Growth behavior and volatile compound production by Brettanomyces bruxellensis in red wine

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GROWTH BEHAVIOUR AND VOLATILE COMPOUND PRODUCTION BY *BRETTANOMYCES BRUXELLENSIS* IN RED WINE

A study on "Brett character"

ŒNO 2007
8°Symposium International d' Œnologie de Bordeaux

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SCOPE OF THE WORK

Wine spoilage caused by the yeast *Brettanomyces bruxellensis* (anamorph of *Dekkera bruxellensis*) is nowadays a major problem for winemakers and interest in *B. bruxellensis* spoilage has resulted in increasingly frequent reports. Nevertheless causes and effects of this spoilage are not fully understood to date. This experimental work aims at:

- Studying the development of *B. bruxellensis* in wine as a function of some environmental parameters, notably pH, sugar content and the stage of winemaking at which spoilage takes place.
- Characterising volatile phenol production in relation to environmental factors and attempting to determine the population level required to trigger Brett character in wine.
- Understanding the complexity of Brett character by tracing a profile of volatile compound production in wine.

EXPERIMENTAL

Strains : three *B. bruxellensis* strains isolated from wine-related environments and belonging to the collection of the Faculté d'Œnologie de Bordeaux).

Culture:

YPG (pH 4.0) → Adaptation on EBB 4-6 days at 25 °C → $5\ 10^3$ cells ml^{-1} → Red wine, 28 days at 25 °C → Plate counts GC-MS analysis

Wine 1 collected before end of alcoholic fermentation was employed to simulate *B. bruxellensis* contamination during stuck alcoholic fermentation.

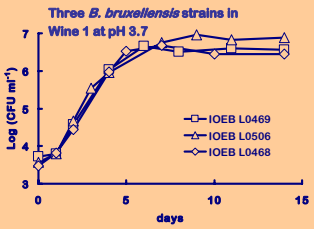
Wine 2 collected after malolactic fermentation.

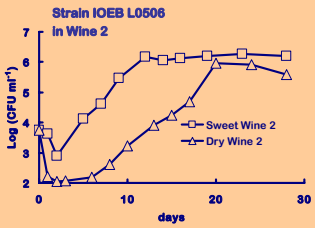
Adjustments were made to the pH and sugar concentration of the wines .

	Wine 1	Wine 2
Vintage	2006	2006
Grape variety	C. Sauvignon	Cabernet / Merlot
pH	3.70 3.50	3.70
Total sugars (g l^{-1})	20.0	1.3* 20.5§
Glucose (g l^{-1})	13.8	0.2* 5.8§
Fructose (g l^{-1})	5.8	0.2* 13.8§
Free SO_2 (mg l^{-1})	12	11
Ethanol (% v/v)	12.48 %	12.55 %

* *Dry;* § *Sweet*

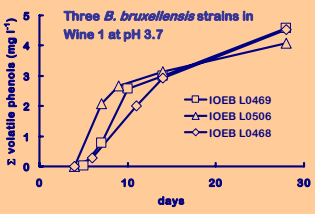
GROWTH

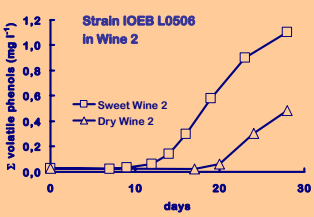




- **Wine 1:** growth was very rapid, with little differences among microbial strains and no effect of pH (results not shown).
- **Wine 2:** biomass levels were much lower, even when wine was supplemented with glucose and fructose.

VOLATILE PHENOLS

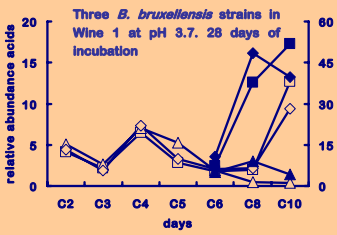




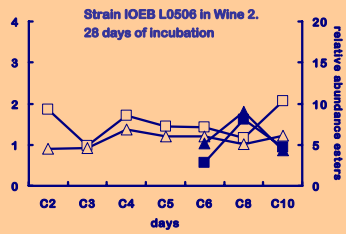
- Volatile phenol production was always characterized by an initial phase that displayed a high rate of synthesis, followed by a second phase of decline or arrest. This was particularly apparent in Wine 1.
- In Wine 1 the correction of pH to 3.5 resulted in an inhibition of volatile phenol synthesis (results not shown).
- Off flavour synthesis was always triggered by population levels between 10^5 and 10^6 cells ml^{-1}, in correspondence of the end of exponential phase.
- The 4-ethylphenol / 4-ethylguaiacol concentration ratio varied between 25:1 (in Wine 1 at pH 3.7) to 11:1 (in Wine 2) and was influenced by pH. This was not influenced by the strain employed and remained constant throughout growth.

OTHER VOLATILE COMPOUNDS

Carboxylic acids (between C_2 and C_{10}) and their ethylic esters (between C_6 and C_{10}) were produced in significant amounts.



C_2-C_{10} acids: (□) IOEB L0468, (◇) IOEB L0469, (△) IOEB L0506
C_6-C_{10} esters: (■) IOEB L0468, (◆) IOEB L0469, (▲) IOEB L0506



C_2-C_{10} acids: (△) dry, (□) sweet
C_6-C_{10} esters: (▲) dry, (■)sweet

The metabolic profiles varied according to each strain and set of conditions.

- **In wine 1** at pH 3.7 up to 0.8 g l^{-1} acetic acid and 1.0 mg l^{-1} isobutyric acid and 2.0 mg l^{-1} isovaleric acid (associated to rancid sensory notes) were obtained. The correction of pH to 3.5 resulted in an inhibition of volatile compound synthesis, this was more marked for strains IOEB L0468 and IOEB L0469 (results not shown).
- Strains IOEB L0468 and L0469 produced short chain fatty acids (up to 0.7 mg l^{-1} hexanoic and octanoic acid and 0.4 mg l^{-1} decanoic acid) that are known to be AF inhibitors in wine.
- **In wine 2** *B. bruxellensis* mainly synthesized ethyl-esters (up to 0.8 mg l^{-1} ethyl-octanoate). These compounds possess "fruity" characteristics but disagreeable "soapy" notes as well.

CONCLUSIONS

- Our data draw attention to the potential of *Brettanomyces* contamination during AF: at this stage, wine is still relatively rich in nutrient factors and growth and off-flavour production are remarkably fast.
- The two-phase reaction course of volatile phenol synthesis is likely to be influenced by the progressive depletion of free hydroxycinnamic acids but this is not the only factor involved.
- For volatile phenol synthesis to be triggered a certain population level is always required.
- Several distinct Brett characters might arise in wine depending on the presence of many different compounds, including 4-ethylphenol, 4-ethylguaiacol, acetic acid and other carboxylic acids and their ethyl-esters.
- Brett character appears to be the result of the interaction among several factors which include microbial strain, wine pH and sugar content and the winemaking stage at which the spoilage occurs.

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