Consiglio Nazionale delle Ricerche
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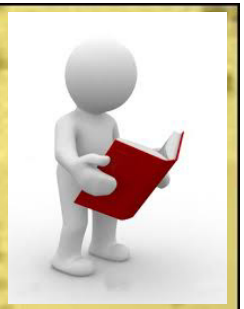
ISTITUTO DI SCIENZE DELLE PRODUZIONI ALIMENTARI

HS-SPME/GC-MS characterization of volatile compounds in Maresco sparkling wine produced in Apulia region

Maria Tufariello

IProViSP

Innovazioni di processo e di prodotto nel comparto dei vini spumanti da vitigni autoctoni pugliesi



Recovery, study and valoritation of native Apulian vine varieties: BIODIVERSITY

Identification of the microflora associated to the most important Apulian grape cultivars and selection of Saccharomyces cerevisiae strain with peculiar oenological properties

Preparation of autochthonous fermentation starters to enhance quality and tipicality of Apulian sparkling wine production

J Ind Microbiol Biotechnol
DOI 10.1007/s10295-011-1002-z

Autochthonous fermentation starters for the industrial production of Negroamaro wines

Mariana Tristezza · Cosimo Vetrano · Gianluca Bleve · Francesco Grieco · Maria Tufariello · Angela Quarta · Giovanni Mita · Giuseppe Spano · Francesco Grieco

2nd MS-Wine Day
MS for grapes, wine, spirits
May 9-10, 2017

IProViSP

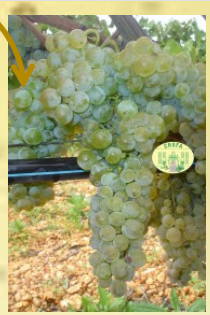
Innovazioni di processo e di prodotto nel comparto dei vini spumanti da vitigni autoctoni pugliesi

ottavianello

maresco

marchione

Bianco d'Alessano



Francavidda

susumaniello

WINEMAKING "Champenoise"

liqueur de tirage

Base wine

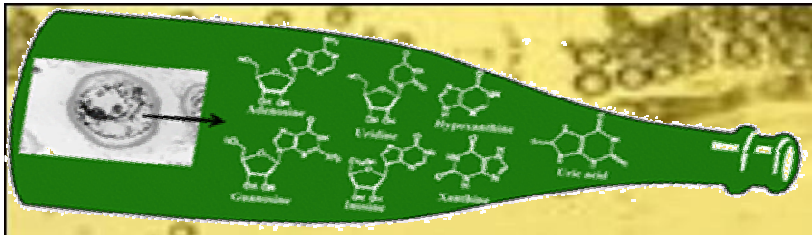
Bottling

"prise de mousse"
Rifermentation
30-60 days

Removing less on
"pupitres"

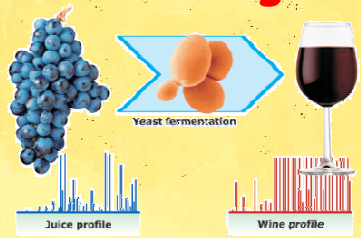
opening

capping

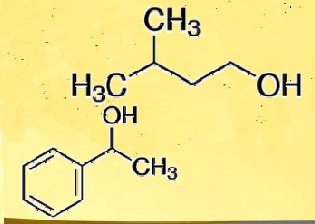


AROMA COMPOUNDS

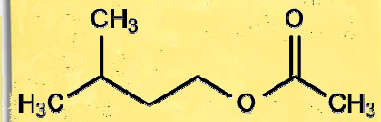
Volatile origins



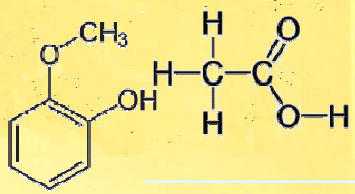
Higher alcohols



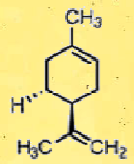
Esters

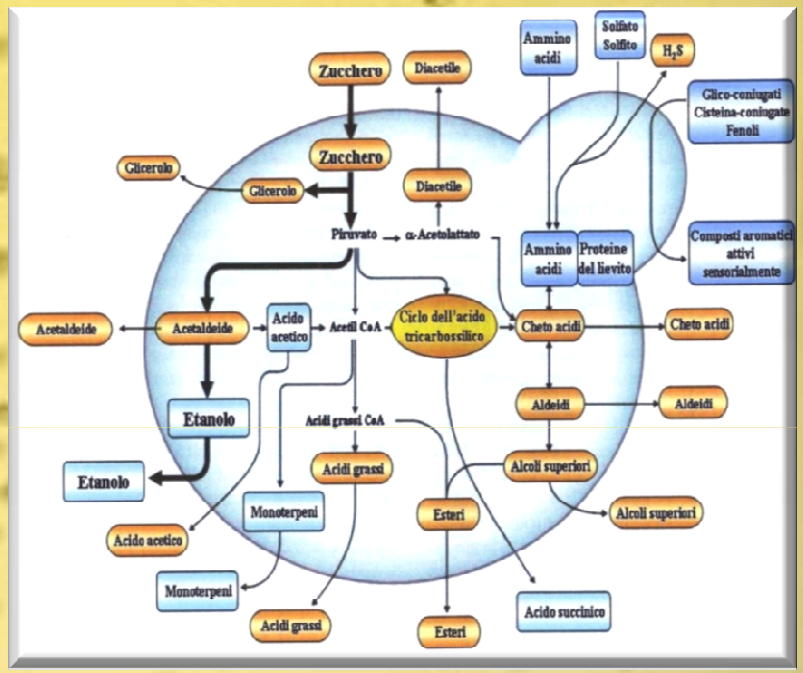


Acids, phenols



Terpenes





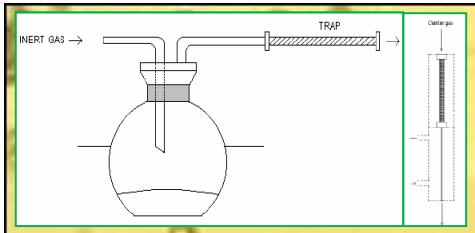
Our Objective:

To define a systematic fingerprinting of the aromatic composition of the sparkling wines elaborated from Maresco autochthonous grape varieties following the "champenoise" method, using autochtonous yeast strains.

To Optimize the SPME sampling and gas chromatographic conditions for the qualitative and quantitative analyses of volatile compounds in the headspace of sparkling wine focusing on sample rapidity preparation and results accuracy.

SPME method validation in terms of the recovery of known quantities of substances (accuracy), precision (repeatability) and the determination of limits of detection and quantification (LODs and LOQs).

Extraction Methods



Food Chemistry 70 (2000) 409–417
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods Section

Characterisation of volatile flavour compounds in Roncal cheese extracted by the 'purge and trap' method and analysed by GC–MS

Jesús M. Izco *, Paloma Torre

Food Research International 53 (2013) 15–23

Aroma of *Aglianico* and *Uva di Troia* grapes by aromatic series

Alessandro Genovese [a,*], Simona A. Lamorte [b], Angelita Gambuti [a], Luigi Moio [a]

J Sci Food Agric 86:922–931 (2006)

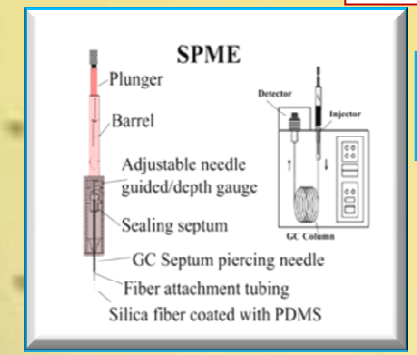
Glycosidic aroma compounds of some Portuguese grape cultivars

Maria João Cabrita,[1]* Ana M Costa Freitas,[1] Olga Laureano[2] and Rocco Di Stefano[3]

Food Research International 43 (2010) 996–1002

Aroma quality improvement of Chardonnay white wine by fermentation and ageing in barrique on lees

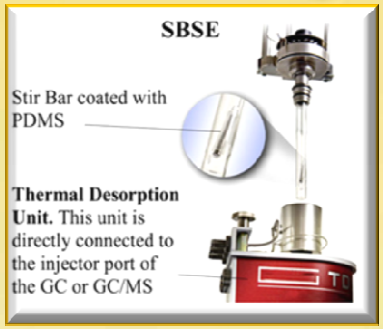
Maria Teresa Liberatore [a], Sandra Pati [b,c,*], Matteo Alessandro Del Nobile [b,c], Ennio La Notte [b,c]



Journal of Chromatography A, 880 (2000) 35–62

Applications of solid-phase microextraction in food analysis

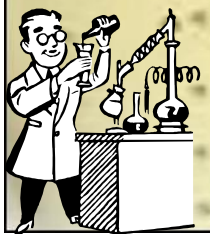
Hiroyuki Kataoka [a,*], Heather L. Lord [b], Janusz Pawliszyn [b]



Journal of Chromatography A, 1098 (2005) 1–6

Stir bar sorptive extraction for the determination of volatile compounds in oak-aged wines

J. Marín [a], A. Zalacain [b], C. De Miguel [c], G.L. Alonso [b], M.R. Salinas [b,*]

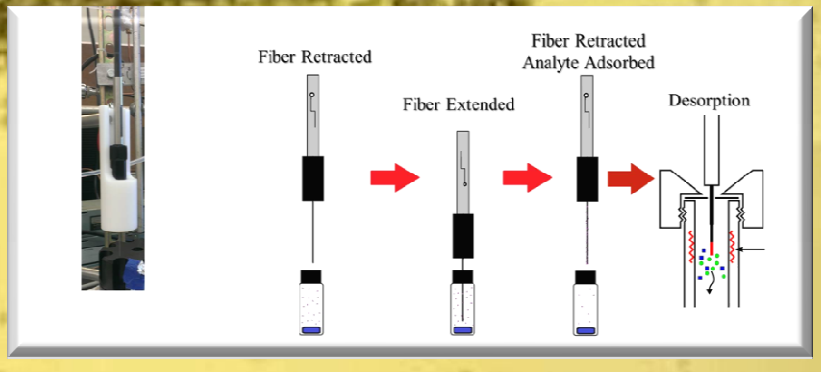


Talanta 79 (2009) 871–876

Comparison of extraction methods for volatile compounds of Muscat grape juice

E. Sánchez-Palomo [a], M.E. Alañón [a], M.C. Díaz-Maroto [a,b,*], M.A. González-Viñas [a], M.S. Pérez-Coello [a]

SPME



Simple, rapid, solvent-free method for extracting volatile and semi-volatile compounds, gives the possibility of extraction and concentration integrated in one step

It's based on equilibrium between the analyte in vapour phase and in silica fibre coated with adsorbent polar or apolar polymer

Not require sample treatments and it allowed us to obtain important volatile compounds of each chemical family

Torrens, J. et al. (2004). Journal of Chromatographic Science, 42(6), 310–316;
J. Bosch-Fuste´ et al. (2007).Food Chemistry 105 (2007) 428–435 ;
Torrens et al. (2010). J. Agric. Food Chem. 58, 2455–2461;
Zhang , M. et al. (2011). Food Chem. 125, 743-749.

DVB/CAR/PDMS 50/30 um

Wine 5 mL

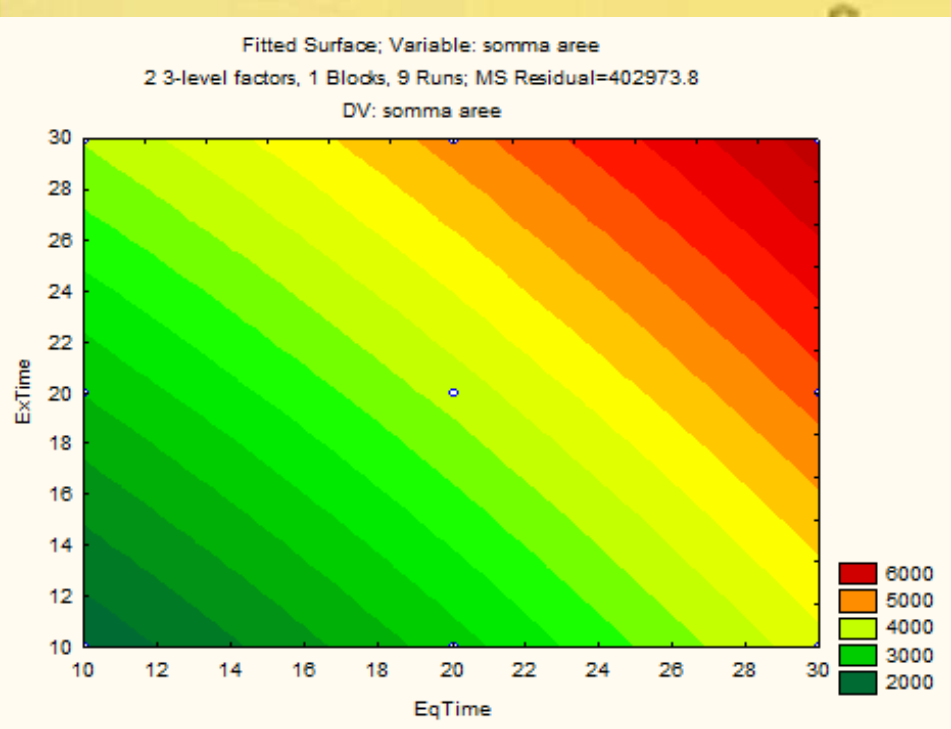
Equilibration Time
Extraction Time….

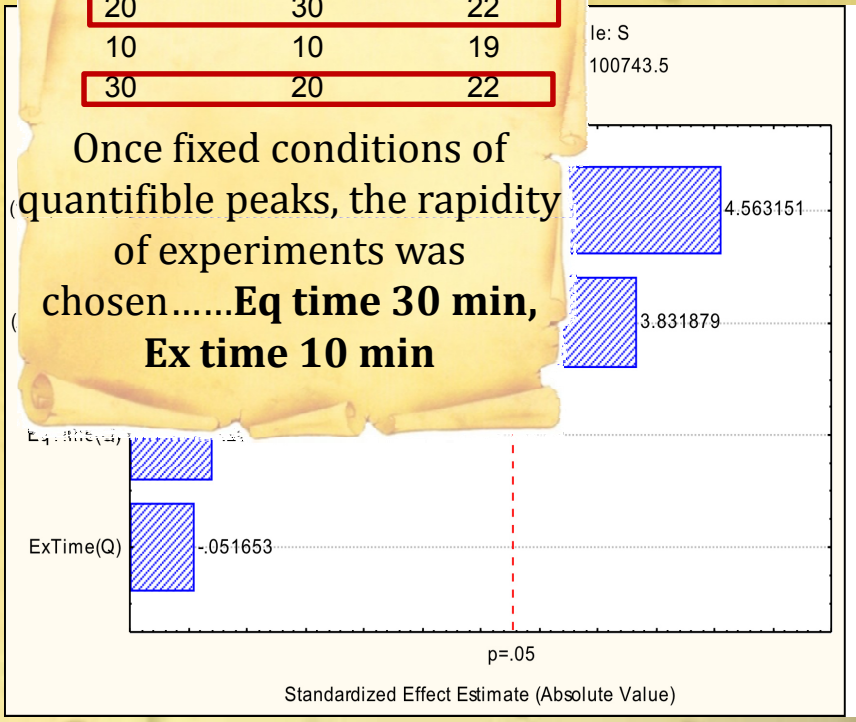
Optimization of Equilibration time and Extraction time by a 2 3-level factor design

Fixed a threshold area of 1500000 (allowing … ably quantifiable … was based on th… with peak area/th… an 1 and on the su…

Eq Time	Extr Time	N. peaks
20	20	19
30	**10**	**22**
10	30	21
10	20	19
30	30	22
20	10	19
20	30	22
10	10	19
30	20	22

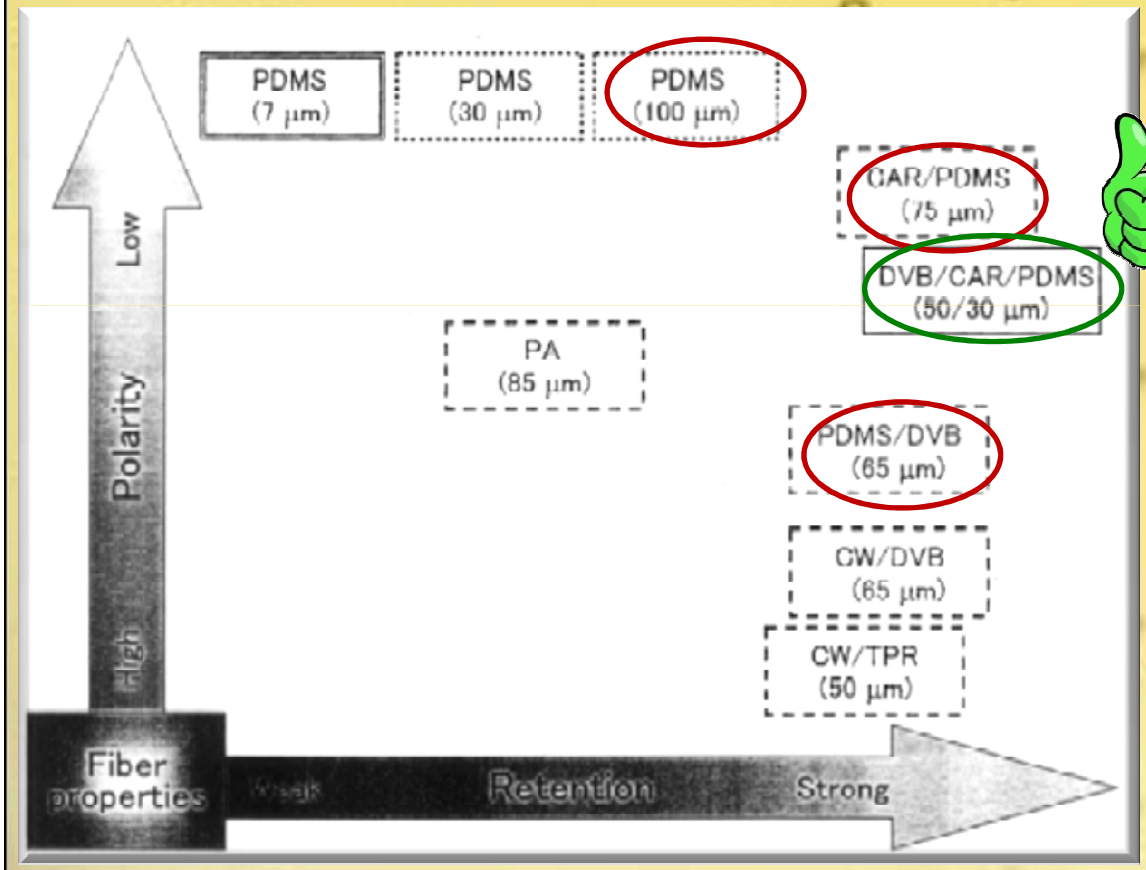
Once fixed conditions of quantifible peaks, the rapidity of experiments was chosen……**Eq time 30 min, Ex time 10 min**





*R. Castro et al. / Analytica Chimica Acta 513 (2004) 141–150; N. M. de Oliveira ARCANJO et. al./ Food Sci. Technol, Campinas, 35(4): 676-682

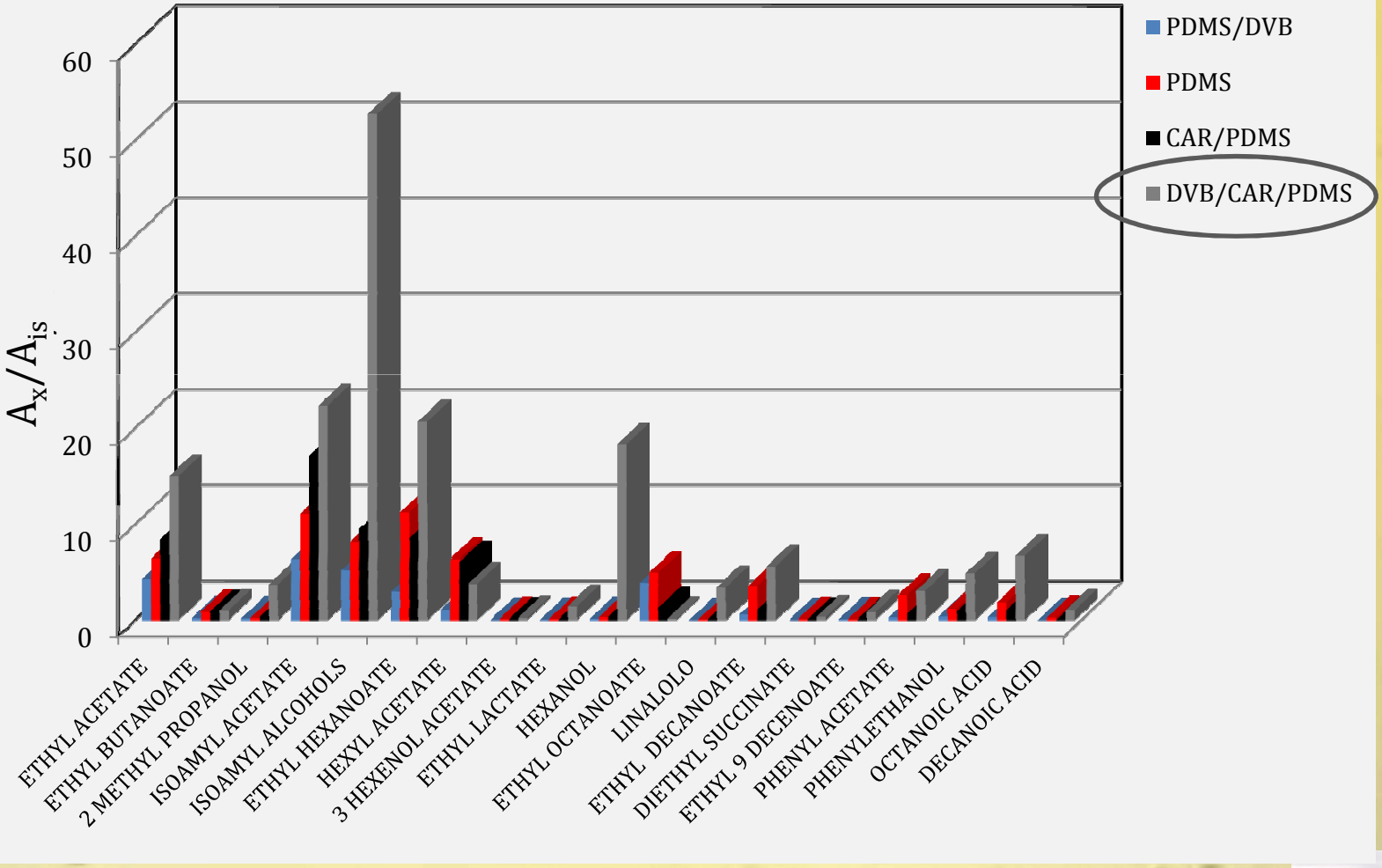
FIBERS TESTED



Mixed coating fibres containing divinylbenzene (DVD), PDMS and carboxen (CAR) or polyethylene glycol (CW), increase the tramping ability of the fibre due to the synergic effect of adsorption and distribution within the stationary phase, producing higher sensitivity than PDMS and PA fibres (E. Sanchez-Palomo et al. / Talanta 66 (2005) 1152–1157

Journal of Chromatography A, 880 (2000) 35–62;

Fibers comparison



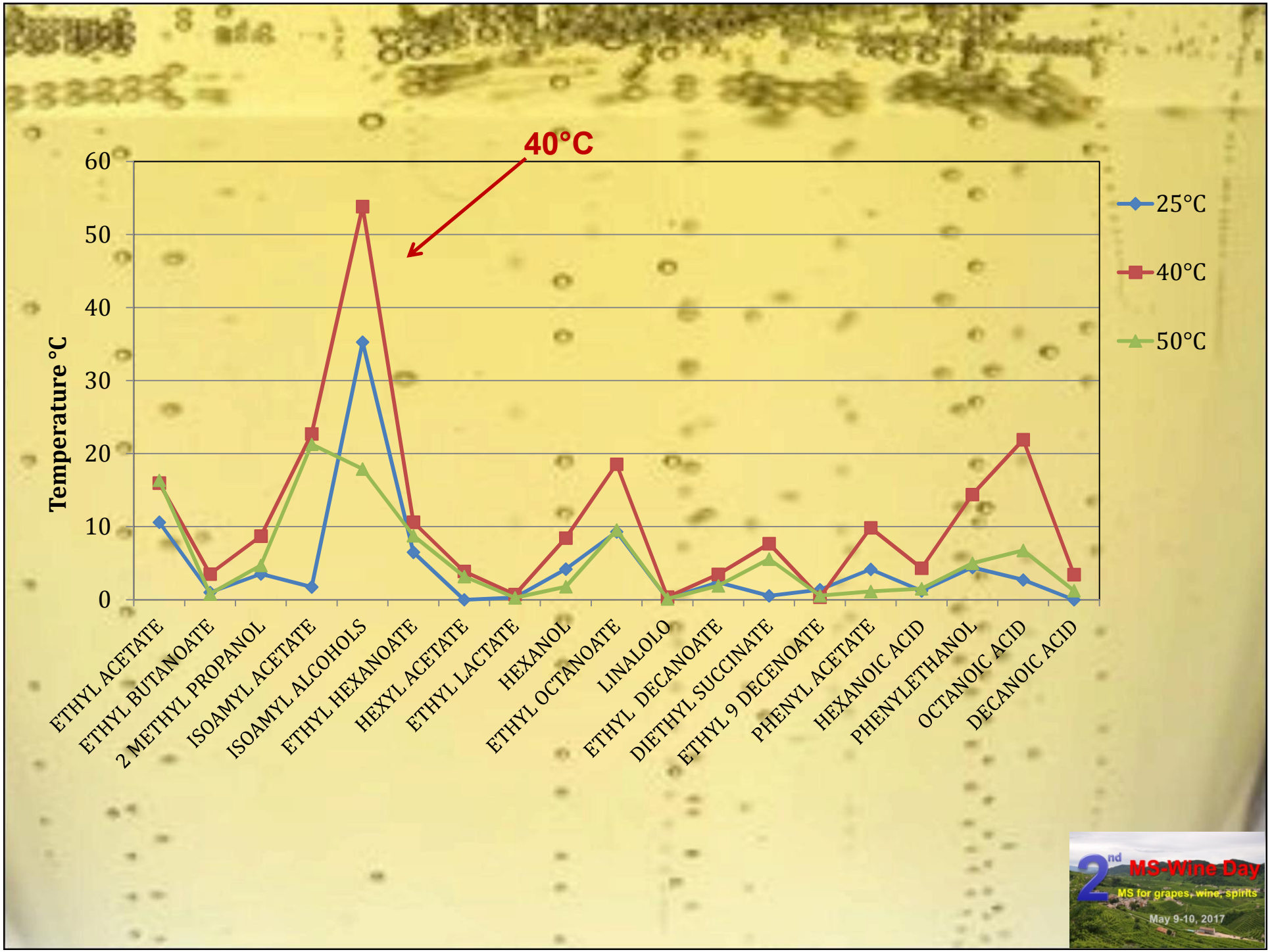
A_x/A_{is}= Analyte Area peak / Internal Standard Area peak

Zhang et al. pointed out that an increase in sampling temperature increases the headspace concentration of aroma compounds, favouring their extraction

SPME analysis is quite sensitive to experimental conditions such as heating temperature and time, sample volume, concentration, and sample matrix and uniformity (Yang and Peppard, 1994).

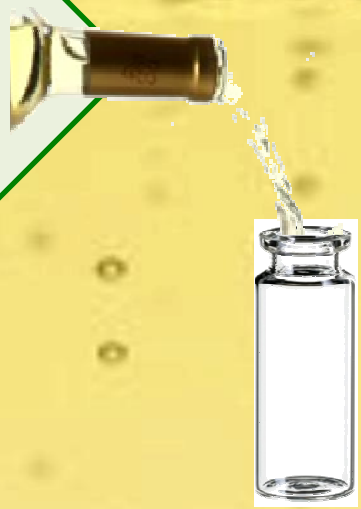
Temperature Extraction-Equilibration:
25°-40°-50°C

Z. Zhang, M.J. Yang, J. Pawliszyn, Anal. Chem. 66 (1994) 844.


40°C
Temperature °C
60
50
40
30
20
10
0
25°C
40°C
50°C
ETHYL ACETATE
ETHYL BUTANOATE
2 METHYL PROPANOL
ISOAMYL ACETATE
ISOAMYL ALCOHOLS
ETHYL HEXANOATE
HEXYL ACETATE
ETHYL LACTATE
HEXANOL
ETHYL OCTANOATE
LINALOLO
ETHYL DECANOATE
DIETHYL SUCCINATE
ETHYL 9 DECENOATE
PHENYL ACETATE
HEXANOIC ACID
PHENYLETHANOL
OCTANOIC ACID
DECANOIC ACID
2nd MS-Wine Day
MS for grapes, wine, spirits
May 9-10, 2017

Conditions Tested

Sample Volumes: 3-**5**-6 mL

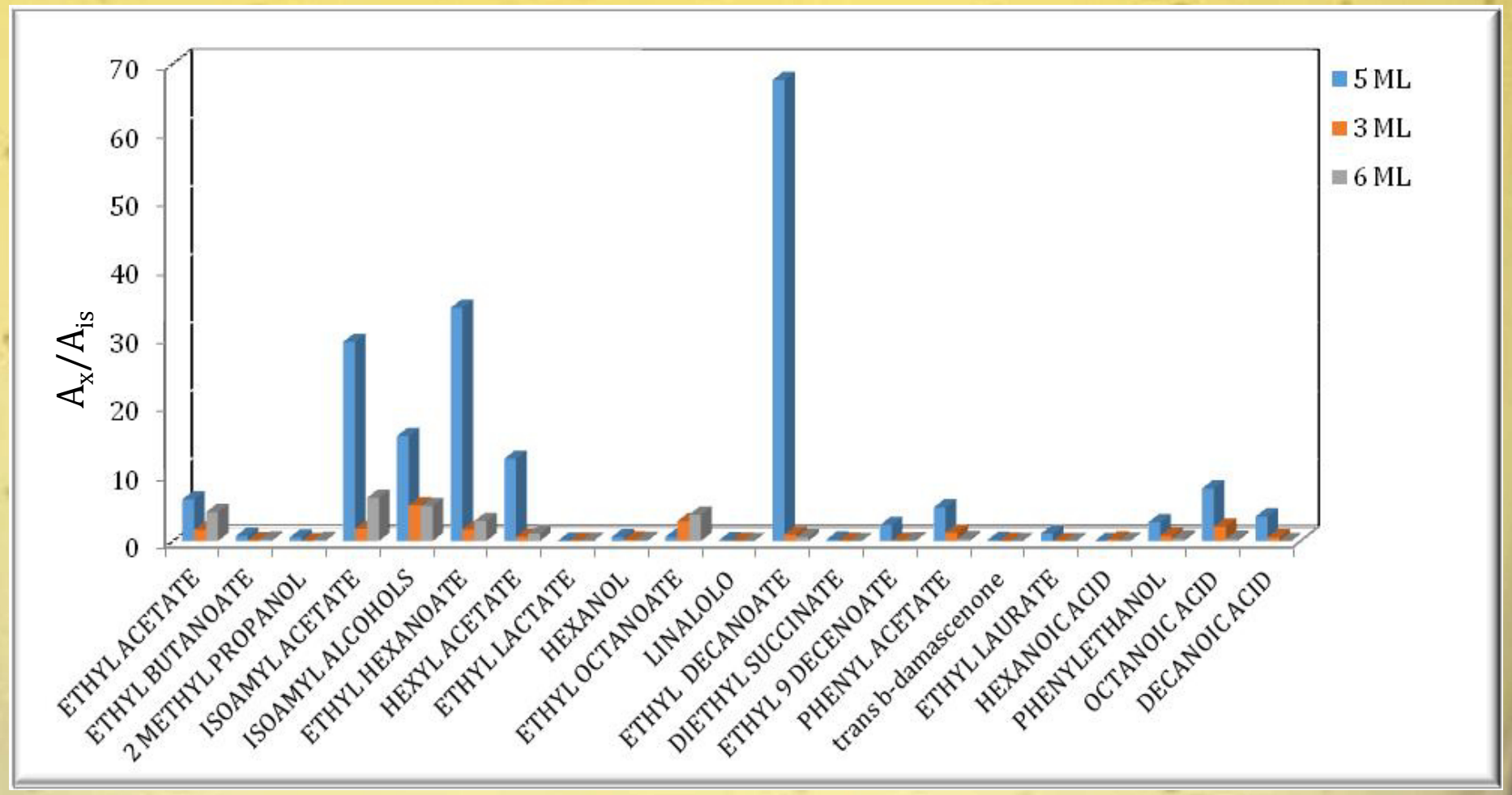


3 STEP

Agitation

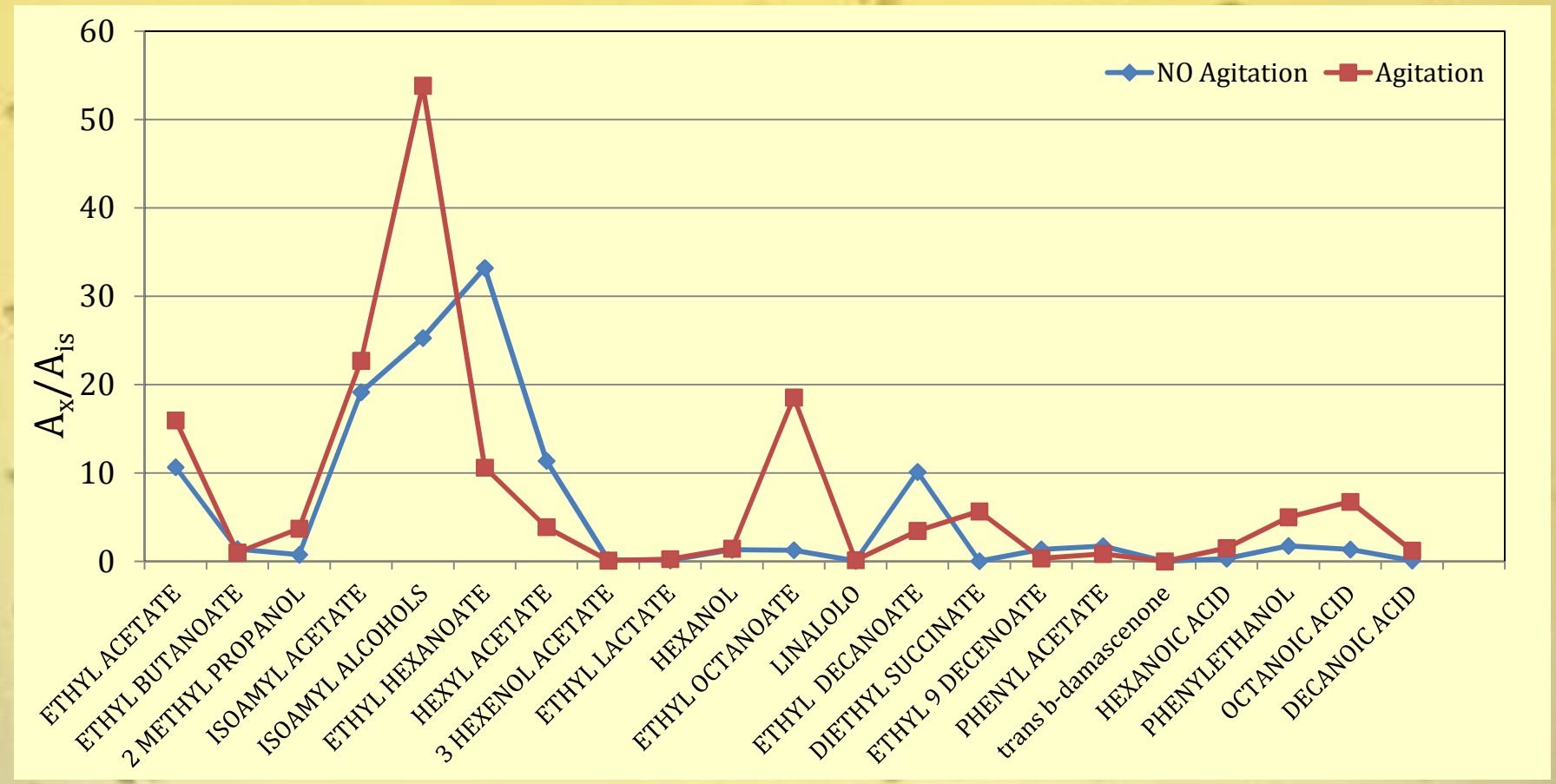
NaCl: 0-1-**2**-3-5 gr

Volume sample



A_x/A_{is}= Analyte Area peak / Internal Standard Area peak

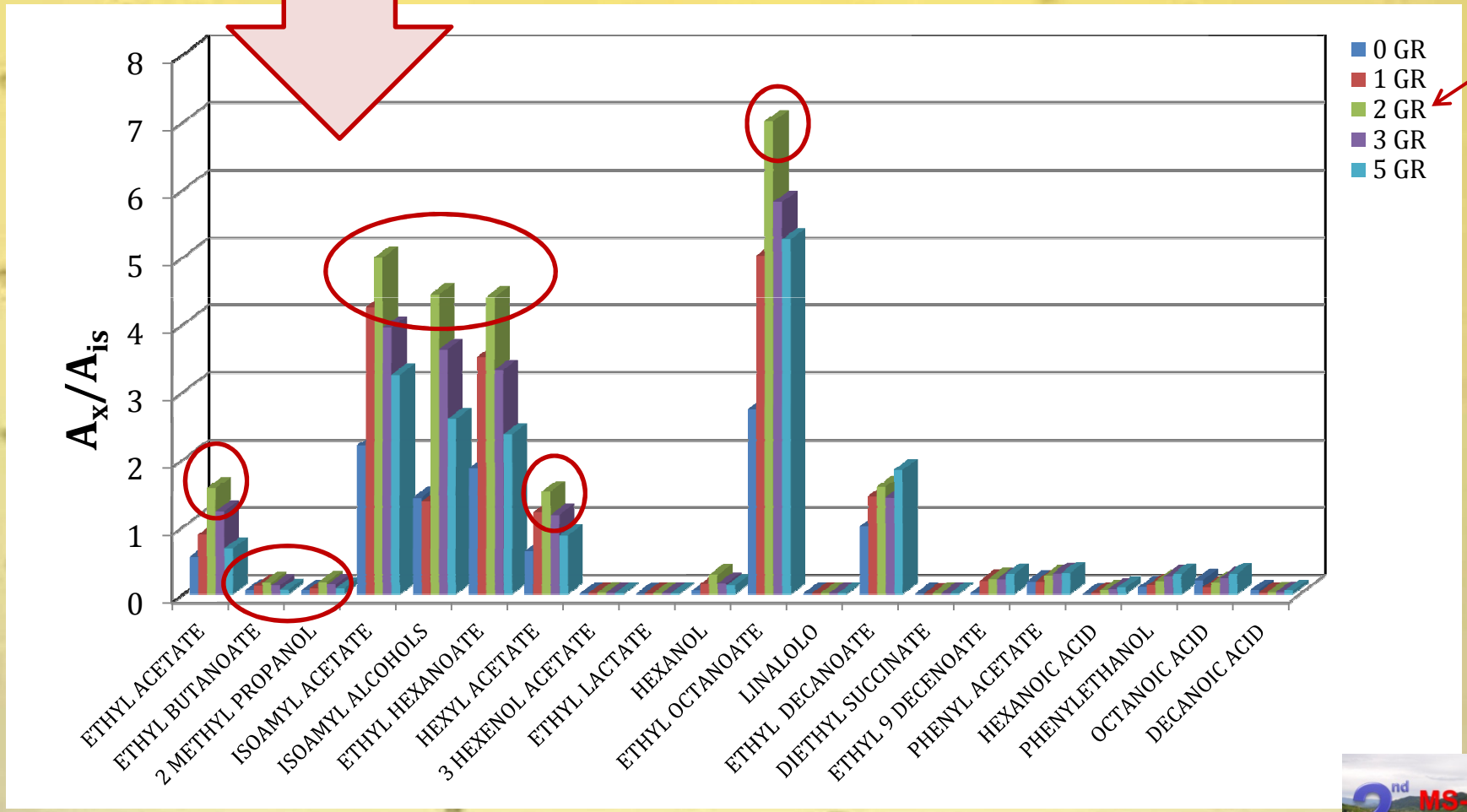
Sample agitation



A_x/A_{is}= Analyte Area peak / Internal Standard Area peak

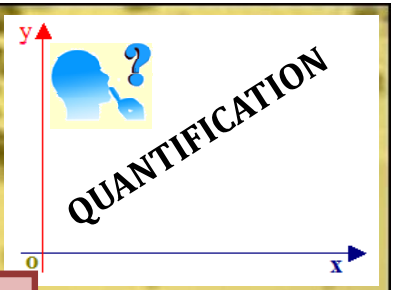
The suitability of the headspace SPME technique depends on the transfer of the analyte from sample to the gaseous phase and therefore to the fibre. This process can be optimised by the increase of the ionic strength.

Salting out
NaCl



A_x/A_{is}= Analyte Area peak / Internal Standard Area peak

According to some authors, the compounds were quantified using the internal standard (ISTD) method and the results were expressed as ISTD equivalents.



According to other authors, the concentration of the volatile compounds was obtained using the calibration curve of one standard compound with the most similar chemical structure, i.e.,belonging to the same class of compounds.

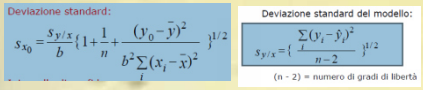
	Peak Area/Peak IS	Internal standard equivalents (mg/L) C=Peak Area x C IS	calibration curves	Conc. (mg/L±SD)	Esters ethyl hexanoate equivalents (mg/L)
2 METHYL PROPANOL	0.37	2.21	y=0.0188x-0.045	21.95±0.5	
ISOAMYL ACETATE	22.30	133.83	y=1.18835x+0.457529	46±7	55.50
ISOAMYL ALCOHOLS	18.87	113.24	y=0.0419x+0.17921	431±70	
ETHYL HEXANOATE	9.42	56.52	y=0.399x+0.1571	23.17±0.35	32.22
HEXYL ACETATE	3.75	22.47	y=0.5447x-0.2331	7.30±0.23	9.0
HEXANOL	2.87	17.22	y=0.3713x-0.07	7.91±0.10	
ETHYL OCTANOATE	5.19	31.16	y=0.9777x+0.6436	5.97±0.67	12.62
LINALOLO	3.42	20.49	Y=0.7506X-0.2543	8.9±1.3	
PHENYL ACETATE	3.04	18.21	y=0.6961x+0.1793	4.10±0.07	7.22
ETHYL DECANOATE	12.97	77.80	y=0.243x+1.903	47±15	32.12
DIETHYL SUCCINATE	1.57	9.42	y=0.133x-0.043	12.13±0.56	3.54
PHENYLETHANOL	4.84	29.06	Y=0.0221X-0.0825	233±98	
OCTANOIC ACID	6.69	40.17	Y=0.0106X-0.727	561±37	
ETHYL ACETATE	1.50	8.97			
ETHYL BUTANOATE	0.98	5.88			
3 HEXENOL ACETATE	0.23	1.39			
ETHYL LACTATE	1.43	8.57			
ETHYL 9 DECENOATE	0.84	5.05			
HEXANOIC ACID	1.42	8.52			
DECANOIC ACID	1.01	6.08			

Validation method parameters

	calibration curve	Conc. (mg/L±SD)	Recovery (%)±SD	Linearity range (mg/L)	LOD Detection limit (mg/L)	LOQ Quantification limit (mg/L)	Precision intra day (n=5) RSD
2 METHYL PROPANOL	y=0.0188x-0.045	21.9±0.5	86±3	6.73-100	6.73	20.4	20
ISOAMYL ACETATE	y=1.188x+0.458	46±7	88±2	3.80-50	3.80	11	17
ISOAMYL ALCOHOLS	y=0.0419x+0.17921	431±70	83±2	30.43-800	30.43	92	11
ETHYL HEXANOATE	y=0.399x+0.1571	23.17±0.35	83±3	0.90-30	0.90	2.7	17
HEXYL ACETATE	y=0.5447x-0.2331	7.30±0.23	83±3	1.10-10	1.10	3.30	18
HEXANOL	y=0.3713x-0.07	7.91± 0.10	83±3	0.90-10	0.90	2.60	13
ETHYL OCTANOATE	y=0.9777x+0.6436	5.97±0.67	85±4	1.40-10	1.40	4.40	19
LINALOLO	y=0.7506x-0.2543	8.9±1.3	86±3	2.30-10	2.30	7.0	26
PHENYL ACETATE	y=0.6961x+0.1793	4.10±0.07	80±2	0.4-100	0.4	1.1	7
ETHYL DECANOATE	y=0.243x+1.903	47±15	80±3	17.58-100	11	33	13
DIETHYL SUCCINATE	y=0.133x-0.043	12.13±0.56	87±3	9.13-100	3.5	10.5	20
PHENYLETHANOL	y=0.0221x-0.0825	233±98	84±3	52.40-800	52.40	159	13
OCTANOIC ACID	y=0.0106x-0.727	561±37	90±4	72.0-800	72.0	218	21

For each volatile compounds the **recovery percentage** was determined by the ratio ***(C1 −C0/C2)×100***, *where C0 is the concentration of the analyte* in the wine, *C1 the concentration of the analyte in spiked* wine sample and *C2 is the concentration of the analyte added to* wine sample;

SD:

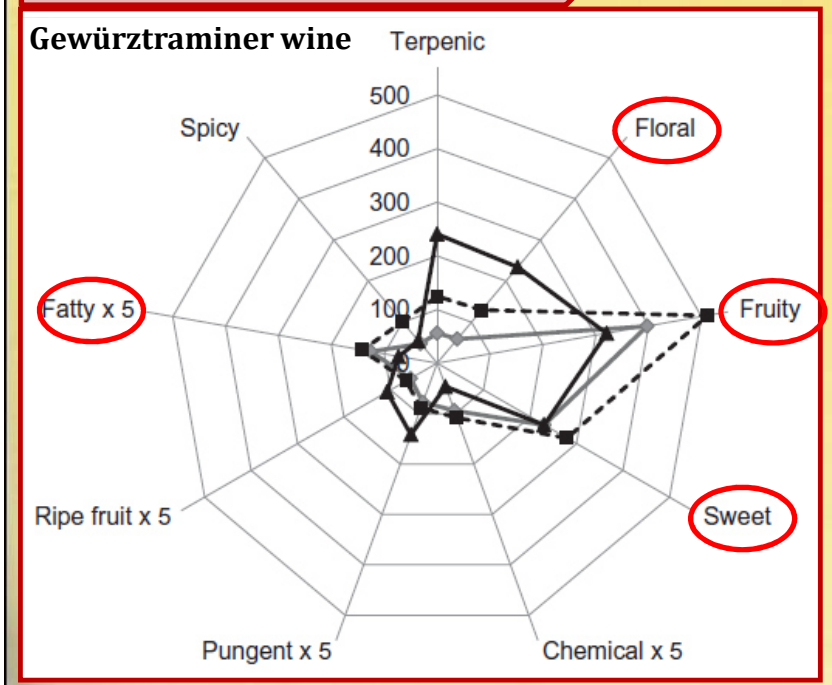


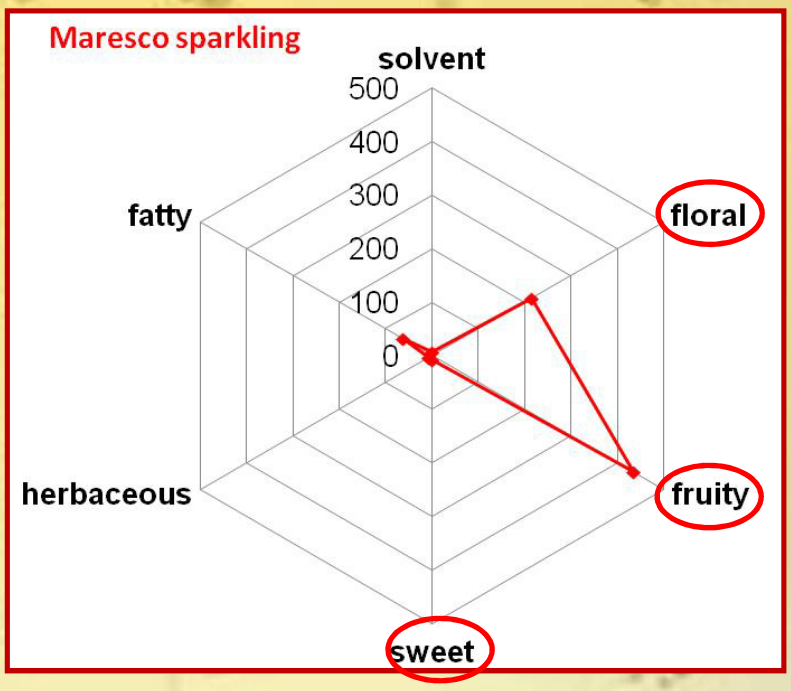
ODOR PROFILE

The contribution of each volatile compound to wine aroma was evaluated qualitatively via its associated descriptor and quantitatively via its OAV.

OAVs were calculated by using the equation OAV = c/oth, where c (mg/L) is the total concentration of each compound in the wine samples and oth (mg/L) is its odour threshold value (Lukic* et al., 2016; Rocha et al. 2004)**

Lukic et al. Food Chemistry 196 (2016) 1048–1057





*:Lukic et al. Food Chemistry 196 (2016) 1048–1057; ** Rocha et al. Analytica Chimica Acta 513 (2004) 257–262

Conclusions

Rapid HS-SPME-GC-MS methodology was found to be fully suitable for the analysis of headspace volatile compounds produced during fermentation process.

An optimised methodology was developed, which was based on 65 um DVB/CAR/PDMS, headspace sampling mode and an equilibration time of 30 min and an extraction time of 10 min, at 40°C.

The range of concentrations of most of the volatile compounds detected in our samples was similar to the values reported in literature.

Autochthonous yeast strains have been shown to be able to produce sparkling wines and could be employed instead of commercial ones, thus enhancing the biodiversity.

Consiglio Nazionale delle Ricerche

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ISTITUTO DI SCIENZE DELLE PRODUZIONI ALIMENTARI

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Sandra Pati

Thank you