CHLOROPHYLL BIODEGRADATION IN *VITIS VINIFERA* VAR. *PINOT NOIR* AUTUMANAL LEAVES

БИОДЕГРАДАЦИЈА ХЛОРОФИЛА КОД *VITIS VINIFERA* VAR. *PINOT NOIR* ЈЕСЕЊЕГ ЛИШЋА

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***Abstract**: Efforts to evaluate the biodegradation of chlorophyll in autumnal leaves continues. The chlorophyll biodegradation pattern was determined in Hamamelidaceae family. The chlorophyll biodegradation in Vitis vinifera var. Pinot noir autumnal leaves is the same as in the Hamamelidaceae species. The study was performed by LC/MS analysis. The chromatogram obtained for the Vitis vinifera var. Pinot noir autumnal leaf extract was compared with the chromatograms obtained for the Hamamelidaceae species, where the chlorophyll biodegradation pattern was determined. Using LC/MS analysis the chlorophyll biodegradation pattern was validated in Vitis vinifera var. Pinot noir autumnal leaves. The LC/MS analysis of autumnal leaf extracts will allow the identification of chlorophyll biodegradation products among other Vitaceae species.*

***Извод**: Делање на разјашњавању биодеградације хлорофила код јесењег лишћа се наставља. Начин биодеградације хлорофила је разјашњен код фамилије Hamamelidaceae. Биодеградација хлорофила код Vitis vinifera var. Pinot noir јесењег лишћа је иста као и код Hamamelidaceae јесењег лишћа. Истраживање је урађено помоћу ТХ/МС. Добијени хроматограми упоређени су са хроматограмима добијеним код Hamamelidaceae врста, где је начин биодеградације хлорофила познат. Употребљавајући ТХ/МС анализу начин биодеградације хлорофила је одређен код Vitis vinifera var. Pinot noir јесењег лишћа. ТХ/МС анализа екстракта јесењег лишћа ће омогућити идентификацију производа биодеградације хлорофила и код осталих Vitaceae врста.*

***Key words**: chlorophyll biodegradation, Vitis vinifera var. Pinot noir, UNCC*
***Кључне речи**: биодеградација хлорофила, Vitis vinifera var. Pinot noir*

INTRODUCTION

Chlorophyll biodegradation products were isolated from *Parrotia persica* (DJAPIC, PAVLOVIC, 2008), Hamamelidaceae and *Hamamelis virginiana* (DJAPIC ET AL., 2009), Hamamelidaceae, *Liquidambar orientalis* (ITURRASPE ET AL., 1995), Altingiaceae and *Liquidambar styraciflua* (ITURRASPE ET AL., 1995), Altingiaceae autumnal leaf extract. The genera Liquidambar was in the Hamamelidaceae family, although recently the genus Liquidambar was transferred to the Altingiaceae family. Several Hamamelidaceae autumnal plant leaves were screened for the presence of chlorophyll biodegradation products. In the following Hamamelidaceae autumnal plant leaves the chlorophyll biodegradation products were identified and systematized: *Forthergilla gardenii*, *Forthergilla major*, *Hamamelis*

mollis, Hamamelis japonica, Hamamelis japonica var. *flavopurpurscens, Corylopsys pauciflora, Corylopsys spicata, Corylopsys willmotiae, Parrotiopsis jacquemoutiana,* etc (DJAPIC, 2007).

The autumnal leaves of the Vitaceae family have the same chlorophyll biodegradation pathway as the autumnal leaves of the Hamamelidaceae family. The autumnal leaves of: *Vitis vinifera* var. Pinot noir, *Vitis riparia, Vitis vinifera ssp. vinifera, Vitis labrusca* and *Parthenocissus tricuspidata,* were screened for the presence of chlorophyll biodegradation products and the chromatograms obtained revealed the same biodegradation pathway as for the autumnal leaves of the Hamamelidaceae family. This work describes the chlorophyll biodegradation products found in *Vitis vinifera* var. Pinot noir autumnal leaves.

MATERIAL AND METHODS

Plant material

Vitis vinifera var. Pinot noir, Vitaceae, autumnal leaves were collected in autumn (2005) from the Botanical Garden of Fribourg, Switzerland. *Vitis vinifera* var. Pinot noir leaves were treated by the gardeners of the Botanical garden of Fribourg with the fungicide on the base of sulphur and sulphurous argil (41%) in the acidic media along with aluminium ions on: June, 17th, 2005 (0.8%); July, 4th, 2005 (0.1%); July, 14th, 2005 (0.1%); August, 4th, 2005(0.1%) and August, 18th, 2005(0.1%). The fungicide uptake by the leaf surface did not influence the chlorophyll biodegradation in *Vitis vinifera* var. Pinot noir green leaves. Those leaves exhibited in autumn the same biodegradation pathway as *Parthenocissus tricuspidata*, Vitaceae whose leaves were untreated with the fungicide.

Extraction

Vitis vinifera var. Pinot noir, Vitaceae leaves (20.00 g "fresh" weight) were chilled by liquid nitrogen, grinded and homogenized in a blender with 0.25 dm^3 methanol, at room temperature, for 10 minutes. The methanol extract was filtered and partitioned between hexane and methanol. Water was added to the methanol phase. Chlorophyll biodegradation products were extracted with dichloromethane from the methanol – aqueous phase. Evaporation of dichloromethane ($t<40^0$C) yielded 18 mg of moderately polar compounds. Moderately polar compounds were subjected to LC/MS. On-line LC/MS analysis were performed on Waters 2695 Separations Module (Milford, MA, USA) coupled to a Waters 2996 PDA UV-Vis detector and connected to Bruker Daltonics esquire HCT (Bruker Daltonik, GmbH, Bremen, Germany) equipped with an electro spray ionization (ESI) source. LC/MS interface operated in the positive electro spray ionization mode. The analytic LC separation was carried on the reverse phase (RP) EC 250x4 mm Nucleosil 100-5 C_4 column together with RP CC 8x4 mm Nucleosil 100-5 C_4 precolumn (Macherey-Nagel, Oesingen, Switzerland). The temperature of the column oven was 22^0 C and the injection volume was 10µl via autosampler injection. Mobile phase consisted of water (0.1 % TFA) and methanol. The proportion of methanol was increased linearly from 10% to 100% in 70 minutes with a flow rate of 0.2 ml/min. LC/MS chromatographic method is depicted in (Table 1).

After each separation the column has been calibrated linearly from 100 % methanol to 90% water (0.1% TFA):10% methanol in 10 minutes and additional 5 minutes at 90% water (0.1% TFA):10% methanol. Data were acquired by HyStar^{TM} and processed by Bruker Daltonics Data Analysis running under Windows NT^{TM} (Microsoft, Redmond, USA). All solvents used were HPLC grade (Acros Organics, Geel, Belgium).

Table 1.

LC/MS timetable for the chromatographic analysis of the chlorophyll biodegradation products

Time	Value of the solvent mixture H_2O(0.1%TFA):MeOH
0	90:10
10	80:20
20	60:40
30	50:50
40	40:60
50	30:70
60	20:80
70	0:100

RESULTS AND DISCUSSIONS

The major chlorophyll biodegradation products found in *Hamamelidaceae* family are: *Parrotia persica* urobilinogenic non – fluorescent chlorophyll catabolite (*Pp*-UNCC) *m/z* 633 (DJAPIC, PAVLOVIC, 2008), *Hamamelis virginiana* UNCC (*Hvir-UNCC) m/z* 633 (DJAPIC ET AL., 2009), three UNCC isomers of *Pp*-UNCC and *Hvir*-UNCC *m/z* 633, and two chlorophyll biodegradation products found in *Cercidiphyllum japonicum*, also known as *Cj*-1, *m/z* 645 and *Cj*-2, *m/z* 629, non – fluorescent chlorophyll catabolite (NCC) (OBERHUBER ET AL., 2003) (Figure 1).

Chlorophyll biodegradation products were studied in *Vitis vinifera* var. Pinot noir autumnal leaves. The chromatogram revealed the presence of one unknown isomer *m/z* 633, *Pp*-UNCC isomer *m/z* 633, *Hvir*-UNCC isomer *m/z* 633 and the traces of *Cj*-1 *m/z* 645 (Figure 2).

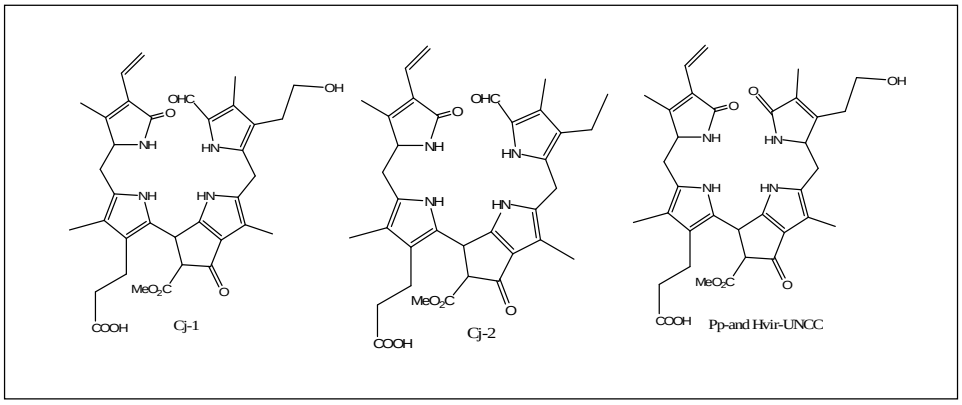

Figure 1. Structure of chlorophyll biodegradation products

The chlorophyll biodegradation, in *Vitis vinifera* autumnal leaves, is the same as in the autumnal leaves of the Hamamelidaceae plant family. The chlorophyll biodegradation products *Cj*-1 (NCC) differs from *Pp*- and *Hvir*- chlorophyll biodegradation product (UNCC) in one lateral group. The aldehyde group present in *Cj*-1 (NCC) is absent in *Pp*- and *Hvir*-UNCCs. The mechanism that can be proposed for the formation of UNCCs from NCCs is the oxidation by Baeyer – Villigerase. The following mechanism, for the biotransformation of NCCs into

UNCCs, can be adopted from the literature (DONOGHUE ET AL., 1976, KELLY, 1996) (Figure 3). The other mechanism that can explain the formation of UNCCs from NCCs is by oxidative decarboxylation of an acid intermediate generated by the oxidation of the aldehyde group.

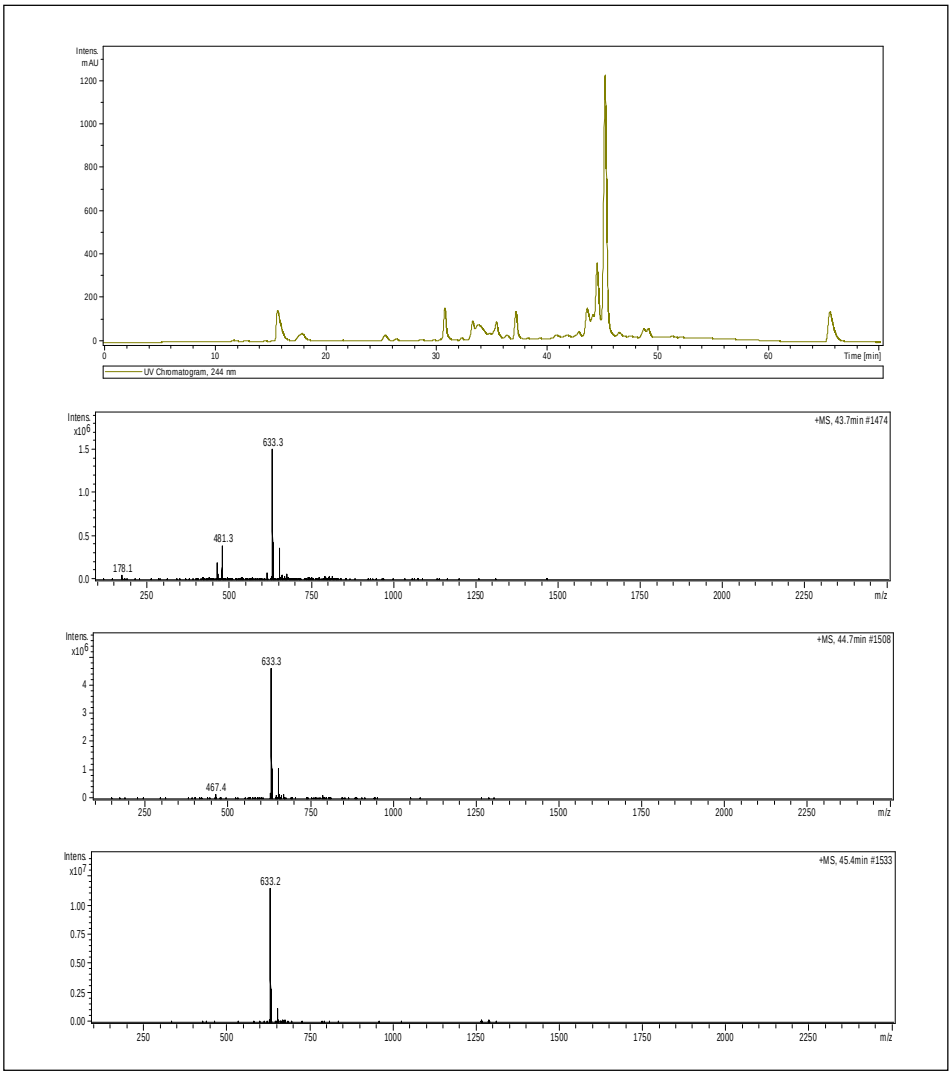

Figure 2: Chromatogram of the *Vitis vinifera* var. Pinot noir autumnal leaf extract, UV detection at λ=244nm and electro spray ionization mass spectra of the UNCCs eluting at 43.7, 44.7 and 45.4 min. on reverse phase EC 250x4 mm Nucleosil 100-5 C_4 column.

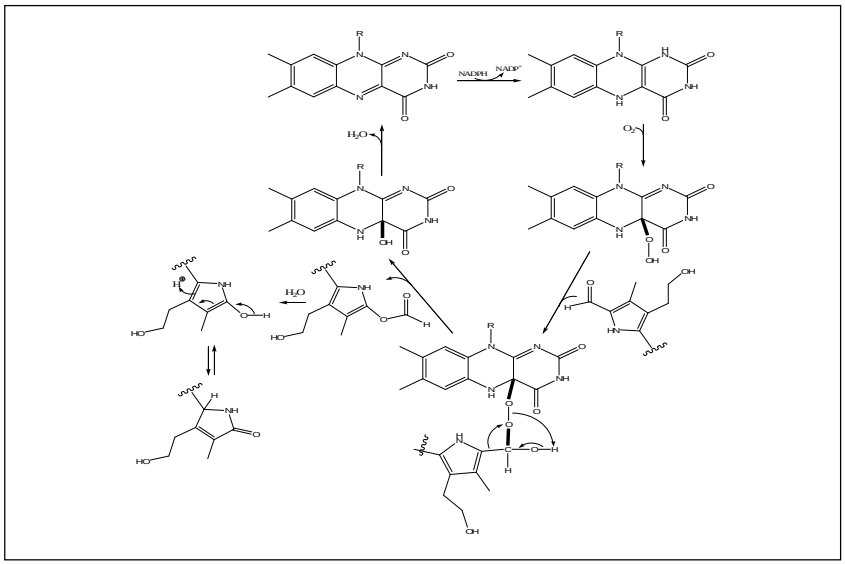

Figure 3. The proposed enzymatic mechanism for the Baeyer – Villiger oxidation

CONCLUSIONS

In conclusion, the information on chlorophyll biodegradation products found in *Vitis vinifera* var. Pinot noir autumnal leaves were obtained using LC/MS analysis. The chromatogram obtained revealed the presence of UNCCs and just a trace of NCCs in *Vitis vinifera* var. Pinot noir autumnal leaves. The mechanism for the formation of UNCCs from NCCs is proposed. Further chlorophyll biodegradation products were not registered. Most probably, further chlorophyll biodegradation products are colorless. The possibility is the derivation of the UNCCs lateral –COOH group into an UV/Vis derivative. In case the research limitations are overwhelmed, even, the faith of nitrogen atoms present in UNCCs can be resolved.

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