hydrogen sulfide, dimethyl trisulfide) has been found, and these derivatives significantly influence the overall perception of the aroma. Other noticeable compounds identified in kiwifruit comprise ethyl 3-methylbutanoate, diethyl carbonate, ethyl 2-butenoate, 1,5-heptadiene-3,4-diol, 2,2-diethyl-1-pentanol, 7-methyl-1-octene, (*E*)-4-hexen-1-ol, 2-methylcyclopentanol and ethyl octanoate.

A. chinensis var. 'Hort16A', i.e. gold kiwifruit, possess a similar volatile profile to that determined for 'Hayward' green kiwifruit, albeit with some remarkable differences, with special reference to dimethyl sulfide, which may be a key odorant in determining the different aromas of these two fruits.

Volatile compounds may also be found as their glycosylated forms stored into the fruit tissue. These compounds may be released upon ripening, processing or storage, and thus modify the aroma of the fruit. Isolation of glycosidically bound volatile from kiwifruit juice, followed by enzymatic hydrolysis with β-glucosidase, resulted in the identification of a wide range of compounds that had not previously been found in fresh kiwifruit aroma, such as octan-3-ol, camphor, 4-methylbenzaldehyde, 2-hydroxybenzaldehyde, neral, geranial, methyl 2-hydroxybenzoate, nerol, geraniol and 2-phenylethanol.

Glycosidically bound volatiles, released by enzymic hydrolysis, have been isolated from kiwifruit juice. Major components found and identified by GC-MS were (*E*)-2-hexenal and benzaldehyde. Compounds not previously identified in kiwifruit include octan-3-ol, camphor, 4-methylbenzaldehyde, 2-hydroxybenzaldehyde, neral, geranial, methyl 2-hydroxybenzoate, nerol, geraniol and 2-phenylethanol.

A very recent study on kiwifruit cultivated in Turkey has shown a general quantitative profile of the main classes of compounds present in fruit flesh (Table 1). Many other researchers have taken on the task of identifying as many components as possible.

Kiwifruits are rich sources of minerals, which are fundamental micronutrients for a balanced diet. In a recent study carried out on four varieties of *A. deliciosa*, namely 'Hayward', 'Bruno', 'Monty', and 'Abbott' grown in Northern Iran, the level of several mineral nutrients have been investigated. Among the macronutrient group (i.e., Ca, Mg, K, and Na) potassium (0.2 w/w%) potassium was found to be the most abundant, regardless of kiwifruit variety, whereas among the mineral micronutrients (Cu, Fe, Mn, Zn), iron and zinc (34 and 31 parts per million (ppm), respectively) are the most abundant. Interestingly, this research also demonstrated that mineral content is independent from soil composition. Other authors reported also that kiwifruit is very rich in Selenium (0.6 mg/100 g lyophilized extract).

Kiwifruit contents	**Amount**
Yield	7.3[a]
Water	83.5 g
Carbohydrates	14.9 g
Protein	0.99 g
Vitamin C	105 mg
Vitamin B group	0.2 mg
Fat	0.44 g
Potassium	332 mg
Chlorine	50 mg
Phosphorus	40 mg
Calcium	35 mg
Magnesium	28 mg
Sulfur	16 mg
Sodium	4 mg
Selenium	0.6 mg
Iron	0.5 mg
Boron	0.2 mg
Zinc	0.16 mg
Copper	0.1 mg
Minerals	0.51 g

[a] The percentage amount (%) of lyophilized aqueous extract obtained from 100 mg kiwifruits.

Table 1. Nutritional values and chemical contents per 100 g lyophilized extract of kiwifruit (*A. deliciosa*).

One of the major antioxidants in fruits and vegetables is L-ascorbic acid (AA), better known as vitamin C by most consumers (Figure 1b). The best known sources of vitamin C are *Citrus* fruits, but fruits from *Actinidia* species are often even richer. Vitamin content in the fruit of various species and cultivars of kiwifruit and other *Actinidia* species was determined by ion-pair reversed-phase high performance liquid chromatography. 'Hayward' kiwifruit where found to contain 65.5 mg/100 g fresh weight (FW) vitamin C. Vitamin C content in *A. deliciosa* fruit varied from 29 mg/100 g FW to 80 mg/100 g FW. Conversely, in most varieties of *A. chinensis* vitamin C content was found to be higher than that determined for 'Hayward', with peaks of 185 mg/100 g FW. In other words, on average one single large fruit may contain up to about

150% of the USDA Recommended Daily Value.

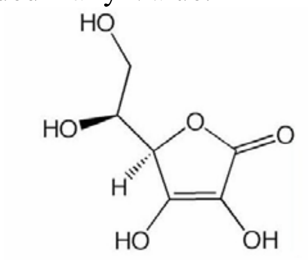


Figure 1b. Ascorbic acid (vitamin C).

Further studies, aimed at evaluating the variation of vitamin C content during kiwifruit storage (extremely important in terms of providing consumers with products containing the highest possible amount of bioactive compounds), showed that 'Hayward' harvested fruits stored at 0 °C and 85-90% relative humidity under static control atmosphere (5% CO_2 + 2% O_2) tend to lose vitamin C faster (up to 70-80% decrease after 5 months) than those stored under normal atmosphere at the same temperature and humidity.

Beyond vitamin C, kiwifruit is an extraordinary source of compounds with antioxidant activity such as phenolics or organic acids. Several studies have been published over the last decades detailing the identification of a wide range of different compounds, ranging from flavonoids, to anthocyanins, cathechins, coumarins, carotenoids, chlorophylls, tocopherols, phytosterols and terpenoids.

Flavonoids are compounds that are currently attracting a lot of attention, given the wide range of health-beneficial actions they exert. They show a strong antioxidant and radical scavenging activity and appear to be associated with reduced risk for certain chronic diseases and certain kinds of cancerous processes, and may prevent some cardiovascular disorders. Flavonoids exhibit also antiviral, antimicrobial, antiulcer, antiallergenic and anti-inflammatory activities; they affect capillary fragility and have the ability to inhibit human platelet aggregation.

Flavonoids are secondary metabolites, ubiquitous in the plant kingdom (Figure 2). To date, more than 5000 naturally occurring flavonoids have been characterized, classified in subclasses according to the structural features of the aromatic core units. Many authors have postulated that their profile can be used as a fingerprint to identify a given fruit, or products derived from it, since their occurrence is often typical of a given species or genus. For instance, to mention some of the most common flavonoid-rich foods, tea (e.g. green tea) is characterized by the abundance of flavanols, in onions flavonols are the main components, and in *Citrus* fruits flavanones by far surpass flavones and other (minor) components. Several flavonoids have been so far identified in kiwifruit, either as their unsubstituted forms, or as their glycosylated derivatives (Figure 3 and Table 2). Interestingly, the flavonoid fraction in the genus *Actinidia* is dominated by flavanols (i.e., flavanone derivatives that bear a hydroxyl substituent at the 3-position) with flavonols following behind (i.e., flavone derivatives again with an hydroxyl substituent at the 3-position).

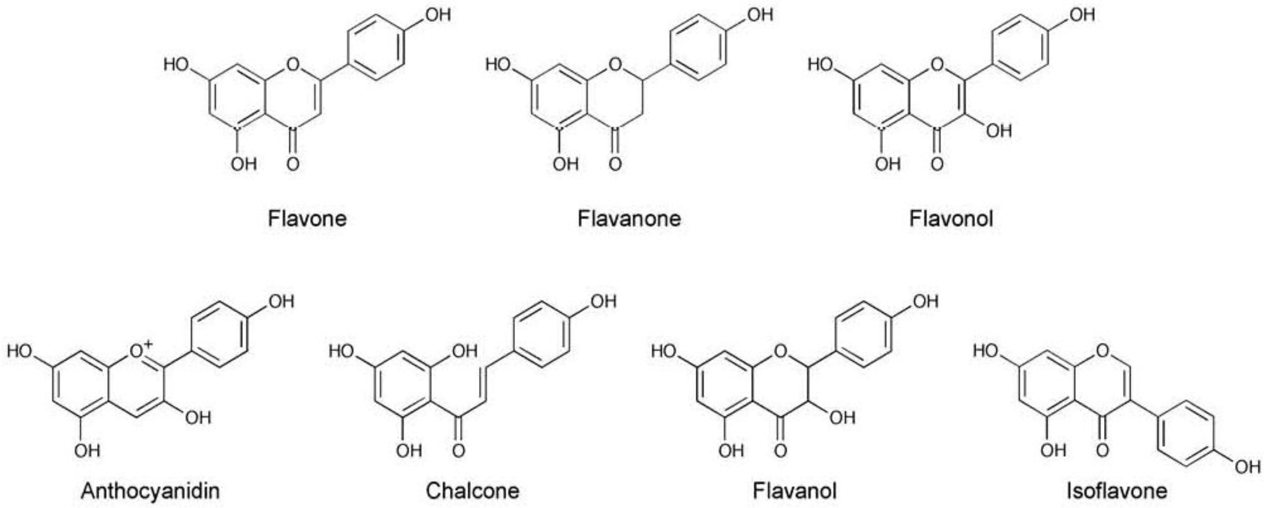


Figure 2. Schematic representation of the flavonoid main subclasses.

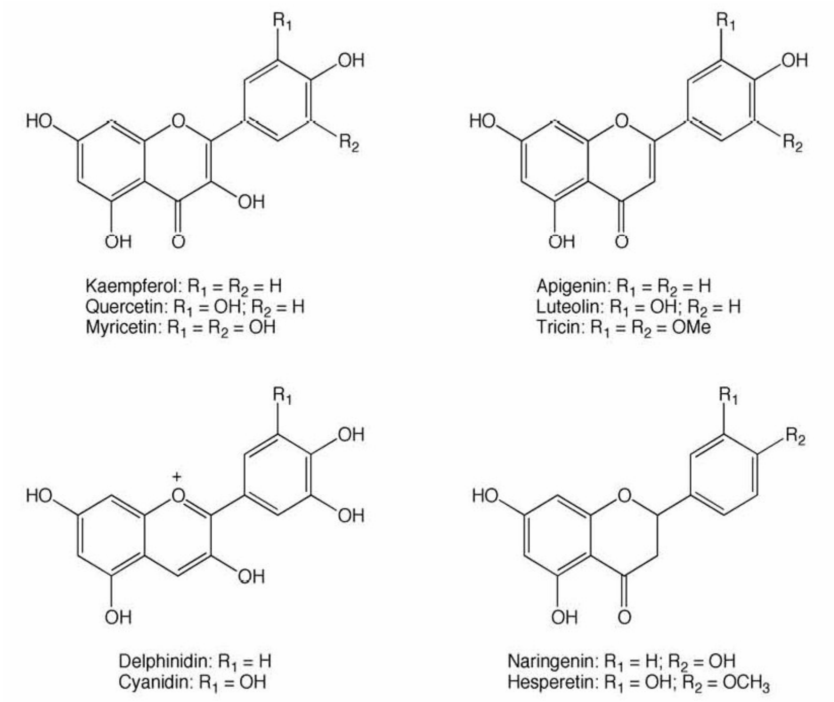


Figure 3. Structure of flavonoid aglycones found in *Actinidia* spp.

Kiwifruit contains significant amounts of procyanidins, with concentrations of individual compounds reaching up to *ca*. 5 mg/kg FW in kiwifruit pulp, and 5 mg/L in pressed juice. Also, range of catechin and epicatechin derivatives (catechin, catechin gallate, epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate, gallocatechin, and gallocatechin gallate) have been identified and quantified. Among the flavonols the most abundant are quercetin- and kaempferol-derived compounds. Different studies have indicated that quercetin-3-*O*-L-rhamnoside (also known as quercitrin) is the most abundant derivative in kiwifruit pulp (1.0–2.8 mg/kg FW) as well as in kiwi juice (0.38–0.45 mg/L).

Aside from the more diffused green-fleshed and yellow-fleshed (gold) kiwifruit, within the *Actinidia* genus a range of different pulp colors occur, including red, purple and orange. The compounds responsible for the pigmentation are mainly carotenoids, chlorophylls and anthocyanins (Tables 2 and 3).

Flavonols	**Flavanols**
Myricetin Quercetin Quercetin-3-*O*-L-rhamnoside (quercitrin) Quercetin-3-*O*-D-glucoside (isoquercitrin) Quercetin-3-*O*-rutinoside (rutin) Kaempferol-3-*O*-L-rhamnoside (afzelin) Kaempferol-3-*O*-D-glucoside (astragalin) Kaempferol-3-*O*-rutinoside Kaempferol-3-*O*-D-galactoside	Catechin Catechin gallate Epicatechin Epicatechin gallate Epigallocatechin Epigallocatechin gallate Gallocatechin Gallocatechin gallate Procyanidins (B3, B2 or B4 and oligomers)
Flavones	**Anthocyanins**
Apigenin Luteolin Tricin	Delphinidin 3-*O*-(xylosyl)galactoside Delphinidin 3-*O*-galactoside Cyanidin 3-*O*-(xylosyl)galactoside Cyanidin 3-*O*-galactoside Cyanidin 3-*O*-glucoside
Flavanones	
Hesperetin Naringenin	

Table 2. Flavonoids and anthocyanins identified in *Actinidia* spp

Carotenoids, xanthophylls and chlorophylls	**Organic and phenolic acids**
β-Carotene	Ascorbic acid
Chlorophyll a	Caffeic acid
Chlorophyll b	3-O-β-D-Gucosyl caffeic acid
Chlorophyll a'	4-O-β-D-Gucosyl caffeic acid
Chlorophyll b'	Ferulic acid
Pheophytin a	Chlorogenic acid
Lutein	Syringic acid
Lutein epoxide	Ellagic acid
Neolutein A	*p*-Hydroxybenzoic acid
Neolutein B	*p*-Coumaric acid
Auroxanthin	Gallic acid

9'-*cis*-Neoxanthin Violaxanthin 9'-*cis*-Violaxanthin Neochrome (*cis*) Neochrome (*trans*) Antheraxanthin Zeaxanthin β-Cryptoxanthin	Tannic acid Protocatechuic acid Vanillic acid Anisic acid Quinic acid Malic acid Citric acid D-isocitric acid Catechol Pyrogallol Curcumin Vanillin
Triterpenoids Oleanolic acid Ursolic acid Actinidic acid Arjunolic acid Asiatic acid 23-Hydroxytormentic acid	**Coumarins** 6-Hydroxy-7-(β-D-glucosyloxy)coumarin 6,8-Dimethoxy-7-(β-D-glucosyloxy)coumarin 6-Methoxy-7-hydroxy-8-(β-D-glucosyloxy)coumarin (fraxin) 7-Hydroxy-6-(β-D-glucosyloxy)coumarin (esculin)
Biogenic amines Putrescine Cadaverine Histamine Tyramine Spermidine	**Tocopherols** δ-Tocomonoenol α-Tocopherol R-Tocopherol δ-Tocopherol
Biogenic amines Putrescine Cadaverine Histamine Tyramine Spermidine	**Phytoterols** β-Sitosterol Stigmasterol Campesterol Stigmast-7-en-3β-ol Ergosterol Ergosterol peroxide 5,7,14,22-Ergostatetraen-3β-ol
Sugars Sucrose Fructose Galactose *myo*-Inositol Cellulose Xyloglucans	

Table 3. Carotenoids, chlorophylls, xanthophylls, organic and phenolic acids, coumarins, tocopherols, triterpenoids biogenic amines and other phenolics identified in *Actinidia* spp.

Carotenoids are a group of lipophilic compounds present in most fruits and vegetables that, beyond contributing to the appearance of fruit, provide additional nutritional value in the form of dietary antioxidants. *A. deliciosa* cv. 'Hayward' was found to contain chlorophylls a and b and the carotenoids normally associated with photosynthesis, e.g. β-carotene, lutein, violaxanthin, and 9'-*cis*-neoxanthin (Figures 4 and 5).

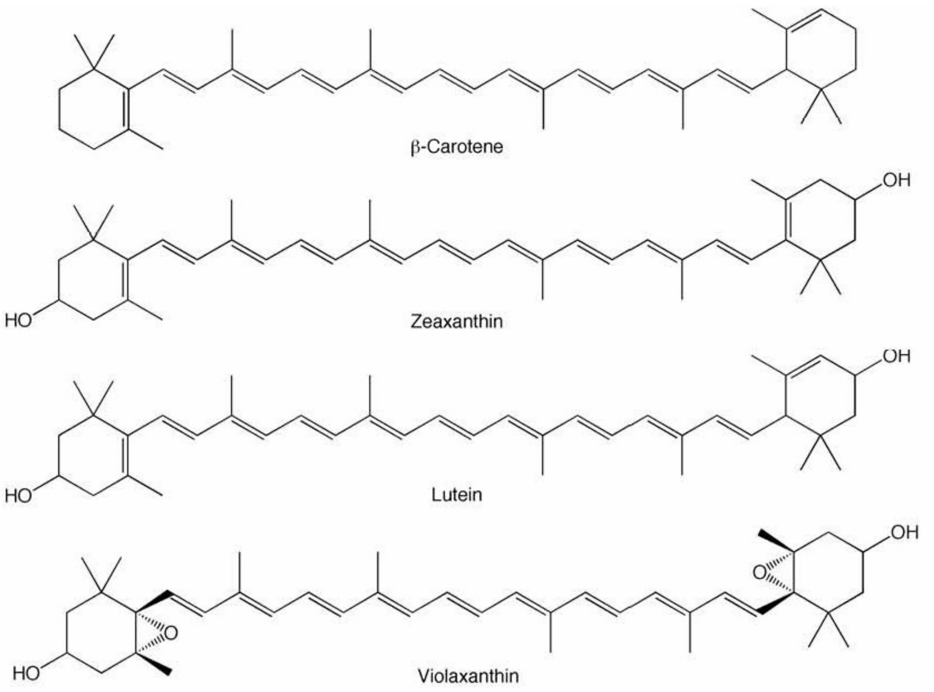


Figure 4. Relevant carotenes and xanthophylls found in *Actinidia* spp.

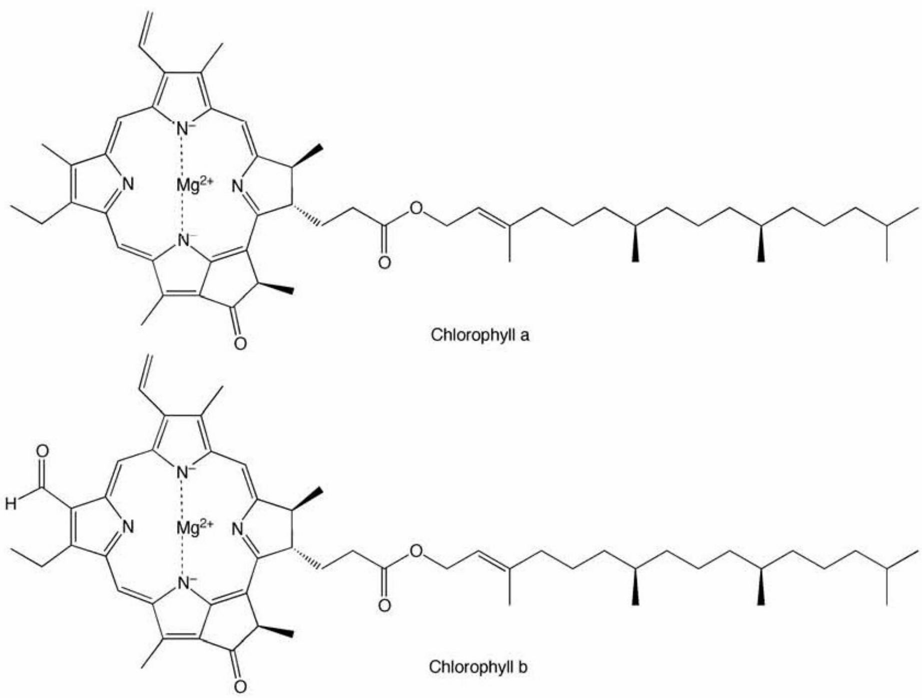


Figure 5. Relevant chlorophylls found in *Actinidia* spp.

A. chinensis cv. 'Hort16A' was also found to contain the same components, albeit with a markedly lower concentration of chlorophylls, and with the noticeable addition of esterified xanthophylls. Orange-fleshed *A. macrosperma* was found to contain mainly β-carotene and no chlorophylls, leading the Authors to conclude that the difference between green-, yellow- and orange-colored flesh lies not in carotenoids differences, but rather in a decreasing amount of chlorophylls.

A small number of genotypes also have red pigments, varying in intensity and in distribution within the fruit. HPLC analysis showed that the red color found mainly in the inner pericarp is due to anthocyanins, allowing to identify five different pigmented compounds, delphinidin 3-*O*-(xylosyl)galactoside, delphinidin 3-*O*-galactoside, cyanidin 3-*O*-(xylosyl)galactoside, cyanidin 3-*O*-galactoside and cyanidin 3-*O*-glucoside. Differences in the relative amounts of cyanidins and delphinidins determine whether the fruit has a red or purple hue. In green kiwifruit, cyanidin 3-*O*-galactoside and cyanidin 3-*O*-glucoside were found as the major anthocyaninis, whereas in yellow-fleshed fruits the major component was cyanidin 3-*O*-(xylosyl)galactoside, with smaller amounts of cyanidin 3-*O*-galactoside. Red- and purple-fleshed kiwis possess the same pigments as green and yellow fruits, albeit in a significantly higher concentration (about 5–90 for the former two *vs.* 200 mg/kg FW for the latter two).

The generic term 'organic acids' is employed to collectively refer to a broad group of compounds generally well represented in plant tissues. These compounds are often discussed together with polyphenols, as they are mostly involved in the same metabolic pathways. Leaving aside ascorbic acid, which has been discussed above, in kiwifruit a variety of structurally very different acids and phenolic compounds has been identified and quantified. Among the pre-eminent ones, there is pyrogallol, found in very high amount in lyophilized aqueous kiwifruit extracts (2.07 g/kg DW), a polyphenol derived from the enzymatic decarboxylation of gallic acid (also present in kiwifruit) operated by the enzyme gallate decarboxylase. Further aromatic acidic compounds identified were a number of compounds formally derived by cinnamc acid (which has never been found in kiwi) such as coumaric, ferulic, chlorogenic, caffeic and glucosyl-caffeic acids, as well as benzoic acid-derived compounds such as anisic, syringic, protocatechuic, *p*-hydroxybenzoic and vanillic acids (Figure 6).

Particularly relevant compounds are the aliphatic derivatives, citric and malic acid, found in kiwifruit along with quinic and isocitric acid. These derivatives have a strong influence on total acidity and on the taste of kiwifruit. Citrate and malate (found in 'Hayward' samples at a 9.9–12.6 and 0.8–1.7 g/kg FW levels, respectively) are sour-tasting compounds that give kiwifruit its typical acidic taste, whereas quinate adds to the overall perception of ‘Hayward’ flavor and banana-like flavors.

Soluble sugars content were determined in various *Actinidia* genotypes at the eating-ripe stage using high performance liquid chromatography. The main soluble sugars in *A. deliciosa* and *A. chinensis* fruits were glucose (29.6–43.8 g/kg FW) and fructose (25.9–41.5 g/kg FW), with sucrose present in smaller (12.8–23.3 g/kg FW, in *A. deliciosa*) to comparable (16.6–39.5 g/kg FW, in *A. chinensis*) amounts. Most *Actinidia* fruits tested also contained at mid development unusually high levels of inositol, up to 40% of the total soluble carbohydrate fraction which falls to 1-2% at full ripeness, mostly as a result of large increases in other sugars content rather than inositol utilization. *myo*-Inositol was found to be the main isomer present.

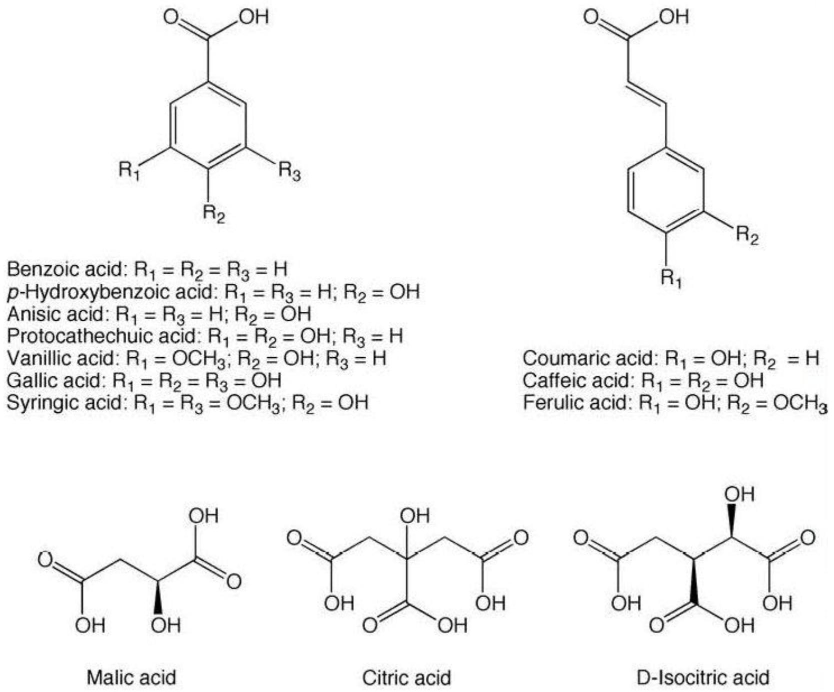


Figure 6. Relevant organic acids found in *Actinidia* spp

In *A. deliciosa*, kiwifruit starch exhibited an apparent amylose content of 43.1% and absolute amylose content of 18.8%. Kiwifruit amylopectins, relative to other starches, had low weight average molecular weight (74,000,000 Da), with long branch chain-length (28.6 average units).

Alcohol-insoluble residues from whole, unpeeled gold and green *A. deliciosa* kiwifruits were analyzed, and the constituent sugar and glycosyl linkage compositions determined. In both cases, residues contained a high proportion of cellulose, with gold kiwifruit containing a higher proportion of hemicellulosic polysaccharides and lower proportion of pectic polysaccharides with respect to green kiwifruit. Also, xyloglucan (which is a hemicellulose present in the primary cell wall) from *A. deliciosa*, was extracted from the pericarp tissues as hemicellulose II (HC-II). Gel permeation chromatography indicated that purified xyloglucan had an average molecular weight of 161,000 Da. α-Amylase/*iso*-amylase digestion showed that it was composed mostly of xylose (32–44%), glucose (37–41%) and galactose (10–17%), with smaller amounts (<5%) of rhamnose, fucose, arabinose and mannose.

In order to study the changes in phenolic composition occurring during kiwifruit juice processing and determine factors affecting the quality of juice products, phenolic compounds in juice were separated and characterized by reversed-phase HPLC. The composition did not differ significantly from that of fresh kiwifruit juice. Among the organic acids, erivatives of coumaric and caffeic acids, including chlorogenic acid, protocatechuic acid, and a derivative of 3,4-dihydroxybenzoic acid were found. The phenolics fraction also contained epicatechin, catechin, and procyanidins (B3, B2, or B4 and oligomers), as well as the flavonols, quercetin and kaempferol (mainly found as their glucoside, rhamnoside, and rutinoside, the former, and as their rhamnoside and rutinoside, the latter). Little change in the concentration of the flavonol glycosides was observed during juice processing, although the overall level of phenolic compounds in kiwifruit juice was found to be low.

However, by-products derived from kiwifruit processing have been found to be a good source of phenolics (in the range of 1–1.5 mg/Kg from the skin) and polysaccharides by means of ethanol/water extraction.

2.3. Kiwifruit Antioxidant Activity

Oxidation is a metabolic process essential for the production of energy for cell activities and survival. However, the metabolism of oxygen in living cells may lead to the formation of undesirable reactive oxygen species (ROS), such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. Oxidants are by-products of normal body metabolism and, if they are not kept under control, may cause extensive damage. A dietary element defined as 'antioxidant' is a compound with the ability to protect cells against the damage produced by ROS. An imbalance between antioxidant and reactive oxygen species usually results in oxidative stress, which causes cellular damage. Oxidative stress has been linked to the insurgence of tumors, aging, atherosclerosis, ischemic injury, inflammation and neurodegenerative diseases such as Parkinson's and Alzheimer's. Antioxidant compounds may help provide protection (i.e., prevention) against these diseases by contributing to the total antioxidant defense system of the human body. Many epidemiological studies have shown that there is a clear connection between antioxidant dietary intake and the risk of cardiovascular and neurodegenerative diseases.

The recognized dietary antioxidants are vitamin C, vitamin E, flavonoids, selenium, and carotenoids. These compounds act by following different radical scavenging mechanism, and may intercept reactive oxygen species at different cellular levels. However, even though their chemical reaction mechanism in several cases has been elucidated, there is still a lot to be discovered about their actual *in vivo* mechanism of action. On top of that, it is still unclear how several of these compounds manage to act in a synergic fashion providing, when taken together, a much higher degree of antioxidative protection than that shown by individual compounds. In light of these facts, presently the best way to take advantage of the entire spectrum of the health-beneficial effects of antioxidants is to obtain them directly from the natural sources, that is, fresh fruits and vegetables, and this is the main reason behind the enormous amount of data appeared in the literature in recent years on the antioxidant activity of raw plant/fruit materials or extracts thereof.

There are several accepted methods for the *in vitro* assessment of antioxidant activity. They mostly rely i) on the quenching of a stable probe radical species, or ii) on the reductive potential of the matrix under investigation. 2,2-Diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation ($ABTS^{\bullet+}$) quenching, as well as Superoxide Radical-anion ($O_2^{\bullet-}$) Scavenging

Activity (SRSA) and Oxygen Radical Absorbance Capacity (ORAC), which provide an estimate of the antioxidant activity by following the disappearance of the probe radical by UV-Vis spectroscopy, belong to the radical-quenching group of assays. Conversely, other routine methods such a Ferric Reducing Antioxidant Power (FRAP) or Mo(VI) reducing power, which monitor the ability of a given food matrix to reduce metals in an oxidized state, belong to the group of assays that rely on the reducing potential of the matrix. It is worth mentioning, however, that many authors agree that no single assay may be regarded as a fully reliable measurement of antioxidant activity, given that raw fruits and fruit extracts are very complex matrices composed of active compounds that may react differently to individual radical probes. To make data comparable, it is common practice to express data as equivalents of known standard radical scavengers such as Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) or gallic acid.

Kiwifruit, as shown in the Section above, is a rich source of polyphenol, vitamin C and other antioxidant compounds. In a recent study, fruits from eight *Actinidia* species were assayed for their antioxidant activity by using a number of different assays (DPPH, ABTS, ORAC, FRAP and SASR). In addition, their total phenolics and vitamin C content were determined, in an attempt to correlate their content to the results obtained by those assays. The significance analysis suggested that the antioxidant capacity of *A. eriantha* and *A. latifolia* fruits were higher than that of the other tested species, reaching up to *ca.* 3,000 μM TE (Trolox Equivalents) against $DPPH^{\bullet}$, and *ca.* 1,600 μM TE against $ABTS^{\bullet+}$. Conversely, the most common *A. deliciosa* var. 'Hayward' showed the (relatively) weakest antioxidant activity (*ca.* 1,000 and 400 μM TE against $DPPH^{\bullet}$ and $ABTS^{\bullet+}$, respectively). These findings significantly correlate with total phenolics (TP) and vitamin C (AA) content determined for all the genotypes, with the wild *A. latifolia* much richer than the widely cultivated 'Hayward' kiwifruit (TP: 710 *vs.* 41 mg gallic acid equivalents (GAE)/100 g FW, respectively; AA: 1322 *vs.* 63 mg/100 g FW, respectively), demonstrating that both total phenolics and vitamin C are major contributors to the total antioxidant capacity of *Actinidia* fruits. In addition, the antioxidant capacity was found to be dependent in part also on the color of kiwifruit flesh. In general, red-fleshed fruits showed higher antioxidant activity than yellow-fleshed ones, probably as a result of the presence of anthocyanins.

A different study has been aimed at the elucidation of the antioxidant activity of individual components identified from *A. deliciosa* var. 'Hayward' pulp and peel. In this case 27 metabolites were isolated, applying a combined extraction/separation methodology, reliant on the fractionation of the fruit material by means of hexane, ethanol, methanol, ethyl acetate and water extraction, followed by chromatographic separation (HPLC, gravity and/or flash column chromatography, thin layer chromatography and gel filtration). By this approach, three vitamin E derivatives, seven sterols, a triterpenoid, three caffeic acid derivatives, two coumarins, eight flavonoids and three catechin derivatives were isolated. All compounds isolated from 'Hayward' kiwifruit fruits were tested for their antioxidant activity by the $DPPH^{\bullet}$ and $O_2^{\bullet-}$ assays, and compared to vitamin C as the reference radical scavenger. As it may be expected, the highest activity was shown by the quercetin and catechin derivatives, which were about twice as efficient as ascorbic acid, closely followed by the kaempferol derivatives.

A common post-harvest process to induce the full ripening of kiwifruit consists of an ethylene treatment, which effectively shortens the ripening time and improves the sensory quality of kiwifruit by increasing fructose, sucrose and soluble solids contents. In this light, studies have been carried out to reveal the variation of antioxidant properties resulting from this treatment. It was observed that the contents of vitamin C and phenolics were higher in ethylene-treated kiwifruit than in the non treated ones, and that the antioxidant activity of total phenolics extracts of treated kiwifruit (as determined by the $ABTS^{\bullet+}$ and FRAP assays) was significantly higher than that of non-treated fruits. Interestingly, correlation coefficients between phenolics and antioxidant activity were significantly higher than between vitamin C and antioxidant activity. The Authors observed that during ethylene treatment the bioactivity of kiwifruit increases, reaching its maximum after six days under an atmosphere containing 100 ppm ethylene, indicating that time as the optimum one for kiwifruit consumption, and concluding that this type of treatment has a positive effect on kiwifruit properties as a health-promoting dietary component.

2.4. Kiwifruit Nutritional and Pharmacological Properties

Given the progressive increase of worldwide consumption of kiwifruit, over the past few years researchers have started to investigate the nutritional properties of the most diffused varieties, that is, the green-fleshed *A. deliciosa* var. 'Hayward' and the yellow-fleshed *A. chinensis* var. 'Hort16A'. Also, given that the health-beneficial effects of diets high in fruits and vegetables are generally not replicated in supplementation trials with isolated antioxidants and vitamins, the emphasis of chronic disease prevention has shifted to whole foods and whole food products. The first epidemiological studies on the actual positive and negative (i.e., allergy, see below) effects of kiwifruit consumption on human health have appeared. This section will cover the main findings in this field, taking into account however only reports based on the consumption (or other uses) of the whole kiwifruit, rather than individual compounds present in it.

Very recently, some authors have reported on the available energy (AE) content of 'Hayward' and 'Hort16A' in terms of ATP yield from digestion. Using growing pig as an *in vivo* model for human upper gastrointestinal tract digestion, followed by incubation with human fecal inocula, to simulate *in vitro* human hindgut fermentation, they performed digestibility assays which allowed themto predict post-absorption ATP production. ATP energy contents of 'Hayward' and 'Hort16A' were quantified as 5.9 and 6.2 kJ g^{-1} DW, respectively, approximately 44–47% of the determined apparent digestible energy content. The AE contents of the kiwifruit, with respect to the one of standard dextrin were 0.57 and 0.61 for 'Hayward' and 'Hort16A', respectively, whereas the comparable ratios for metabolizable energy (ME) were 0.74 and 0.73. From these data, the authors concluded that the relatively low energy content (along with its high water content) make kiwifruit an ideal food for weight-loss diets.

The same research groups reported also on the dietary effect on digestion of actinidin, a cysteine protease abundant in 'Hayward' kiwifruit (but almost absent in 'Hort16A'). After prior studies on the *in vitro* action of actinidin under simulated gastric conditions, in a test study on rats fed with 'Hayward' and 'Hort16A' kiwifruit together with different protein sources, it was observed that, dietary actinidin significantly enhanced gastric digestion of the main proteins of beef muscle, soy protein isolate, gelatin and gluten but not of whey protein isolate or zein.

In a human intervention trial, the markers of antioxidant status, DNA stability, plasma lipids, and platelet aggregation were measured after the dietary supplementation of fresh *A. chinensis* kiwifruit, to gather insight on the potential effect of the bioactive components of gold kiwifruit. After supplementation of a normal diet, for two to four weeks, with one or two kiwifruits per day, plasma vitamin C increased, as did resistance towards H_2O_2-induced DNA damage, whereas plasma triglycerides and blood platelet aggregation decreased. Purine oxidation in lymphocyte DNA decreased significantly after one kiwifruit per day, pyrimidine oxidation decreased after two fruits per day. Interestingly, biomarkers relevant to both cancer and cardiovascular diseases indicate that there is no significant difference between one and two kiwifruits per day. These findings however demonstrated that *A. chinensis* kiwifruit consumption strengthens resistance towards endogenous oxidative damage, thus benefiting public health by reducing the risk of thrombotic events mediated by platelet activation.

Other studies have shown that kiwifruit posses remarkable immunopotentiating activity. In a study aimed at clarifying whether the level of this activity is consistent with the content of vitamins or other major nutrients in the fruit, mice were fed with kiwifruit juice obtained from different genotypes. After oral administration, cytokine production increased, and urinary oxidative stress markers revealed a significant inhibition of *in vivo* oxidative stress, showing in this case the antioxidative protection provided by kiwifruit consumption. It is worth also adding that green-kiwifruit was found to be slightly more active than yellow-fleshed one.

In an attempt to expand the potential applications of the whole kiwifruit plant (not just the fruit), an investigation was carried out on *A. deliciosa* leaves extracts. This study led to some interesting observations: oral administration of a 90% aqueous methanol extract induced a substantial suppression of the postprandial blood glucose level in mice fed with soluble starch or sucrose solutions. The Authors suggested that the mechanism of action might involve the inhibition of α-amylase and α-glucosidase (albeit by separate components, according to independent *in vitro* experiments). Therefore, kiwifruit leaves may be regarded as a new resource for preventing metabolic disorders such as diabetes.

It is known, at an anecdotal level, that kiwifruit is an excellent remedy for constipation. To verify this hypothesis, kiwifruit (one kiwifruit per 30 kg bodyweight) was implemented in the diet of a population of healthy elderly adults (> 60 years) for three weeks, followed by a further 3-week crossover period. Based on the observations reported, kiwifruit consumption resulted in a significant improvement of all tested measures of laxation in these subjects. Given that 'Hayward' kiwifruit has been reported as one of the most nutrient dense fruits, it is difficult to isolate mechanisms, and there are a number of different agents that may enhance laxation (either separately or in concert). In general, laxatives are divided in four classes: bulkers, osmotic regulators, stimulant laxatives and fecal softeners. Kiwifruit seems to be acting through both increasing and softening fecal bulk.

Recently, a study that describes the use of kiwifruit pulp as a topical remedy has been reported. This research originates from the fact that since World War II the chemical debridement of wounds had been carried out with the aid of enzymes (proteases) from plant extracts (e.g., *Carica papaya*, the melon tree), and from the fact that kiwifruit, owing to its high actinidin content, is used as an efficient meat tenderizer. In this case, application of kiwifruit pulp mixed with kiwifruit juice on acute burn wounds on mice led to faster healing, as demonstrated by the accelerated separation of eschar without surgical intervention or bleeding. The Authors suggested that kiwi could be useful for limited second-degree or deep full-thickness burn wounds, and lowering burn wound care expenses. In addition, kiwi pulp appears to act on devitalized tissue rather than on the underlying tissues of the healing wound, and this may help in controlling bacterial growth under the devitalized tissues that are difficult to access with local or injectable antibiotics.

A last topic worth reviewing in this short survey is kiwifruit allergy. Kiwifruit allergy was first described in 1981 and since then there have been reports of the allergy presenting with a wide variety of symptoms, ranging from localized oral allergy syndrome (OAS) to life-threatening anaphylaxis. Also, in recent years kiwifruit allergy has been often associated to allergies to pollen and latex. To date, eleven of the major allergens responsible for kiwi allergy have been isolated and characterized from green kiwifruit (*A. deliciosa*). Act d 1 (actinidin) has been established as a major kiwifruit allergen, and a link between IgE level to Act d 1 and anaphylaxis has been revealed. Act d 2 (a thaumatin-like protein) seems to be a relevant kiwifruit allergen in the Mediterranean region. Act d 3, a 40 kD protein, is a clinically relevant allergen in the Spanish population of allergic subjects. Clinical relevance of other identified kiwifruit allergens such as Act d 4 (phytocystatin), Act d 5 (kiwellin), Act d 6 (a pectin methylesterase inhibitor), Act d 7 (a pectin methylesterase), Act d 8 (a pathogenesis-related protein PR-10) [16], Act d 9 (profilin), Act d 10 (nsLTP1) and Act d 11 (major latex protein) has not been yet established.

In gold kiwifruit (*A. chinensis*) only three allergens (i.e., kiwellin, PR-10 and nsLTP1, marked in this case as Act c 5, Act c 8 and Act c 10) have been found so far.

Symptoms associated to kiwifruit allergy include contact urticaria, wheeze and laryngeal edema after handling the fruit, as well as oral symptoms, including swelling of the lips and tongue accompanied by pruritus and a burning sensation in the mouth after ingestion. Hand and face dermatitis may follow handling of the fruit or the vines. In addition, some subjects have shown more generalized reactions including vomiting, respiratory compromise and cardiovascular collapse.

3. Annona

3.1. Facts and Figures about Annona

Plants of the Annonaceae family are important economic crops in many areas of Africa and Asia as well as in South, North and Central America, and their social and economic values are rapidly increasing. The name derives from the Latin word, 'annona', which means 'annual harvest'. Annonaceae fruits have been fundamental dietary components for the Andean-highland valley populations for thousands of years, and they even influenced their culture and art, to the point that cherimoyas (i.e., *Annona cherimola* Mill.) were often depicted in their ceramics and used as ornamental elements of their buildings.

Figure 7. Annona

To date, botanists have assigned 119 species to the Annonaceae family, five of which (along with a hybrid) are grown for domestic/commercial use. Cherimoya (*A. cherimola*), soursop (*A. muricata*, also known as 'graviola' or 'guanabana') and sugar apple (*A. squamosa*) are the crops with higher consumers' demand and hence commercial relevance, whereas the remaining two, custard apple (*A. reticulate*) and wild soursop (*A. senegalensis*) are until now only of local interest. A further crop, mainly diffused in Taiwan, is the 'atemoya' (*Annona* × *atemoya* or *Annona squamosa* × *Annona cherimola*), a hybrid of the sugar apple and the cherimoya, known also as the 'pineapple sugar apple'. Even though fruit quality of custard apple and wild soursop is generally low and trees are grown by subsistence farmers, limited quantities of fresh fruits are sold in the local markets in developing countries.

Annona plants are not difficult to cultivate, as they are fast-growing and require little care. The life of an *Annona* tree can span fifteen years, depending on the species. Among the *Annonacae* varieties, cherimoya is the only that is well adapted to subtropical climates or tropical highland areas. It is indigenous of Ecuador and Peru, but it grows up flourishing and producing a good fruit yield in the Mediterranean area (Spain, Italy, Egypt, Israel), in southern California, South Africa, Argentina and Chile. The remaining species are mainly adapted to the tropical lowlands, although they may also be found in subtropical areas. Sugar apple, in particular, it is the most drought-tolerant among *Annona* genotypes, and grows well both in moist tropical climates and in drier, subtropical ones. Compared to cherimoya and soursop, this variety is more resistant to large atmospheric temperature ranges.

Annonas plants usually grow up as shrubs or small trees (up to 5–7.5 m high). They have erect or spreading crowns and a grey-brown, rough and corrugated bark. With few exceptions, annonas fruits are deciduous, even the tropical species, especially when cultivated without irrigation in areas with a pronounced dry season. The fruits vary from species to species with differences in shape (round/oval/oblong/heart-shaped), size (from 2–3 to 30 cm) and color (mostly green, though some varieties have pink or red fruits). The fruit is a syncarp with spirally arranged carpels, with each segment of flesh surrounding a single hard, black seed. Fruit size is generally proportional to the number of seeds within. The flowers are pollinated by insects, but fruit production is usually very poor unless farmers resort to hand pollination.

Due to the easy perishability, the fruits of most Annona species are commercialized mainly in local, regional or national trade, and only rarely internationally. The edible fruit component is pulp that is sold fresh or used in semi-processed and processed products. The fruit denied of peel is usually simply eaten or cut in slices or cubes and added to fruit cups or salads or various dessert recipes. It is also blended with orange juice, lime juice and cream and freeze as sherbets or ice cream. Products such as juice, jam and ice cream are widely commercialized in Latin America and occasionally exported. The juice is also used to prepare drinks and fermented liquors. Among the cultivated crops, cherimoya is mostly eaten fresh because of its superior taste and exported to international markets. Soursop fruit is much larger than the other species and is ideal for processing in part as a result of the high recovery of pulp, but also because of its exotic taste and smell. Sugar apple is seldom processed as most fruit is consumed fresh. The fresh fruit of custard apple is considered to be of poor quality and of little commercial importance.

Annona trees are 'multipurpose' plants utilized in all their components. The fruits, as above mentioned, are widely consumed and the leaves, the root and stem are also a source of products for medicinal or industrial use. The fruits are used in traditional medicine to counteract rickets, root, bark and leaves are used to treat the diabetes (infusion) as antidiarrheal drugs and also as an antispasmodic. The seeds are used as insecticide to kill lice and cure parasitic skin disorders. The resin, isolated from the seeds, induces symptoms similar to those provoked by atropine, and used in small amounts it acts as a potent emetic and cathartic.

3.2. Annona Composition

The *Annona* genus seems to be one of the least chemically as well as pharmacologically known genera, when compared with the large number of species belonging to it. Earlier studies on *Annona* species have shown that this family is an excellent source of a wide variety of secondary metabolites belonging to several categories. The fruits of *Annona* genotypes are a source of essential macro- and micronutrients, such as sugars, vitamins, lipids, fibers, proteins, minerals and amino acids. The nutritional composition of cherimoya fruits is described in Table 4. The composition of the fruits is generally influenced by a great variety of external factors, such as climate, cultivar variety, salinity of the soil. Therefore, it is possible to observe remarkable inter-species variations in the detected amounts of analytes. However, cherimoya pulp is characterized, as can be expected for sweet fruits, by a high content of carbohydrates

(18–25 mg/100 g FW) and a low content of acids (mainly citric and malic acid). It contains also vitamin C, thiamine, riboflavin, pantothenic acid, vitamin B-6, vitamin E, folate, vitamin K and niacin in good amounts, whereas its content in vitamin A is quite modest. It is a good source of minerals such as calcium, phosphorous, iron and potassium. This last element is particular abundant in the pulp (> 250 mg/100 g FW) and, according to the policy of the Food Drug Administration (FDA, 1993), it may be regarded as a high contribution source of mineral, providing more about 20–30% of the recommended potassium daily intake for an adult (1000 mg/day). Phosphorous is the second most abundant mineral, followed closely by calcium. Zinc is the only detected and quantified trace minerals and its concentrations are under 0.2 mg/100 g FW.

***Annona cherimola* contents**	**Amount**
Water	71.0–81.0 g
Proteins	1.0–2.1 g
Fats	0.1–0.6 g
Carbohydrates	16.0–25.0 g
Fibers	1.3–4.4 g
Ashes	0.6–1.0 g
Calcium	17.0–30.0 mg
Phosphorous	21.0–47.0 mg
Iron	0.4–0.8 mg
Potassium	250.0–380.0 mg
Sodium	2.0–9.0 mg
Magnesium	14.0–22.0 mg
Zinc	0.1–0.2 mg
Copper	0.1–2.4 mg
Selenium	0.6–0.7 μg
Vitamin A	0.1–6.0 (IU)
Tiamine	0.06–0.12 mg
Riboflavin	0.05–0.14 mg
Niacine	0.5–1.0 mg
Pantothenic acid	0.14–0.27 mg
Vitamin B-6	0.06–0.22 mg
Folate	10.0–14.0 μg
Vitamin K (phylloquinone)	0.4–0.6 μg
Vitamin E (α-tocopherol)	0.08–0.2 mg
Ascorbic acid	19.0–43.0 mg
β-carotene	1.0–5.0 μg
α-carotene	10.0–12.0 μg
Citric acid	0.1–0.6 mg
Malic acid	0.1–0.3 mg
Coline	7.6–10.0 mg

Table 4. Nutritional values and chemical contents per 100 g FW of cherimoya (*A. cherimola*).

The carotenoids components are limited to the identified α-carotene and β-carotene, both present at a microgram level.

The pulp is very rich in water (reaching more than 70% of the total fruit weight), whereas the fat contribution to total composition of the fruit is modest, although the distribution of saturated fatty acids (about 33%), mono-unsaturated fatty acids (about 15%) and polyunsaturated fatty acids (about 32%) has been reported.

Annona pulp is also an excellent source of energy able to supply living organisms with quickly metabolized simple sugars. In fact, sucrose, glucose and fructose are the main saccharides identified and quantified in the edible part of annona, amounting to about 23, 37 and 33% of total sugars, respectively, while the remaining percentage is starch.

Annona fruits also exhibit a fairly high content in amino acids, although not as high as that observed for their seeds. Seventeen amino acids have been identified, including the essential ones arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanin, tyrosine and alanine.

In the cultivated varieties of *Annona,* the distribution of volatile aroma components is quite different from species to species. They are mainly esters (mainly methyl hexanoate, methyl and ethyl butanonate) and terpenoids, but up to one hundred and eighty individual compounds have been identified. The main compounds, according to a series of recent studies, are methyl benzoate, methyl 2-hexenoate, methyl 3-phenyl-2-propanoate, hexadecanoic acid, methyl 2-hydroxy-2-methyl valerate, α-pinene, β-pinene, sabinene, myrcene, limonene, terpinenol, d-terpineol and germacrene D.

The proximate composition of annona seeds shows that they contain 12.20% moisture, 12.10% ash, 24.00% fat, 17.60% crude fiber, 8.50% crude protein and 25.3% carbohydrate. Calcium, potassium, magnesium, zinc, iron, copper, manganese, lead and chromium are the main mineral detected in at a mg/g level. The phytochemical screening indicates the presence of saponins, steroid, flavonoid and glycosides. The seeds contain also *N*-fatty acyl tryptamines including *N*-nonadecanoyltryptamine, *N*-behenoyltryptamine, *N*-lignocereyltryptamine, *N*-cerotoyltryptamine, *N*-octacosanoyl tryptamine, *N*-tricosanoyl-4,5-dihydroxytryptamine, *N*-lignoceroyl-4,5-dihydroxytryptamine, *N*-pentacosanoyl-4,5-dihydroxytryptamine, *N*-heptacosanoyl-4,5-dihydroxytryptamine and alkaloids as atemoine and cleistopholine, besides a broad number of acetogenin. The seeds contain also seventeen essential and non- essential amino acids in varying amount. In particular, they are rich in all the essential amino acids that play fundamental roles in body development and growth. The essential amino acids observed in the sample are comparable with Food and Agricultural Organization/World Health Organization (FAO/WHO) standards (2007). In particular phenylalanine, threonine, valine, isoleucine and leucine are above the reference standard dietary intake of amino acids profile for children and adults (WHO/FAO/UNU, 1985). In general, amino acids are the building block of protein and are essential for a healthy body growth and development. These macronutrients perform multiple tasks: they regulate blood sugar concentration, growth and repairs of muscles (leucine), they are the starting material for the production of neurotransmitters (e.g., norepinephrine) and hemoglobin (isoleucine), and they stimulate adequate absorption of calcium and help in the formation of collagen (lysine).

As far as *Annona* juice is concerned, few studies have been published in the literature. *A. muricata* (soursop) freshly-prepared fruit juice is used in traditional medicine to stimulate appetite, for the treatment of hip stiffness and pain, urine bladder disease and hemorrhoids. However, studies on soursop juice showed that processing significantly affects the physical and chemical composition of the juice, even though it was found to be microbiologically safe for consumption.

3.3. Annona Antioxidant Activity

The genus Annona contains a considerable amount of antioxidant compounds, mainly vitamin C and polyphenolic compounds that help to prevent diseases associated with oxidative stress, such as cancer, heart disease, atherosclerosis, neurodegenerative diseases and in the process of aging.

Cherimoline, cherinonaine, kauranes, lignans, amides, acetogenins, lactam amide, purines, steroids, alkaloids, *p*-quinone, benzenoids, polyamines and flavonoids are the main health promoting compounds present in *Annona* fruits.

Skin, flesh and juice from several genotypes have been analyzed to test their antioxidant potential. The juice showed the highest antioxidant activity in the oxygen radical absorbance capacity (ORAC) assay, whereas the pulp exhibited the lowest. Moreover, Raji (Burkitt's Lymphoma) and HT-29 (colon cancer) cell lines, incubated in the presence of *Annona* skin, flesh and juice showed an increase in the antioxidant uptake. In particular, the highest uptake is obtained incubating both cell lines with Annona juice. Such uptake is further increased when the cells are exposed to AAPH (2,2'-azobis-2-methyl-propanimidamide dihydrochloride, a radical initiator), that simulates the conditions experienced by cells under oxidative stress, indicating that, in this event, cells maximize their antioxidant potential to counteract the onset of oxidative stress.

Analysis of organic extracts (ethanol, methanol and dimethylformammide) obtained from the pulp of ripe fruits detected the presence of significant amounts of total phenols, which determined a very high antioxidant, antiperoxidative and cytoprotective activity on lymphocyte treated with *tert*-butyl hydroperoxide. In particular, these organic extracts gave a strong antioxidant response when tested towards $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and $O_2^{\bullet-}$ radicals, as well as manifesting remarkable reducing activity when subjected to the FRAP assay. They are also able to counteract lipid peroxidation induced by a strong oxidant and cytotoxic compound (*tert*-butyl hydroperoxide) reaching over 70% of inhibition. The protective activities of these extracts are evident also on isolated human peripheral blood lymphocytes exposed to the same compound, enhancing cell survival and decreasing the release of lactate dehydrogenase, a common marker of cytotoxicity. Furthermore, a positive correlation was found between the antioxidant activity and total phenols content of the extracts. Much more evident are the correlation between FRAP-$ABTS^{\bullet+}$, FRAP-$DPPH^{\bullet}$ and $DPPH^{\bullet}$-$ABTS^{\bullet+}$ measured activities, indicating the potential of these organic extracts, albeit with different degrees of efficiency, in scavenging different radicals, including neutral, cationic and anionic ones.

Among the compounds identified in *Annona* fruits, the procyanidins and the flavan-3-ols are among the major responsible of antioxidant activity showed by pulp extracts. Proanthocyanidins are condensed tannins, and they are the second most abundant natural phenolics after lignin. They are oligomers or polymers of flavan-3-ols linked mainly *via* C4→C8 bond (Figure 8), even though in some cases also C4→C6 linkages can be found (so-called B-type linkages). An additional ether bond between C2→C7, resulting in a double connection of the flavan-3-ol units, is usually termed A-type linkage. Proanthocyanidins consist of different flavan-3-ol monomers, also called catechins. The most abundant type of proanthocyanidins in plants, the procyanidins, exclusively consist of (epi)catechin units.

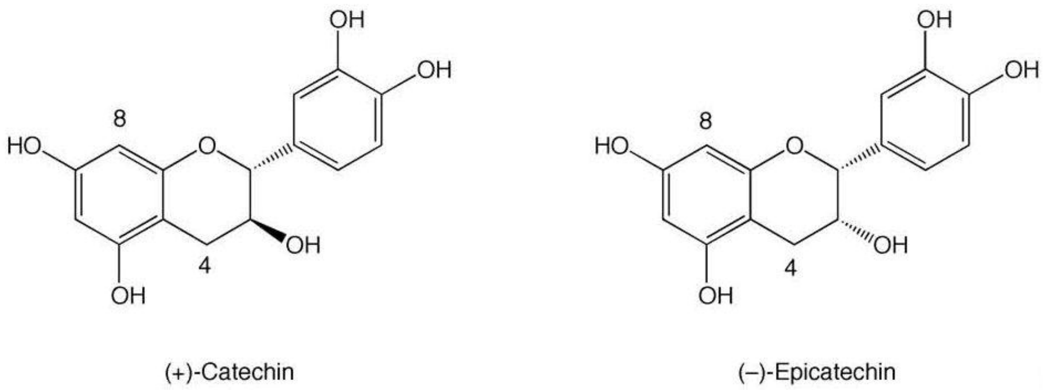


Figure 8. Structures of catechin and epicatechin. Positions 4 and 8 are highlighted.

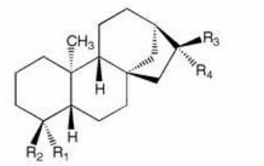
In a recent study, four B-type epicatechin dimers and trimers have been identified in cherimoya pulp extracts (along with a further flavonoid derivative, quercitrin). These compounds possess a large number of hydroxyl groups and are, therefore, among the most powerful antioxidant and radical scavenger compounds in the phenolics family. Furthermore, epicatechin/epicatechin polymers are even better antioxidants than catechin/catechin ones, and the B procyanidins present higher activity than the A procyanidins.

3.4. Annona Pharmacological Properties

The fruit (but also the leaves) of *Annona* species contain aporphine alkaloids, namely, isoquinoline derivatives. They are biosynthesized from the aromatic amino acid tyrosine, which represent the precursor of isoquinoline ring. The isoquinolines are the largest group of plant alkaloids, divided into about twenty classes with different activity. Morphine and its derivatives, that are member of this class, with their influence the central nervous system are the most studied and well documented isoquinoline derivatives. However, other isoquinoline alkaloids have antimicrobial, anthelminthic, cardiovascular, gastrointestinal, oxytocic, spasmolytic, insecticidal, tumor inhibiting, cytotoxic, anti-inflammatory and neuromuscular blocking effects. Three alkaloids (annonaine, nornuciferine and asimilobine) have been isolated from fruits of *Annona*, fruits that have been exploited in traditional medicine for their tranquillizing and sedative properties. The effects of isoquinoline derivatives are unlikely to be produced by action on a single type of receptor, but it is believed that one of such sites of interaction may modify the intracellular level of secondary messengers such as cAMP. In fact, the three compounds found in *Annona*, showed moderate *in vitro* affinity for the 5-HT_{1A} receptor. Annonaine, in particular, is an agonist of the 5-HT_{1A} receptor, but also has inhibitory activity on dopamine re-uptake. However, the interactions with dopaminergic, adrenergic and other 5-HT receptors, in addition to the inhibition of the re-uptake systems, might also account for the tranquillizing and sedative properties of *Annona*.

Annona stems are also rich sources of *ent*-kaurane diterpenoids with a range of interesting biological activities. Diterpenoids are a vast class of natural isoprenoids, biosynthesized from mevalonic acid through 2*E*,6*E*,10*E*-geranylgeranyl pyrophosphate. Kauranes (Figure 9) represent an important group of tetracyclic diterpenes, and the most representative *ent*-kaurane diterpenoids isolated from plants belonging to *Annona* genus are: annomosin A, annosquamosin C, annosquamosin D, annosquamosin E, annosquamosin F, annosquamosin G, annoglabayin, *ent*-kaur-16-en-19-oic (kaurenoic) acid and 16α,17-dihydroxy-*ent*-kauran-19-oic acid. Kaurane diterpenes have many different diverse biological activities including plant growth regulating, antimicrobial, antiparasitic, insect antifeedant, cytotoxic, antitumor, anti-HIV, steroidogenic, antifertility, hipotensive and antiinflamatory activities. Kaurenoic acid, in particular, is relatively abundant in some *Annona* species and it shows a wide spectrum of bioactivities such as antiinflammatory, antibacterial, antifungal and molluscicidal properties. Annoglabayin, a derivative containing a unique carbon bridge between two nor-*ent*-kaurane monomeric units, induces apoptosis in Hep G2 treated cells.

Graviola (*A. muricata*) and cherimoya seeds and leaves are also an important source of acetogenins, which are a large group of compounds with antiparasitic and cytotoxic activity used in pharmaceutical sciences (Figure 10). So far, they have only been found in various species of the family Annonaceae (the family that contains the genus *Annona*). Acetogenins (ACGs), a class of polyketides, are a family of secondary metabolites isolated from the plant family *Annonaceae*, derived from the polyketide pathway. The first acetogenin isolated was uvaricin, and up till this day more than 400 members of the family have been isolated and analyzed for their potential biological effects. They are characterized by long-chain fatty acids (C32 or C34), and the terminal carboxylic acid is combined with a 2-propanol unit at the C-2 position to form a methyl-substituted terminal γ-lactone subunit, either saturated or unsaturated, with one to three tetrahydrofuran (THF) rings (located along the hydrocarbon chain) and a long aliphatic regions bearing a number of hydroxyl, acetoxyl, ketones and epoxides groups and/or double bonds. The annonaceous acetogenin are generally as classified into mono-THF, adjacent bis-THF, nonadjacent bis-THF, non-THF ring, tri-THF (based on the THF number) and non classical acetogenin (THP and ring –hydroxylated THF compounds). This classification is not conclusive and these compounds are sub-classified based on the γ-lactone, substituted γ-lattone or ketolactone variations. Some important acetogenins of cherimoya seeds are annonacin, annonasin, artemoin-A, artemoin-B, artemoin-C, artemoin-D, artemoyin, asimicin, atemotetrolin, bulladecin, bullatacin, cherimolin-1, cherimolin-2, atemoyacin E, cleistopholine, desacetyluvaricin, isodesacetyluvaricin, molvizarin, motrilin, neoannonin, perviflorin, rolliniastatin-1, rolliniastatin-2, rollinicin, almenequin and squamocin.



	R_1	R_2	R_3	R_4
Annosquamosin C	OH	CH_3	CH_2OH	H
Annosquamosin D	OH	CH_3	$OCOCH_3$	CH_2OH
Annosquamosin E	OCHO	CH_3	OH	CH_2OCOCH_3
Annosquamosin F	CH_3	OOH	OH	CH_2OCOCH_3
Annosquamosin G	CH_3	OOH	OH	CH_2OH
16α,17-Dihydroxy-*ent*-kauran-19-oic acid	COOH	CH_3	CH_2OH	OH
ent-Kaur-16-en-19-oic acid	COOH	CH_3	=CH_2	

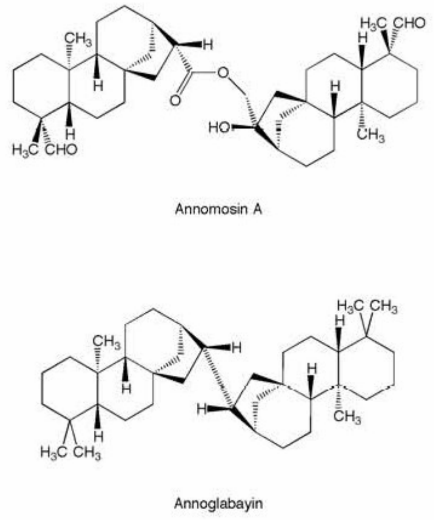


Figure 9. Kauranes found in *Annona* genus.

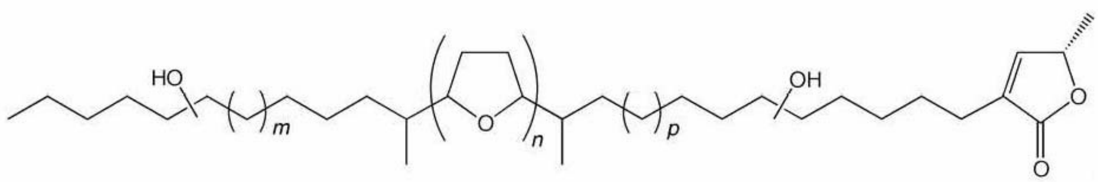


Figure 10. General structure of acetogenins.

Acetogenins are unique, not only for their chemical features, but also for their several interesting biological activity. Several studies showed their efficiency as immunosuppressive, antimalarial, insecticidal, antifeedant and antitumor drugs. For instance, acetogenins are the most powerful inhibitor known to date (effective even in nanomolar concentrations) of mitochondrial NADH-ubichinone oxidoreductase (complex I), a membrane-bound protein, at the level of oxidative phosphorylation. They are also strong inhibitors of NADH oxidase in cells with high metabolic levels, like cancerous ones, leading to apoptosis. The inhibitory effects of ACGs even stronger than those of classical respiratory inhibitors such as rotenone or piericidin A. Moreover, some acetogenins show growth-inhibition activity against multidrug resistant (MDR) cancer cells. These compounds show promising future utilization as cytotoxic agents against cancer cells, as well as pesticidal and antimicrobial compounds.

4. Concluding Remarks

Taking the lead from traditional folk medicine, modern medicinal research is currently shifting its focus from fully synthetic pharmaceuticals to naturally derived ones, as a result of the growing awareness that the plant kingdom is a largely unexplored source of bioactive compounds whose properties may prove fundamental in fighting diseases as yet undefeated. In this context, research on nutraceuticals is moving at a fast pace, driven also by the growing attention consumers are putting in what they eat. Therefore, knowledge of hitherto unknown or underexploited potential sources of health promoting compounds is a strategic target.

Tropical fruits have been for millennia a pillar in the diet of tropical and subtropical population. They are natural treasure troves, full of active compounds yet to be identified, as demonstrated by the increasing number of reports on the composition and on the remarkable nutritional, pharmacological and medicinal properties of plant species that until recently have been ignored by the scientific community. In this chapter, the composition and properties of fruits from two genera, Actinidia and Annona, have been reviewed, with the goal of providing the reader with a basis of knowledge that covers up the main findings on these two genera, which may also be used as starting point for further reading in this field.

Glossary

Acetogenins : Phytochemicals found mainly in *Annona* genotypes, possessing a polyketide structure, and manifesting antitumor activity.

Anthocyanins : Red, purple or blue pigments, belonging to the broader flavonoids class, manifesting strong antioxidant activity.

Antioxidant activity : The ability to intercept (scavenge) free radicals.

Carotenoids : A class of yellow to red polyunsaturated pigment compounds, able to intercept free radical, and participating to a wide range of biological processes.

Complex I : A membrane enzyme that catalyzes the transfer of electrons from NADH to coenzyme Q in the mitochondrial oxidative phosphorylation.

Flavonoids : A large group of phenolics, ubiquitous in the plant kingdom, occurring generally as such or as their glicosilated forms, and possessing a wide range of health-beneficial activities.

Isoquinolines : A group of plant alkaloids biosynthesized from the aromatic amino acid tyrosine.

Kauranes : Tetracyclic diterpenes identified in numerous medicinal plants.

Nutraceuticals : Food product containing functional nutrition ingredients with physiological effects.

Polyamines : Organic polycationic compound having two or more primary amino groups, which bind to DNA and are involved in cellular growth, in modulating, are important modulators of a variety of ion channels and senescence in plants.

Proanthocyanidins : Condensed (oligomeric) tannins, composed of catechin end/or (epi)catechin, belonging to the broader flavonoids class, present in many plant species, and manifesting very high antioxidant activity.

Terpenes : A large and diverse class of isoprenoid phytochemicals possessing medicinal properties and biological activity.

Tocopherols : A group of lypophilic polyunsaturated radical scavengers related to vitamin E.

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Biographical Sketches

Giuseppe Gattuso studied chemistry at the Università di Messina, Italy, where he received his Laurea (M.Sc.) in Chimica in 1993. He then moved to the University of Birmingham, UK, where he obtained a Ph.D. in Chemistry under the supervision of Professor Sir J. F. Stoddart, in 1997. He currently holds the position of Associate Professor of Organic Chemistry at the Università di Messina. His most recent research has been carried out in the field of host-guest and supramolecular chemistry and in the area of natural compounds. In addition, he is conducting research on themes focused on the design and synthesis of macrocyclic compounds (calixarenes, heteracalixarenes, cyclodextrins, and cyclic amides) and their use as receptors/sensors, and as building blocks for the self-assembly of ordered supramolecular aggregates (1D and 2D supramolecular oligo/polymers). He is also carrying out research on the isolation and characterization of natural compounds with bioactive potential from plant sources, such as flavonoids and furocoumarins, and their *in vitro* antioxidant and biological properties. He is the author of numerous publications in high-impact factor international journals in the fields of organic chemistry, supramolecular chemistry, and agrofood chemistry.

Davide Barreca studied Biological Science at the Università di Messina, Italy, where he received his Laurea (M.Sc.) in Biology in 2003. He received his Ph.D. degree in 2006 from the University of Messina, where he afterward obtained a Post-doctoral fellowship with research grants for two years, discussing a thesis on the antioxidant and pro-oxidant activity of some poliphenols from natural sources on cell cultures. Since 2011, he has held the position of Ricercatore (Assistant Professor) in Biochemistry and Clinical Biochemistry, at the Università di Messina. His most recent research has been carried out in the field of isolation, identification and analysis of antioxidant and biological function of flavonoids present in natural matrix, with particular regard to *Citrus* juice and tropical and subtropical fruits, and in process of protein biostabilization and interaction with natural molecules, utilizing several enzymatic, chromatographic, electrophoresis and spectroscopic techniques, such as HPLC, Circular Dichroism, UGGE (Urea Gradient Gel Electrophoresis), Fluorescence, IR and UV-Visible spectroscopy, Small Angle Neutron Scattering (SANS) and HPLC-DAD-MS-MS. He is the author of numerous publications in high-impact factor international journals in the fields of biochemistry, cell biology, chemical-physic, and agrofood biochemistry. He is a member of the Italian Society of Neutron Scattering and American Chemical Society and acts as a reviewer for several prestigious international journals.