Genes, Genomes and Genomics ©2011 Global Science Books

Chemistry of the Chilean Strawberry (*Fragaria chiloensis* spp. *chiloensis*)

Guillermo Schmeda-Hirschmann[1*] • Mario Simirgiotis[2] • José Cheel[3,4]

[1] Laboratorio de Química de Productos Naturales, Instituto de Química de Recursos Naturales, Universidad de Talca, Casilla 747, Talca, Chile
[2] Laboratorio de Productos Naturales, Facultad de Ciencias Básicas, Universidad de Antofagasta, Casilla 170, Antofagasta, Chile
[3] Department of Pharmacognosy, Faculty of Pharmacy, Charles University, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic
[4] Department of Research and Development, CPN Ltd., Dolní Dobrouč 401, 56102 Dolní Dobrouč, Czech Republic

***Corresponding author*: * schmeda@utalca.cl**

ABSTRACT

The Chilean strawberry (*Fragaria chiloensis* spp. *chiloensis*) is one of the progenitors of the cultivated strawberry, *Fragaria* × *ananassa*. Recent studies have started to disclose the chemical composition of the native fruit, the secondary metabolite occurrence and distribution in different plant parts as well as the biological activity of the main fruit constituents. This article revises the chemistry of the Chilean strawberry and compares some of the most characteristic constituents with that of the highly cultivated and worldwide known *F.* × *ananassa*. The main phenolic in the white strawberry are ellagic acid or hexahydroxydiphenolic acid-based hydrolysable tannins and procyanidins as well as flavonoid glycosides from quercetin and kaempferol. The general trend in the plant is the accumulation of condensed tannins of increasing molecular weight in the rhizomes while the leaf contains mainly hydrolysable tannins and flavonoids.

Keywords: HPLC-DAD-MS, leaf, phenolics, rhizome, secondary metabolites

Abbreviations: DAD, diode array detector; **DPPH**, diphenylpicrylhydrazyl radical; **ESI**, electron spray ionization; **HHDP**, hexahydroxydiphenolic acid; **HPLC**, high performance liquid chromatography; **LC**, liquid chromatography; **MS**, mass spectrometry; **MS/MS** and **MS^n**, mass spectrometry-mass spectrometry analysis; **MW**, molecular weight; **UV-vis**, ultraviolet visible

CONTENTS

INTRODUCTION

Gathering of wild fruits and berries for food was a relevant activity for the hunter-gatherer societies that can be traced back to prehistoric times all over the world. In Chile, collection of wild fruits, berries and mushrooms was common in pre-Colombian times and provided essential nutrients during the summer and food reserves for the winter time.

In Southern Chile and the southern Andean region of Argentina, the sweet and aromatic fruits from the native strawberry (**Fig. 1**) known under the Mapuche name Kellén or Llahuén were appreciated as food and medicine (de Mösbach 1992; Ladio *et al.* 2007). The Mapuche aborigines consumed the fresh fruits during the summer time and dried the berries for the winter season. The fruits were also fermented to prepare "chicha", a very popular alcoholic beverage, which is still consumed. All parts of the plant were considered to have medicinal properties. The leaves' infusion was used as a digestive, to stop bleeding and diarrhea as well as to treat eye complaints (Murillo 1889); de Mösbach (1992) refers to the use of the flower calyx in infusion to treat indigestion and diarrhea. The roots were indicated as astringent, to treat dysentery and chronic diarrhea as well as a diuretic. The root infusion was also recommended as eye drops for sore eyes. The fruits were gathered as a seasonal food by the already extinct ethnic groups of the Kawashkar ("Alacalufes") and Selknam ("Onas") in the southernmost part of America. At present, only the fruits are considered of interest and peasants in the coastal region of central Chile have selected the white variety as a crop for several positive agronomic traits. *F. chiloensis* is characterized for having an intense aroma, and a remarkable pest and disease resistance spectrum, along with other characteristics that makes it an attractive species for plant research, such as, manipulation tolerance and long post-harvest life (Potter and Dale 1994; Hancock *et al.* 1999). Indeed, research on this species has been focused on its development as a new exotic berry, as well as for genetic improvement of *F.* × *ananassa* (Hancock *et al.* 2001; Retamales *et al.* 2005). The Chilean native strawberry *Fragaria chiloensis* ssp. *chiloensis* (L.) Mill. (Rosaceae) occurs in two different botanical forms, namely f. *patagonica* and f. *chiloensis*. The *patagonica* form has red-coloured fruits and grows in the wild at latitudes from 35º to 45º, while the *chiloensis* form

Received: 20 August, 2010. Accepted: 15 January, 2011.

Invited Mini-Review

Fig. 1 (**A**) White strawberry culture in Contulmo, Region del Bio-Bio, Chile. (**B**) Fruiting white strawberry. (**C**) *Fragaria chiloensis* ssp. chiloensis f. *chiloensis* (white strawberry) fruit. (**D**) Flowering *Fragaria chiloensis* ssp. chiloensis f. *patagonica* plant growing at the Andean slopes of Chillan. **A-C**: Rudy Montenegro, **D**: Cristina Theoduloz.

has white fruits and is cultivated by peasants in a small coastal area in central Chile, between latitudes 35º and 39º. *F. chiloensis* is one of the progenitors of the cultivated strawberry *Fragaria* × *ananassa* Duch, originated by hybridization of the North American *F. virginiana* Mill. and *F. chiloensis* (L.) Mill. (Retamales *et al.* 2005).

The chemistry of *Fragaria chiloensis* (L.) Duch.

Studies on chemical composition of the Chilean strawberry were first focused on the fruit constituents using antioxidant tests, some comparison by HPLC-UV-vis detection and bioassay-guided isolation of the bioactive compounds searching for differences in the antioxidant activity and phenolic composition with the commercial and worldwide used strawberry *F.* × *ananassa*. The ripe fruits of *Fragaria chiloensis* subsp. *chiloensis* used for the studies were obtained from commercial plantations located in the Province of Arauco, VIII Region, Chile.

Early studies of the fruits

Cheel *et al.* (2005) reported the isolation and identification of *E*-cinnamic acid glycosides, cyanidin-3-*O*-glucoside and the amino acid tryptophan from the fruits of the "white"-coloured *F. chiloensis* ssp. *chiloensis*. The free radical scavenging effect of the extracts and isolated compounds was assessed by the bleaching of the diphenylpicrylhydrazyl radical (DPPH), scavenging of the superoxide anion and lipoperoxidation in human erythrocytes. The compounds were also investigated for cytotoxicity on human lung fibroblasts (MRC-5, ATTC CCL-171). Most of the antioxidant compounds were polar constituents with ellagic acid as the most active product, followed by cyanidin-3-*O*-glucoside. The *E*-cinnamic acid glycosides presented low antioxidant effect and comprised 1-*O*-*E*-cinnamoyl-β-D-rhamnopyranoside, 1-*O*-*E*-cinnamoyl-α-xylofuranosyl-(1→6)-β-D-glucopyranose and 1-*O*-*E*-cinnamoyl-β-D-xylopyranoside (Cheel *et al*. 2005).

HPLC comparison of phenolics in receptacles (thalamus) and achenes. Total content of polyphenols and antioxidant activity

An HPLC comparison of the compounds' distribution in the fruit indicated that the red coloured cyanidin-3-*O*-glucoside and free ellagic acid occurs mainly in the achenes while the *E*-cinnamic acid glycosides and tryptophane in the thalamus (Cheel *et al*. 2005). Further indication on the presence of hydrolysable tannins in the thalamus was provided by the identification of gallic acid from the hydrolyzed extract. The total content of phenolics, anthocyanins and flavonoids of the receptacles (thalamus) and the achenes from both forms of *F. chiloensis* ssp. *chiloensis* (f. *patagonica* and f. *chiloensis*) was compared with a commercial accession of *F* × *ananassa* and *F. vesca* (Cheel *et al.* 2007). Differences in the phenolic contents were positively correlated with the superoxide anion scavenging effect and antioxidant activity measured by the DPPH discoloration assay. The total phenolic, flavonoid and anthocyanin content in fruits of *Fragaria chiloensis* ssp. *chiloensis* form *chiloensis* and the commercial strawberry *F.* × *ananassa* is compared in **Table 1**.

The cultivars of *F.* × *ananassa* and the wild growing *F. chiloensis* ssp. *chiloensis* f. *patagonica* contains 7-15 times more anthocyanins than the f. *chiloensis* fruits. A clear compartmentalization can be observed for the different phenolic antioxidants in the fruits, with most of the total anthocyanins (85%) and total flavonoids (77.2%) found in the achenes of the f. *chiloensis*. For *F.* × *ananassa* and the f. *patagonica* of the Chilean strawberry, most of the anthocyanins (99 and 81.6%) and flavonoids (74.7 and 66.5%), respectively, occurs in the thalamus (Cheel *et al.* 2007).

Table 1 Phenolic, flavonoid and anthocyanin content in fruits of *Fragaria chiloensis* ssp. *chiloensis* form *chiloensis* and the commercial strawberry *F.* × *ananassa*. Data are presented as mg equivalent/100 g of fresh fruit.

	F. chiloensis ssp. *chiloensis* form *chiloensis*	*F.* × *ananassa*
Phenolic	106.3 mg gallic acid equivalents (Cheel *et al.* 2007)	159-289 mg catechin equivalents (Cordenunsi *et al.* 2002) 161-330 mg gallic acid equivalents (Heinonen *et al.* 1998; Proteggente *et al.* 2002; Scalzo *et al.* 2005)
Flavonoid	30.0 mg quercetin equivalents (Cheel *et al.* 2007)	46.2 - 78.0 mg catechin equivalents (Meyers *et al.* 2003) 123.2 mg quercetin equivalents for *F.* × *ananassa* cv. 'Chandler' (Cheel *et al.* 2007)
Anthocyanin	2.3 mg cyanidin-3-glucoside equivalents (Cheel *et al.* 2007)	22.0 - 80.0 mg cyanidin 3-glucoside equivalents (Woodward 1972; Montero *et al.* 1996; Meyers *et al.* 2003; Skupien and Oszmianski 2004; Cheel *et al.* 2007; Lopes da Silva *et al.* 2007) 13.0 - 55.0 mg pelargonidin 3-glucoside equivalents (Cordenunsi *et al.* 2002)

Full HPLC-DAD-ESI-MS^n analysis

High performance liquid chromatography with diode-array detector hyphenated with electrospray ionization mass spectrometry detector has been used and resulted an excellent method for analysis and fast fingerprinting of red strawberry (Seeram *et al.* 2006; Aaby *et al.* 2007; Lopes da Silva *et al.* 2007) and white strawberry polyphenols (Simirgiotis *et al.* 2009, 2010). A comparison of the constituents of fruit extracts from the forms *chiloensis* and *patagonica* of *F. chiloensis* ssp. *chiloensis* was undertaken by HPLC-DAD and HPLC-ESI-MS^n (Simirgiotis *et al.* 2009, 2010). The fruits of both forms of the Chilean strawberry contained flavonoid glycosides, tannins (hydrolizable and condensed) and ellagic acid derivatives (**Table 2**). The flavonoids were identified as glycosides of kaempferol and quercetin. The sugars moieties were identified as pentoses and hexoses but also coumaroyl derivatives of kaempferol were identified (Simirgiotis *et al.* 2009). A methyl quercetin glucuronide and two quercetin glycosides were found only in the *chiloensis* form. Anthocyanins found in both strawberry forms included glycosides of cyanidine and pelargonidin as well as the corresponding malonyl glucosides. Catechin occurred in the *chiloensis* form while ellagic acid, its rhamnoside and a pentoside were present in both forms. The hydrolysable tannins in the fruit included several compounds based on ellagic acid as well as on HHDP (hexahydroxydiphenolic acid, the ellagic acid precursor), the number of compounds detected were higher for the red coloured fruits of the *patagonica* form. Condensed tannins including polymers of flavan 3-ol monomeric units were present mainly in the *patagonica* form. Overall, the main phenolic constituents of the white fruits were ellagic acid and quercetin glucuronide. The phenolics occurring in the fruits of the white and red Chilean strawberries are summarized in **Table 2** (Simirgiotis *et al.* 2009, 2010) Molecular weights (deprotonated molecules) and diagnostic daughter ions obtained by MS-MS can be found in our previous work (Simirgiotis *et al.* 2009, 2010). Relevant information on HPLC analysis of phenolic compounds in berries can be found in Määtä-Riihinen *et al*. (2003) and Määtä-Riihinen *et al*. (2004). The phenolic constituents of the white strawberry leaf and rhizomes are summarized in **Table 3** (Simirgiotis *et al.* 2010).

Antioxidant properties and HPLC-DAD-ESI-MS^n analysis of leaves and rhizomes

The composition of polyphenols in rhizomes and leaves of the wild growing *F. chiloensis* ssp. *chiloensis* was carried out with samples collected in the western Andean slopes at Las Trancas, Termas de Chillán, VIII Región, Chile, in the distribution area of the plant.

The chemistry of the Chilean strawberry leaves was determined by HPLC-DAD-ESI-MS^n and comprised several condensed and hydrolizable tannins. Four condensed tannins, corresponding to procyanidin tetramers were identified in the leaves, one of them also occurring in rhizomes. Procyanidin tetramers were the most frequent procyanidins found in the rhizomes. The hydrolysable tannins occurring in the leaves were ellagic acid or HHDP-based compounds and include isomers of castalagin/vescalagin, casuarictin/potentillin, sanguiin H-6/lambertianin A and agrimoniin isomers. Ellagic acid also occurred in the leaves and this occurrence can also be a consequence of hydrolysis during the work-up of the samples. Two quercetins and a kaempferol coumaroyl hexoside were also found in the leaves. The quercetin (pentoside and glucuronide) derivatives occurring in leaves and fruits were different from the derivatives detected in the rhizomes (pentoside). However, kaempferol coumaroyl hexosides were found in the three plant parts studied, leaves, fruits and rhizomes. The total phenolic content of Chilean strawberry leaves was similar to that of cultivars of *Fragaria* × *ananassa* Duch. (Skupien and Oszmianski 2004).

The rhizomes presented mainly condensed tannins based on cyanidin/procyanidin. Sixteen out of the nineteen compounds identified in rhizomes were procyanidins. Three procyanidin trimers, eight tetramers, a pentamer and four hexamers were tentatively identified by HPLC MS-MS (Simirgiotis *et al.* 2010). A quercetin pentoside and a kaempferol coumaroyl hexoside are the flavonoid derivatives identified from the rhizomes, the latter also occurring in leaves and fruits. The general trend is the accumulation of condensed tannins of increasing molecular weight in the rhizomes (**Table 3**). Molecular weights (deprotonated molecules) and MS^n ions can be found in our previous work (Simirgiotis *et al.* 2010). Several tannins have been identified in red strawberry fruits (Seeram *et al.* 2006; Aaby *et al.* 2007) and red strawberry leaves (Oertel *et al.* 2001; Hukkanen *et al.* 2007) including agrimoniin, sanguiin H-6, and small ellagic acid derivatives.

Tannin-containing plants have been traditionally used to cure diarrhea, relieve gastric complains and as astringent. Ferreira *et al*. (2005), Haslam (2007), Yoshida *et al*. (2000) and Yoshida *et al*. (2005) revised relevant chemical and biological aspects of this group of natural products. The chemical compounds found in the leaves and rhizomes of the Chilean strawberry can, at least in part, explain the traditional use of the herb infusion to treat indigestion, diarrhea and dysentery. As further studies with the infusions and proper biological assays should be undertaken to clearly confirm the traditional indications of use, the association with the tannin constituents should be considered a working hypothesis.

The antioxidant properties of several cultivars of red strawberries are well known (Asami *et al.* 2003; Skupien and Oszmianski 2004). A comparison of the antioxidant activity between the red strawberry cultivar Chandler and the white strawberry growing in the same location in Chile was reported (Simirgiotis *et al.* 2009). In different strawberry cultivars, Tulipani *et al.* (2008) reported that the main antioxidant compounds were derivatives of ellagic acid. Flavonoids based on quercetin and kaempferol were also detected, but they showed a moderate antioxidant effect. Recently, newer approaches for the treatment of obesity have involved inhibition of dietary triglyceride absorption via inhibition of pancreatic lipase as this is the major source of excess calories (Birari and Bhutani 2007). The activity of extracts from different berries as inhibitors of pancreatic lipase in vitro was recently published (McDougall *et al.* 2009). The tannin-rich strawberry extracts tested were rich in polyphenols composed of ellagitannis and proanthocy-

Table 2 Phenolic compounds identified in *Fragaria chiloensis* ssp. *chiloensis* (f. *chiloensis* and f. *patagonica*) fruits by HPLC-DAD, LC-MS and LC-MS/MS data. x: Presence

Tentative identification	f. *chiloensis*	f. *patagonica*
Ellagitannin isomer 1 (MW = 784)	x	x
Ellagitannin isomer 2 (MW = 784)	x	x
Procyanidin tetramer isomer 1 (MW = 1154)		x
Procyanidin tetramer isomer 2 (MW = 1154)		x
Cyanidin-3-*O* glucoside* (MW = 449)	x	x
Ellagitannin isomer 3 (MW = 934)		x
Ellagitannin isomer 4 (MW = 934)		x
Pelargonidin-3-*O* glucoside* (MW = 433)	x	x
Catechin* (MW = 290)	x	
Ellagitannin isomer 5 (MW = 934)		x
Ellagitannin isomer 6 (MW = 934)		x
Cyanidin-malonyl-glucoside (MW = 534)	x	x
Pelargonidin-malonyl- glucoside (MW = 518)	x	x
Ellagitannin isomer 7 (MW = 936)		x
Ellagitannin isomer 8 (MW = 936)		x
Ellagitannin isomer 9 (MW = 936)		x
Ellagitannin isomer 10 (MW = 936)	x	
Ellagic acid pentoside (MW = 434)	x	x
Ellagic acid rhamnoside (MW = 448)	x	x
Ellagic acid* (MW = 302)	x	x
Quercetin hexoside (MW = 464)	x	
Quercetin glucuronide* (MW = 478)	x	x
Quercetin pentoside (MW = 436)	x	x
Ellagitannin isomer 11 (MW = 934)	x	x
Quercetin pentoside (MW = 434)	x	
Kaempferol glucuronide (MW = 462)	x	x
Methyl-quercetin-glucuronide (MW = 492)	x	
Kaempferol coumaroyl-hexoside (MW = 594)	x	x
Kaempferol coumaroyl-hexoside (MW = 594)	x	x

*Identified by spectroscopic means and comparison with standard compounds.

Table 3 Compounds identified in *Fragaria chiloensis* ssp. *chiloensis* leaves and rhizomes by HPLC-DAD, LC-MS and LC-MS/MS data. x: Presence

Tentative compound identification	Leaves	Rhizomes
Tetragalloylglucose (MW = 964)	x	
Procyanidin trimer isomer 1 (MW = 866)		x
Procyanidin trimer isomer 2 (MW = 866)		x
Bis-HHDP-glucose isomer 1 (MW = 784)	x	
Procyanidin tetramer isomer 1 (MW = 1154)		x
Procyanidin tetramer isomer 2 (MW = 1156)	x	
Procyanidin tetramer isomer 3 (MW = 1154)		x
Bis-HHDP-glucose isomer 2 (MW = 784)	x	
Procyanidin tetramer isomer 4 (MW = 1154)		x
Procyanidin tetramer isomer 5 (MW = 1154)		x
Procyanidin trimer isomer 3 (MW = 866)		x
Procyanidin tetramer isomer 6 (MW = 1154)	x	
Castalagin or vescalagin (MW = 934)	x	
Procyanidin tetramer isomer 7 (MW = 1154)		x
Procyanidin tetramer isomer 8 (MW = 1154)	x	x
Vescalagin or Castalagin (MW = 934)	x	
Procyanidin tetramer isomer 9 (MW =1154)		x
Procyanidin tetramer isomer 10 (MW = 1154)	x	
Casuarictin or potentillin (MW = 936)	x	
Procyanidin tetramer isomer 11 (MW = 1154)		x
Sanguiin H-6 or lambertianin A (MW = 1870)	x	
Procyanidin pentamer isomer 1 (MW = 1442)		x
Agriimoniin isomer 1 (MW = 1870)	x	
Procyanidin hexamer isomer 1 (MW = 1730)		x
Procyanidin pentamer isomer 2 (MW = 1442)		x
Agriimoniin isomer 2 (MW = 1870)	x	
Procyanidin hexamer isomer 2 (MW = 1730)		x
Agriimoniin isomer 3 (MW = 1870)	x	
Procyanidin hexamer isomer 3 (MW = 1730)		x
Quercetin pentoside (MW = 434)		x
Quercetin glucuronide (MW = 462)	x	
Quercetin pentoside (MW = 434)	x	
Kaempferol coumaroyl-hexoside (MW = 594)	x	x

anidins. Polyphenols contained in the diet plays a preventive role against infections and improves oral health (Petti and Scully 2009). However, studies on humans are needed to confirm the studies published so far, including the positive impacts against periodontal diseases (Petti and Scully 2009). In a revision on the biological implications of ellagitannins, Clifford and Scalbert (2000) critically examined the evidence on absorption, bioavailability and effect of this group of compounds in animals. As the hydrolysis product of ellagitannins is usually ellagic acid, several effects ascribed to those hydrolizable tannins may have some relationship with this particular compound or its metabolites.

The main sources of ellagitannins in our diet are berries, including strawberries. However, it is noteworthy that the polyphenols that are the most common in the human diet are not necessarily the most active within the body, either because they have a lower intrinsic activity or because they are poorly absorbed from the intestine, highly metabolized, or rapidly eliminated (Manach *et al*. 2004). Extensive knowledge of the bioavailability of polyphenols and the effects of the food matrix of Chilean strawberry is thus essential if their health effects are to be understood. Clifford and Scalbert (2000) reported the occurrence of casuarictin in strawberry fruits while other ellagitannins have been found in the leaves, as sanguiin H-6, casuarictin, potentillin and pedunculagin. The information available does not allow a clear conclusion about the possible benefits, risks or harm for human beings due to ellagitannins contained in food. The beneficial effect of dietary flavonoids, however, is better known. The antioxidant and chemopreventive effects of anthocyanins is well documented and was recently revised by Wang and Stoner (2008).

The potential benefit of strawberry extracts as ingredients in functional foods and cosmetics was considered to develop a formulation of a synergistic blend (OptiBerry) containing wild blueberry, bilberry, cranberry, elderberry, strawberry, and raspberry seed extracts (Lau *et al.* 2009). The safety and efficacy properties of this formulation were demonstrated using standard toxicology and whole-body antioxidant status procedures (Yasmin *et al*. 2003; Bagchi *et al*. 2006). The benefit of Chilean strawberry on skin health remains to be explored.

Oxidation of exogenous flavonoids by laccases secreted by the roots can play a role in allelophatic plant interactions (Pourcel *et al.* 2007). The autooxidation of quercetin can led to the antifungal compound 3,4-dihydroxybenzoic acid (Pourcel *et al.* 2007).

In the Rosaceae family, several ellagitannins are usually based on a primary unit of bis-HHDP glucopyranose and gallotannins on galloyl-bis-HHDP glucopyranose (Okuda *et al*. 1992; Okuda 2005; Hager *et al.* 2008). As in the Chilean strawberry, Hager *et al.* (2008) found in blackberries a series of tannins including castalagin/vescalagin, lambertianin A/sanguiin H-6 but also pedunculagin, casuarictin/potentillin, and most of the compounds were found in the seed tissues. In the roots of medicinal Rosaceae plants, Oszmianski *et al.* (2007) found large amounts of procyanidins based either on (+) catechin or (-) epicatechin after thioacidolysis of polymers of proanthocyanidins and ellagic acid after acid hydrolysis of the plant material. A correlation of the oxidative changes in hydrolysable tannins and the plant evolution was published by Okuda *et al.* (2000) with special emphasis in the subclass Rosidae.

One of the most important postharvest problems in commercially cultivated strawberry is gray mold, caused by the fungus *Botrytis cinerea* Pers (González *et al*. 2009). The antifungal activity against *B. cinerea* declines with advancing strawberry fruit maturity (Terry *et al*. 2004). Schaefer *et al*. (2008) reported that grape varieties infected with *B. cinerea* decreased fungal damage with increasing anthocyanin content. Moreover, anthocyanin-enriched extracts directly inhibited growth rates of nine fruit-rot fungi on agar plates. Anthocyanins reduced fungal growth by 50% in the concentrations that typically characterise unripe blackberries and by 95% in the concentrations that typify ripe blackberries. Current control of *Botrytis cinerea* by pre-harvest fungicide application present some practical disadvantages due to fungicide resistance and difficulties to target the infection at the point of harvest (Wurms *et al*. 1999). An alternative approach is to improve host resistance, but requires further research to realise its potential.

Studies on the enzymes involved in tannin biosynthesis have shown the different steps in the formation of complex molecules including gallotannins and ellagitannins (Grundhöfer *et al.* 2001; Niemetz and Gross 2005; Gross 2008). The proposed starting compound for the biosynthesis, β–glucogallin (1-*O*-galloyl-β-D-glucopyranose) led after specific galloylation steps to the different mono- to penta-galloylglucoses. Enzyme studies with cell-free leaf extracts from different tannin-rich plants and immunohistochemical studies with antibodies allowed a much more complete picture of the hydrolysable tannin biosynthesis. According to Grundhöfer *et al.* (2001), hydrolysable tannins are formed in the walls of leaf mesophyll cells and the tannins are deposited in these cells. *In vitro* acylation of glucose to yield pentagalloylglucose is carried out by four galloyltransferases to derivatives with higher galloyl substitution. For ellagitannins, Niemetz and Gross (2005) proved that a partially purified enzyme fraction was able to catalyze the oxidation of 1,2,3,4,6-pentagalloylglucose to tellimagrandin II and the possible formation *in vitro* of casuarictin or peduncularin. The hydrolysable tannins identified so far in the white strawberry and the recent advances in enzymology of both groups of hydrolysable tannins (ellagitannin and gallotannin) opens new perspectives in molecular boilogy studies looking for the genes regulating biosynthesis of this group of compounds in strawberry. This approach was applied to understand the flavonoid biosynthesis in *F. chiloensis* (Saud *et al.* 2009) and correlate the chemical phenotype with the plant molecular background.

CONCLUSION

The information presented in this article can provide to chemists, plant breeders and molecular biologists information on the secondary metabolism, antioxidant activity, chemical diversity and distribution of different phenolic compounds in the native white strawberry, opening possibilities for more accurate studies on the biosynthesis of selected compounds with desirable biological properties. Knowing the type and quantity of beneficial compounds in this local strawberry can be also a starting point for food and plant biotechnologists, to produce a higher-antioxidant or high-yield polyphenolic strawberry, managing phenol contents by plant breeding and selection, to cope the local and partially global demand for optimized levels of beneficial phenolic compounds and nutraceutics in crop plants. However, much more research work should be performed, including fruits in different stages of development (immature, turning, ripe fruits), influence of geographical location, environmental conditions and different agronomic practices to obtain a clear picture of the secondary metabolites variation and content within the white strawberry. The difference in fruit colour between the two botanical forms of *Fragaria chiloensis* was recently shown to be coincident with different transcriptional patterns in the anthocyanin-related genes (Salvatierra *et al.* 2010).

ACKNOWLEDGEMENTS

We are grateful to the Programa de Investigación en Productos Bioactivos, Universidad de Talca, for financial support. Mario Simirgiotis is a research member of CONICET. José Cheel thanks the project MSM 0021620822.

REFERENCES

Aaby K, Ekeberg D, Skrede G (2007) Characterization of phenolic compounds in strawberry (*Fragaria × ananassa*) fruits by different HPLC detectors and contribution of individual compounds to total antioxidant capacity. *Journal of Agricultural and Food Chemistry* **55**, 4395-4406

Asami DK, Hong Y J, Barrett DM, Mitchell AE (2003) Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. *Journal of Agricultural and Food Chemistry* **51 (5)**, 1237-1241

Bagchi D, Roy S, Patel V, He G, Khanna S, Ojha N, Phillips C, Ghosh S, Bagchi M, Sen CK (2006) Safety and whole-body antioxidant potential of a novel anthocyanin-rich formulation of edible berries. *Molecular and Cellular Biochemistry* **281**, 197-209

Birari RB, Bhutani KK (2007) Pancreatic lipase inhibitors from natural sources: Unexplored potential. *Drug Discovery Today* **12**, 879-889

Cheel J, Theoduloz C, Rodríguez J, Saud G, Caligari PDS, Schmeda-Hirschmann G (2005) *E*-cinnamic acid derivatives and phenolics from Chilean strawberry fruits, *Fragaria chiloensis* ssp. *chiloensis*. *Journal of Agricultural and Food Chemistry* **53**, 8512-8518

Cheel J, Theoduloz C, Rodríguez J, Caligari P, Schmeda-Hirschmann G (2007) Free radical scavenging activity and phenolic content in achenes and thalamus from *Fragaria chiloensis* ssp. *chiloensis*, *F. vesca* and *F.* × *ananassa* cv. Chandler. *Food Chemistry* **102**, 36-44

Cordenunsi B, Oliveira do Nascimento JR, Genovese MI, Lajolo FM (2002) Influence of cultivar on quality parameters and chemical composition of strawberry fruits grown in Brazil. *Journal of Agricultural and Food Chemistry* **50**, 2581-2586

Clifford MN, Scalbert A (2000) Ellagitannins - nature, occurrence and dietary burden. *Journal of the Science of Food and Agriculture* **80**, 1118-1125

de Mösbach EW (1992) *Botánica Indígena de Chile* (2nd Edn), Alfabeta, Santiago de Chile, 140 pp

Ferreira D, Gross GG, Kolodziej H, Yoshida T (2005) Tannins and related polyphenols: Fascinating natural products with diverse implications for biological systems, ecology, industrial applications and health protection. *Phytochemistry* **66 (18)**, 2124-2126

González G, Moya M, Sandoval C, Herrera R (2009) Genetic diversity in Chilean strawberry (*Fragaria chiloensis*): Differential response to *Botrytis cinerea* infection. *Spanish Journal of Agricultural Research* **7**, 886-895

Gross GG (2008) From lignins to tannins: Forty years of enzyme studies on the biosynthesis of phenolic compounds. *Phytochemistry* **69**, 3018-3031

Grundhöfer P, Niemetz R, Schilling G, Gross GG (2001) Biosynthesis and subcellular distribution of hydrolyzable tannins. *Phytochemistry* **57**, 915-927

Hager TJ, Howard LR, Liyanage R, Lay JO, Prior RL (2008) Ellagitannin composition of blackberry as determined by HPLC-ESI-MS and MALDI-TOF-MS. *Journal of Agricultural and Food Chemistry* **56**, 661-669

Hancock JF, Lavin A, Retamales JB (1999) Our southern strawberry heritage: *Fragaria chiloensis* of Chile. *HortScience* **34**, 814-816

Hancock JF, Callow PW, Dale A, Luby JJ, Finn CE, Hokanson SC, Hummer KE (2001) From the Andes to the Rockies: Native strawberry collection and utilization. *HortScience* **36**, 221-225

Haslam E (2007) Vegetable tannins-lessons of a phytochemical lifetime. *Phytochemistry* **68 (22)**, 2713-2721

Heinonen MI, Meyer AS, Frankel EN (1998) Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. *Journal of Agricultural and Food Chemistry* **46**, 4107-4112

Hukkanen AT, Kokko HI, Buchala AJ, McDougall, GJ, Stewart D, Karenlampi SO, Karjalainen RO (2007) Benzothiadiazole induces the accumulation of phenolics and improves resistance to powdery mildew in strawberries. *Journal of Agricultural and Food Chemistry* **55**, 1862-1870

Ladio A, Lozada M, Weigandt M (2007) Comparison of traditional wild plant knowledge between aboriginal communities inhabiting arid and forest environments in Patagonia, Argentina. *Journal of Arid Environments* **69**, 695-715

Lau FC, Bagchi M, Zafra-Stone S, Bagchp D (2009) The benefits of antioxidant-rich fruits on skin health. In: Tabor A, Blair RM (Eds) *Nutritional Cosmetic, Beauty from Within*, Elsevier Inc., pp 217-232

Lopes da Silva F, Escribano-Bailón MT, Pérez Alonso JJ, Rivas-Gonzalo JC, Santos-Buelga C (2007) Anthocyanin pigments in strawberry. *LWT Food Science and Technology* **40**, 374-382

Määtä-Riihinen KR, Kamal-Elin A, Törrönen AR (2003) High-performance liquid chromatography (HPLC) analysis of phenolic compounds in berries with diode array and electrospray ionization mass spectrometric (MS) detection: *Ribes* species. *Journal of Agricultural and Food Chemistry* **51**, 6736-6744

Määtä-Riihinen KR, Kamal-Elin A, Törrönen AR (2004) Identification and quantification of phenolic compounds in berries of *Fragaria* and *Rubus* species (Family Rosaceae). *Journal of Agricultural and Food Chemistry* **52**, 6178-6187

Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L (2004) Polyphenols: Food sources and bioavailability. *American Journal of Clinical Nutrition* **79**, 727-747

McDougall GJ, Kulkarni NN, Stewart D (2009) Berry polyphenols inhibit pancreatic lipase activity *in vitro*. *Food Chemistry* **115 (1)**, 193-199

Meyers KJ, Watkins CB, Pritts MP, Liu RH (2003) Antioxidant and antiproliferative activities of strawberries. *Journal of Agricultural and Food Chemistry* **51**, 6887-6892

Montero TM, Mollá EM, Esteban RM, López-Andréu FJ (1996) Quality attributes of strawberry during ripening. *Scientia Horticulturae* **65**, 239-250

Murillo A (1889) *Plantes Médicinales du Chili. Exposition Universelle de Paris, Section Chilienne*, Biblioteca Nacional, Paris, 234 pp

Niemetz R, Gross GG (2005) Enzymology of gallotannin and ellagitannin biosynthesis. *Phytochemistry* **66 (17)**, 2001-2011

Oertel B, Keutgen N, Lenz F (2001) Responses of strawberry to long-term elevated atmospheric ozone concentrations II. Changes of soluble phenol contents in leaves. *Gartenbauwissenschaft* **66**, 164-171

Okuda T, Yoshida, T, Hatano T, Iwasaki M, Kubo M, Orime T, Yoshizaki M, Naruhashi N (1992) Hydrolysable tannins as chemotaxonomic markers in the *Rosaceae*. *Phytochemistry* **31 (9)**, 3091-3096

Okuda T (2005) Systematics and health effects of chemically distinct tannins in medicinal plants. *Phytochemistry* **66**, 2012-2031

Okuda T, Yoshida T, Hatano T (2000) Correlation of oxidative transformations of hydrolyzable tannins and plant evolution. *Phytochemistry* **55**, 513-529

Oszmianski J, Wojdylo A, Lamer-Zarawska E, Swiader K (2007) Antioxidant tannins from Rosaceae plant roots. *Food Chemistry* **100 (2)**, 579-583

Petti S, Scully C (2009) Polyphenols, oral health and disease: A review. *Journal of Dentistry* **37 (6)**, 413-423

Potter J, Dale A (1994) Wild and cultivated strawberry with tolerance or resistance to root-lesion nematode. *HortScience* **29**, 1074-1077

Pourcel L, Routaboul JM, Cheynier V, Lepiniec L, Debeaujon I (2007) Flavonoid oxidation in plants: From biochemical properties to physiological functions. *Trends in Plant Science* **12 (1)**, 29-36

Proteggente AR, Sekher Pannalaa A, Paganga GA, Van Buren L, Wagner E, Wiseman S (2002) The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Radical Research* **36**, 217-233

Retamales JB, Caligari PDS, Carrasco B, Saud G (2005) Current status of the Chilean native strawberry and the research needs to convert the species into a commercial crop. *HortScience* **40**, 1633-1634

Salvatierra A, Pimentel P, Moya-Leon MA, Caligari PDS, Herrera R (2010) Comparison of transcriptional profiles of flavonoid genes and anthocyanin contents during fruit development of two botanical forms of *Fragaria chiloensis* ssp. *chiloensis*. *Phytochemistry* **71**, 1839-1847

Saud G, Carbone F, Perrotta G, Figueroa CR, Moya M, Herrera R, Retamales JB, Carrasco B, Cheel J, Schmeda-Hirschmann G, Caligari PDS (2009) Transcript profiling suggests transcriptional repression of the flavonoid pathway in the white-fruited Chilean strawberry, *Fragaria chiloensis* (L.) Mill. *Genetic Resources and Crop Evolution* **56**, 895-903

Scalzo J, Politi A, Pellegrini N, Mezzetti B, Battino M (2005) Plant genotype affects total antioxidant capacity and phenolic contents in fruit. *Nutrition* **21**, 207-213

Schaefer HM, Rentzsch M, Breuer M (2008) Anthocyanins reduce fungal growth in fruits. *Natural Product Communications* **3**, 1267-1272

Seeram NP, Lee R, Scheuller HS, Heber D (2006) Identification of phenolic compounds in strawberries by liquid chromatography electrospray ionization mass spectroscopy. *Food Chemistry* **97**, 1-11

Simirgiotis MJ, Theoduloz C, Caligari PDS, Schmeda-Hirschmann G (2009) Comparison of phenolic composition and antioxidant properties of two native Chilean and one domestic strawberry genotypes. *Food Chemistry* **113**, 377-385

Simirgiotis MJ, Schmeda-Hirschmann G (2010) Determination of phenolic composition and antioxidant activity in fruits, rhizomes and leaves of the white strawberry (*Fragaria chiloensis* spp. chiloensis form chiloensis) using HPLC-DAD-electrospray tandem mass spectrometry and free radical quenching techniques. *Journal of Food Composition and Analysis* **23**, 545-553

Skupien K, Oszmianski J (2004) Comparison of six cultivars of strawberries (*Fragaria* × *ananassa* Duch.) grown in northwest Poland. *European Food Research and Technology* **219**, 66-70

Terry LA, Joyce DC, Adikaram NKB, Khambay BPS (2004) Preformed antifungal compounds in strawberry fruit and flower tissues. *Postharvest Biology and Technology* **31**, 201-212

Tulipani S, Mezzetti B, Capocasa F, Bompadre S, Beekwilder J, Ric de Vos CH, Capanoglu E, Bovy A, Battino M (2008) Antioxidants, phenolic compounds, and nutritional quality of different strawberry genotypes. *Journal of Agricultural and Food Chemistry* **56**, 696-704

Wang LS, Stoner GD (2008) Anthocyanins and their role in cancer prevention. *Cancer Letters* **269 (2)**, 281-290

Woodward JR (1972) Physical and chemical changes in developing strawberry fruits. *Journal of the Science of Food and Agriculture* **23**, 465-473

Wurms KV, Long PG, Sharrock KR, Greenwood DR (1999) The potential for resistance to *Botrytis cinerea* by kiwifruit. *Crop Protection* **18**, 427-435

Yasmin T, Sen CK, Hazra S, Bagchi M, Bagchi D, Stohs SJ (2003) Antioxidant capacity and safety of various anthocyanin berry extract formulations. *Research Communications in Pharmacology and Toxicology* **2**, 25-33

Yoshida T, Hatano T, Ito H (2005) High molecular weight plant polyphenols (tannins): Prospective functions. *Recent Advances in Phytochemistry* **39**, 163-190

Yoshida T, Hatano T, Ito H, Okuda T (2000) Chemical and biological perspectives of ellagitannin oligomers from medicinal plants. *Studies in Natural Products Chemistry* **23**, 395-453