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vitivinicultural Systems for CoOperation**

(GiESCO 2013)

**Porto, Portugal
7th – 11th July 2013**

PROCEEDINGS / *COMPTES RENDUS*

TOME I

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All texts have been reviewed and corrected by the Editorial Review Board, members of the Scientific Committee of GiESCO 2013 and Editors.

We apologize for errors that could have arisen during the editing process despite our careful vigilance.

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PREFACE

18^{èmes} Journées Internationales GiESCO 2013, Porto, Portugal

PORTUGAL - Diversité, Patrimoine, Innovation

Le 18^{ème} Symposium International GiESCO 2013 (Groupe *internationale* d'Experts en Systèmes vitivinicoles pour la CoOpération) s'étend entre 7 et 11 Juillet, à la Faculté des Sciences de l'Université de Porto - Portugal, sous le Haut Patronage de Son Excellence Monsieur le Président de la République Portugaise. A ce événement sont associés l'OIV (Organisation Internationale de la Vigne et du Vin), le Rector de l'Université de Porto, l'Institut d'Agronomie - Université Technique de Lisbonne, l'Institut National de la Recherche Agronomique et Vétérinaire IP, la Fondation pour Science et Technologie, l'Institut de la Vigne et du Vin, IP, la Commission de la Viticulture de la Région des Vinhos Verdes, l'Institut des Vins du Douro et de Porto, IP, le ViniPortugal, la « Casa do Douro » et la "*Chaire UNESCO Culture et Traditions du Vin*".

Le grand succès auprès de la communauté scientifique se traduit par la présentation de plus de 220 articles scientifiques (orales et posters) d'environ 250 chercheurs et scientifiques de 23 pays. Pendant quatre jours et neuf séances, seront abordés les sujets: Méthodologie et écophysiologie, Relations Hydriques; Viticulture de montagne et des régions chaudes; Environnement: climat et sol ; Système de culture, Rendement, Qualité ; Systèmes de Conduite; Nouveaux concepts et Technologies avancées en Viticulture, Viticulture Générale; Gestion des territoires. Viticulture durable; Académie de la Vigne et du Vin.

Cette réunion sera également l'occasion pour que les participants puissent connaître les dernières avancées technologiques de l'industrie de la vigne et du vin Portugais et sa diversité, à travers des visites techniques dans la REGION DES VINHOS VERDES et la région de la HAUT DOURO VITICOLE, classé Patrimoine Mondial par l'UNESCO en 2001.

Dans ce colloque on désire aussi honorer le Prof. Rogério de Castro pour sa contribution à l'enseignement, de la Viticulture et de leur collaboration avec GiESCO, raison pour laquelle ce jour et ouvert à la communauté scientifique et technique.

Saisissant l'occasion pour souhaiter la bienvenue à tous les participants, nous remercions à toutes les personnes impliquées dans l'organisation de cet événement, en particulier à Dra. Teresa Mota, Eng^a. Anabela Carneiro, à Eng^a. Susete Melo et Eng.^o António Fonseca, les membres du Comité Organisateur et du Comité Scientifique par la révision des articles, ainsi que toutes les institutions et les entreprises que, d'une manière ou d'une autre, ont contribué à l'organisation de ce colloque.

Finalement, je remercie ma famille pour leur soutien.

Jorge B. Lacerda de Queiroz

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ARSENIC IN SOIL AND GRAPES AND CHANGES IN ELEMENTAL CONTENT DURING WINEMAKING

ARSENIC DANS LE SOL ET LES RAISINS, ET MODIFICATIONS DU CONTENU ELEMENTAIRE PENDANT LA VINIFICATION

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SUMMARY

Healthiness is a prerequisite for all agricultural products, foods and beverages. The presence of arsenic (As) in these products creates concern, due to its carcinogenicity. In the case of vines grown on soils naturally rich in As, investigation of its content in grapes and the relative wines, as well as its possible effects on the winemaking process, may be appropriate.

28 different vineyards planted with Chardonnay in Trentino (north-eastern Italy) were studied. 10 of these were close to an ancient mining area with deposits of arsenopyrite and other sulfide minerals containing As traces. Soil and grapes from each vineyard were analysed to determine As content. The content of aqua regia extractable As was measured in the soil samples using the ISO 11466/1995 method, whereas As in grapes was determined after acid mineralisation in a closed vessel.

White wines were produced from 7 vineyards characterised by the highest As content using 10 yeast strains. Furthermore, the same grape samples were fermented with skin-contact during fermentation using only 1 yeast strain.

The content of As in the soil and grapes was higher in vineyards near the ancient mining area. Skin-contact increased As in wine and statistically significant differences were found among yeast strains. Nevertheless, in the conditions of this study, the content of As in wines was always below the limit (200 µg/L) suggested by the OIV, by at least 100 times.

RÉSUMÉ

L'état sanitaire est l'élément-clé de tout produit issu de l'agriculture, nourriture ou boisson. La présence d'arsenic (As) dans ces derniers génère des inquiétudes, compte-tenu de son effet cancérigène. Dans le cas de la viticulture sur des sols naturellement riches en As, une étude de sa présence dans les raisins et des effets possible au processus de vinification semble approprié.

28 différents vignobles de Chardonnay dans la région du Trentino (nord-est de l'Italie) ont été suivis. 10 d'entre eux étaient à proximité d'exploitations minières contenant traces de arsenopyrite et minéraux sulfurés contenant des traces d'As. Pour chaque vignoble, le sol et les baies de raisins ont été analysées afin de détecter la présence d'As. Le teneur d'As extractible parmi eau régale a été mesuré par la méthode ISO 11466/1995, tandis que les analyses sur les raisins ont été effectués après minéralisation.

Des vins blancs ont été produits à partir des raisins de 7 vignobles, ayant la plus grande teneur en As, en utilisant dix souches différentes de levures. En outre, les mêmes raisins ont été fermentés avec macération pelliculaire, en utilisant une seule souche de levure.

Le teneur d'As dans le sol et dans les baies étaient en plus grande quantité dans les vignobles proches d'anciens mines. La macération pelliculaire a augmenté la présence d'As dans le vin et de différences statistiquement significatives ont été révélées parmi les levures. Cependant, la teneur d'As dans les vins produits dans les conditions de ce travail s'est révélée en-dessous de la limite (200 µg/L) fixée par l'OIV, d'au moins 100 fois.

Keywords: mining areas, soil, grape, skin-contact, yeast strains

Mots-clés: zone d'exploitation minière, sol, raisin, macération pelliculaire, souches de levures

INTRODUCTION

Arsenic (As) is naturally present in the Earth's crust, being widespread in various types of rocks and especially in mineral sulphides. Its origin in grapes and wine can be both natural/geological and anthropogenic (Kabata-Pendias, 2001; Arai, 2010). The International Agency for Research on Cancer (IARC) classifies As and its inorganic and organic compounds as carcinogenic or possibly carcinogenic to humans (group 1 or 2B).

In soils, As concentration ranges from <0.1 to 600 mg/kg, with an average content <10 mg/kg. Its bioavailability varies widely, being affected mainly by pH, redox potential and the presence of Mn, Al and Fe oxides (Adriano, 2001).

In vine leaves, As ranges from 60 to 410 µg/kg dry weight (dw) (Ko *et al.*, 2007, Bertoldi *et al.*, 2013) whereas in grape berries not treated with As-containing pesticides, a concentration varying between an undetectable level and 70 µg/kg dw has

been reported (Ko *et al.*, 2007; Fang *et al.*, 2010; Bertoldi *et al.*, 2013).

Nowadays, As concentration in wines varies from <0.5 to 17 µg/L (Aguilar *et al.*, 1987; Galani-Nikolakaki *et al.*, 2002; Herce-Pagliai *et al.*, 2002; Kment *et al.*, 2005; Chanthai *et al.*, 2007; Fiket *et al.*, 2010) but in the past a much higher content had been reported (Creelius, 1977). Its presence is affected by oenological treatments such as the use of bentonites or fossil shell flours (Castañeira Gómez *et al.*, 2004; Nicolini *et al.*, 2010).

In this study we investigated the presence of As in soils, grapes and wines coming from vineyards located in an old mining area naturally rich in As and the roles of yeast strain and skin contact in modifying the final content of this element in wine.

MATERIALS AND METHODS

Twenty-eight vineyards planted with Chardonnay (*Vitis vinifera* L.) in Trentino (north-eastern Italy) were studied. Ten of those were close to an ancient mining area with deposits of arsenopyrite and other sulphide minerals containing As traces. Soil and grapes from each vineyard were analysed to determine As content.

The soil (0-60 cm) was sampled near the vine roots, air-dried, sieved to 2mm, ground (<0.2mm) and extracted with aqua regia using the ISO 11466/1995 method. At ripeness, grape samples (5 berries from 20 vines) were collected, washed with 1% HNO₃, rinsed with milliQ water, frozen, homogenised and mineralised with ultrapure nitric acid using a microwave system (Mars Express, CEM, Matthews, USA) as reported in Bertoldi *et al.* (2013).

White wines were produced from grape samples of 7 vineyards characterised by the highest As content, using 10 different yeast strains (Perdomini

only FR95 strain. Wines were processed avoiding treatments and materials potentially releasing As.

Soil and grape extracts were analysed to determine total As content with an inductively coupled mass spectrometer (ICP-MS) equipped with a collision cell (Helium gas) to remove interferences and using the Sc as internal standard. As quantification in wine was carried out after dilution against standard solutions prepared in 5% ethanol.

Basic analysis (alcohol, titratable and volatile acidity, pH, glycerol) of wine was performed using an FT-IR Grapescan 2000 (Foss, Hillerød, Denmark).

The data were statistically evaluated using the software package STATISTICA 8.0 (Statsoft Inc, Tulsa, USA).

RESULTS AND DISCUSSION

Considering all 28 vineyards, the median aqua regia-extractable As naturally present in the soils was 11.4 mg/kg (min-max 3.7-283 mg/kg), similar to the average worldwide content in uncontaminated soils reported in the literature (Hooda, 2010). Seven soils showed As values higher than 20 mg/kg and three of these exceeded 50 mg/kg. The 10 soils near the mining area, where six of the soils with the highest As content were located, had a median value significantly higher than the others (Table I; Mann-Whitney U test, $p < 0.05$). A similar result was observed for the grapes: the median As content in grapes collected in vineyards near the mining area was significantly higher than in grapes of the other areas (Mann-Whitney U test, $p < 0.001$). Despite the high As content in the soil, 1% HNO₃ washed grapes had a limited endogenous content.

Taking into account only the 7 soils used for the winemaking experiment, the median As content

Table I: Minimum, median and maximum values of As in soils and grapes (dw: dry weight)

Minimum, médiane et maximum d'As dans les sols et les raisins (dw: poids sec)

	soil (mg/kg)	grapes (□g/kg dw)
samples from mine area (N=10)	4.3 - 26.4 - 283	<0.1 - 6.81 - 32.6
samples used for winemaking (N=7)	7.6 - 43.7 - 283	4.06 - 7.18 - 32.6
samples from outside mine area (N=18)	3.7 - 10.5 - 31.8	<0.1 - 1.04 - 7.28

Blastosel FR95, La Claire EM2, AEB Fermol Arom plus (FAP), Ferrari WP and SN6, Oliver Ogar VP5, Springer Oenologie CK S102, Laffort Zymaflore VL1, Lallemand Vitilevure DV10 and AWRI 1503). Furthermore, the same grape samples were fermented with a 7-day skin-contact vinification as for red wine (punching the cap twice a day, maximum fermentation temperature 28°C) using

was 43.7 mg/kg and a median value of 7.18 µg/kg dw was measured in the relative grapes.

The mean composition (N=70; i.e. 7 soils × 10 strains) of the white wines produced was: alcohol 9.63 ± 0.93 % vol., pH 2.83 ± 0.24, titratable acidity 10.97 ± 1.53 g/L, volatile acidity 0.25 ± 0.07 g/L, residual sugars 1.85 ± 6.26 g/L, glycerol 6.59 ±

1.07 g/L and As $0.68 \pm 0.35 \mu\text{g/L}$. Figure 1 shows the effect of yeast strain on some wine chemical

Using the same grapes and yeast strain, a comparison between white wines (W) and wines

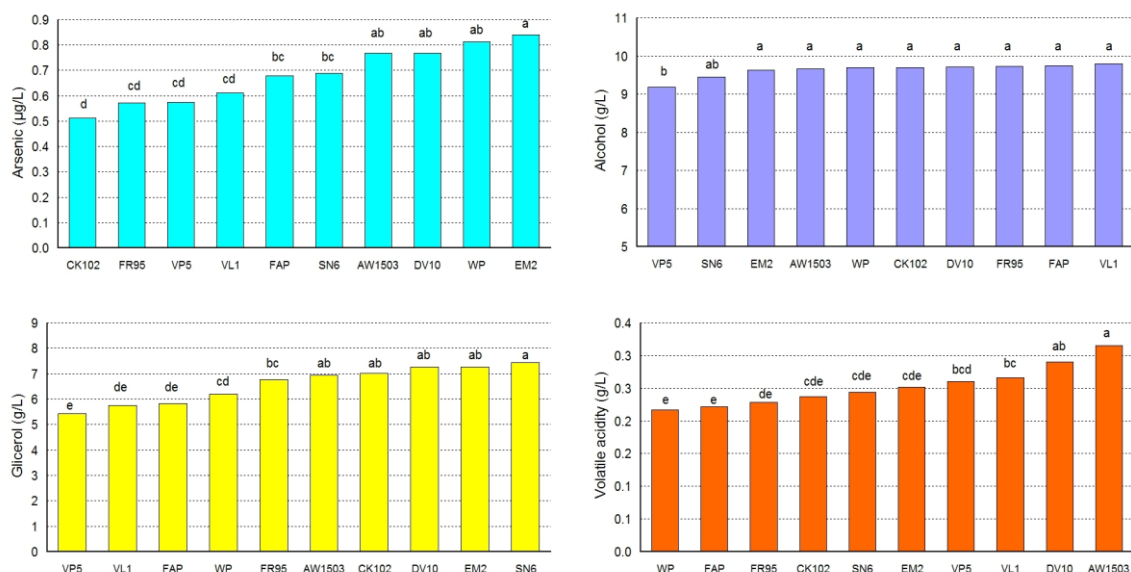


Figure 1 : Effect of yeast strains on some basic compositional parameters of wine and As content (values correspond to the mean of 7 soils)
Effet des souches de levure sur certains paramètres de base de la composition du vin et sa teneur en As

parameters and As content along with the statistical differences between strains assessed by Fischer's Least Significant Difference test ($p < 0.05$).

Some yeast strains, particularly EM2, were associated with a higher As content in wine while others (e.g. CK102, FR95, VP5 and VL1) seemed to reduce wine As content in wine. The difference

processed according to the skin-contact protocol (SK) typical for red wines was carried out. W wines had a significantly lower As content (LSD test, $p < 0.05$) than SK wines. For the latter, no significant differences were observed when measuring As content before and after malolactic fermentation (Figure 2).

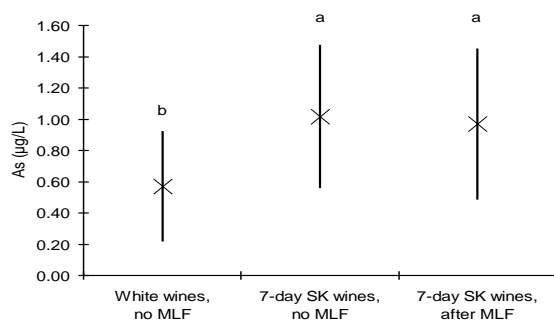


Figure 2: Winemaking effect on As content (values correspond to the mean \pm standard deviation of 7 fermentations; SK: skin-contact fermentation)

Effet de la vinification sur la teneur en As (SK: fermentation avec macération pelliculaire)

range between strains in percentage terms was about 40%. The observed differences could be due to electrostatic interactions of As - e.g. with protein sulphide groups, wine colloids and yeast cell wall - as well as to vacuolar sequestration or volatilisation during fermentation.

CONCLUSION

The content of As in the soil and grapes was higher in vineyards near the ancient mining area investigated. Skin-contact vinification increased As

in wine and statistically significant differences due to yeast strains were found. The concentration observed in wines was well below the limit suggested by the OIV (200 µg/L) even if produced from grapes grown on soils naturally rich in As.

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