Exploring Douro Biome Terroir Complexity: Constructing Quality Port Wines using Selected Endogenous Non-Saccharomyces

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Introduction

Port wine is a fortified wine produced in the Douro Appellation (Portugal) having intrinsic aroma characteristics such as the terroir particularities, varieties and winemaking procedures, including the yeast strains.

Traditionally, Port wines have been produced by spontaneous fermentation, incorporating the diversity and complexity of the biome terroir.

Recent advances (Goddard M.R., 2008) have shown non-*Saccharomyces* species (NSAC) to dominate the early phase of fermentation, establishing these yeasts as "key-players", contributing metabolic complexity to Port Wine.

Aims

- Study of biome terroir Douro
- Isolation & characterization of dominant strains
- Quality evaluation of Port produced using best NSAC strains
- Top 2 Douro grape varieties: Touriga Nacional (TN) & Touriga Franca (TF)
- Sensory Evaluation and Aroma Profiling

Methodology

Overview

- Fast track yeast screening of spontaneous fermentations 2012-2016 – "**Best Dominant Strains**"
- Characterization using stress factor tests.
- Biomass production & micro-scale fermentations – **Best Enological Strains**
- Sensory Evaluation – Port wines
- Wine Volatile Profiling – SPME-GCxGC-ToFMS

Yeast Isolation, Characterization

- Cryopreserved must samples 2012-2016
  - Sampling mid/end-fermentation – "dominant strains"
  - Hedonic sensory screening – "wild" fermented Ports
- Lysine culture medium plating
- Colony morphology
- ITS-PCR identification
- PCR specie specific primer confirmation
- Phenotypic characterization – stress factor tests

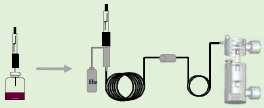
Sensorial analysis

- Sensory panel comprised of 8 Symington expert tasters + 3 commercial tasters from Ângelo Coimbra.
- Wines scored and ranked using hedonic preference (0-20).
- FIZZ software statistical evaluation.

Port Production

- Grapes from **2 vineyard parcels** (1 TN & 1 TF)
- Port production used **8 yeasts consortium:**
  - 5 Non-*Saccharomyces* Douro (2 LT; 2MP; 1HU);
  - 1 mixture (LT+MP+HU);
  - 2 Commercial Non-Saccharomyces (MP.com; LT.com).

Volatile composition



- SPME-GC×GC-ToFMS Pegasus 4D.
- PCA of Volatile Organic Compounds.

Results

- **Yeast Profiling – 500 NSAC strains**
- **Yeast Characterization – 96 strains from 3 principal species**
- **Best Strain Selection: 15 strains; 5 from each principle specie**
- **Fermentation – 15 best strains**
- **Wine Volatile Profiling – best 5 stains (2 KT; 2MP; 1HU) + Mix (KT+MP+HU)**

Yeast population profiling

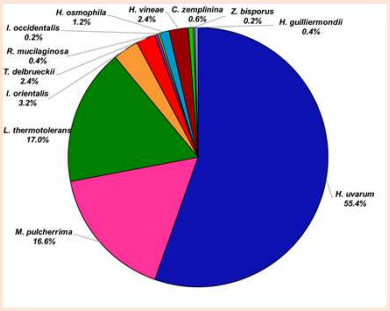
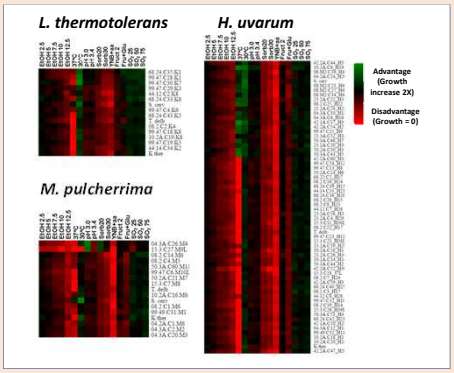


Figure 1: Clonal diversity within NSAC yeast species (RAPD-PCR)

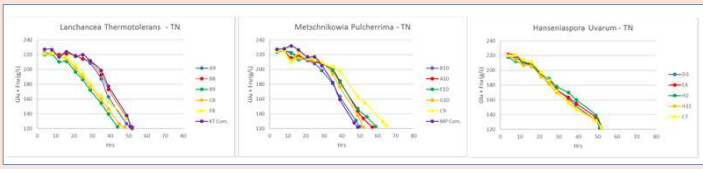
- **500 NSAC strains isolated from 12 species**
- **3 species dominate: HU; LT; MP**

Phenotypic Characterization



- **5 Best/most diverse strains were selected for biomass production**
- **Selection criteria: Enological performance**

Fermentation Kinetics



- 5 strains of each species
- MP with greatest variance
- Fortification ~120g/L G+F

Hedonic Preference

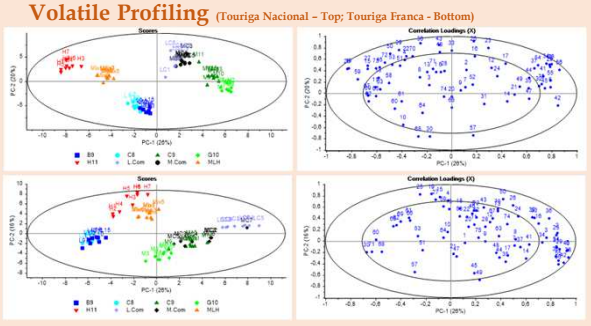
Rank	T. Nacional - "LT"	
	LT Strain	Score (0-20)
1	B9	14.74 ± 1.88
2	C8	14.57 ± 2.24
3	A9	14.16 ± 2.10
4	B8	14.06 ± 2.45
5	F8	13.61 ± 2.24
6	Commercial	13.00 ± 4.11

Rank	T. Nacional - "MP"	
	MP Strain	Score (0-20)
1	G10	14.97 ± 1.90
2	B10	14.43 ± 2.45
3	A10	14.10 ± 2.28
4	C9	13.95 ± 2.02
5	E10	13.61 ± 3.27
6	Commercial	13.60 ± 2.45

Rank	T. Nacional - "HU"	
	HU Strain	Score (0-20)
1	H11	13.07 ± 3.16
2	E4	12.90 ± 2.70
3	C7	7.80 ± 3.82
4	H2	6.14 ± 4.03
5	D3	4.17 ± 3.10
6		

- Wines fortified with EtOH
- Random order presentation
- Douro strains preferred on ranking.
- 5 Best strains selected for volatile profiling (2LT; 2MP; 1HU)

Volatile Profiling (Touriga Nacional – Top; Touriga Franca - Bottom)



- Douro *Lanchancea thermotolerans* (B9 & C8) very different to commercial strain (L.Com)
- Commercial *Lanchancea thermotolerans* volatile profile more similar to MP
- Douro *Metschnikowia pulcherrima* (C9 & G10) similar in profile to Commercial MP (**M.Com**)
- Mix (MLH) clustered between NSAC Douro (MP + LT + HU)

nº	VOC	nº	VOC
1	Acetaldehyde	39	Ethyl hexanoate
2	3-Methylbutanal	40	Diethyl butanedioate
3	Hexanal	41	Ethyl octanoate
4	Benzaldehyde	42	Ethyl phenyllactate
5	Benzeneacetaldehyde	43	2-Phenylethyl acetate
6	Nonanal	44	Ethyl nonanoate
7	Decanal	45	Methyl decanoate
8	Undecanal	46	1-HO-2,6,6-trime.ph.-2-ene-propanoate
9	Dodecanal	47	Ethyl decanoate
10	1-Propanol	48	Ethyl dodecanoate
11	2-Methyl-1-propanol	49	Ethyl Miristoleate
12	1-Butanol	50	Isopropyl Myristate
13	3-Pentene-2-ol	51	Ethyl pentadecanoate
14	3-Methyl-1-butanol	52	Methyl hexadecanoate
15	1-Pentanol	53	Ethyl hexadecanoate
16	2,3-Butanediol	54	3-Methylbutanoic acid
17	3-Methyl-1-pentanol	55	Hexanoic acid
18	3-Hexen-1-ol	56	Octanoic acid
19	1-Hexanol	57	Decanoic acid
20	1-Octen-3-ol	58	α-Pinene
21	2-Ethyl-1-hexanol	59	β-Pinene
22	Benzyl Alcohol	60	α-Terpinene
23	1-Octanol	61	Limonene
24	Benzeneethanol	62	α-Terpinolene
25	1-Nonanol	63	Linalool
26	1-Decanol	64	m/z 69, 81, 71, 95
27	Ethyl propanoate	65	α-Terpineol
28	2-Methylpropyl ethanoate	66	Geraniol
29	Ethyl butanoate	67	Nerol
30	Ethyl Lactate	68	Dodecanyl acetate
31	Ethyl 2-methylbutanoate	69	4,11-Safosadene
32	Ethyl 3-methylbutanoate	70	Farnesol
33	Isoamyl acetate	71	Ethylene
34	Ethyl hexanoate	72	β-Damascenone
35	Ethyl 3-hexenoate	73	β-Ionol
36	Hexyl acetate	74	α-iso-methyl ionone
37	Ethyl heptanoate	75	2,6-bis(1,1-dimethylethyl)-phenol
38	Methyl octanoate		

VOC NSAC DOURO: HU; LT; MP

Descriptive Character – NSAC Aroma Modulation – TN Ports

(Professional Taster)

- ***Lanchancea thermotolerans*** – Aroma: **fresh, floral, citrus bergamot**; Palate: **good acidic freshness** with long finish **with creamy lactate notes**
- ***Metschnikowia pulcherima*** – Aroma: **floral with blackberry fruit notes**; Palate: **black fruit with cacao**.
- ***Hanseniaspora uvarum*** – Aroma: **caramel and chocolate** with hints of acetaldehyde and ethyl acetate; Palate: **chocolate and caramel**.
- **LT + MP + HU** – Complex Aroma: **citrus fruity, floral & chocolate**; Palate: **good fresh acidity, black fruit with chocolate**

Conclusions

- **NSAC Douro yeast strains add complexity modulating Port wine aroma**
- **LT contributes citrus floral notes, and acidic freshness (lactic acid)**
- **Best HU strain produces significantly lower [ethyl acetate]**
- **HU contributes chocolate/caramel aroma/taste**
- **MP favours fruity profile**
- **LT+MP+HU "Douro Terroir Blend" preferred with greatest complexity**
- **Indigenous Port wine Terroir yeast strains, which respect regional typicity, present enormous oenological potential**

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