A study of chemichal role on ageing of red wine minor components: Flavano-ellagitannins

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Written for presentation at the
2007 CIGR Section VI International Symposium on
FOOD AND AGRICULTURAL PRODUCTS: PROCESSING AND INNOVATIONS
Naples, Italy
24-26 September 2007

Abstract

This study have demonstrated the evolution of C-Glucosidic Ellagitannins (Vescalagin and Castalagin) through nucleofilic substitution reaction in other products , during wine ageing , occurred in presence of oak wood. Flavano-ellagitannins synthesis as Acutissimins A e B and Epiacutissimins A e B was gained by means organic solutions, which simulated the conditions in wine, with endocondensation of oak wood ellagitannins and catechins or epicatechins. The same reaction has occurred also during the artificial ageing of red wine by American Oak staves. LC-MS was used to characterize these compounds. Aqueous solutions, simulating wine, were prepared with 12% ethanol, 5 g/l tartaric acid and pH 3,2 and added with American oak staves. The solutions had two different quantity of oak (5-10%) and an increasing concentration of catechin (100-500 mg/l). Maceration of these solutions lasted 42 days. Altought was analysed samples of commercial wine aged with oak staves(0.5g/l) for 6 weeks. An extract of each solution and wine at the end of maceration time was injected into HPLC system. HPLC-DAD analysis was carried out using an HPLC Agilent 1100 with a quaternary pump system (Agilent Technologies, Canada).

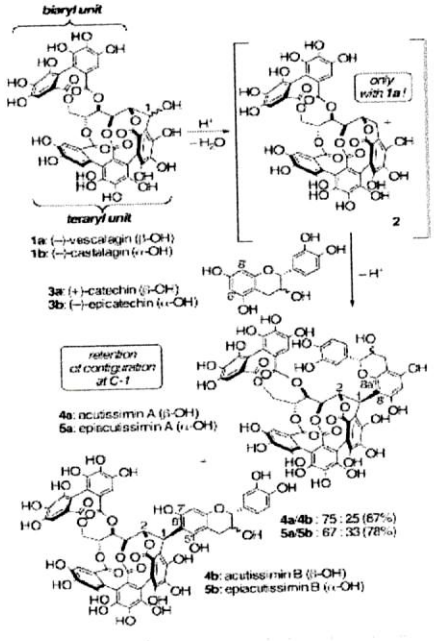
The analytical column (150 x 4.6mm,5µm) was PHENOMENEX GEMINI C18. The same extracts were concentrated and injected through the same HPLC system to collect the fractions containing ellagitannins. The recovered fractions were injected into HPLC-MS system, HP 1100 (Agilent Technologies, Canada) equipped with a 1100series LC-MSD with a Multimode Ion Source. A typical cromatographic profile of a model solution extract showed in addition to catechin peak, also the presence of three new peaks. The model solutions containing great quantity of oak wood had the strongest presence of these new compounds: by LC-MS the collected fractions resulted to have m/z ratio corresponding to ellagitannins and Acutissimins. Also the simulation of wine ageing with oak chips produced the presence of the same compounds.The analysis of phenolic compounds, anthocyanins and colour evolution of final product described a wine having similar characteristics of a wine aged with Quercus Alba barrels

Keywords: flavano-ellagitannins, Acutissimins, red wine, HPLC, mass spectrometry, oak wood.

Introduction

Recently some products are largely diffused on the international markets as an alternative to the employment of the oak barrels in winemaking. It was referred in particular to use of oak wood and wood fragment of various shapes and proportions toasted more or less intensely. Chips and staves are the more commonly known products (Meca G., 2006). Their use has been approved by the OIV, and currently REGULATIONS (CE) N1507/2006 has regulated their employment in wine making. Oak wood chips are employed in order to accelerate the wine aging process. The chemical effects on wine aged in contact with oak chips are similar to a wine aged in oak barrels. The long time contact of wine in the barrel allows to this hydroalcoholic acid solution the extraction of various substances, where the ellagitannins :vescalagin and castalagin, are the main components yielded from wood (Vivas N.,1995). The work presented herein concerns the formation in red wine aged with oak chips of acutissimin A and B(4a) and related flavano-ellagitannins from flavan-3-ols and C-glycosidic ellagitannins such as (-)-vescalagin (1a) and its C-1 epimer (-)-castalagin (1b) that feature an unusual open-chain glucose core C-C-linked to a galloyl-derived teraryl unit (Scheme 1).(Jourdes, 2004)

Acutissimins hemisyntesis chemical path



These C-aryl glycosides are characteristic metabolites of durable hardwood species. Acutissimin A (4a) was first isolated from the bark of the sawtooth oak (Quercus acutissima),[Quideau S., 2003]. The compound was later found to be an inhibitor of human DNA topoisomerase II that is 250-fold more potent in vitro (concentration required for 100% inhibition, IC100=0.2 mm) than the clinically used anticancer drug etoposide (VP-16).[13] The chemistry of the formation of 4a is simple and involves an acid-catalyzed nucleophilic substitution reaction between either (-)-1a or (-)-1b at the C-1 center and (+)-catechin (3a) at its nucleophilic C-8 center (Scheme 1). During the aging time catechin and the epicatechin (flavonoidi substances to

isomere of the wine) react with ellagitannins of the wood, forming Acutissimins (3-4).The aim of the experimentation has been the study of Acutissimins evolution during the shortening aging period with oak chips In this purpose they have been used for the experimentation alcoholic acid model solutions and commercial wine.

Materials and Methods

Flavano-Ellagitannins "Acutissimin A and B" hemisynthesis has carried out trough an 12% v/v ethanolic solutions containing 5g L^{-1} Tartaric acid pH 3.2 added with oak chips and catechin (Puech, J.). Four model solutions were prepared:

- Sol. 100 ppm di catechin-5% Oak chips (100/5).
- Sol. 500 ppm di catechin-5% Oak chips (500/5).
- Sol. 100 ppm di catechin-10% Oak chips (100/10).
- Sol. 500 ppm di catechin-10% Oak chips (500/10).

The evolution of chemical reaction has been clearly demonstrated trough acutissimins identification and quantitative evaluation of catechin content. The Acutissimins hemisyntesis have been monitored over a period of 42 days by HPLC gradient reversed fase analysis.(AGILENT 1100 SERIES, Column Phenomenex Gemini C18 150 * 4,6 mm, 5 micron i.d. Detector UV-VIS, DAD). As eluting phases has been used a water and Trifluoroacetic acid (TFA) at 0,5% solution(Sol. A) and methanol at 0,5% of TFA (Sol. B). Absorbance traces are obtained at 230, 280 and 308 nm (9). Although has been simulated a short aging process of commercial red wine with oak chips hardly toasted, added in the same surface/volume ratio of a 225 lt oak barrel. Aging process has been monitored over a period of 6 weeks , during this time were determinate : Totals pholyphenols content, antocyanins, colour values , pholyphenols by HPLC and the flavano-ellagitannins formation has been shown trough HPLC-MS analysis. Working conditions was : HPLC AGILENT 1100 SERIES, Coloumn ZORBAX C18 100 * 2.1 mm, 3.5 µm i.d. Detector LC-MS ESI-APCI. The flow was 0.2ml/min with elution program Water-Methanol-0.5% acetic acid (60%-40%) in isocratic mode. Analysis was carried out in SIM modality.

Mass Spectrometry operating conditions

MSD-Spray chamber: Dryng gas flow= 12 l/min, Nebulizer pressure=60 psi, Dryng gas temperature=300 °C , Vaporaizer temperature= 250 °C, Capillary voltage=2000 V, Corona current=2 µA, Changing voltage=2000 V.

Qualifier Ions for Acutissimins identification.

m/z=180 = Glucosio

m/z=271 = Acutissimins-Ellagitannins

m/z=291 = Catechin

m/z=754 = Ellagitannini-Glucosio

m/z=914 = Acutissimine-Catechin

m/z=934 = Ellagitannini

m/z=1025 = Acutissimine-Glucosio

m/z=1205 = Acutissimine

.Each of the four compounds has a molecular mass of 1205 Da, and their presence was validated by mass spectrometry and comparison of their chromatographic retention times and mass fragmentation patterns with those of the hemisynthetic compounds. Quantitative analysis has been executed using catechin as esternal standard.

Results and Discussions

In figure 1 is illustrated a typical HPLC chromatographic profile at 230,280,308 nm of model solutions. It is possible to observe the presence of the peak of catechin and others three peaks, corresponding to new formation substances by catechin and woods reaction. In particular peaks n°2 and 3 had mass spectrum with fragment ions corresponding to acutissimin, instead for the peak n°1 the mass spectra corresponds to m/z ratio of wood ellagitannins.

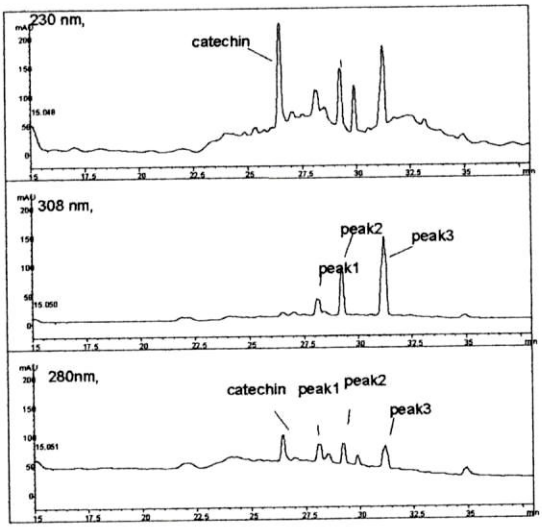
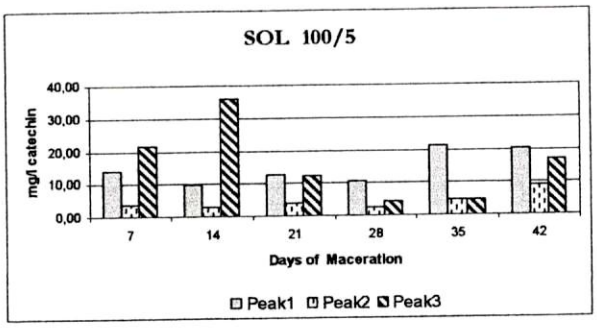


Fig.1 HPLC profile of model solutions (Peak 1:ellagitannins, Peak 2: Acutissimin B, Peak 2 Acutissimin A)

The peak 1 had an amount between 20,19 and 37,32 ppm at 42th day. Peaks 2 and 3 which had a fragmentation spectrum corresponding to acutissimin A and B respectively, had a variable values durino maceration period. An higher ratio catechin/oak wood(SOL100/10-500/10) has promoted Acutissimins formation. In the model solution 500/10 has been found an high amount of acutissimins, -30,78 ppm for peak 2 and 31,68 ppm for peak 3 (Fig. 2a,b-3a,b).

A



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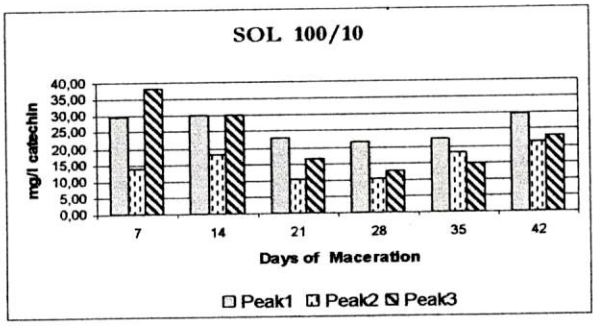
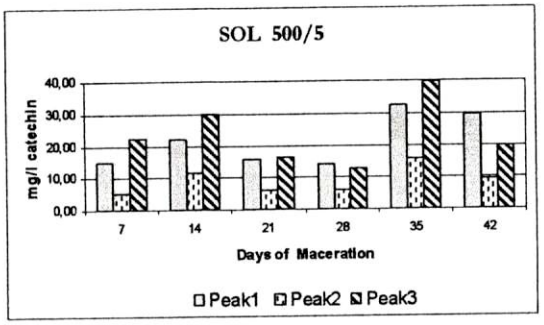


Figure 2.a,b Ellagitannins trend in model solutions with catechin 100 ppm.

A



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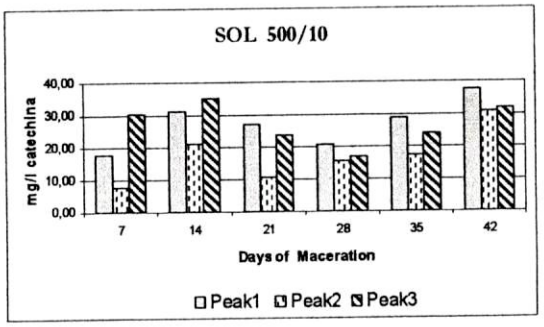

Figure 3.a,b Ellagitannins trend in model solutions with catechin 500 ppm.

Trough HPLC-MS fitted with electrospray and APCI sorgent , has been evidenced the presence of fragments corresponding to m/z ratio of Ellagittanins and Acutissimins in shortening aged wine extracts . (figure 4.)

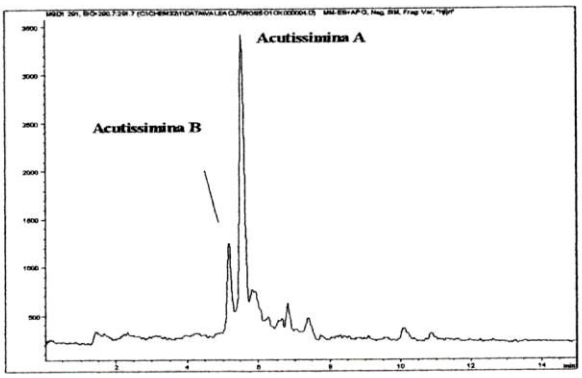

Fig.4 LC-MS chromatogram of wine Acutissimin.

In Table 3 it was monitored acutissimins trend during rapid ageing. Their hemisyntesis in red wine aged with oak chips had a concentration between 0.10 -0.36 mg/L.

Tab.3 Acutissimins amount in red wine aged with oak chips.

t	acutissimin A	acutissimin B
Week	mg/l catechin	mg/l catechin
1	0,36 ± 0,030	0,11 ± 0,021
2	0,33 ± 0,040	0,18 ± 0,030
3	0,35 ± 0,045	0,16 ± 0,036
4	0,20 ± 0,048	0,13 ± 0,039
5	0,19 ± 0,048	0,23 ± 0,039
6	0,22 ± 0,051	0,15 ± 0,040

Wine treated with oak chips during ageing undergoes to a series of modification which are similar to transformations of a wine aged in a oak barrel(5). Total polyphenols content , initially at 437 ppm, increased at the end of ageing period at 2126ppm. They reach these values as a consequence of ellagittanins formation but even for wood release of its compounds. It has been also evidenced a decrease of total free antocyanins content which reduced their values from 178ppm to 63.58ppm(6). The ageing leads to a wine colour variation, in fact it changes to yellow-orange tonality, red colour decreases from 77,54% to 10%(Tab.4.).

Determination	Time (Days)	
	0	42
pH	3,43±0,08	3,38 ±0,06
Total acidity (g/l tartaric acid)	3,46 ±0,07	6,21 ±0,09
Volatile acidity (g/l acetic acido.)	0,67 ±0,06	0,90 ±0,06
Alcoholic grade (%v/v)	11,00 ±0,19	11,00 ±0,17
Total Poliphenols (mg/l gallic acid)	713,67±0,22	2.126,43±0,44
Total Antocyanin (mg/l malvidin)	178,03 ±0,38	63,50 ±0,35
Colour Intensity (u.a.)	1,64 ±0,12	1,78 ±0,12
Tonality (Abs 420/520)	1,06 ±0,12	1,12 ±0,16
Red colour purity (dA%)	77,64 ±0,71	10,00 ±0,75
Tonality (% 420nm)	37,90 ±0,23	41,00 ±0,25
Tonality (% 520nm)	38,20 ±0,14	49,9 ±0,21
Tonality (% 620nm)	24,93 ±0,12	10,08 ±0,18

Tab.4 Oenological parameters of wine aged with oak chips.

Conclusions

The study of a model system has well demonstrated the hemisynthesis of flavano-ellagitannins since the 7th day of maceration . At the end of maceration, there is an high amount of acutissimins coupled with an higher amount of catechin. Instead at the beginning (7th day) their formation is influenced by oak quantity added to solutions. Acutissimins determination in wine aged with oak chips represents the first verified hypothesis of a general chemical transformation pathway of wine c-glucosydic ellagitannins, and the first step for a qualitative and quantitative evaluation of wine rapid ageing.

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