

Ethyl Carbamate Content in Wines Produced and Commercialized in Southern Brazil

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SUMMARY. Although the production of wines in Brazil is well established, there is little information on the characteristics and composition of these products. The results presented are an initial report on the chemical determination of ethyl carbamate in Brazilian wines.

RESUMEN. “Contenido de carbamato de etilo en vinos producidos y comercializados en el Sur de Brasil”. Aunque la producción de vinos en Brasil está bien establecida, hay escasa información acerca de las características y composición de estos productos. Los resultados presentados en esta oportunidad constituyen un informe inicial sobre la determinación química de carbamato de etilo en vinos brasileiros.

INTRODUCTION

Although grapes were first introduced into Brazil very early on by the Portuguese colonizers, most present day Brazilian wine production can be traced to the activities of the Italian immigrants that settled in the Serra Gaúcha of Northeastern Rio Grande do Sul in the second half of the XIX Century ^{1,2}. At present the state of Rio Grande do Sul represents 90% of the total Brazilian wine production ³.

About 80% of the total regional production of Rio Grande do Sul is still derived from non-*Vitis vinifera* grapes ^{3,4} and the final products (defined as “common wines” in the Brazilian legislation ⁵) have a restricted local and regional consumption. *V. vinifera* (“European”) grape production is increasing, and their wines are now being introduced into international markets with a certain measure of success ³. Sparkling wines of good quality are being produced for national consumption and could enter the international trade ³.

The main products include red wines (80%

non-*vinifera* grapes of Niagara, Bordo, Herbe-
mont, Seibel varieties; 20% *V. vinifera* grapes of Cabernet Franc, Cabernet Sauvignon, Barbera, Merlot, Chardonnay and Pinot Noir varieties ^{2,3}), rosé and white wines (Italian Riesling, Moscato, Semillon, Chardonnay, Gewürztraminer and Trebbiano varieties ^{2,3}), and sparkling wines. Of these products, the “common wines” of non-*vinifera* grapes ⁵ are characteristic of the region ^{3,4}, with vast production of generic reds and whites. Sparkling wines have been the most successful, and are usually produced from wines of Italian Riesling, Chardonnay and Pinot Noir varieties ².

All the wines have been poorly described in analytical terms, and little is published about their components or quality ⁴. The present results, although limited to ethyl carbamate (EC) content, represent an initial contribution to the characterization of Brazilian regional wines, and we hope will be the first of a series further extending the characterization of the Brazilian products.

KEY WORDS: Brazil; wines; ethyl carbamate

PALABRAS CLAVE: Brasil, Carbamato de Etilo, Vinos

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EC is formed naturally in wines and other fermented foods ⁶. It is derived from the fermentation processes of amino acids, in particular arginine ^{7,8}. The final EC content of a wine will be variable, depending on the amino acid concentration in the grape juice, on the fermentation process to which the must is subjected and to the stockage conditions of the product ⁹. As EC is potentially toxic ^{10,11}, there are maximum levels accepted in the different countries ^{12,13}. The European Union (EU) accepts 10-15 µg/L EC for national wines and 15 µg/L EC for imported wines. FAO recommends maximum levels of 30 µg/L EC in wines ¹⁴.

MATERIAL AND METHODS

Reagents

Solvents (Merck) were of analytical and chromatographic grade (pesticide free). Celite 454 was also purchased to Merck. Standards of ethyl carbamate (EC) and propyl carbamate (PC) were 99.9% purity (Aldrich).

Samples

Wine samples were purchased in commercial outlets at Caxias do Sul and Bento Gonçalves (both in Rio Grande do Sul), covering all main brands produced in the counties of Bento Gonçalves, Garibaldi, Cotiporã, Farroupilha, Santana do Livramento, Caxias do Sul and Flores da Cunha. They were numbered and their register is kept at the Instituto de Biotecnologia.

GC-MS Equipment

A Hewlett Packard HP 6890 Plus GC with a HPS 5973 MSD was used. Column: HP Innnowax (30 m, id 0.25 mm; film thickness 0.2 µm); initial temperature, 40°C (5 min); ramp, 40-200°C (3 °C/min); final temperature, 200 °C (30 min); injector temperature, 200 °C; injection mode, split/splitless. Carrier gas, He; Auxiliary gas, N₂. MSD temperature, 250 °C; EI mode, 70 eV; SIM M/Z 62, 74 and 89.

Techniques

The technique of the Official Method of OIV (1996) was used throughout ¹⁴. The method was tried to achieve adequate conditions for repeatability (0.1295 standard deviation and 0.76% variation coefficient, with a correlation coefficient of R = 0.99 for linearity). The recommended EC recovery of over 90% was obtained using 2 x 90 mL CH₂Cl₂. Average recoveries of 94.95% EC were achieved. Further increases in the volume of CH₂Cl₂ used gave marginal improvements. A hydroalcoholic 15% v/v blank was prepared from pure ethanol and water. 1.0 mg/mL stock solutions of EC and PC were prepared. They were diluted to 10.0 µg/mL and used to calibrate the equipment. A PC solution of 400 ng/mL was used as internal standard. 2 µL of extract were injected in the GC-MS.

RESULTS AND DISCUSSION

The results obtained for wines are presented in Table 1.

Wine variety	Number of samples	Minimum values (µg/L)	Maximum values (µg/L)	Mean	Number of Samples over 15 µg/L	Number of Samples over 30 µg/L
Cabernet Sauvignon	30	2.01	31.82	10.62	8	3
Merlot	17	1.84	32.41	6.63	1	1
Gamay	3	3.36	6.54	4.47		
Pinot Blanc	5	2.66	10.13	7.35		
Generic Reds	9	2.35	36.20	16.55	4	1
Gewüztraminer	12	1.19	30.49	10.09	3	1
Italian Riesling	10	1.02	39.08	13.00	3	1
Chardonnay	5	1.69	70.01	19.25	1	1
Semillon	3	3.54	20.49	14.50	2	
Generic Whites	3	4.65	5.10	4.80		
Common Reds	10	2.07	9.02	5.14		
Sparkling Wines	17	2.08	24.61	7.60	1	

Table 1. Ethyl carbamate content in wines from the Serra Gaúcha.

The range of EC concentrations was from 1.02 µg/L to 70.0 µg/L, with means ranging from 4.47 µg/L to 19.25 µg/L for the different wines. Of the 124 products sampled, 23 were over the EU limits, but only 8 were also over the FAO limits. Of these 7 were just over the 30 µg/mL limit and one sample was very clearly over the limit.

The EC concentrations for 17 Sparkling wine samples are presented in Table 2.

Wine sample Register N°	Type	Ethyl carbamate content (µg/L)	County of origin
1	Brut	2.27	Bento Gonçalves
2	Brut	2.49	Bento Gonçalves
3	Brut	5.63	Bento Gonçalves
4	Brut	5.91	Bento Gonçalves
5	Brut	6.11	Bento Gonçalves
6	Brut	2.08	Bento Gonçalves
7	Brut	3.23	Garibaldi
8	Brut	2.96	Garibaldi
9	Brut	2.14	Garibaldi
10	Brut	26.26	Garibaldi
Mean Bruts		5.94	
Variation coefficient (%)		49.78	
11	Demi-Sec	6.42	Garibaldi
12	Demi-Sec	8.36	Garibaldi
13	Demi-Sec	14.31	Garibaldi
14	Demi-Sec	8.19	Garibaldi
15	Demi-Sec	9.22	Garibaldi
16	Demi-Sec	12.01	Garibaldi
17	Demi-Sec	13.42	Garibaldi
Mean Demi-Secs		10.72	
Variation coefficient (%)		7.59	

Table 2. Ethyl carbamate content in Sparkling wines from the Serra Gaúcha.

In this group the range was from 2.08 µg/L to 24.6 µg/L, with an average value of 7.60 µg/L. All samples were within the FAO limits and only one sample was over the EU limits.

As seen in Table 1, the average EC concentrations observed in the wines that are used as base for the Sparkling wines were 13.0 µg/L for Italian Riesling, 19.3 µg/L for Chardonnay and 7.44 µg/L for Pinot Blanc. These results are interesting in showing that the EC content for the

Sparkling wines is lower on average than those in the wines of origin. This situation is probably due to the consumption of some of the nitrogenous compounds still present in the wine of origin during the second fermentation, but could also indicate other production practices.

CONCLUSIONS

The EC content of Brazilian sparkling wines is generally within the accepted values for this compound in wines both in FAO and OMS regulations^{9,13}, but further controls should be established to improve the results for wines. The Sparkling wines presented lower levels of EC when compared with the wines produced of grapes of the same varieties, but there was no direct correlation of the products so this is an observation that needs further direct proof.

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REFERENCES

1. Nör Paz, I & I. Baldissierotto (1997) "A história da Estação Experimental de Viticultura e Enologia 1921-1990". Cadernos de Pesquisa da Universidade de Caxias do Sul **5**, N° 3. UCS, Caxias de Sul.
2. UVIBRA (1999) *Relatório anual da produção de Uvas do Rio Grande do Sul, 1999*. União dos Vitivinicultores do Brasil, Bento Gonçalves
3. Lapolli, J.N., L.M.R. de Mello, C. Trarbach, G. Brenner, A.N. Teixeira, R.W. Santiago & J.C. Comin (1995) "A competitividade da vitivinicultura brasileira. Análise Setorial e Programa de Ação com destaque para o Rio Grande do Sul". Banrisul/Embrapa, Porto Alegre.
4. Johnson, H (1986) "El Vino. Atlas Mundial de Vinos y Licores". 3ª Ed. Blume, Barcelona.
5. República Federativa do Brasil (1988) *Lei N° 7678. Dispõe sobre a produção, circulação e comercialização do vinho e derivados da uva e do vinho, e da outras providências. November 8, 1988. Art. 9, inc. 4.* (<http://www.agricultura.gov.br>).
6. Ough, S.C. (1976) *J. Agric. Food Chem.* **24**: 323-8.
7. Monteiro, F.F, K.E. Trousdale & F.L. Bisson (1989) *Amer. J. Enol. Vitic.* **40**: 1-8.
8. Mora de Orduña, R., S.Q. Liu, M.L. Patchett &

- G.J. Pilone (2000) *FEMS Microbiol. Letters* **183**: 331-5.
9. Bertrand, A (1997) El carbamato de etilo en los vinos. Observaciones sobre su origen. Posibilidades de disminuir su contenido. *XXII Congress de la Vigne et du Vin*. Buenos Aires, Argentina, págs. 2-13.
10. Liu, S.Q., G.G.Pritchard, M.J. Hardman & G.J. Pilone (1994) *Amer. J. Enol. Viticulture* **45**: 235-42.
11. Zimmerli, B. & J. Schlatter (1991) *Mutation Res.* **259**: 325-50.
12. Colborn, T., D. Dumanoski & J. Peterson Myers (1997) *O Futuro Roubado*. Editora L&PM, São Paulo.
13. de la Torre, M.C. (1997) *Analisis* **25**: 21-26.
14. OIV - Office International de la Vigne et du Vin (1996) *Recueil d'Analyses*. Dosage du carbamate d'ethyle dans les boissons alcoolisées. oeno/scma/96/43, Paris.