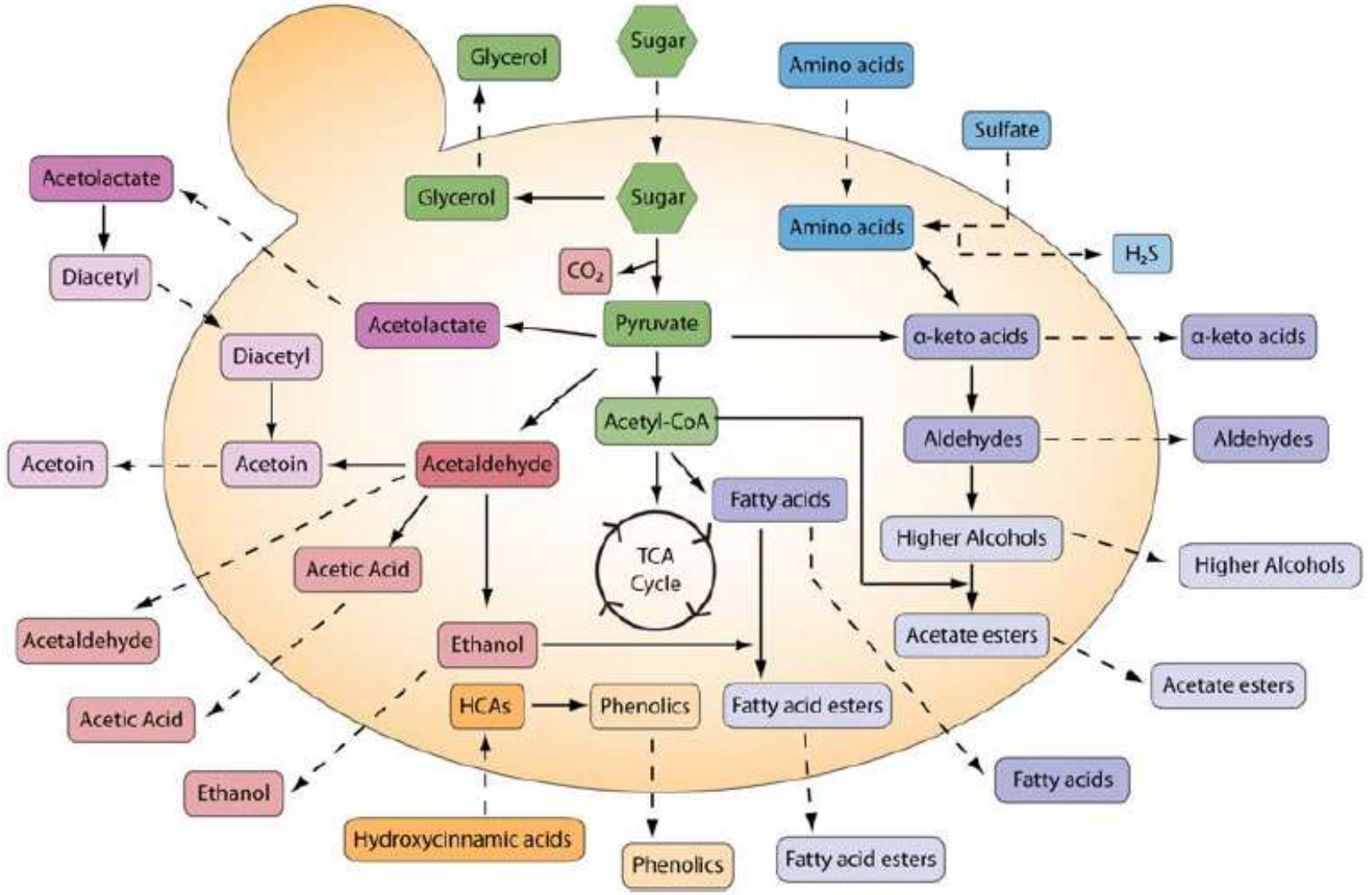
Yeasts and aroma

Nils Arneborg, FOOD-KU
na@food.ku.dk

UNIVERSITY OF COPENHAGEN

Aroma compound metabolism in yeast



Dzialo et al., 2017

Based on the results from a Master thesis by:

Mirian S.K. Jensen

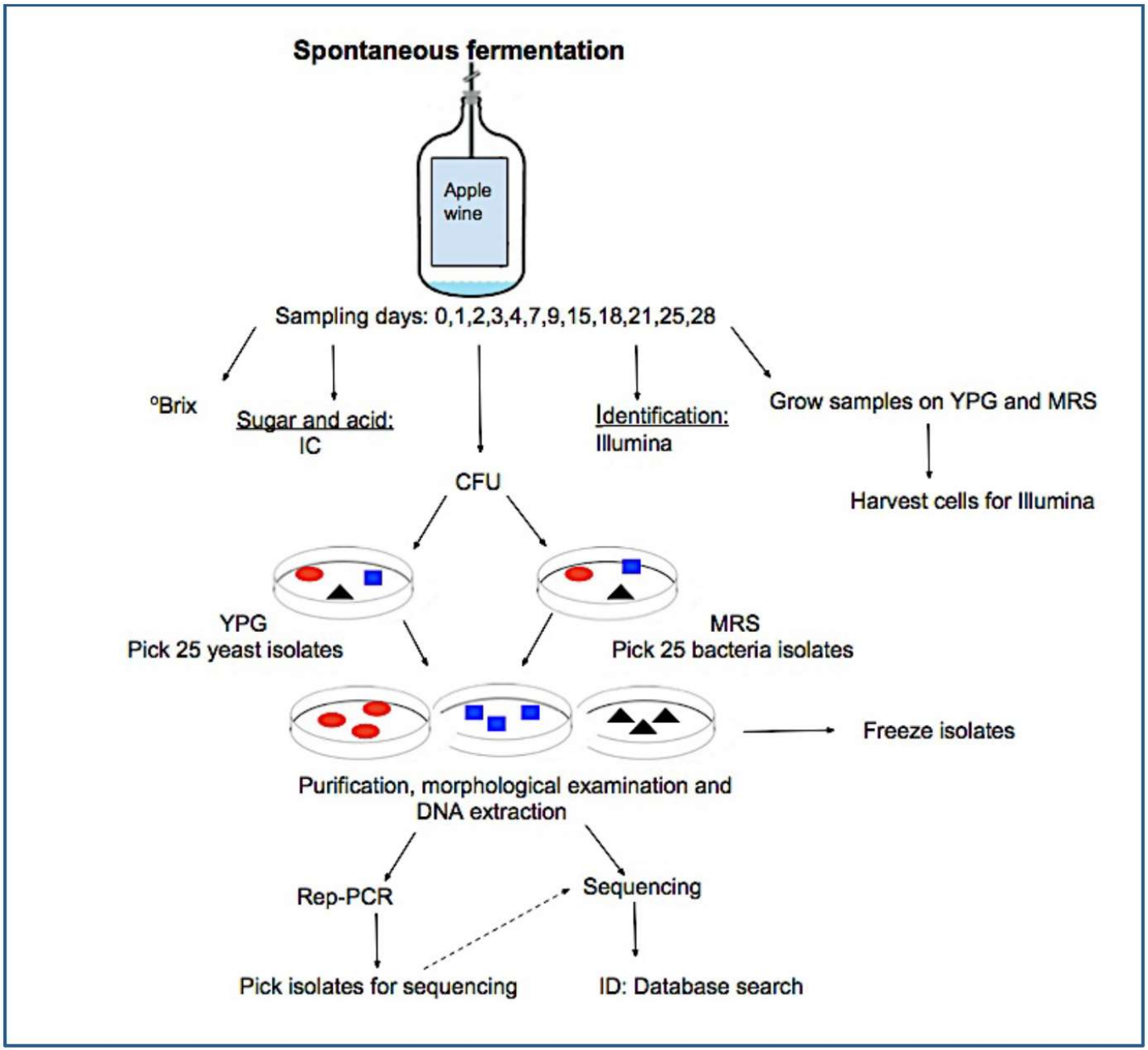
Outline of work

1. Cryo-must production. 16 °Brix
2. Spontaneous fermentation of apple wine from cryo-must. 16 °C
3. Illuminating the succession of indigenous yeast and bacteria.
4. Purification and identification of indigenous yeast and bacteria isolates.
5. Start fermentation series with identified isolates, in sequential and co-inoculations with commercial yeast strain as standard. 16 °Brix, 16 °C
6. Investigate fermentation potential and impact on aroma profile of isolates in single, sequential and co-inoculations (CFU, HPLC, IC, DHS-GC-MS).

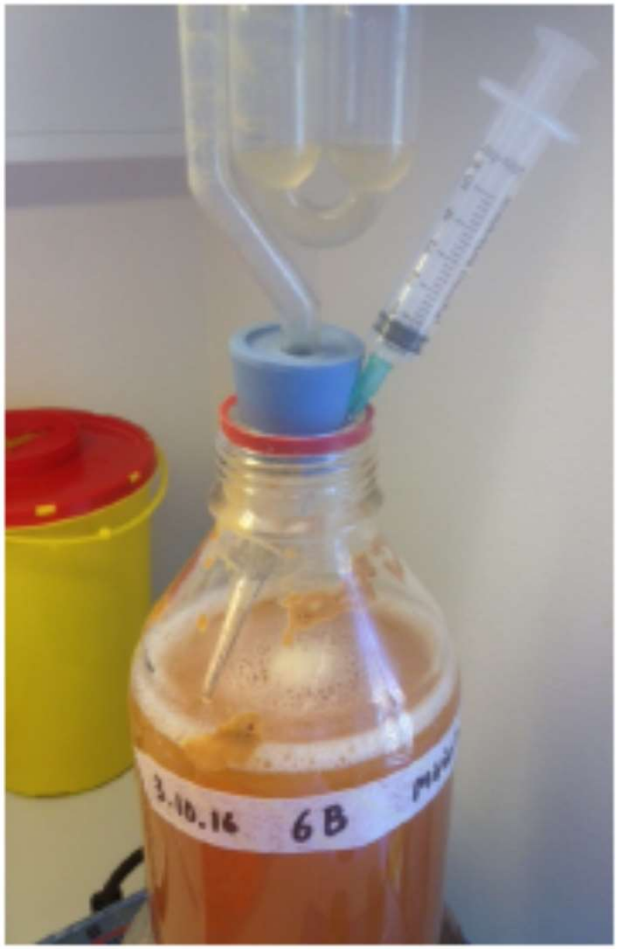
Cryo-must

Picture 2.1: Ice cider, produced with cryo-concentration from bulk freezing, relying on outside temperatures (Picture from Eve's Cidery, 2017).

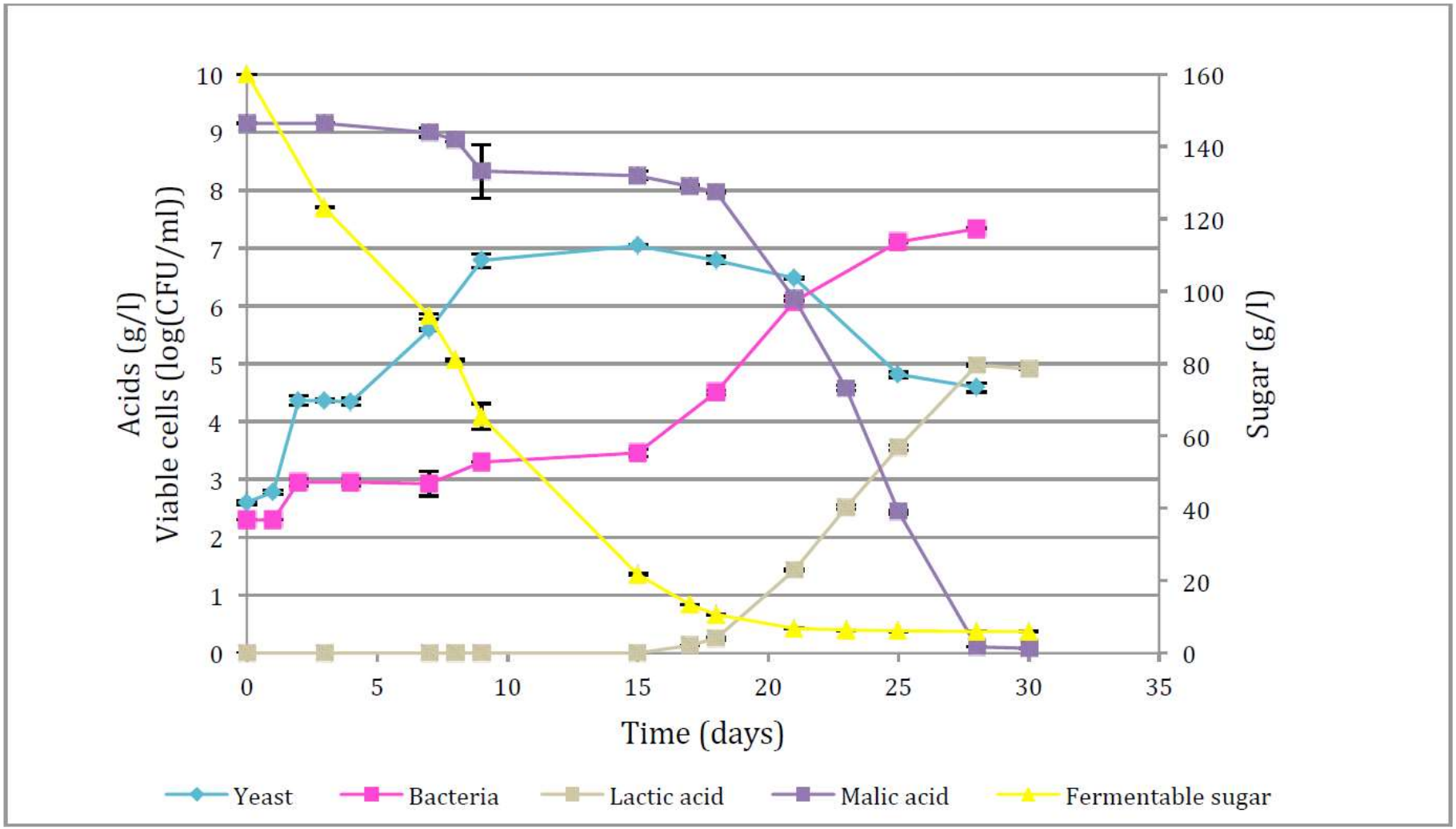
Experimental setup



Fermentation setup



Spontaneous fermentation



Yeasts and bacteria isolated and identified from spontaneous fermentation

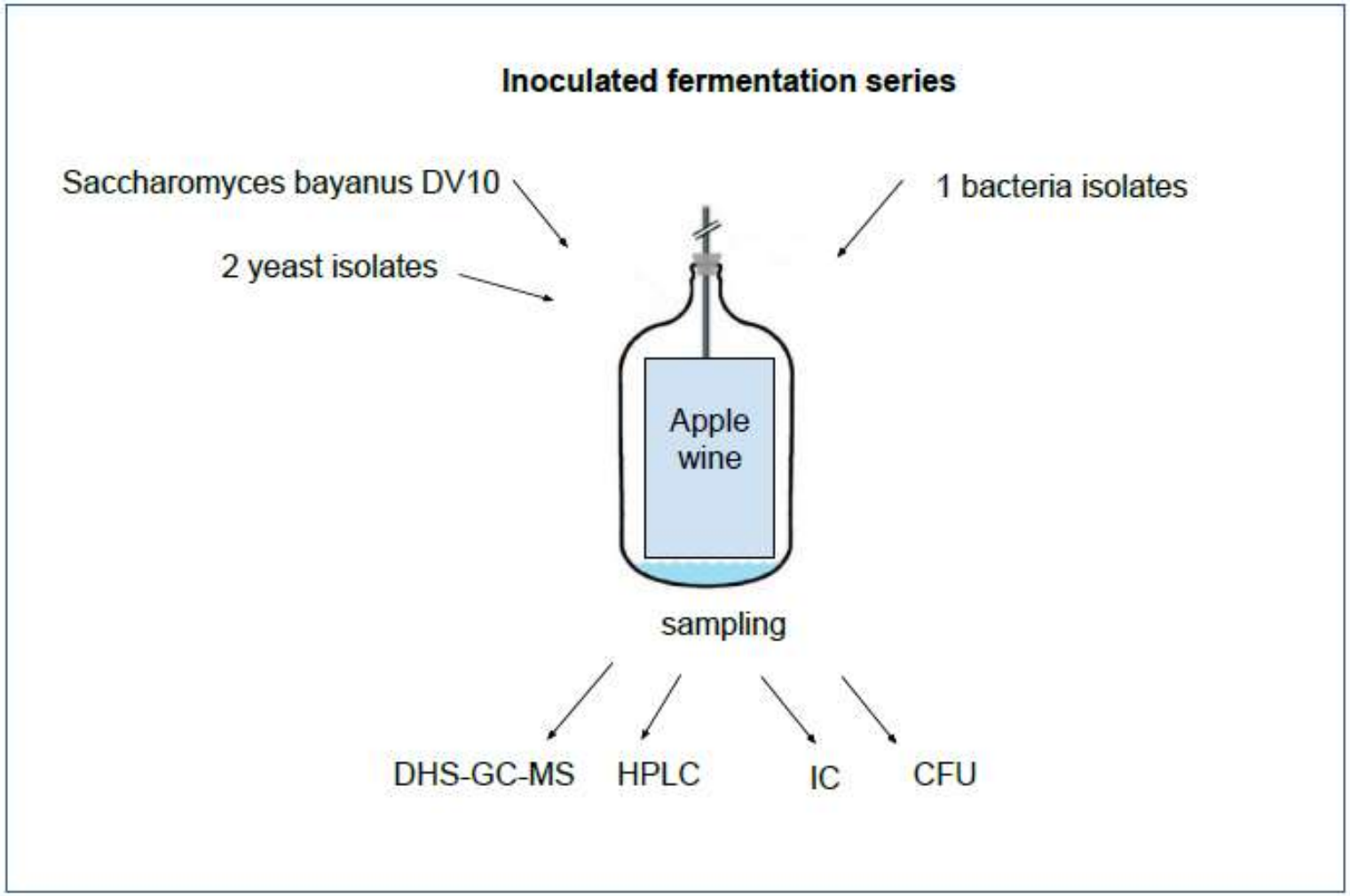
Yeast day 3

rep-PCR group	Isolates per group	Id no. of isolates sequenced	Blastet on	Blast identification	% identity	% of yeast population
1	2	20	NL1 + NL4	*Metschnikowia sp.*	100	8,33 %
2	4	12	NL1 + NL4	*Hanseniaspora sp.*	100	16,67 %
3	2	24	NL1 + NL4	*Torulaspora delbrueckii*	100	8,33 %
4	2	4		Not a pure culture		
5	8	14	NL4	*Pichia membranifaciens*	100	33,3 %
6	2	22	NL1 + NL4	*Lindnera misumaiensis*	100	8,33 %
7	2	8	NL1 + NL4	*Metschnikowia sp.*	99 - 100	8,33 %
8	2	10	NL1 + NL4	*Metschnikowia sp.*	100	8 %

Bacteria day 18

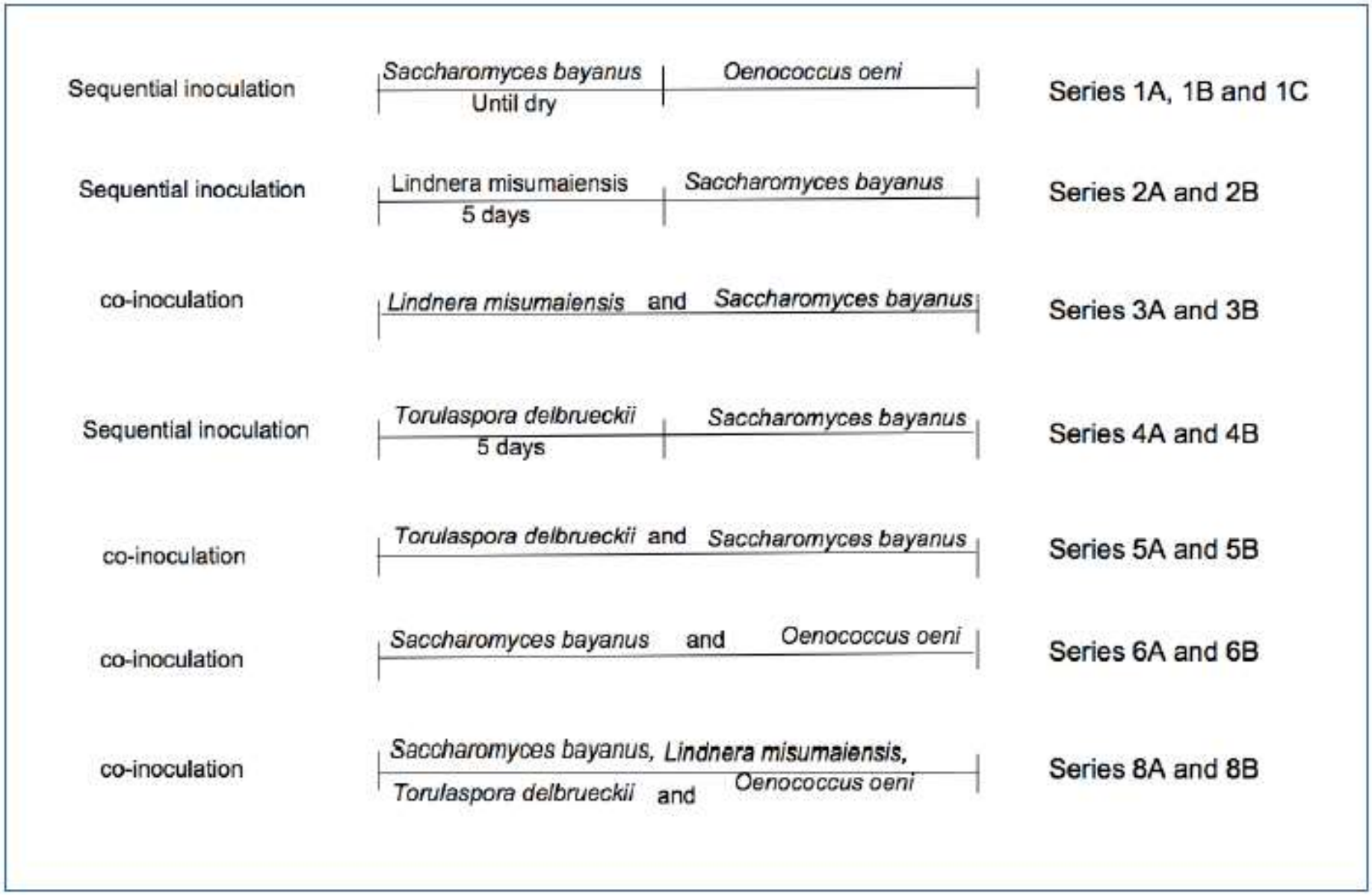
rep-PCR group	Isolates per group	Id no. of isolates sequenced	Blastet on	Blast identification	% identity	% of yeast population
1	14	11	27F	*Oenococcus oeni*	100	56.0%
		3	27F	*Oenococcus oeni*	98.6	
		18	800R	*Oenococcus oeni*	100	
2	11	8	27F	*Oenococcus oeni*	100	44.0%
		23	27F	*Oenococcus oeni*	99 - 100	
		14	27F	*Oenococcus oeni*	99 - 100	

Experimental setup

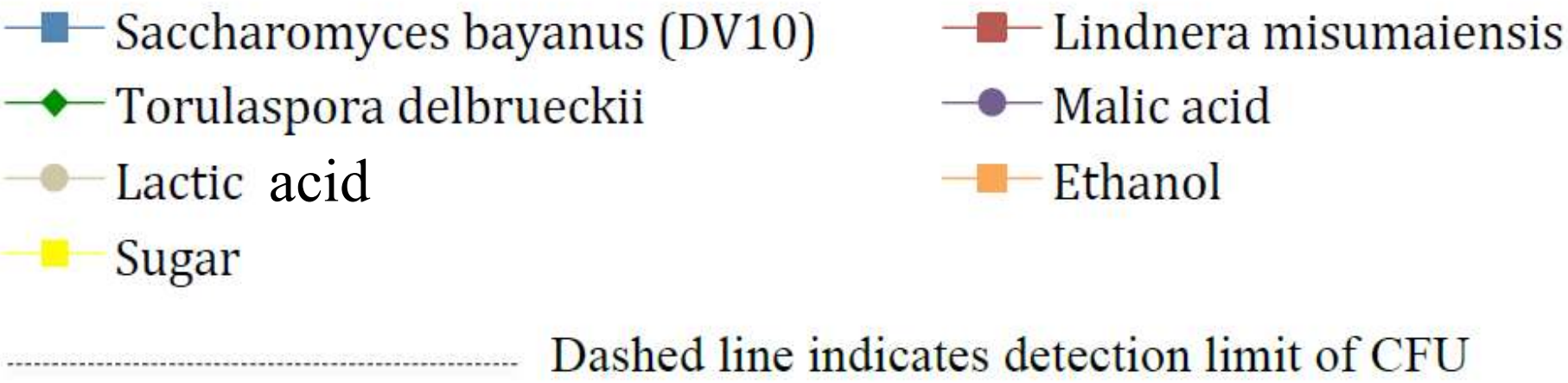


Inoculated fermentation series

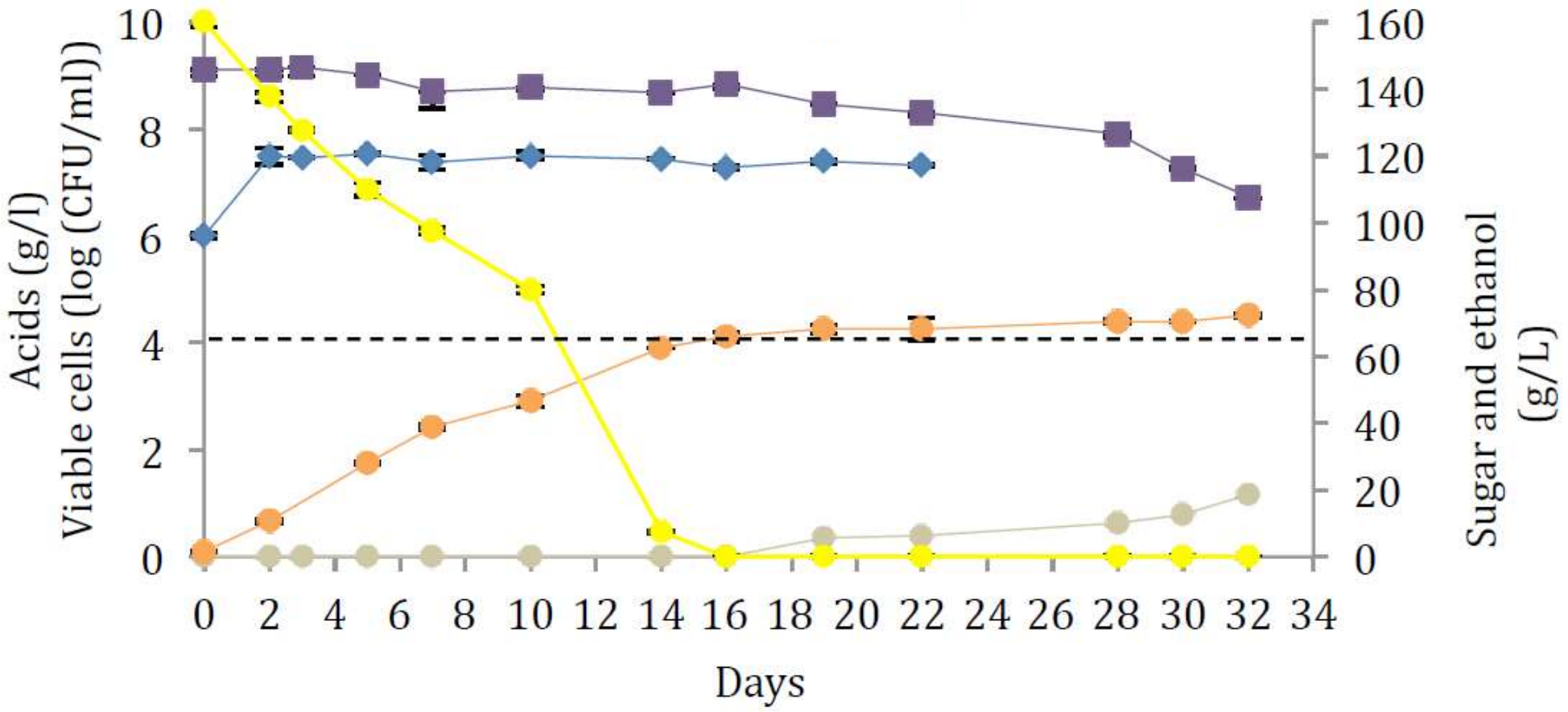
Experimental plan



Symbol explanation (for next 5 slides)

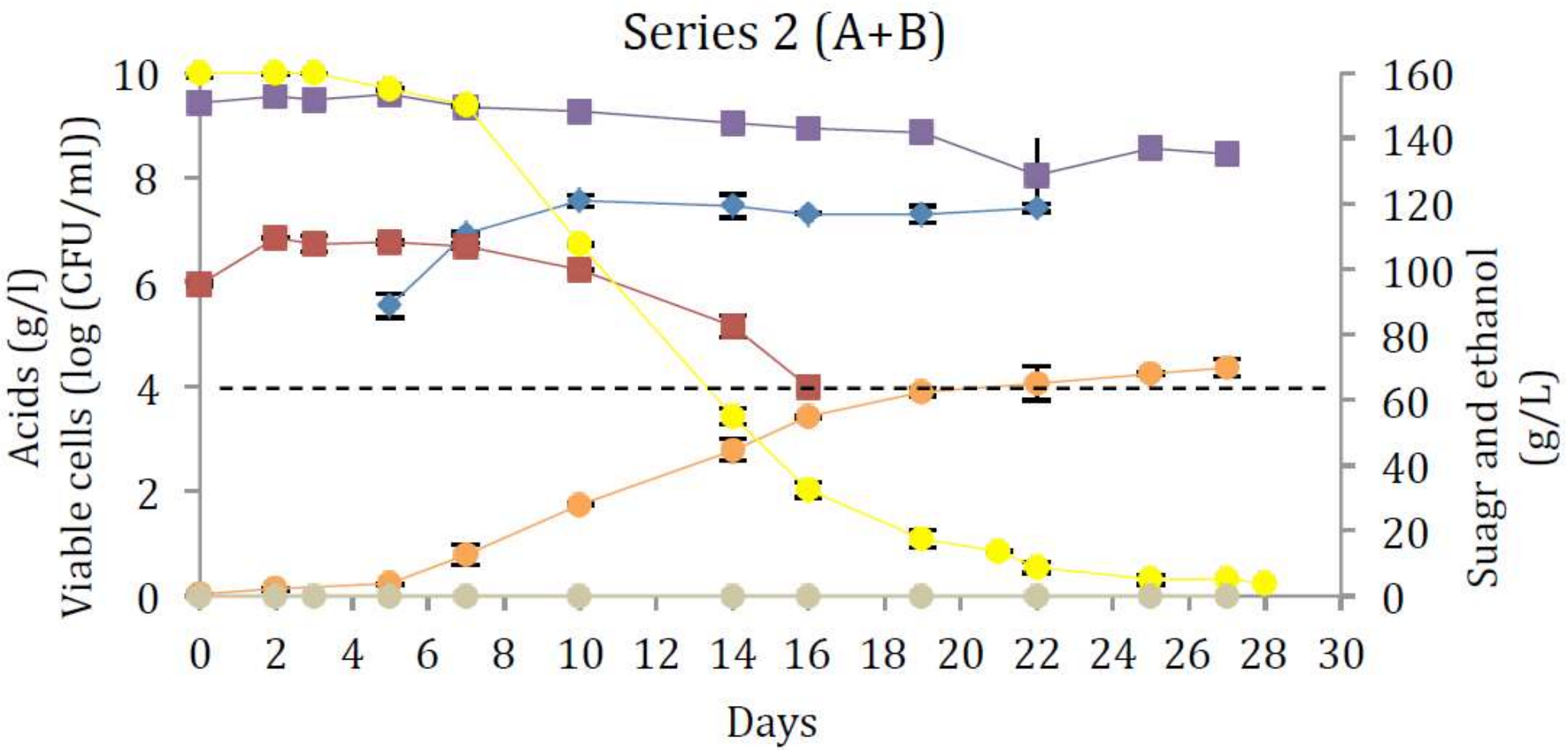


Control fermentation
Sb-Oo



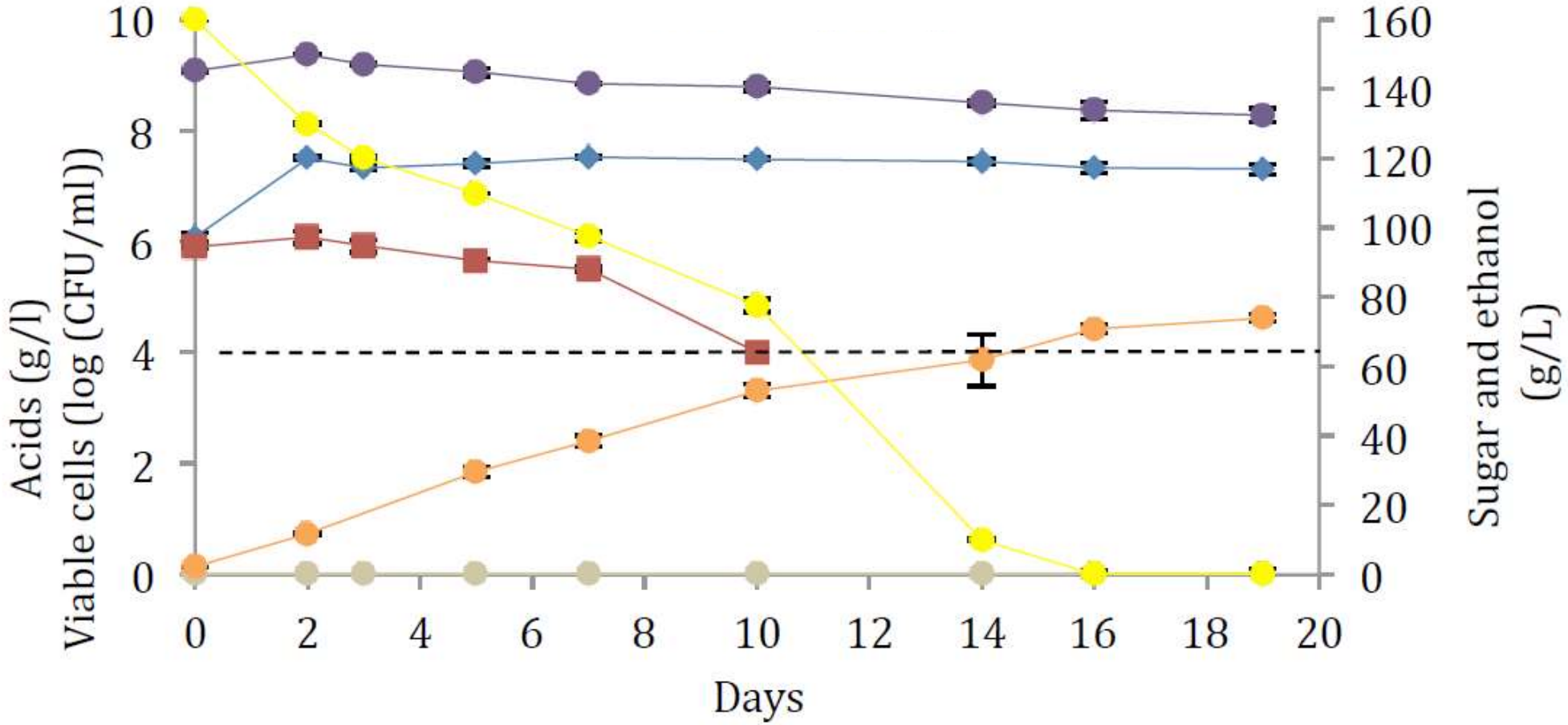
Sequential fermentation
Lm-Sb

Yeast ID: WLN agar



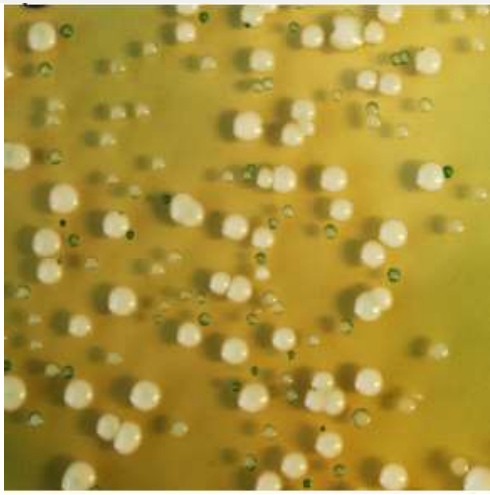
Co-inoculation fermentation

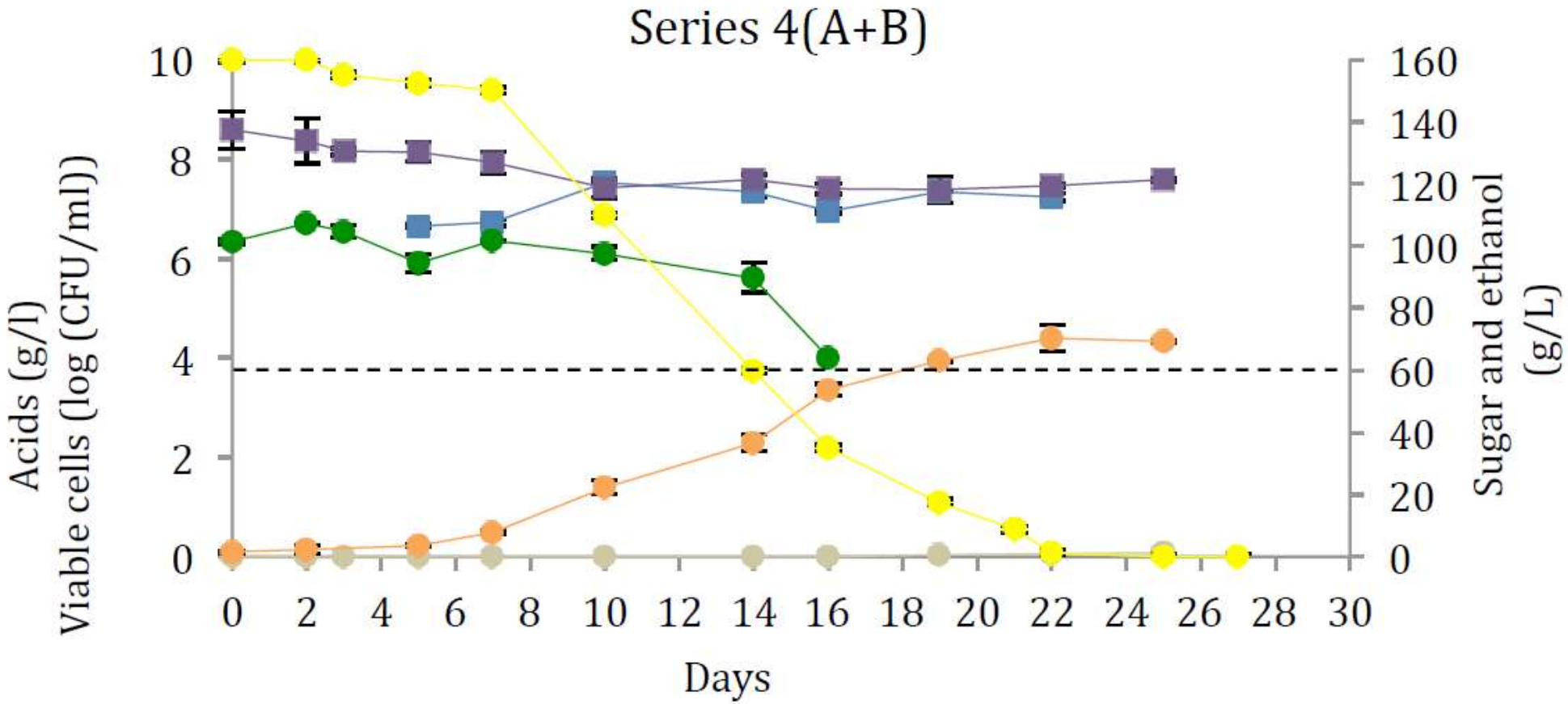
Lm+Sb



Sequential fermenation
Td-Sb

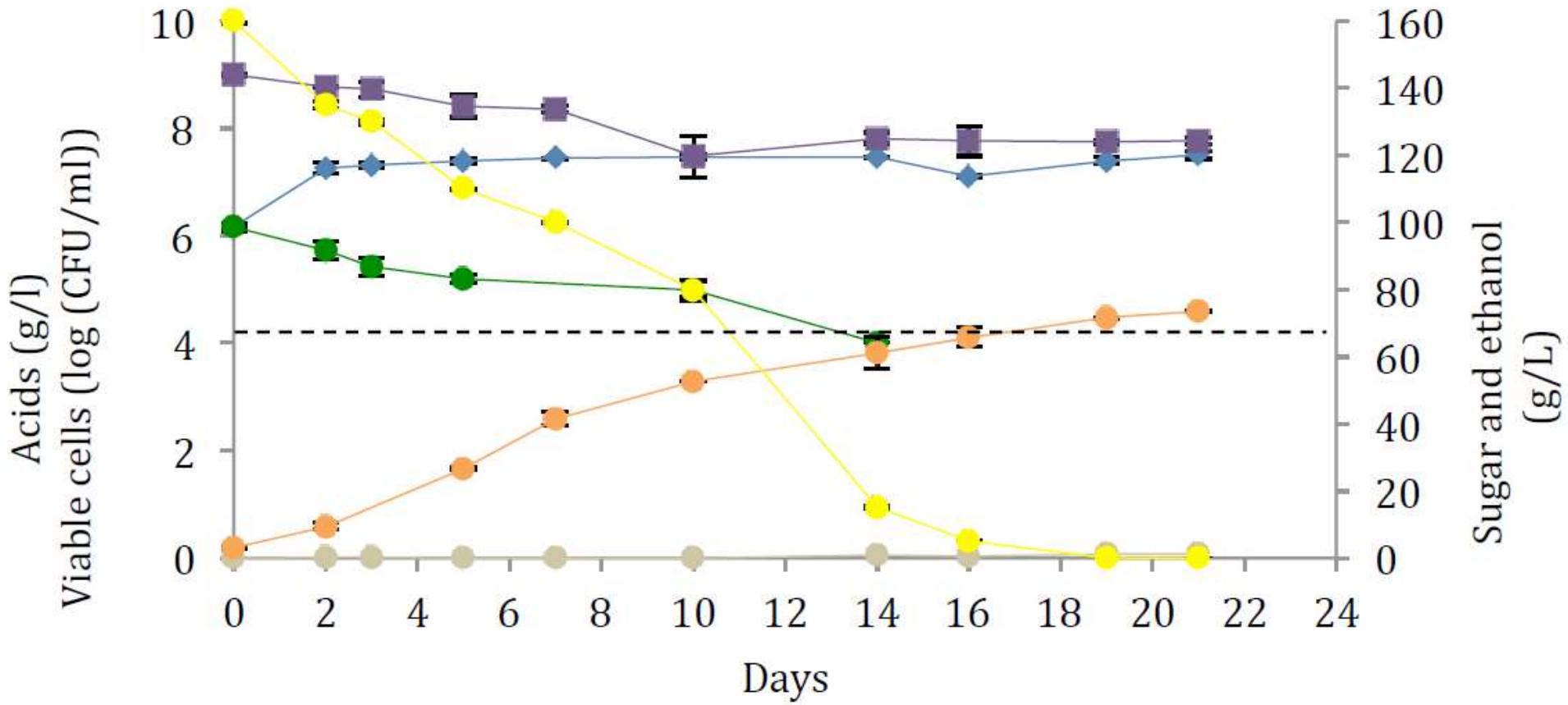
Yeast ID: WLN agar



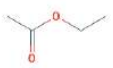
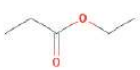
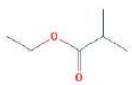
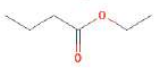
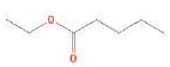
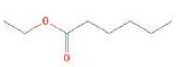
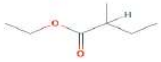
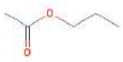


Co-inoculation fermentation

Td+Sb

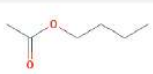
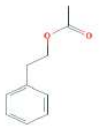
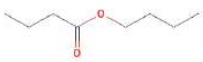
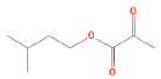
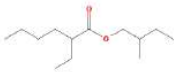


Aroma compounds in finished apple wines

	Compound	Molecular formula	Structure	*Sb*[A]	*Sb-Oo*	*Lm-Sb*	*Lm+Sb*	*Td-Sb*	*Td+Sb*	*Sb+Oo*	*Sb+Lm+ Td+Oo*	Significance[B]
Esters												
	Ethyl esters											
1	Ethyl acetate	$C_4H_8O_2$		56300	57759	60430	58498	71634	65681	40758	67111	ns
2	Ethyl propanoate	$C_5H_{10}O_2$		13505	14292	6603	13223	11200	10636	9204	9790	ns
3	Ethyl 2-methylpropanoate	$C_6H_{12}O_2$		379	532	267	354	199	289	292	369	ns
4	Ethyl butanoate	$C_6H_{12}O_2$		32722	33964	37755	34230	32313	30290	21114	28026	ns
5	Ethyl pentanoate	$C_7H_{14}O_2$		158	191	177	144	113	120	71	159	ns
6	Ethyl hexanoate[F]	$C_8H_{16}O_2$		61885 a	52664 ab	66005 a	61029 a	59448 a	54778 ab	33205 b	54468 ab	*
7	Ethyl octanoate[F]	$C_{10}H_{20}O_2$		50604	38782	47681	40813	40632	19184	23081	34272	ns
8	Ethyl decanoate	$C_{12}H_{24}O_2$		35762 a	21556 bc	33394 ab	26830 abc	26504 abc	29280 ab	6024 d	14170 cd	***
9	Ethyl dodecanoate[F]	$C_{14}H_{28}O_2$		9349	5063	10000	7553	8869	7723	490	3422	ns
10	Ethyl 2-methylbutanoate	$C_7H_{14}O_2$		251 b	380 a	222 b	213 b	159 b	178 b	280 ab	276 ab	**
	Acetates											
1	Propyl acetate	$C_5H_{10}O_2$		4715 bc	4036 bc	5543 b	5029 bc	9663 a	6358 b	2549 c	3681 bc	***
2	2-Methylpropyl acetate	$C_6H_{12}O_2$		5051 cd	4384 cd	15933 a	6088 c	8910 b	6219 c	2929 d	5648 c	***

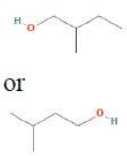
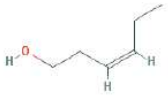
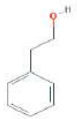
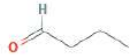
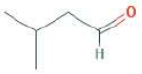
(continued)

Table 5.10 continued

	Compound	Molecular formula	Structure	*Sb*[A]	*Sb-Oo*	*Lm-Sb*	*Lm+Sb*	*Td-Sb*	*Td+Sb*	*Sb+Oo*	*Sb+Lm+ Td+Oo*	Significance[B]
3	Butyl acetate	$C_6H_{12}O_2$		93973 a	81225 ab	69014 ab	90267 ab	67941 ab	63613 ab	56725 bc	22010 c	***
4	3-Methylbutyl acetate	$C_7H_{14}O_2$		0 c	0 c	0 c	0 c	114672 a	0 c	68316 b	98061 a	***
5	Pentyl acetate	$C_7H_{14}O_2$		1926 a	1551 abc	1265 abc	1701 ab	1603 abc	1118 bcd	1043 cd	538 d	***
6	3-Hexenyl acetate [C]	$C_8H_{14}O_2$		1130 bc	873 cd	1662 ab	1085 bc	2018 a	1273 bc	417 d	807 cd	***
7	2-Phenylethyl acetate	$C_{10}H_{12}O_2$		2666 b	2854 ab	5439 a	3248 ab	3535 ab	3100 ab	1267 b	2444 b	*
	Other esters											
1	Butyl butanoate[E]	$C_8H_{16}O_2$		0	0	0	0	0	0	0	0	ns
2	3-Methylbutyl 2-oxopropanoate[F]	$C_8H_{14}O_3$		81326 a	79713 ab	114428 a	85373 a	93672 a	84893 ab	34521 b	83137 ab	*
3	2-Methylbutyl 2-ethylhexanoate[F]	$C_{13}H_{26}$		537 ab	209 bc	613 a	330 abc	290 abc	305 abc	24 c	137 c	**
4	3-Methylbutyl decanoate[F]	$C_{15}H_{30}O_2$		246	147	341	225	233	169	0	75	ns
Higher alcohols												
1	2-Methyl-1-propanol	$C_4H_{10}O$		24909	21214	33880	24236	20048	24146	13124	20612	ns
2	1-Butanol	$C_5H_{12}O$		35513	32841	31733	35416	25329	30880	17458	25468	ns

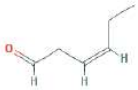
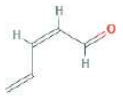
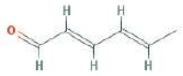
(continued)

Table 5.10 continued

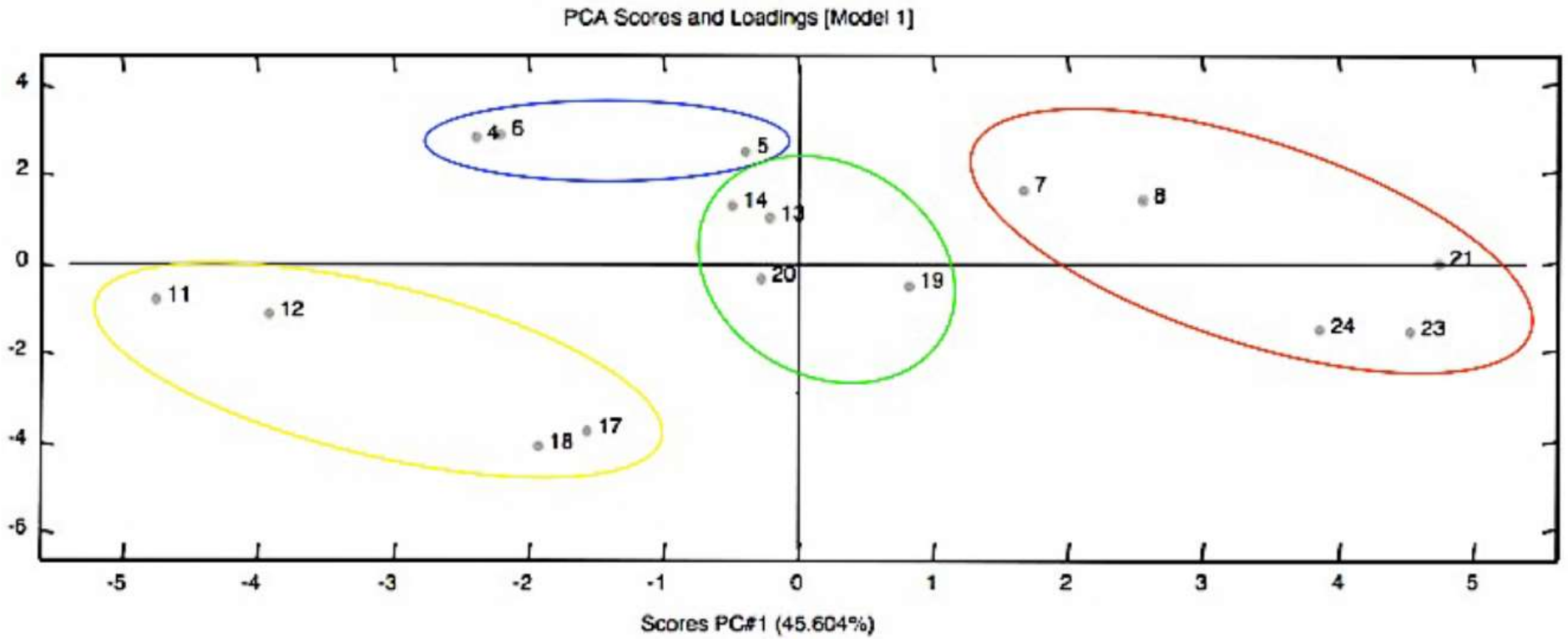
	Compound	Molecular formula	Structure	*Sb*[A]	*Sb-Oo*	*Lm-Sb*	*Lm+Sb*	*Td-Sb*	*Td+Sb*	*Sb+Oo*	*Sb+Lm+ Td+Oo*	Significance[B]
3	2/3 Methyl-1-butanol [D]	$C_5H_{12}O$		171256	167132	154927	168220	147594	159398	84279	150928	ns
4	1-Pentanol	$C_5H_{12}O$		1025	995	1006	1139	1051	1032	51611	853	ns
5	1-Hexanol	$C_6H_{14}O$		0 b	0 b	0 b	0 b	5358 a	0 b	0 b	0 b	***
6	3-Hexen-1-ol[F]	$C_6H_{12}O$		1231	1310	1144	1312	1233	1214	957	1098	ns
7	6-Methylhept-5-en-2-ol	$C_8H_{16}O$		78	93	76	76	102	83	39	98	ns
8	Octanol	$C_8H_{18}O$		142	251	133	150	109	131	143	265	ns
9	2-Phenylethanol	$C_8H_{10}O$		5034	6931	5543	5040	4975	6589	4634	6963	ns
10	1-propanol[E]	C_3H_8O		0	0	0	0	0	0	0	0	ns
Aldehydes												
1	Butanal	C_4H_8O		85	22	17	15	6	13	9	14	ns
2	3-Methylbutanal	$C_5H_{10}O$		692	223	250	344	297	436	149	185	ns
3	Hexanal	$C_6H_{12}O$		261	0	0	20665	10	0	148	83	ns

(continued)

Table 5.10 continued

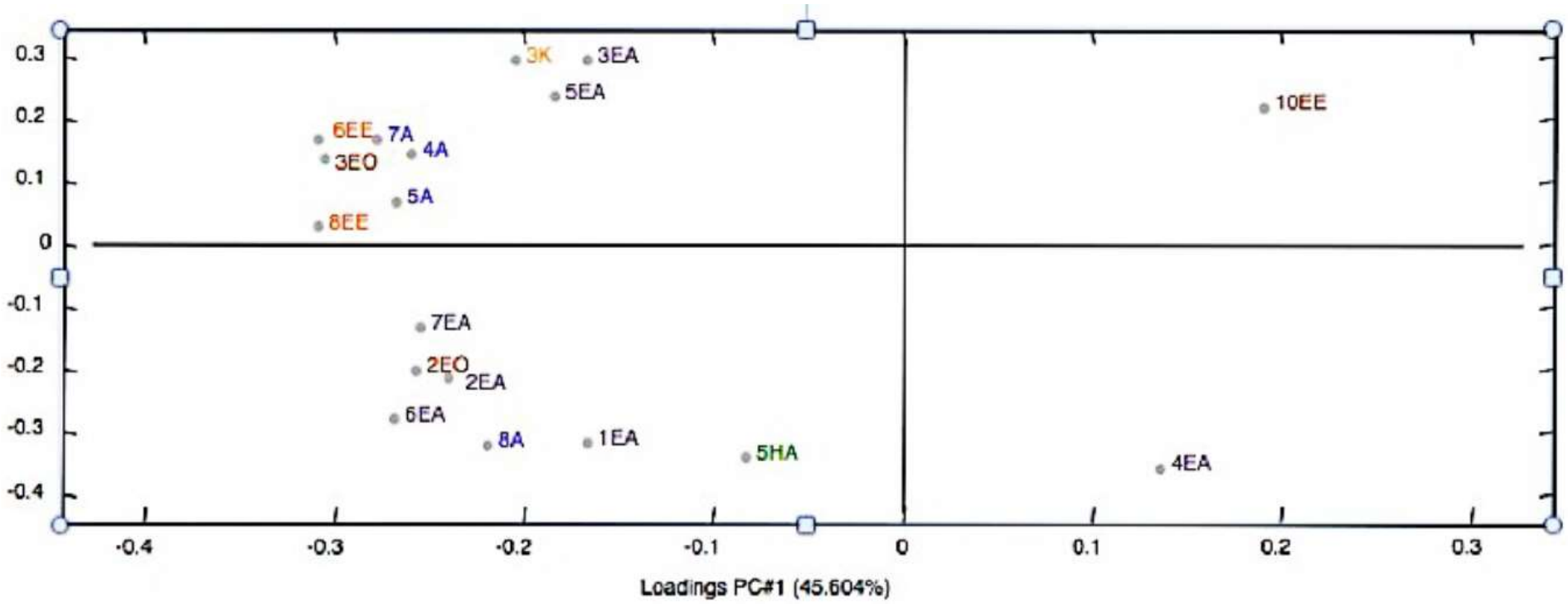
	Compound	Molecular formula	Structure	*Sb*[A]	*Sb-Oo*	*Lm-Sb*	*Lm+Sb*	*Td-Sb*	*Td+Sb*	*Sb+Oo*	*Sb+Lm+Td+Oo*	Significance[B]
4	3-Hexenal	$C_6H_{10}O$		96 bc	125 ab	146 a	96 bc	75 c	81 bc	6 d	0 d	***
5	Octanal	$C_8H_{16}O$		51 a	26 ab	42 ab	38 ab	48 ab	38 ab	12 b	16 ab	*
6	(2E)-2,4-Pentadienal	C_5H_6O		546	620	553	526	413	502	245	429	ns
7	Nonanal	$C_9H_{18}O$		298 a	169 ab	314 a	246 ab	203 ab	190 ab	88 b	160 ab	*
8	2,4-Hexadineal	C_6H_8O		0 b	0 b	14 a	0 b	14 a	5 ab	0 b	0 b	**
9	Benzaldehyde	C_7H_6O		159	80	79	99	71	76	47	80	ns
Ketones												
1	1-Hydroxy-2-butanone	$C_4H_8O_2$		495	523	397	474	221	392	290	367	ns
2	6-Methyl-5-Hepten-2-one	$C_8H_{14}O$		195	215	130	151	54	101	34	79	ns
3	1-Phenylethanone	C_8H_8O		222 a	133 ab	164 a	146 ab	128 ab	144 ab	51 b	125 ab	**
Terpinolene												
1	4(8)-Menthene	$C_{10}H_{18}$		51	57	63	61	70	50	7	28	ns

PCA plot (scores) of aroma samples from finished apple wines



4,5 and 6: End of primary fermentation with *Saccharomyces bayanus* (series 1A, 1B and 1C). 7 and 8: End of primary and "MLF" in sequential fermentation with *Saccharomyces bayanus* and *Oenococcus oeni* (series 1A and 1B). 11 and 12: Finished sequential fermentation with *Lindnera misumaiensis* and *Saccharomyces bayanus* (series 2A and 2B). 13 and 14: Finished co-inoculation fermentation with *Lindnera misumaiensis* and *Saccharomyces bayanus* (series 3A and 3B). 17 and 18: Finished sequential fermentation with *Torulaspora delbrueckii* and *Saccharomyces bayanus* (series 4A and 4B). 19 and 20: Finished co-inoculation fermentation with *Torulaspora delbrueckii* and *Saccharomyces bayanus* (series 5A and 5B). 21 and 22: Finished co-inoculation fermentation with *Saccharomyces bayanus* and *Oenococcus oeni* (series 6A and 6B). 23 and 24: Finished co-inoculation fermentation with *Lindnera misumaiensis, Torulaspora delbrueckii, Saccharomyces bayanus* and *Oenococcus oeni* (series 8A and 8B).

PCA plot (loadings) of aroma samples from finished apple wines



Aroma compounds significantly different between samples are plotted with group code (color and letters) and numbers. Group code: HA = higher alcohol (green), EE = ethyl ester (red), EA = acetate ester (purple), EO = other esters (brown), A = aldehyde (blue), and K = ketone (orange). Number in front of letters refers to the compounds listed in table 5.10.

Conclusion

Torulaspora delbrueckii

Sequential inoculations schemes:

- Production of 3-methylbutyl acetate and 1-hexanol

Sequential- or co-inoculation schemes:

- Production of 2,4-hexadineal
- Increase of 2-phenylethyl acetate
- Increase of propyl-acetate

Lindnera misumaiensis

Sequential fermentation schemes:

- Production of 2,4-hexadineal
- Increase in 3-hexenal and 2-methylpropyl acetate

Sequential- or co-inoculation schemes:

- Increase in 2-phenylethyl acetate

Oenococcus oeni

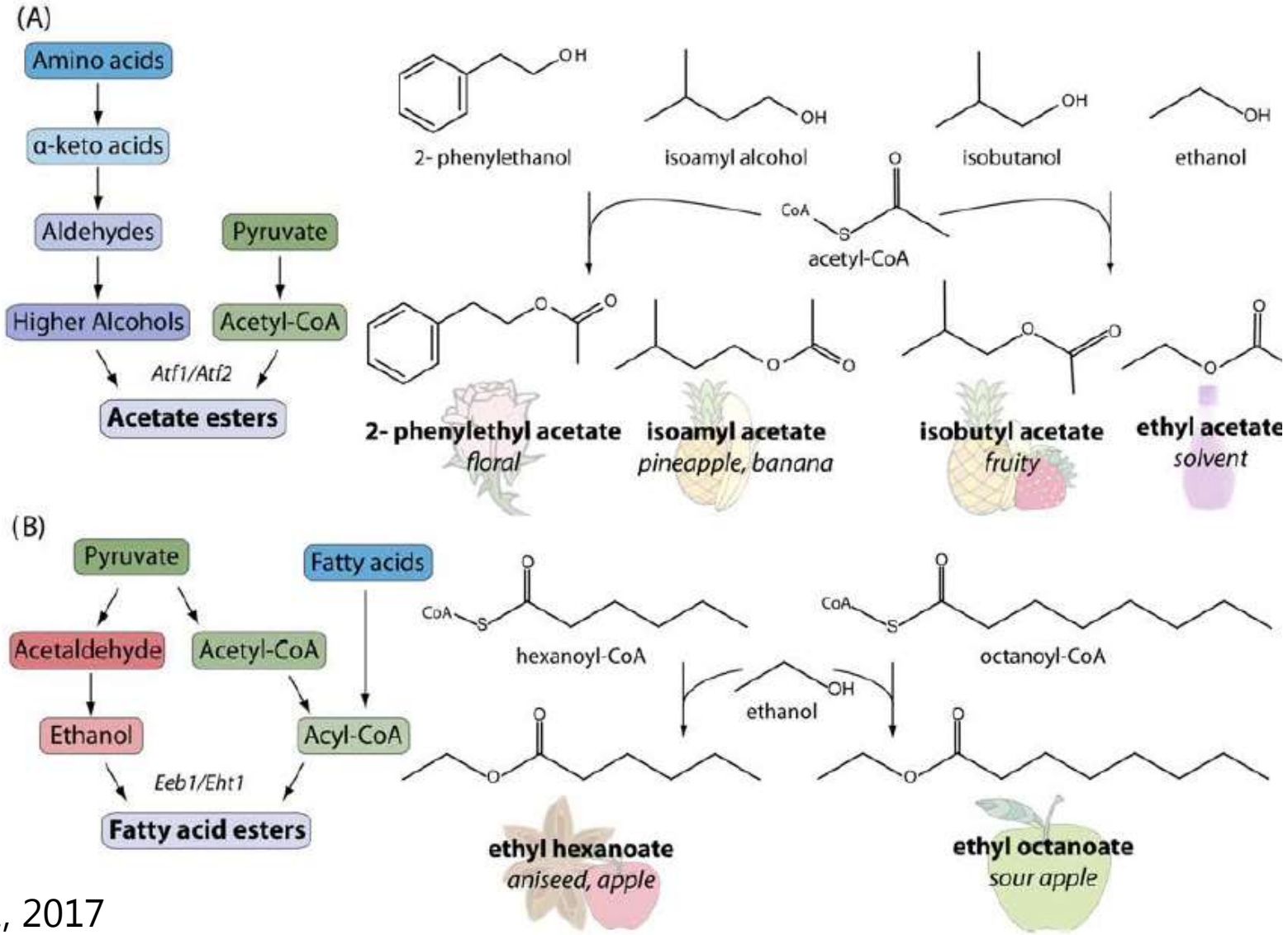
Co-inoculation schemes:

- Production of 3-methylbutyl acetate
- Decrease of 3-methylbutyl 2-oxopropanoate and 3-hexenal

Sequential- or co-inoculation schemes:

- Increase in ethyl 2-methylbutanoate
- Decrease in ethyl hexanoate ,ethyl decanoate, octanal, nonanal and 2-methylbutyl 2-ethylhexanoate

Ester synthesis in yeast



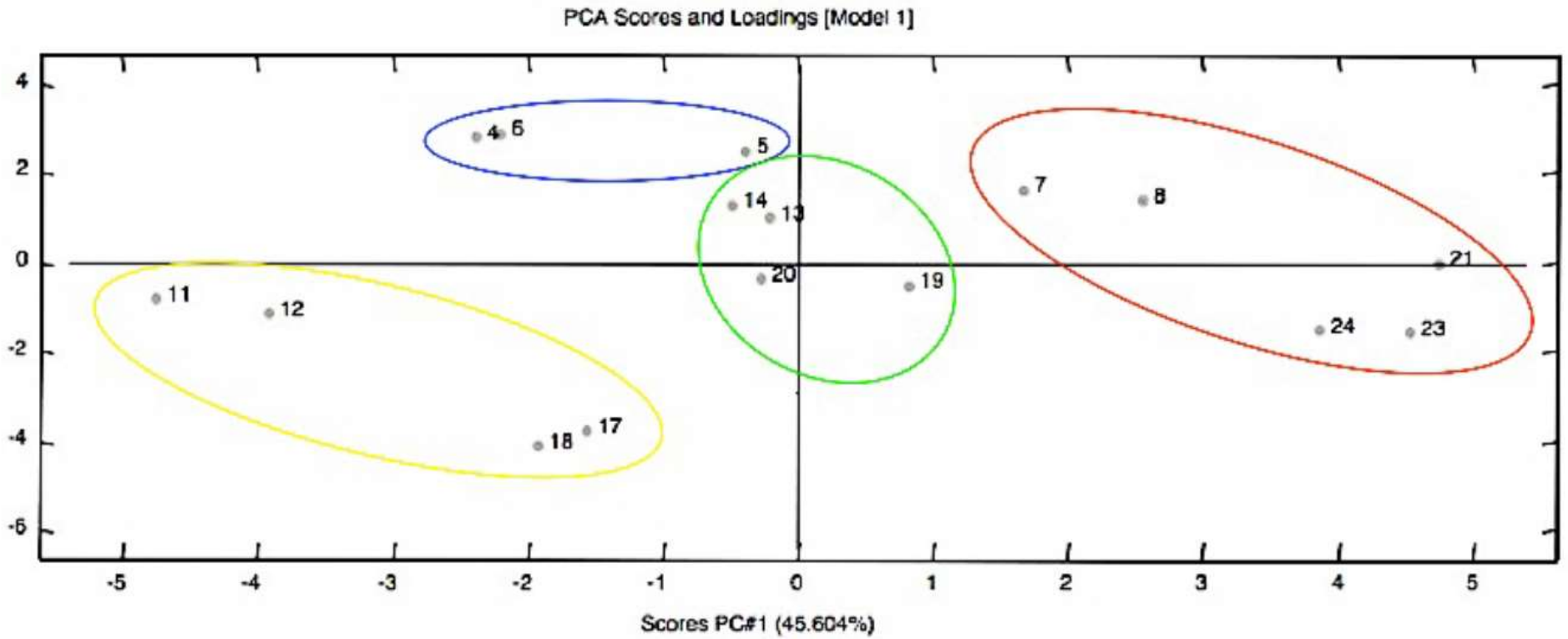
Dzialo et al., 2017

Table 1. Esters, higher alcohols and other aroma and flavour compounds commonly found in wine (Soles et al. 1982, Etiévant 1991, Fleet and Heard 1993, Martineau et al. 1995, Guth 1997, Lambrechts and Pretorius 2000, Swiegers and Pretorius 2005, Siebert et al. 2005, Smyth 2005).

Compound	Concentration in wine (mg/L)	Aroma threshold (mg/L)	Aroma descriptor
Ethyl acetate	22.5–63.5	7.5*	VA, nail polish, fruity
Isoamyl acetate	0.1–3.4	0.03*	Banana, pear
2-Phenylethyl acetate	0–18.5	0.25*	Flowery, rose, fruity
Isobutyl acetate	0.01–1.6	1.6****	Banana, fruity
Hexyl acetate	0–4.8	0.7**	Sweet, perfume
Ethyl butanoate	0.01–1.8	0.02*	Floral, fruity
Ethyl hexanoate	0.03–3.4	0.05*	Green apple
Ethyl octanoate	0.05–3.8	0.02*	Sweet soap
Ethyl decanoate	0–2.1	0.2*****	Floral, soap
Propanol	9.0–68	500**	Pungent, harsh
Butanol	0.5–8.5	150*	Fusel, spiritous
Isobutanol	9.0–174	40*	Fusel, spiritous
Isoamyl alcohol	6.0–490	30*	Harsh, nail polish
Hexanol	0.3–12.0	4**	Green, grass
2-Phenylethyl alcohol	4.0–197	10*	Floral, rose
Acetic acid	100–1150	280*	VA, vinegar
Acetaldehyde	10–75	100**	Sherry, nutty, bruised apple
Diacetyl	<5	0.2** / 2.8***	Buttery
Glycerol	5–14 g/L	5.2 g/L**	Odourless (slightly sweet taste)
Linalool	0.0017–0.010	0.0015******/0.025*****	Rose
Geraniol	0.001–0.044	5******/30*	Rose-like
Citronellol	0.015–0.042	8******/100*	Citronella
2-acetyl-1-pyrroline (ACPY)	Trace	0.0001******	Mousy
2-acetyltetrahydropyridine (ACPTY)	0.0048–0.1	0.0016******	Mousy
4-ethylphenol	0.012–6.5	0.14*/0.6***	Medicinal, barnyard
4-ethyl guaiacol	0.001–0.44	0.033*/0.11***	Phenolic, sweet
4-vinyl phenol	0.04–0.45	0.02******	phamaceutical
4-vinyl guaiacol	0.0014–0.71	10******	Clove-like, phenolic

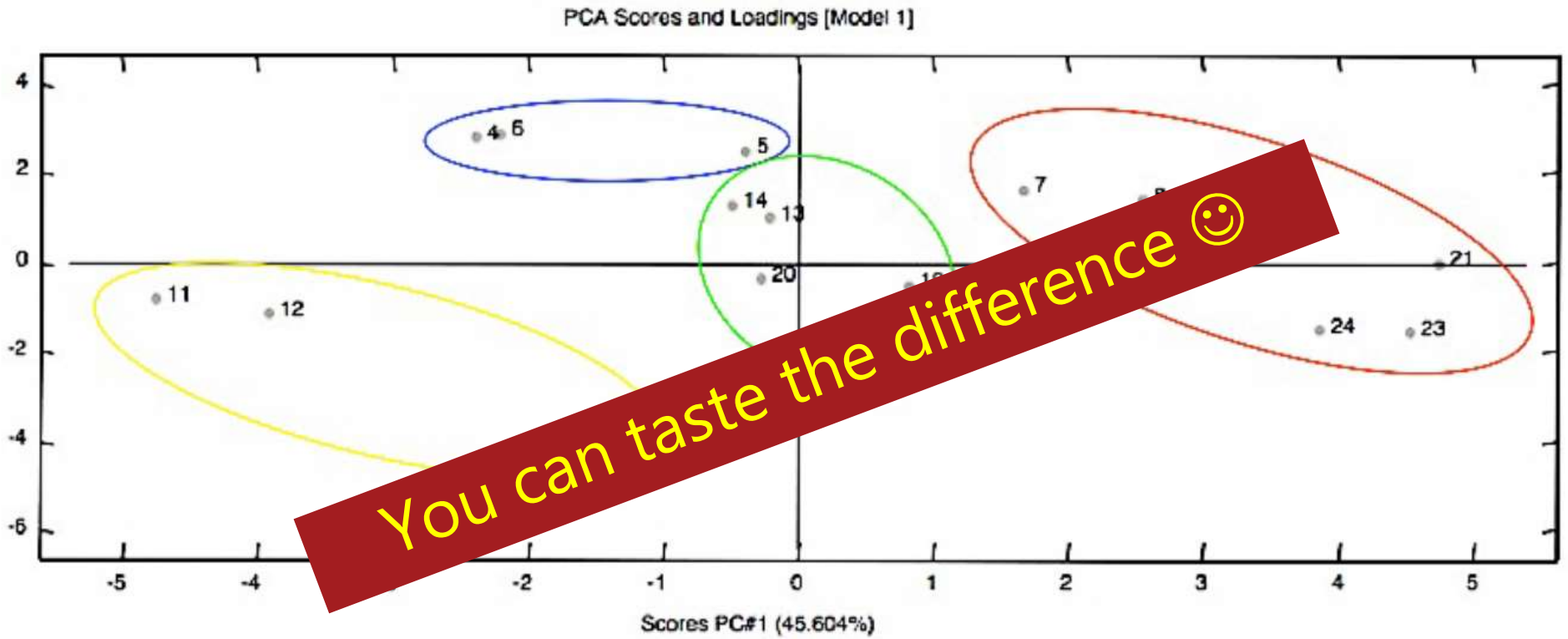
* 10% ethanol, ** wine, *** red wine,**** beer, ***** synthetic wine, ****** water

PCA plot (scores) of aroma samples from finished apple wines



4,5 and 6: End of primary fermentation with *Saccharomyces bayanus* (series 1A, 1B and 1C). 7 and 8: End of primary and "MLF" in sequential fermentation with *Saccharomyces bayanus* and *Oenococcus oeni* (series 1A and 1B). 11 and 12: Finished sequential fermentation with *Lindnera misumaiensis* and *Saccharomyces bayanus* (series 2A and 2B). 13 and 14: Finished co-inoculation fermentation with *Lindnera misumaiensis* and *Saccharomyces bayanus* (series 3A and 3B). 17 and 18: Finished sequential fermentation with *Torulaspora delbrueckii* and *Saccharomyces bayanus* (series 4A and 4B). 19 and 20: Finished co-inoculation fermentation with *Torulaspora delbrueckii* and *Saccharomyces bayanus* (series 5A and 5B). 21 and 22: Finished co-inoculation fermentation with *Saccharomyces bayanus* and *Oenococcus oeni* (series 6A and 6B). 23 and 24: Finished co-inoculation fermentation with *Lindnera misumaiensis, Torulaspora delbrueckii, Saccharomyces bayanus* and *Oenococcus oeni* (series 8A and 8B).

PCA plot (scores) of aroma samples from finished apple wines



4,5 and 6: End of primary fermentation with *Saccharomyces bayanus* (series 1A, 1B and 1C). 7 and 8: End of primary and "MLF" in sequential fermentation with *Saccharomyces bayanus* and *Oenococcus oeni* (series 1A and 1B). 11 and 12: Finished sequential fermentation with *Lindnera misumaiensis* and *Saccharomyces bayanus* (series 2A and 2B). 13 and 14: Finished co-inoculation fermentation with *Lindnera misumaiensis* and *Saccharomyces bayanus* (series 3A and 3B). 17 and 18: Finished sequential fermentation with *Torulaspora delbrueckii* and *Saccharomyces bayanus* (series 4A and 4B). 19 and 20: Finished co-inoculation fermentation with *Torulaspora delbrueckii* and *Saccharomyces bayanus* (series 5A and 5B). 21 and 22: Finished co-inoculation fermentation with *Saccharomyces bayanus* and *Oenococcus oeni* (series 6A and 6B). 23 and 24: Finished co-inoculation fermentation with *Lindnera misumaiensis, Torulaspora delbrueckii, Saccharomyces bayanus* and *Oenococcus oeni* (series 8A and 8B).

Take-home messages

- Nowadays, there is still a plethora of unexplored habitats out there from which yeasts can be isolated and used for e.g. new fermented alcoholic beverages

- The style/aroma of a fermented beverage may be controlled by the choice of yeast and/or inoculation strategy