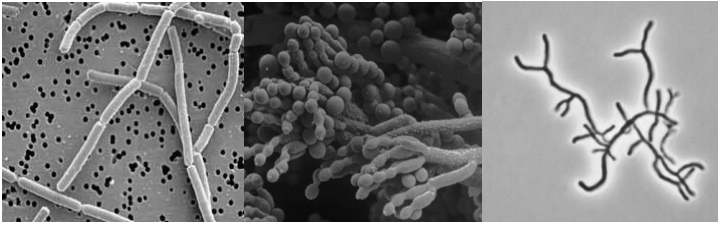


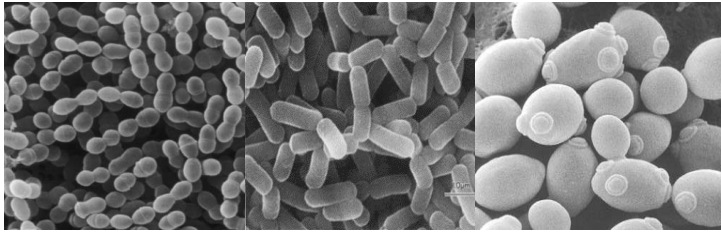
# **Direct Injection Mass Spectrometry for the Real-Time Volatilomics in Food System Microbiomes: the Potential of Providing Temporal Dimension in Multi-Omics Studies**

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# Fermentation: the leading example of bioprocess



# 'Omics' technologies: **Metabolomics studies**

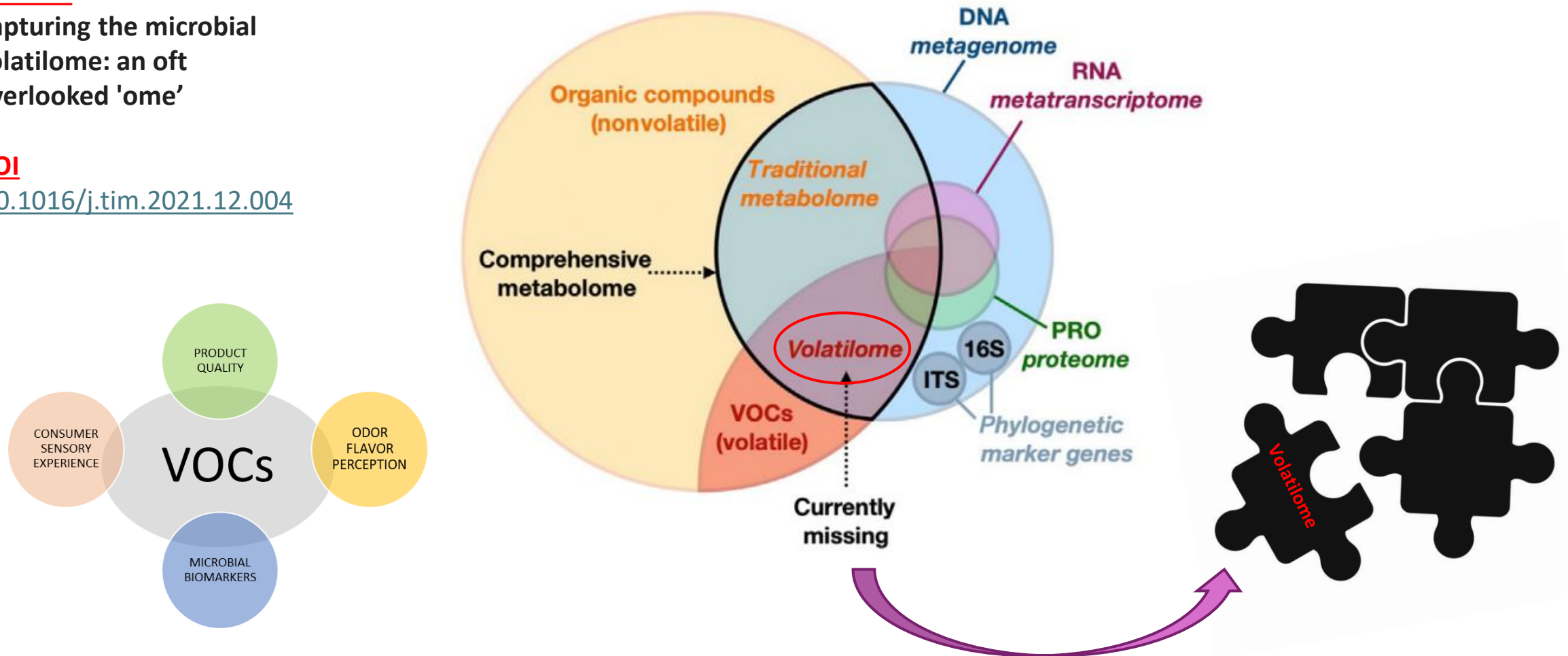
## Review

Capturing the microbial  
volatilome: an oft  
overlooked 'ome'

## DOI

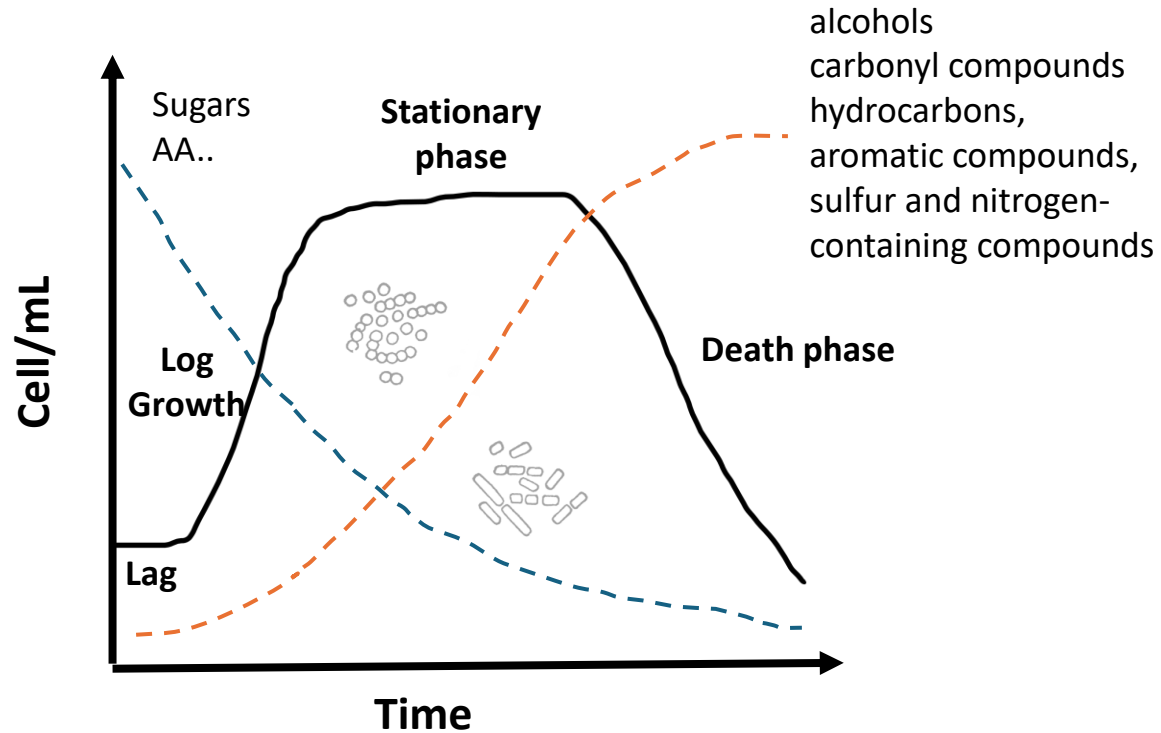
[10.1016/j.tim.2021.12.004](https://doi.org/10.1016/j.tim.2021.12.004)

## (A) Comprehensive metabolome & multi-omics





# Microbial growth curve



	Primary metabolism	Fatty acid pathway	Aromatic	Sulfur	Pyrazines	Terpenoid
Common	<div><chem>CC(C)CO</chem> 3-Methylbutanol</div> <div><chem>CC(C)OC(=O)C</chem> 3-Methyl-4-butanolide</div> <div><chem>CC(C)C(=O)O</chem> Acetoin</div> <div><chem>CC(C)O</chem> Butane-2,3-diol</div> <div><chem>CC(C)C(=O)O</chem> 3-Methylbutanoic acid</div> <div><chem>CC(C)C(=O)OCC</chem> Ethyl acetate</div> <div><chem>CC(=O)O</chem> Acetic acid</div> <div><chem>CCCCC(=O)OCC</chem> Butyl 2-methylbutanoate</div> <div><chem>N</chem> (ammonia) <chem>N#C</chem> (hydrogen cyanide)</div>	<div><chem>CCCCCCCCC(=O)O</chem> Nonan-2-one</div> <div><chem>CCCCCCCCC=O</chem> Nonanal</div> <div><chem>CC(C)CCCCCCCCC(=O)O</chem> 10-Methylundecan-2-one</div> <div><chem>CCCCCCCCC(=O)O</chem> Nonanoic acid</div>	<div><chem>c1ccc(cc1)CO</chem> 2-Phenylethanol</div> <div><chem>c1ccc2c(c1)c(c[nH]2)</chem> Indol</div> <div><chem>c1ccc(cc1)C(=O)OC</chem> Methyl benzoate</div>	<div><chem>CSC</chem> Dimethyl sulfide</div> <div><chem>CS</chem> CH<sub>3</sub>SH</div> <div><chem>CSSC</chem> Dimethyl disulfide</div> <div><chem>CSSS</chem> Dimethyl trisulfide</div>	<div><chem>CN1C=NC=CC=C1</chem> Methylpyrazine</div> <div><chem>CN1C=NC(=C(C)N1)C</chem> 2,5-Dimethylpyrazine</div> <div><chem>CN1C=NC(=C(C)N1)C</chem> Trimethylpyrazine</div>	
Group	<div><chem>CC(C)N(C)C(=O)Cc1ccccc1</chem> Schleiferone A</div> <div><chem>CC(C)N(C)C(=O)Cc1ccccc1</chem> Schleiferone B</div>	<div><chem>CCCCCCCC=CC</chem> 1-Undecene</div> <div><chem>CC(C)CCCCCCC(O)C</chem> (S)-9-Methyldecan-3-ol</div> <div><chem>C1CCC2(C1)CCC3(C2)CCC4(C3)CCC5(C4)CCC6(C5)CCC7(C6)CCC8(C7)CCC9(C8)CCC10(C9)CCC11(C10)CCC12(C11)CCC13(C12)CCC14(C13)CCC15(C14)CCC16(C15)CCC17(C16)CCC18(C17)CCC19(C18)CCC20(C19)CCC21(C20)CCC22(C21)CCC23(C22)CCC24(C23)CCC25(C24)CCC26(C25)CCC27(C26)CCC28(C27)CCC29(C28)CCC30(C29)CCC31(C30)CCC32(C31)CCC33(C32)CCC34(C33)CCC35(C34)CCC36(C35)CCC37(C36)CCC38(C37)CCC39(C38)CCC40(C39)CCC41(C40)CCC42(C41)CCC43(C42)CCC44(C43)CCC45(C44)CCC46(C45)CCC47(C46)CCC48(C47)CCC49(C48)CCC50(C49)CCC51(C50)CCC52(C51)CCC53(C52)CCC54(C53)CCC55(C54)CCC56(C55)CCC57(C56)CCC58(C57)CCC59(C58)CCC60(C59)CCC61(C60)CCC62(C61)CCC63(C62)CCC64(C63)CCC65(C64)CCC66(C65)CCC67(C66)CCC68(C67)CCC69(C68)CCC70(C69)CCC71(C70)CCC72(C71)CCC73(C72)CCC74(C73)CCC75(C74)CCC76(C75)CCC77(C76)CCC78(C77)CCC79(C78)CCC80(C79)CCC81(C80)CCC82(C81)CCC83(C82)CCC84(C83)CCC85(C84)CCC86(C85)CCC87(C86)CCC88(C87)CCC89(C88)CCC90(C89)CCC91(C90)CCC92(C91)CCC93(C92)CCC94(C93)CCC95(C94)CCC96(C95)CCC97(C96)CCC98(C97)CCC99(C98)CCC100(C99)CCC101(C100)CCC102(C101)CCC103(C102)CCC104(C103)CCC105(C104)CCC106(C105)CCC107(C106)CCC108(C107)CCC109(C108)CCC110(C109)CCC111(C110)CCC112(C111)CCC113(C112)CCC114(C113)CCC115(C114)CCC116(C115)CCC117(C116)CCC118(C117)CCC119(C118)CCC120(C119)CCC121(C120)CCC122(C121)CCC123(C122)CCC124(C123)CCC125(C124)CCC126(C125)CCC127(C126)CCC128(C127)CCC129(C128)CCC130(C129)CCC131(C130)CCC132(C131)CCC133(C132)CCC134(C133)CCC135(C134)CCC136(C135)CCC137(C136)CCC138(C137)CCC139(C138)CCC140(C139)CCC141(C140)CCC142(C141)CCC143(C142)CCC144(C143)CCC145(C144)CCC146(C145)CCC147(C146)CCC148(C147)CCC149(C148)CCC150(C149)CCC151(C150)CCC152(C151)CCC153(C152)CCC154(C153)CCC155(C154)CCC156(C155)CCC157(C156)CCC158(C157)CCC159(C158)CCC160(C159)CCC161(C160)CCC162(C161)CCC163(C162)CCC164(C163)CCC165(C164)CCC166(C165)CCC167(C166)CCC168(C167)CCC169(C168)CCC170(C169)CCC171(C170)CCC172(C171)CCC173(C172)CCC174(C173)CCC175(C174)CCC176(C175)CCC177(C176)CCC178(C177)CCC179(C178)CCC180(C179)CCC181(C180)CCC182(C181)CCC183(C182)CCC184(C183)CCC185(C184)CCC186(C185)CCC187(C186)CCC188(C187)CCC189(C188)CCC190(C189)CCC191(C190)CCC192(C191)CCC193(C192)CCC194(C193)CCC195(C194)CCC196(C195)CCC197(C196)CCC198(C197)CCC199(C198)CCC200(C199)CCC201(C200)CCC202(C201)CCC203(C202)CCC204(C203)CCC205(C204)CCC206(C205)CCC207(C206)CCC208(C207)CCC209(C208)CCC210(C209)CCC211(C210)CCC212(C211)CCC213(C212)CCC214(C213)CCC215(C214)CCC216(C215)CCC217(C216)CCC218(C217)CCC219(C218)CCC220(C219)CCC221(C220)CCC222(C221)CCC223(C222)CCC224(C223)CCC225(C224)CCC226(C225)CCC227(C226)CCC228(C227)CCC229(C228)CCC230(C229)CCC231(C230)CCC232(C231)CCC233(C232)CCC234(C233)CCC235(C234)CCC236(C235)CCC237(C236)CCC238(C237)CCC239(C238)CCC240(C239)CCC241(C240)CCC242(C241)CCC243(C242)CCC244(C243)CCC245(C244)CCC246(C245)CCC247(C246)CCC248(C247)CCC249(C248)CCC250(C249)CCC251(C250)CCC252(C251)CCC253(C252)CCC254(C253)CCC255(C254)CCC256(C255)CCC257(C256)CCC258(C257)CCC259(C258)CCC260(C259)CCC261(C260)CCC262(C261)CCC263(C262)CCC264(C263)CCC265(C264)CCC266(C265)CCC267(C266)CCC268(C267)CCC269(C268)CCC270(C269)CCC271(C270)CCC272(C271)CCC273(C272)CCC274(C273)CCC275(C274)CCC276(C275)CCC277(C276)CCC278(C277)CCC279(C278)CCC280(C279)CCC281(C280)CCC282(C281)CCC283(C282)CCC284(C283)CCC285(C284)CCC286(C285)CCC287(C286)CCC288(C287)CCC289(C288)CCC290(C289)CCC291(C290)CCC292(C291)CCC293(C292)CCC294(C293)CCC295(C294)CCC296(C295)CCC297(C296)CCC298(C297)CCC299(C298)CCC300(C299)CCC301(C300)CCC302(C301)CCC303(C302)CCC304(C303)CCC305(C304)CCC306(C305)CCC307(C306)CCC308(C307)CCC309(C308)CCC310(C309)CCC311(C310)CCC312(C311)CCC313(C312)CCC314(C313)CCC315(C314)CCC316(C315)CCC317(C316)CCC318(C317)CCC319(C318)CCC320(C319)CCC321(C320)CCC322(C321)CCC323(C322)CCC324(C323)CCC325(C324)CCC326(C325)CCC327(C326)CCC328(C327)CCC329(C328)CCC330(C329)CCC331(C330)CCC332(C331)CCC333(C332)CCC334(C333)CCC335(C334)CCC336(C335)CCC337(C336)CCC338(C337)CCC339(C338)CCC340(C339)CCC341(C340)CCC342(C341)CCC343(C342)CCC344(C343)CCC345(C344)CCC346(C345)CCC347(C346)CCC348(C347)CCC349(C348)CCC350(C349)CCC351(C350)CCC352(C351)CCC353(C352)CCC354(C353)CCC355(C354)CCC356(C355)CCC357(C356)CCC358(C357)CCC359(C358)CCC360(C359)CCC361(C360)CCC362(C361)CCC363(C362)CCC364(C363)CCC365(C364)CCC366(C365)CCC367(C366)CCC368(C367)CCC369(C368)CCC370(C369)CCC371(C370)CCC372(C371)CCC373(C372)CCC374(C373)CCC375(C374)CCC376(C375)CCC377(C376)CCC378(C377)CCC379(C378)CCC380(C379)CCC381(C380)CCC382(C381)CCC383(C382)CCC384(C383)CCC385(C384)CCC386(C385)CCC387(C386)CCC388(C387)CCC389(C388)CCC390(C389)CCC391(C390)CCC392(C391)CCC393(C392)CCC394(C393)CCC395(C394)CCC396(C395)CCC397(C396)CCC398(C397)CCC399(C398)CCC400(C399)CCC401(C400)CCC402(C401)CCC403(C402)CCC404(C403)CCC405(C404)CCC406(C405)CCC407(C406)CCC408(C407)CCC409(C408)CCC410(C409)CCC411(C410)CCC412(C411)CCC413(C412)CCC414(C413)CCC415(C414)CCC416(C415)CCC417(C416)CCC418(C417)CCC419(C418)CCC420(C419)CCC421(C420)CCC422(C421)CCC423(C422)CCC424(C423)CCC425(C424)CCC426(C425)CCC427(C426)CCC428(C427)CCC429(C428)CCC430(C429)CCC431(C430)CCC432(C431)CCC433(C432)CCC434(C433)CCC43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Fig. 5 | Major biosynthetic pathways of microbial volatile organic compounds. Examples of common, group and specific volatile compounds. Strain-specific signatures are difficult to find among the primary metabolism-derived compounds. There are no commonly emitted terpenes.

Weisskopf *et al.*, 2021

# Volatile Organic Compounds Monitoring

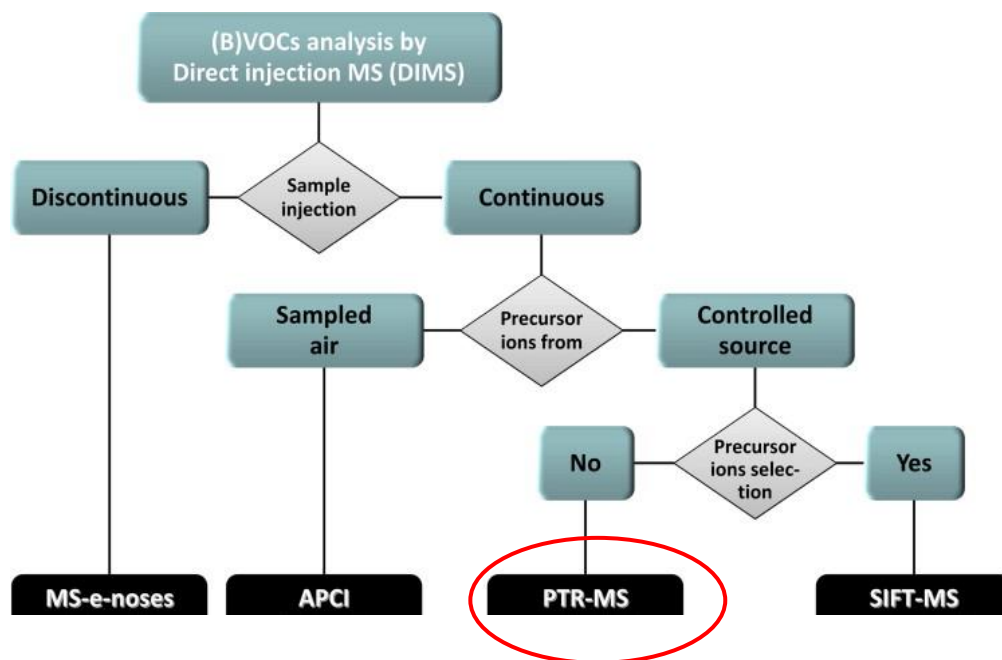
# Proton Transfer Reaction (PTR), combined with a Time-of-Flight (ToF) Mass Spectrometer (MS)

## Review

PTR-MS: a green alternative for food volatile profiling

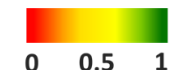
## DOI

10.1016/j.greeac.2022.100041



**GREEN**

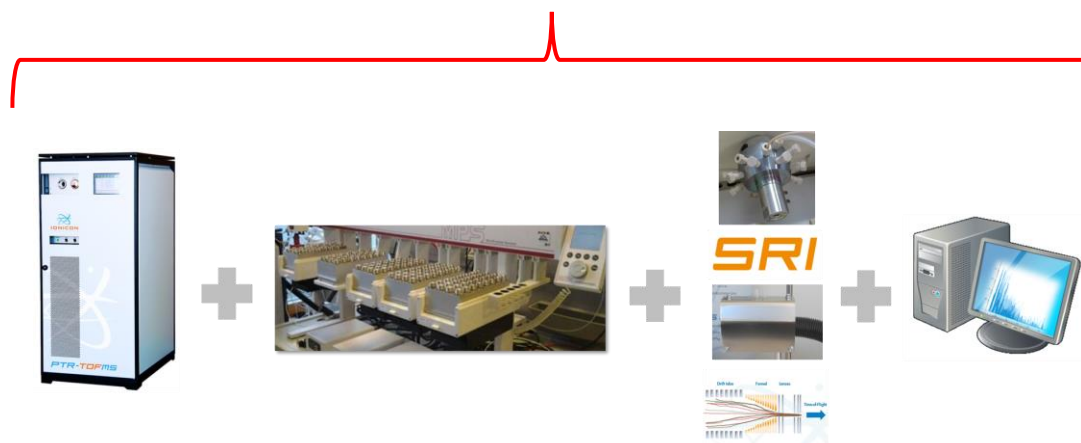
GC-MS



PTR-ToF-MS



1. Sample treatment
2. Sample amount
3. Device positioning
4. Sample prep. stages
5. Automation, miniaturization
6. Derivatization
7. Waste
8. Analysis throughput
9. Energy consumption
10. Source of reagents
11. Toxicity
12. Operator's safety



- **RAPID**
- ON LINE MONITORING
- MASSIVE SCREENINGS
- BIOPROCESS STUDY
- VERSATILE APPROACH

# Applications of PTR-MS in Food Science and Technology

## Chapter

Real-Time  
Monitoring of  
Flavoring Starter  
Cultures for  
Different Food  
Matrices Using  
PTR-MS

## DOI

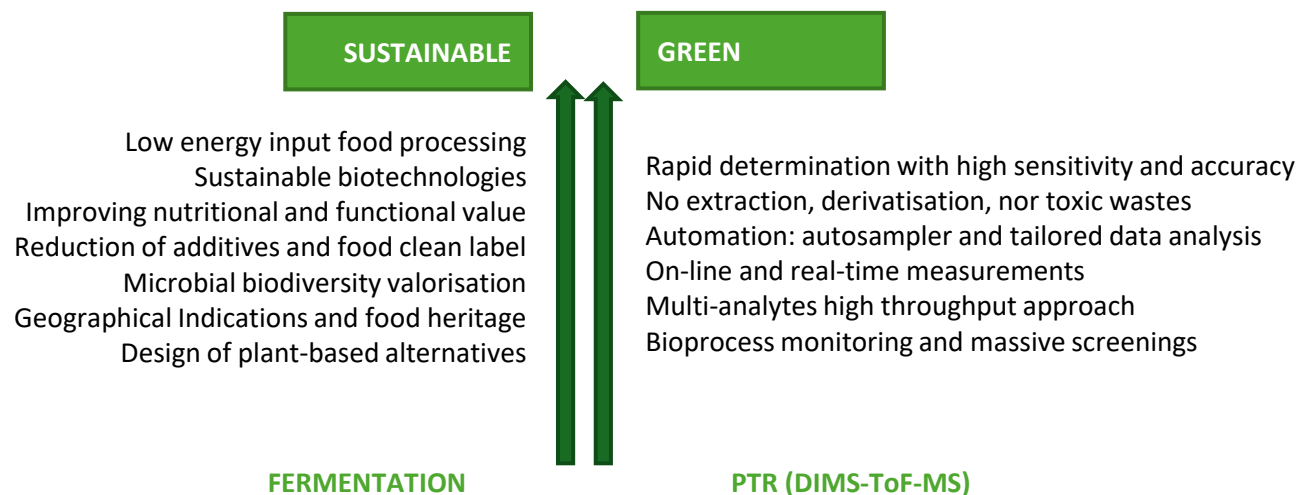
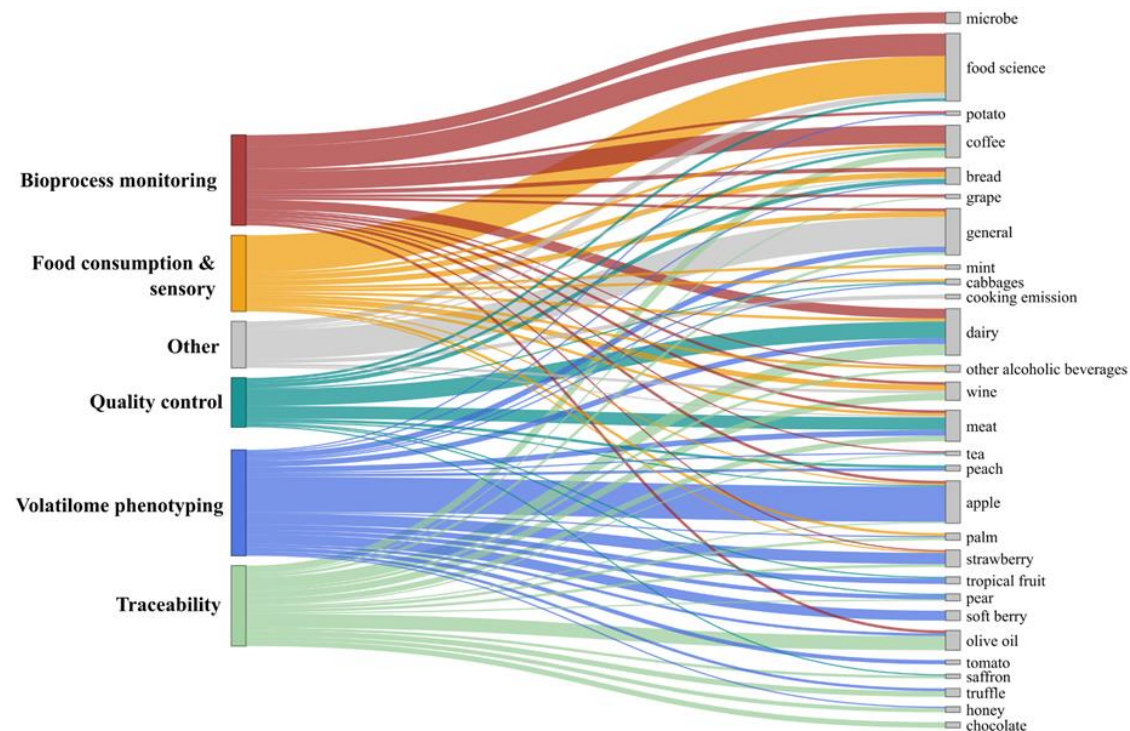
10.1021/bk-2021-  
1402.ch010

## Review

PTR-MS: online  
and rapid  
determination of  
volatile organic  
compounds of  
microbial origin

## DOI

10.1007/s00253-  
015-6528-y



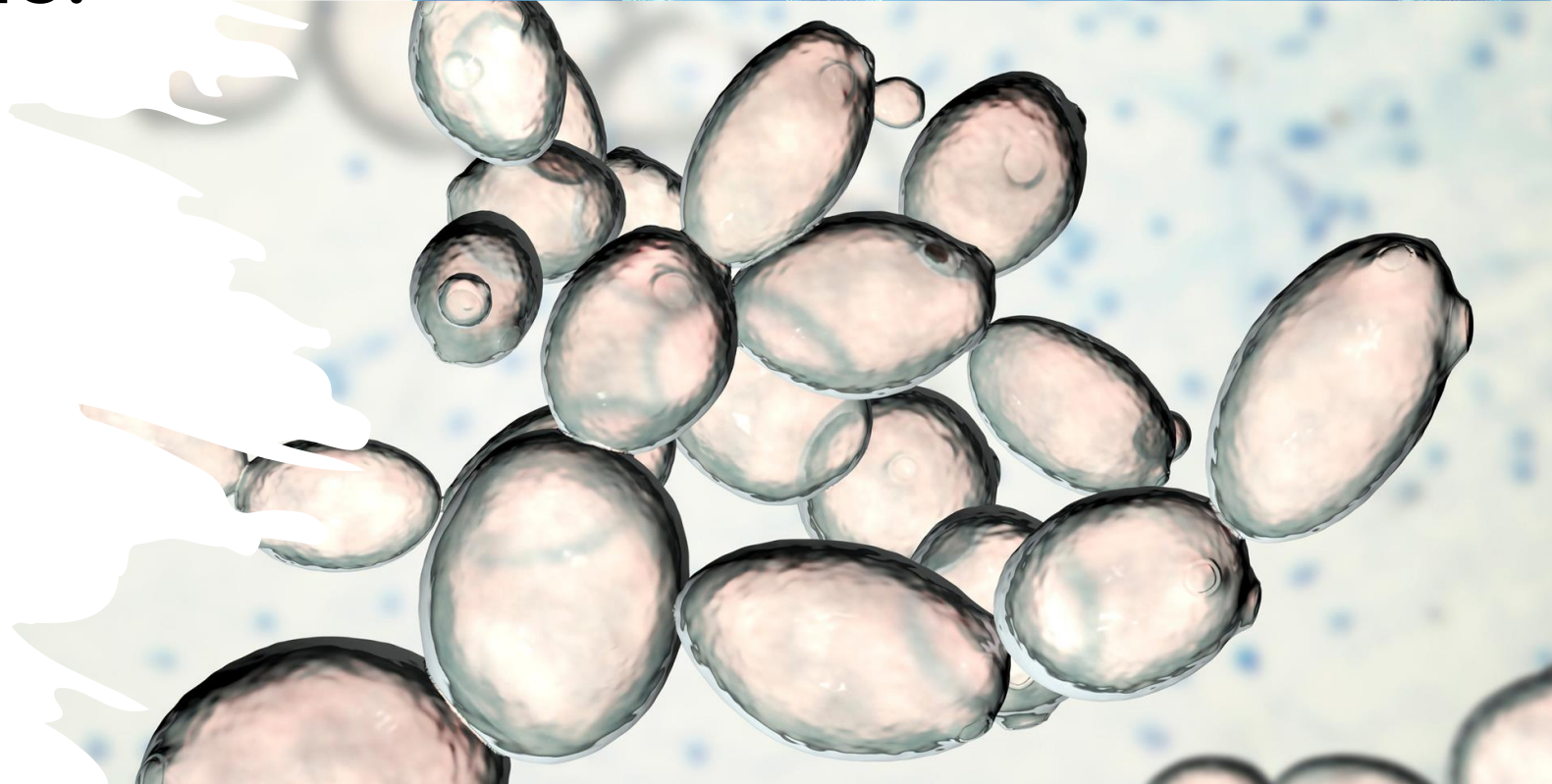


# CASE STUDIES

## Volatilome of single culture

### BIOPROCESS MONITORING:

1. Lactic acid bacteria
2. Yeasts

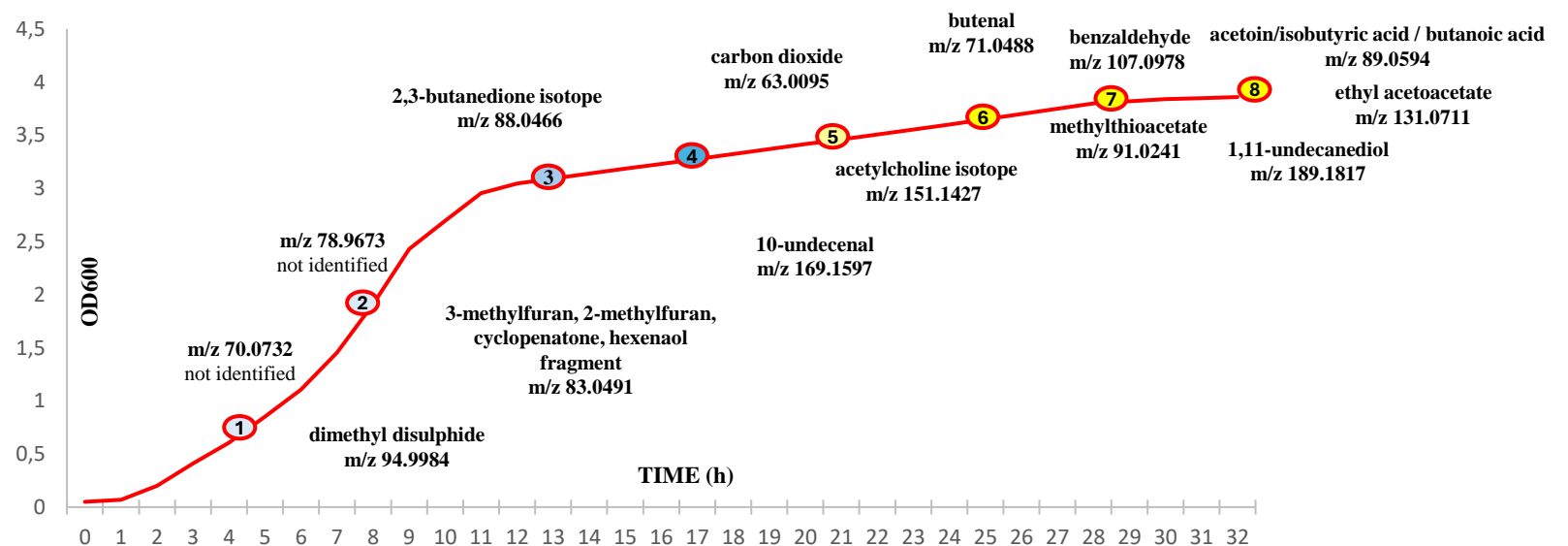
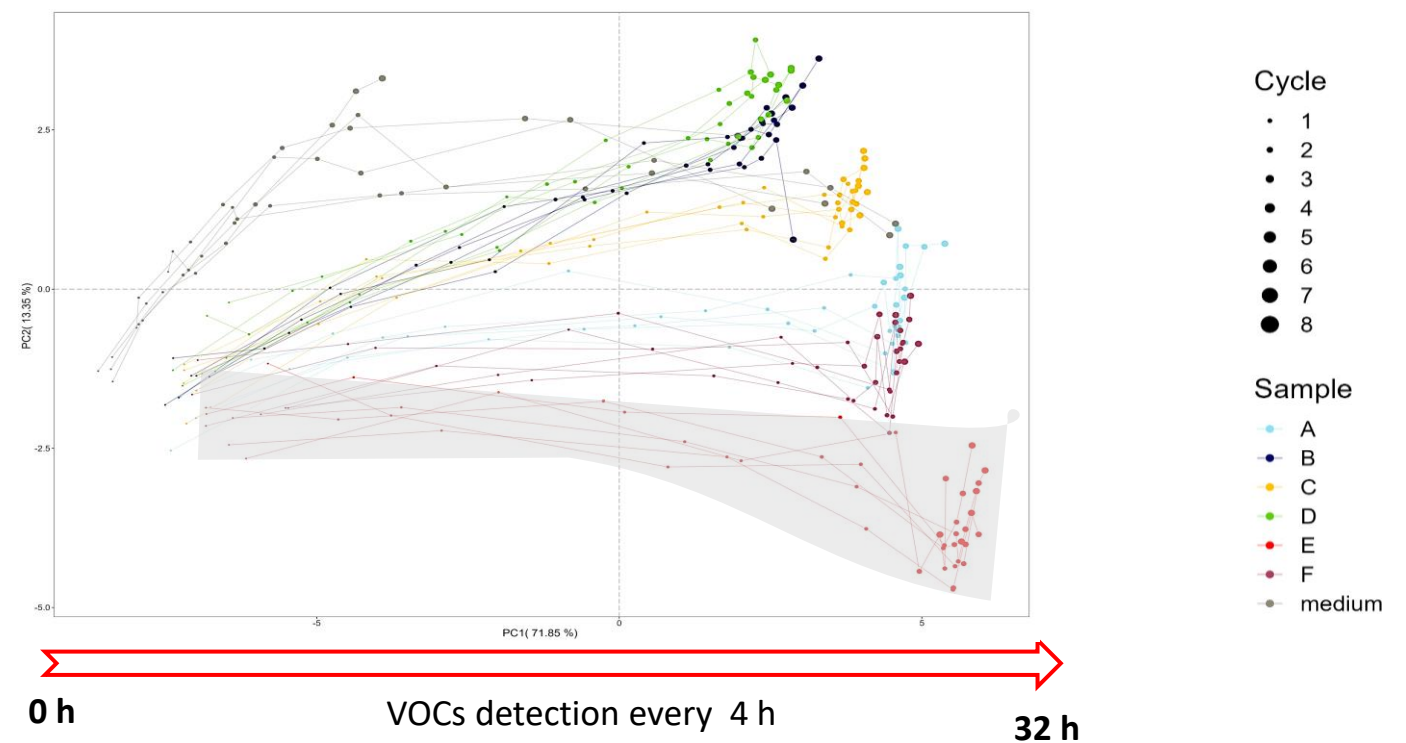
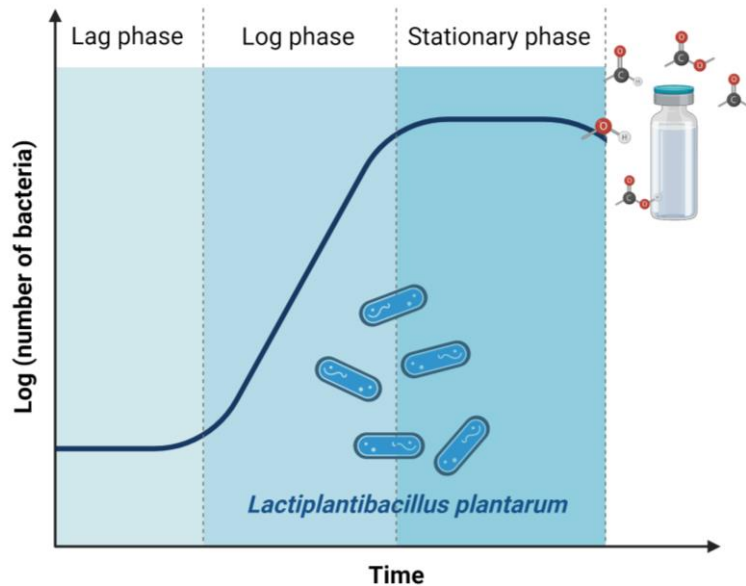


1

Case study  
Volatome  
different LAB  
strains  
Matrix  
Microbiological  
medium

Six strains of  
*Lactiplantibacillus*  
*plantarum*  
A B C D E F  
(two laboratory  
strains and four from  
different ecological  
niches)

Optimal condition: T 30°C & pH 6.2

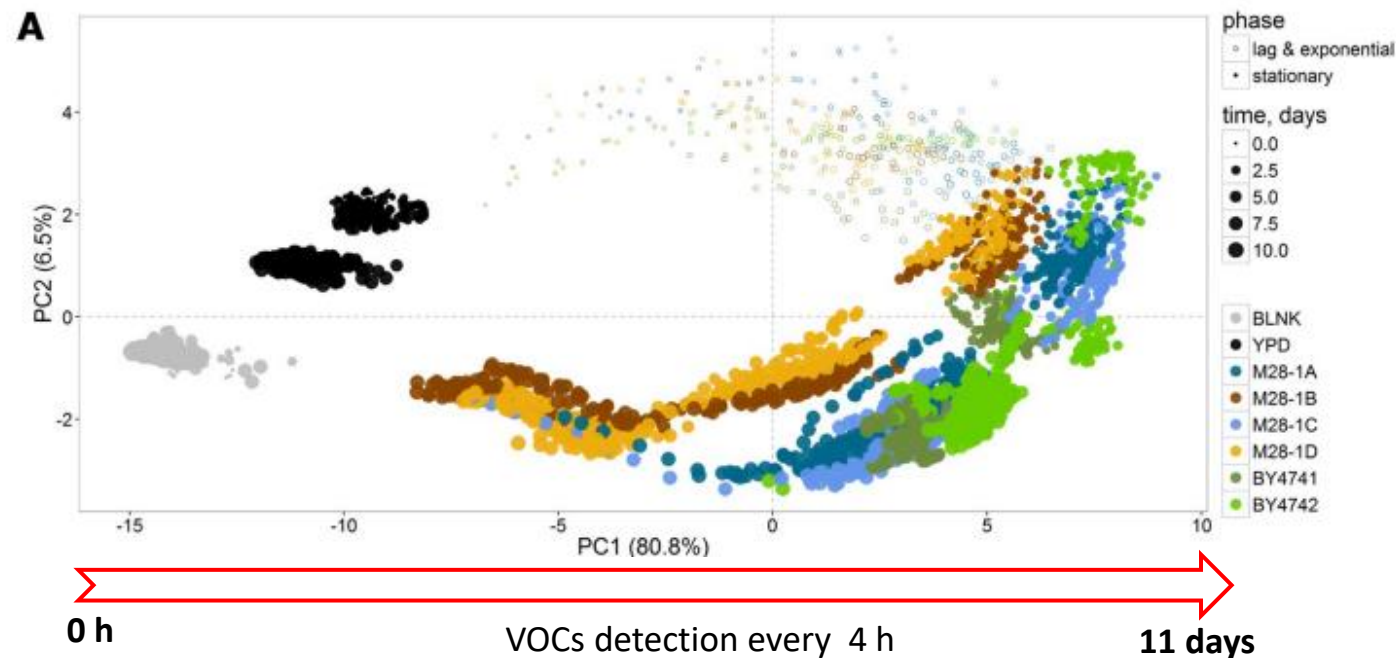
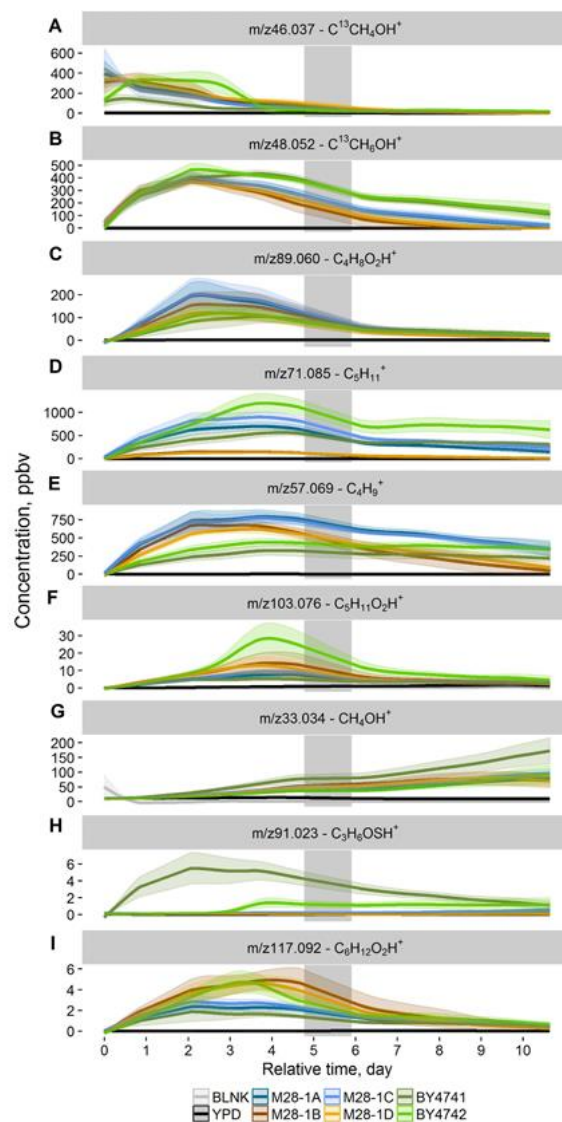




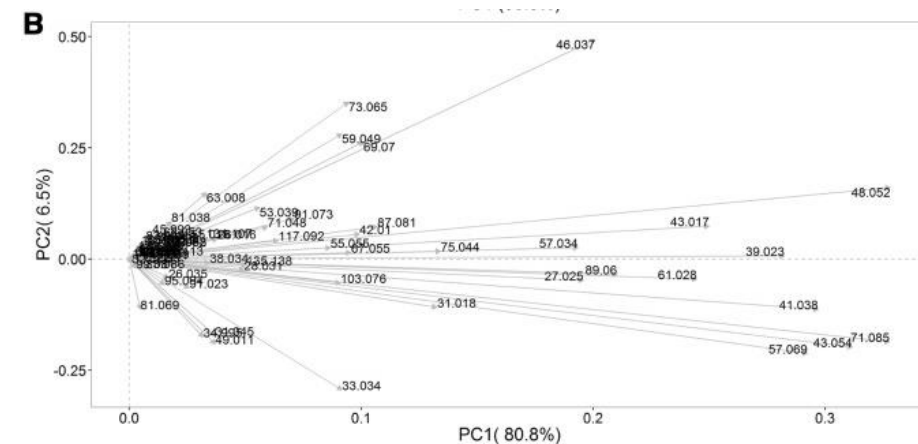
**Case study**  
**Volatome**  
 different yeast  
 strains  
**Matrix**  
 Microbiological  
 medium  
**DOI**  
 10.1007/s11306-  
 017-1259-y

Six different  
*Saccharomyces cerevisiae* strains  
 (four meiotic  
 segregants of a  
 natural strain and  
 two laboratory  
 strains)

Curves of selected mass  
 peaks tentatively identified



General picture  
 of yeast  
 colonies' VOC  
 profiles  
 evolution during  
 the 11 days  
 showing the  
 drastic  
 differences with  
 growth



# CASE STUDIES

## Volatilome of microbiome

### SCREENING/CHARACTERIZATION ANALYSIS:

1 Bread

2 Table olive

### BIOPROCESS MONITORING:

3 Grape juice and must

4 Milk and cream





**Case study**  
**Volatilome of  
sourdoughs**

**Matrix**  
**Bread**

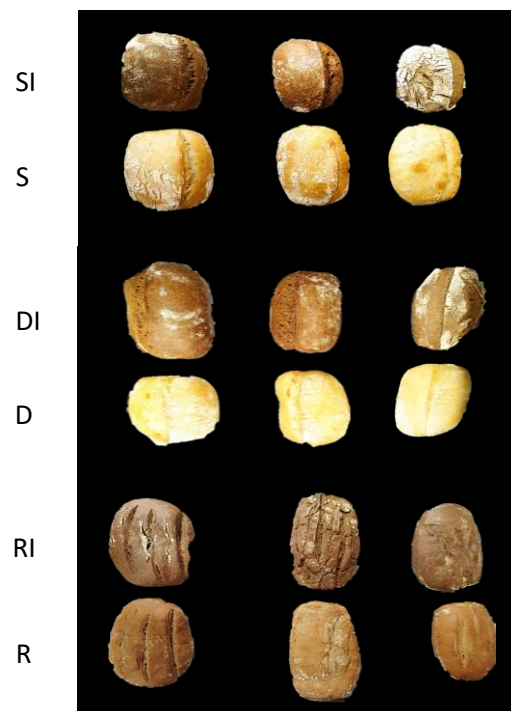
**18 types of dough** (200 g), with different combinations of cereal flours, microbial resources and insect flour.

Leavening agent  
Brewer's yeast (Y)  
Commercial sourdough (CS)  
Traditional Trentino sourdough (TS)

## LEAVENING AGENT

TS                      CS                      Y

## INSECT (I) AND CEREAL FLOUR (S, D, R)

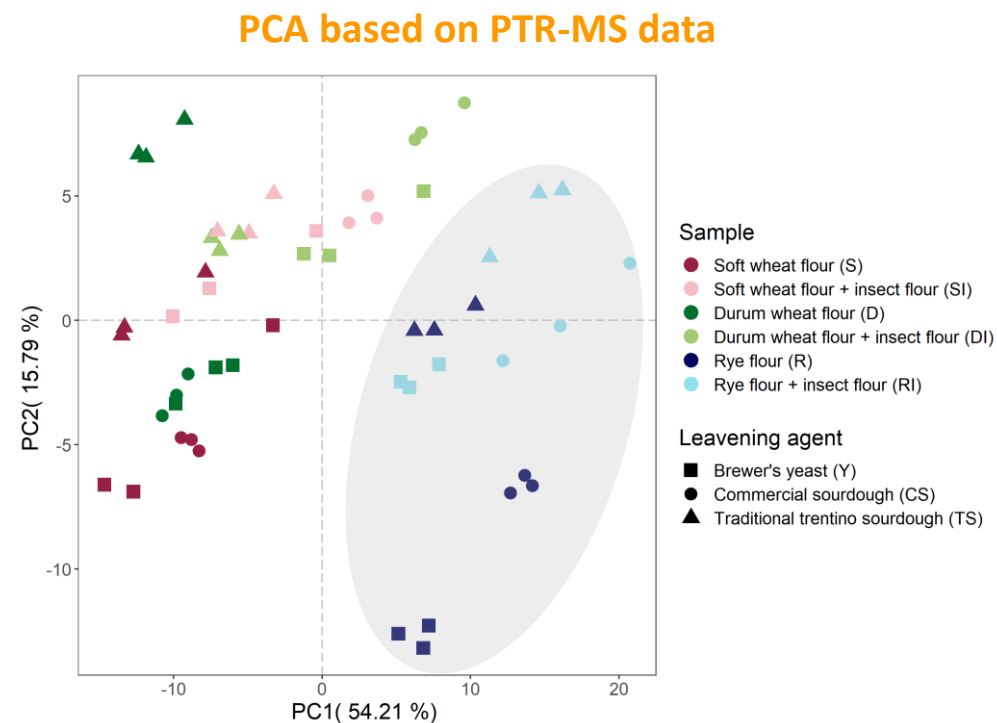


## Flour

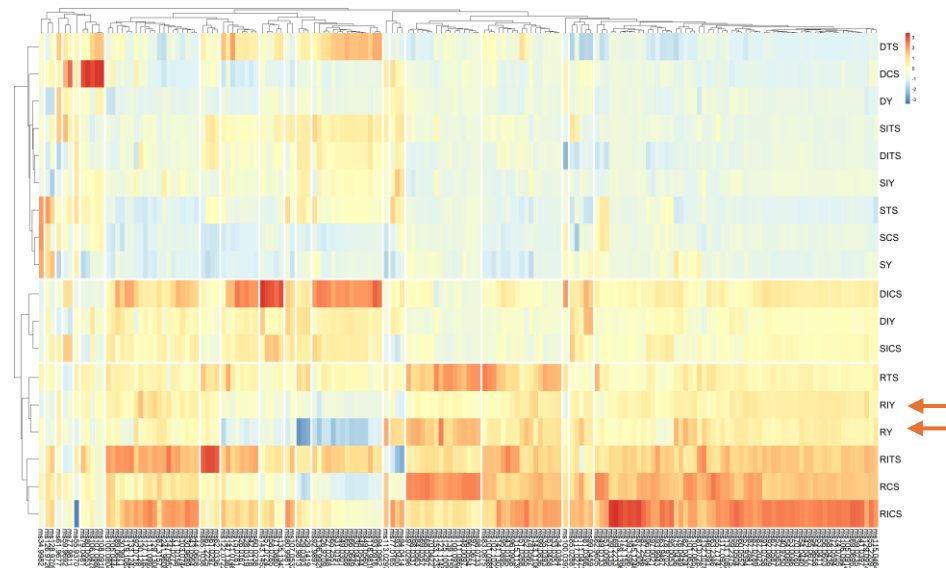
## Cereal

Soft wheat (S)  
Durum wheat (D)  
Rye (R)

Insect  
Cricket 15% (I)



## Heatmap based on PTR-MS data





## Case study

Volatilome of  
two different  
fermentation

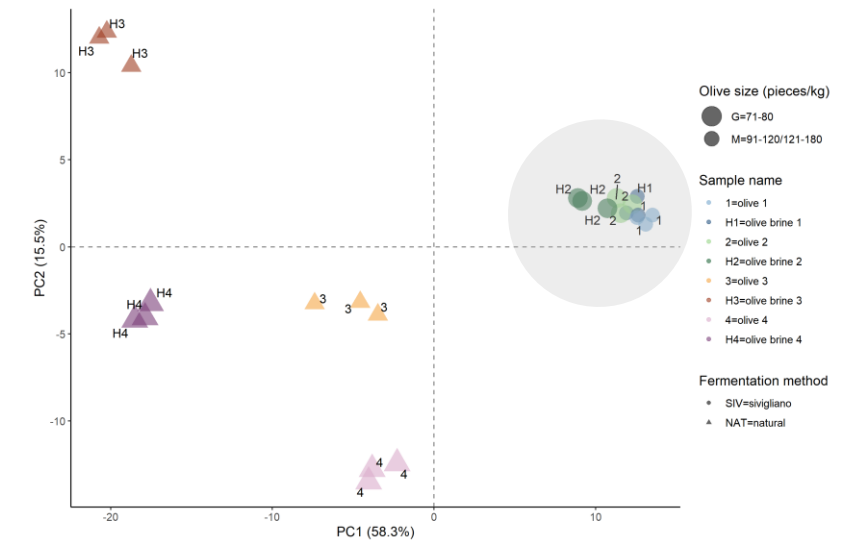
## Matrix

Table olive and  
brine

Description of table olives (1, 2, 3 and 4) and respective brines (H1, H2, H3 and H4) under evaluation.

Sample name	Matrix	Size (pieces/kg)	Fermentatio n mode
1	olive fruit	M	SIV
H1	brine	-	SIV
2	olive fruit	G	SIV
H2	brine	-	SIV
3	olive fruit	M	NAT
H3	brine	-	NAT
4	olive fruit	G	NAT
H4	brine	-	NAT

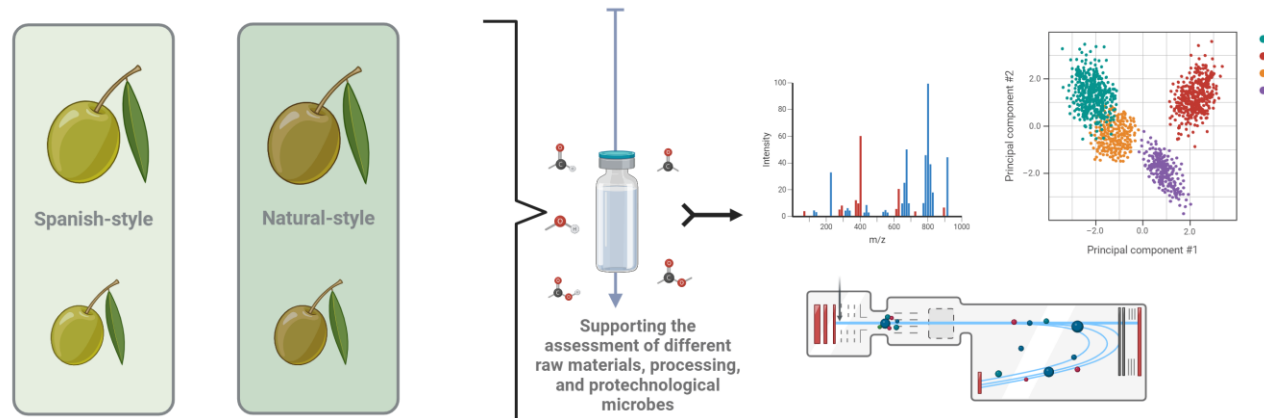
## PCA based on PTR-MS data



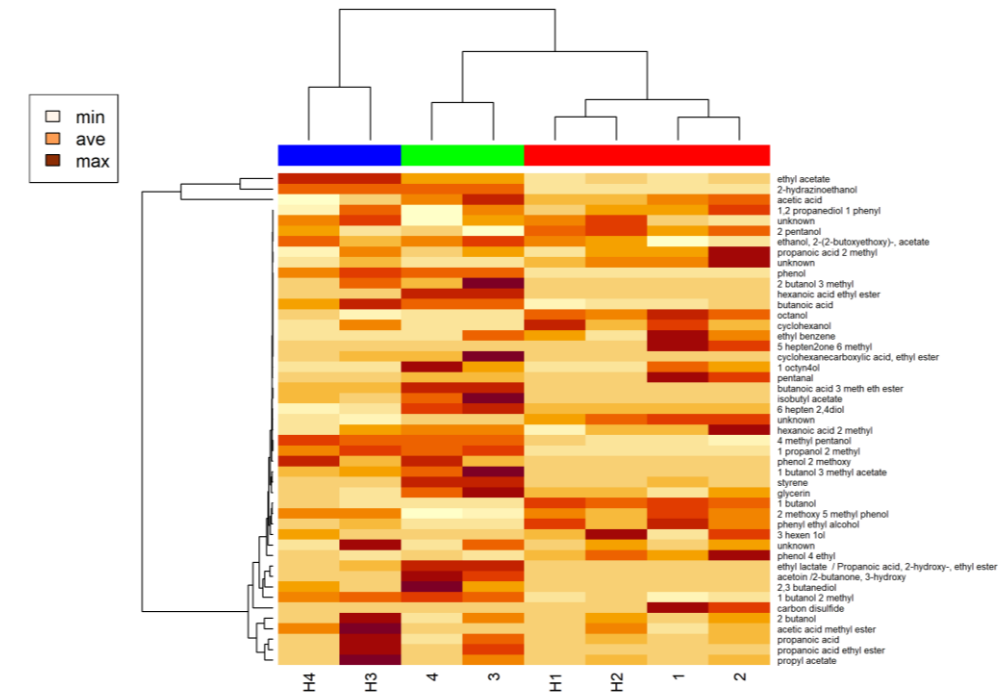
## Apulian olives, 'Bella di Cerignola' variety

**G**  
71-80  
pieces/kg

**M**  
91-120/  
121-180  
pieces/kg



## Heatmap based on GC-MS data



## Case study

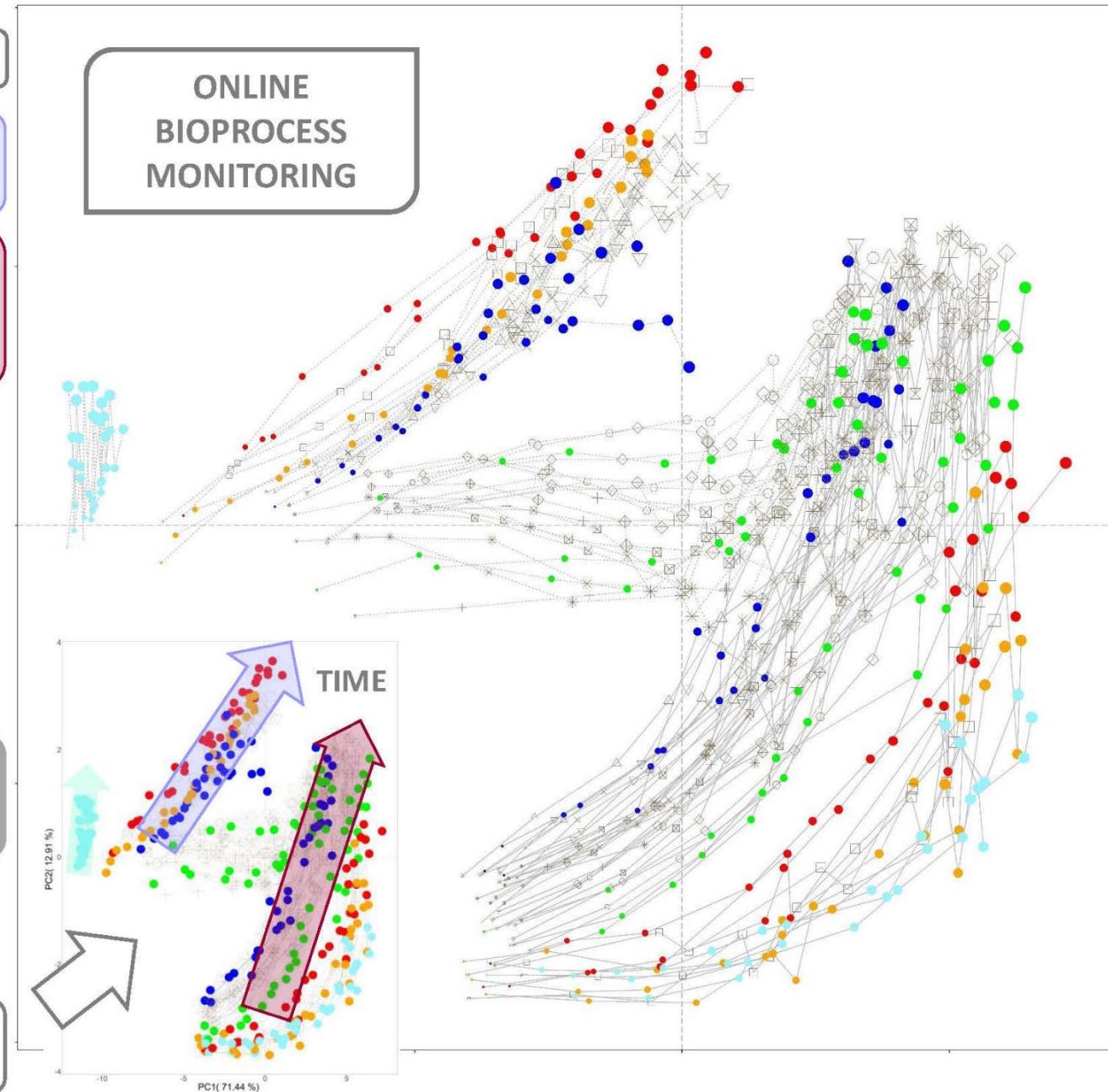
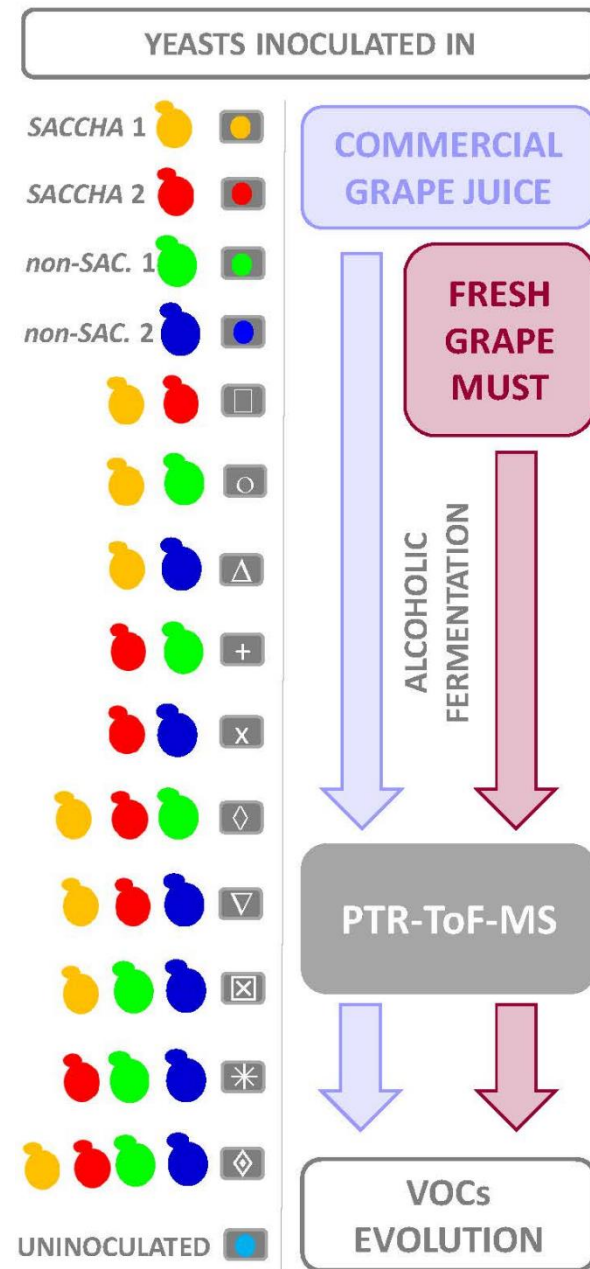
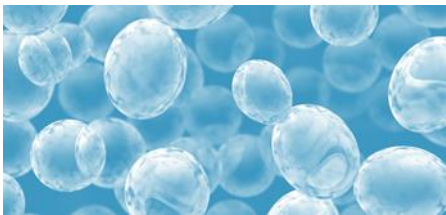
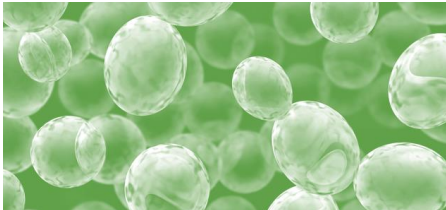
Interaction  
among starter  
cultures:  
alcoholic  
fermentation

Matrix

Wine

DOI

10.3390/fermentatio  
n6020055



4

Case study  
Volatilome of  
starter  
Matrix  
Milk/Cream

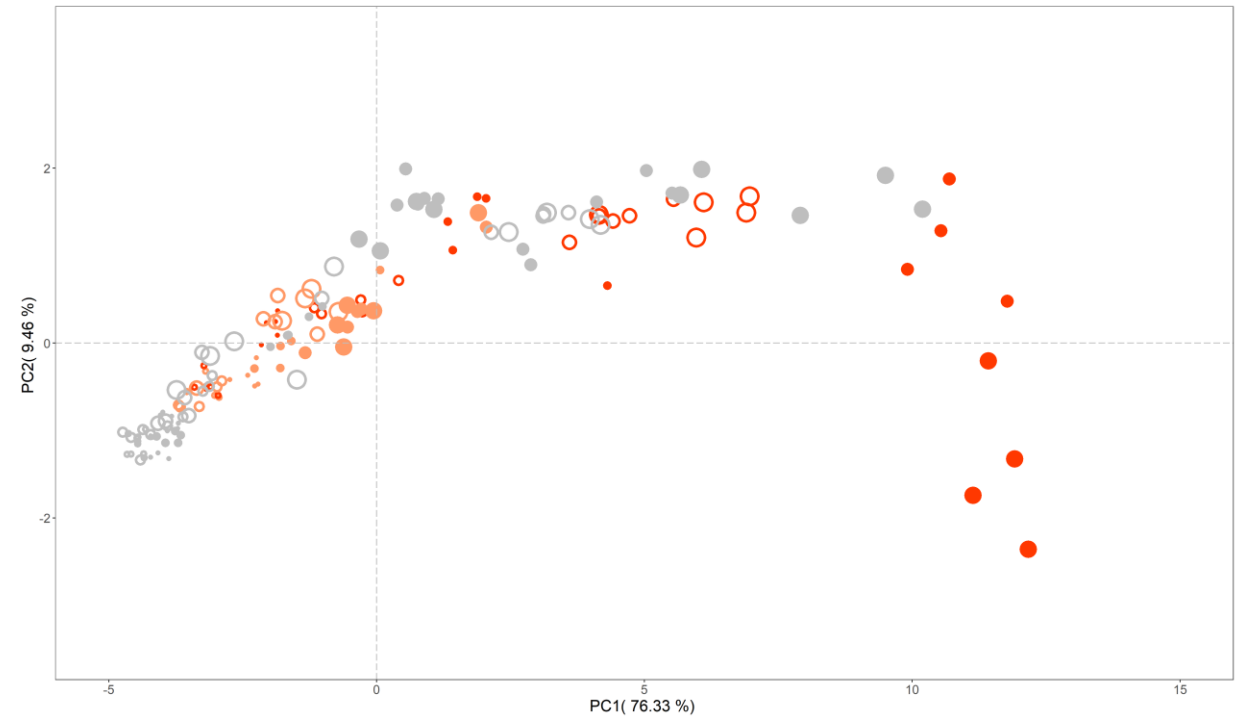
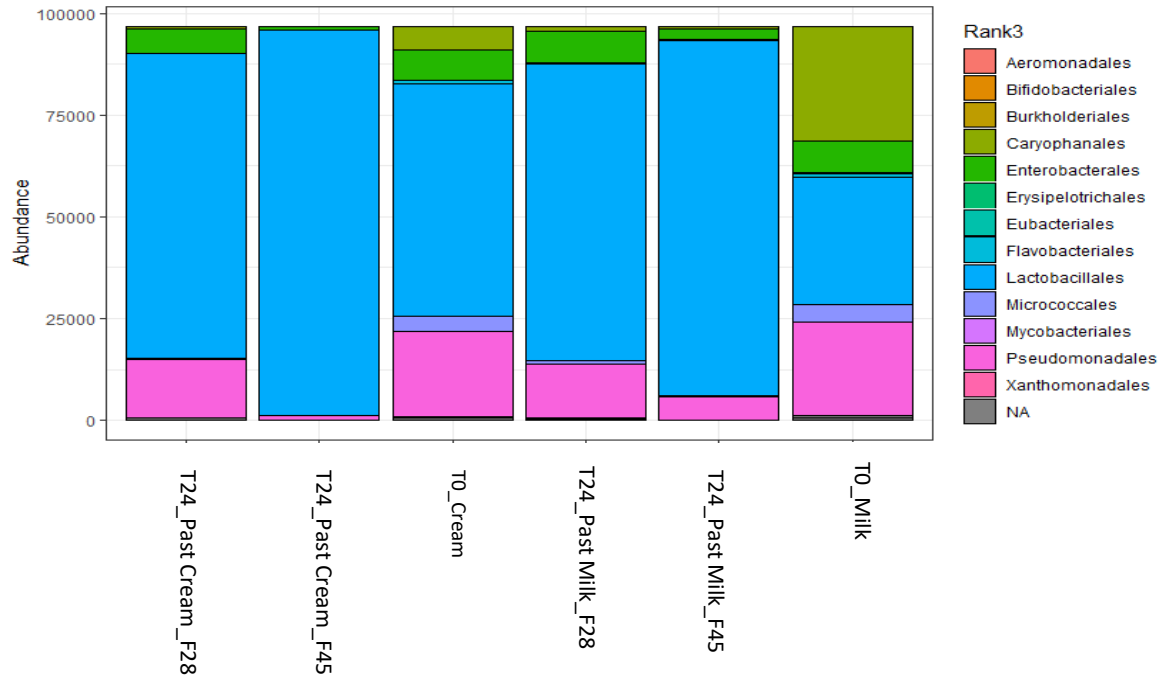


**Matrix**  
Milk  
Cream  
(50% diluted with milk)

**Treatment**  
Pasteurization  
Not pasteurization

**Fermentation temperature**  
28°C  
45°C

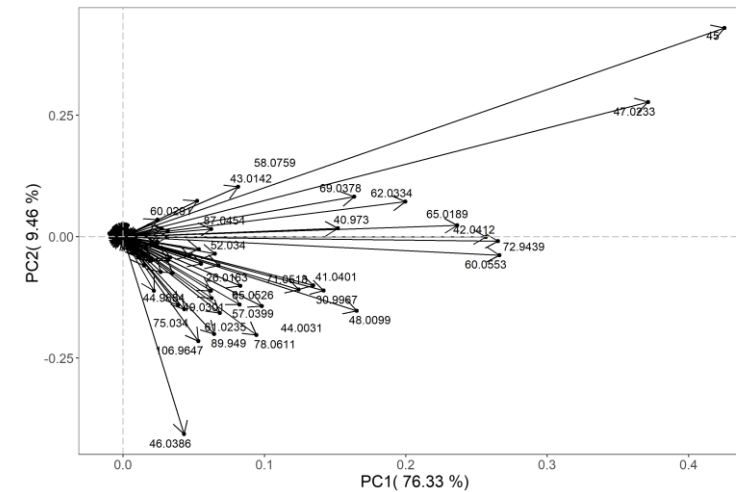
Taxa\_V3-V4



0 h

VOCs detection every 6 h

24 h



Fermentation Temperature

○ 28°C  
● 45°C

Cycle

• 1  
• 2  
• 3  
• 4

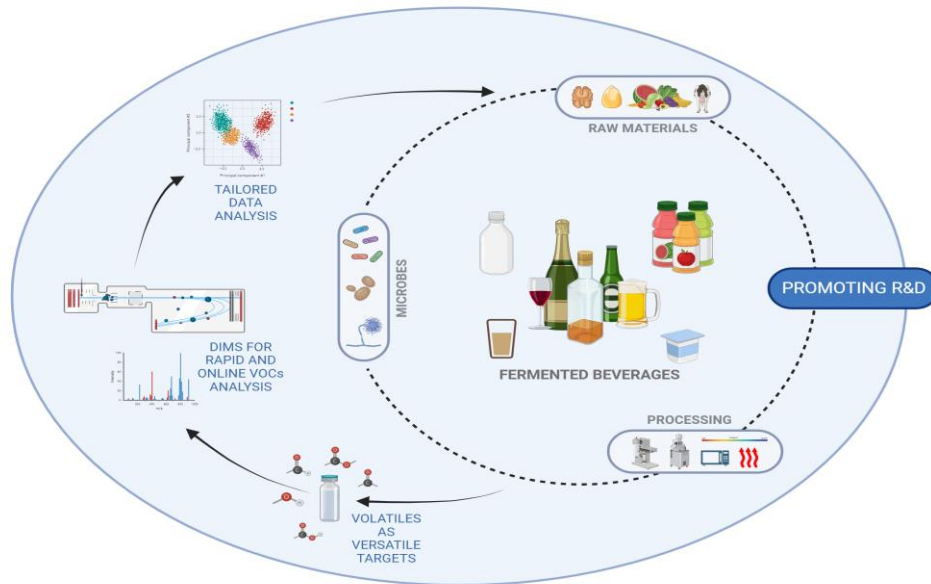
Sample

• Pasteurized milk (M\_P)  
• Non-pasteurized milk (M\_NP)  
• Pasteurized cream (C\_P)  
• Non-pasteurized cream (C\_NP)



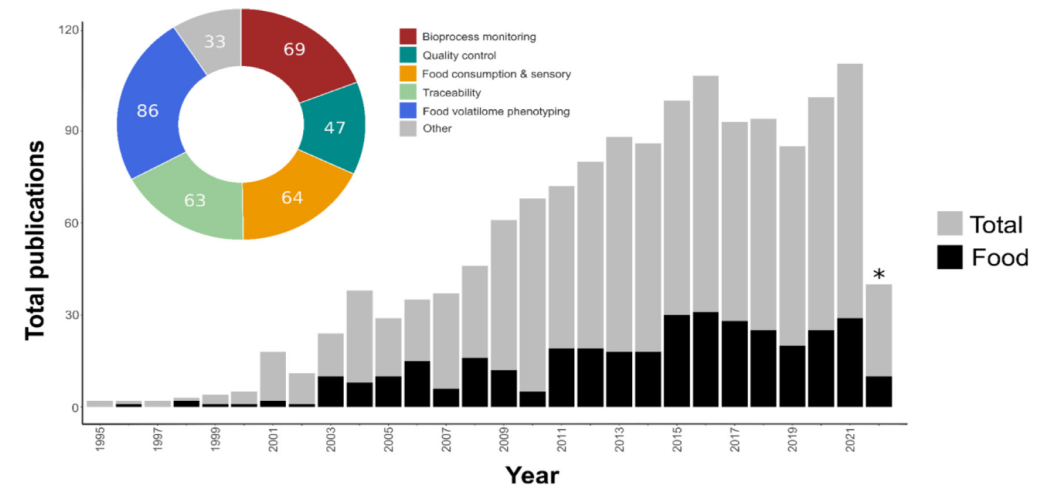
# Studies on 'Volatilome' for a deeper understanding

Variables involved in a fermented food process and analysis.



Corvino *et al.*, 2024  
DOI: 10.1111/ijfs.17398

The total number of manuscripts by year related to the general PTR-MS topic in grey and to food science and technology in specific in black.



Mazzucotelli *et al.*, 2022  
DOI:10.1016/j.greeac.2022.100041



Thank to my colleagues:

Maria Mazzucotelli  
Emanuela Betta  
Andrea Dell'Olio  
Massimo Pindo

# Thank you for attention!

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