Volatile compound fingerprinting of mixed-culture fermentations.

published in
Applied and Environmental Microbiology
2011

DOI (link to publisher)
10.1128/AEM.00352-11

document version
Publisher's PDF, also known as Version of record

Link to publication in VU Research Portal

citation for published version (APA)

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

E-mail address:
vuresearchportal.ub@vu.nl

Download date: 23. Apr. 2024
Volatile Compound Fingerprinting of Mixed-Culture Fermentations

Frank A. M. de Bok,1,2* Patrick W. M. Janssen,1,2 Jumamurat R. Bayjanov,3 Sander Sieuwerts,1,2,4 Arjen Lommen,5 Johan E. T. van Hylckama Vlieg,1,2† and Douwe Molenaar1,2‡

Top Institute Food and Nutrition, P.O. Box 557, 6700 AN Wageningen, The Netherlands1; NIZO food research, P.O. Box 20, 6710 BA Ede, The Netherlands2; Center for Molecular and Biomolecular Informatics, Radboud University Nijmegen Medical Centre, Geert Grooteplein Zuid 26-28, 6525 GA Nijmegen, The Netherlands3; Wageningen University, Laboratory of Microbiology, Dreijenplein 10, 6703 HB Wageningen, The Netherlands4; and RIKILT—Institute of Food Safety, Bornsesteeg 45, Postbus 230, 6700 AE Wageningen, The Netherlands5

Received 16 February 2011/Accepted 30 June 2011

With the advent of the -omics era, classical technology platforms, such as hyphenated mass spectrometry, are currently undergoing a transformation toward high-throughput application. These novel platforms yield highly detailed metabolite profiles in large numbers of samples. Such profiles can be used as fingerprints for the accurate identification and classification of samples as well as for the study of effects of experimental conditions on the concentrations of specific metabolites. Challenges for the application of these methods lie in the acquisition of high-quality data, data normalization, and data mining. Here, a high-throughput fingerprinting approach based on analysis of headspace volatiles using ultrafast gas chromatography coupled to time of flight mass spectrometry (ultrafast GC/TOF-MS) was developed and evaluated for classification and screening purposes in food fermentations. GC-MS mass spectra of headspace samples of milk fermented by different mixed cultures of lactic acid bacteria (LAB) were collected and preprocessed in MetAlign, a dedicated software package for the preprocessing and comparison of liquid chromatography (LC)-MS and GC-MS data. The Random Forest algorithm was used to detect mass peaks that discriminated combinations of species or strains used in fermentations. Many of these mass peaks originated from key flavor compounds, indicating that the presence or absence of individual strains or combinations of strains significantly influenced the concentrations of these components. We demonstrate that the approach can be used for purposes like the selection of strains from collections based on flavor characteristics and the screening of (mixed) cultures for the presence or absence of strains. In addition, we show that strain-specific flavor characteristics can be traced back to genetic markers when comparative genome hybridization (CGH) data are available.

Metabolomic analysis, i.e., the measurement of (relative) concentrations of a large number of metabolites in a biological sample, is essential to come to a comprehensive understanding of living organisms, since phenotypic properties result from the history, genotype, environment, and their interactions (7). But even in the absence of a complete understanding of the causal chain leading to a particular metabolic profile, these profiles have the potential for use as fingerprints or biomarkers in a large variety of applications, like diagnosis in medicine (10) or in food sciences as biomarkers of taste and health properties (3). These applications depend on the ability to collect high-quality metabolome data and on the proper normalization and analysis of the resulting high-dimensional data. The technology for collecting metabolome data has advanced rapidly in the past years (3). In particular, gas chromatography-mass spectrometry (GC-MS) and liquid chromatography (LC)-MS are increasingly utilized for profiling of biological samples because of their inherent robustness, sensitivity, and large dynamic range (23). However, the development of data analysis techniques is lagging behind, with the consequence that the novel techniques are probably not used to their full potential. Bottlenecks in data analysis concern the normalization and alignment of GC-MS data to allow the comparison of samples, the chemical identification of compounds, and the identification of biomarkers. To tackle the normalization and alignment problems, tools have been developed that facilitate user-assisted preprocessing of multiple mass spectra to correct retention time drifts that are inherent in chromatography (8, 14). Basically these packages perform baseline correction, smoothing, and alignment of mass spectra to enable peak comparison and subsequent multivariate analysis. Together with technological advances, such as ultrafast GC coupled to time-of-flight (TOF) detection of ions (19, 30), these software packages significantly reduce the time required to analyze and compare detectable compounds in large numbers of samples. One of the tools that is widely applied is MetAlign (http://www.metalign.wur.nl/UK/) (16), a package that initially was developed to support LC-MS-based metabolomics and was, for instance, used as such to study the metabolite profiles of Arabidopsis thaliana and fruits of the tomato plant (Lycopersicon esculentum) (2, 15).

Methods for the subsequent identification of biomarkers, by investigation of correlations of metabolome data with other sample properties, are sometimes included in the packages for data preprocessing. However, these are not suitable for all challenges in data mining and the researcher then depends on other methods. A “Swiss knife” among the methods for the detection of such correlations is the Random Forest algorithm.

* Corresponding author. Present address: KBBL Wijhe B.V., Industrialweg 16, 8131 VZ Wijhe, The Netherlands. Phone: 31(0)570-522324; Fax: 31(0)570-522325; E-mail: fdebok@gmail.com.
† Present address: Danone Research, Avenue de la Vauve, 91767 Palaiseau Cedex, France.
‡ Present address: University of Amsterdam, Faculty of Earth and Life Sciences, Systems Bioinformatics, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands.

† Published ahead of print on 8 July 2011.
The Random Forest algorithm was introduced by Breiman (4) as a method for supervised or unsupervised data classification and regression. It has properties that make it very suitable as an exploratory tool for high-dimensional data gathered on relatively small sets of samples, like transcriptome or metabolome data. The method is relatively insensitive to noise and outliers, thereby avoiding overfitting. Also, data transformations like normalizations and rescaling are usually not necessary. Furthermore, the method indicates which of the variables are highly correlated with sample properties by so-called variable importance measures. Since only a small number of algorithm parameter values, to which the algorithm is not very sensitive, have to be set by the user, it is also a very user-friendly method. These properties have made the Random Forest algorithm a popular tool in life sciences, and in this paper we show how it can be applied to target differences in profiles of volatile compounds present in food products.

Mixed-culture fermentation represents an interesting case in which to apply metabolite profiling techniques, since the organoleptic properties of fermented food products are strongly influenced by microbial activity during and after fermentation and directly relate to the strains involved and their population dynamics. As a consequence, small changes in microbial composition or population dynamics may have a large effect on product characteristics and on flavors in particular, since small differences in the levels of some of these can have a significant impact on the aroma of the product (5, 11, 20). Hence, there is a clear need for fast and predictive methods to screen for flavor development in mixed-culture food fermentations (21, 24).

We investigated an ultrafast GC/TOF-MS approach to perform untargeted profiling of volatile compounds detected in food using MetAlign and subsequent mining of the data using the Random Forest algorithm. To reduce analysis times, samples were not concentrated and the usefulness of the approach for screening purposes was examined by analyzing the volatile compounds produced by various mono- and mixed cultures of lactic acid bacteria (LAB) during fermentation of milk.

### MATERIALS AND METHODS

Ultrafast GC/TOF-MS. A Thermo Finnigan Tempus ultrafast GC/TOF-MS system was used for separation and detection of volatile compounds in the headspace of fermented milk samples. Chromatography was performed using a capillary column (10 m by 0.18 mm; UFM PH-RTX200; Interscience, Breda, Netherlands) with a 0.4-
m film thickness. The column oven, which was cooled by injection of liquid carbon dioxide, was held at 10°C for 0.5 min, programmed to 200°C at 100°C/min, and then held at 200°C for 0.5 min. The injector, transfer line, and detector temperatures were kept constant at 250°C. Before injection, sample vials were transferred from a cooled sample tray (4°C) into a dynamic headspace of fermented milk samples. Chromatography was performed using a.

**Culture conditions.** Streptococcus thermophilus strains CNZRJ06, LMG18311, and LMD9 and 40 Lactococcus lactis strains (Table 1) were precultured in GM-17 broth containing 1% glucose at 42°C and 38°C, respectively. Lactobacillus delbrueckii subsp. bulgaricus strain ATCC BAA-365 and Lactobacillus plantarum strain WCFS1 were precultured in MRS broth at 42 and 37°C, respectively. Cultures were adapted to growth in reconstituted ultra-high-heat-treated (UHT) skim milk by transferring 1% from the broth cultures. The lactobacilli and streptococci were incubated at 37°C for 24 h, and the lactococci were incubated at 30°C for 48 h. Acidification of each milk batch was recorded using the CINAC system (9), and CFU counts were determined by plating on selective agar media. Samples for ultrafast GC/TOF-MS analysis were prepared by transferring 5-ml fermented milk samples to 10-ml glass crimp-cap vials or by inoculating the milk within these vials for the lactococci. The vials were sealed immediately after inoculation or filling with magnetic crimp caps with Teflon inserts.

**Data analysis.** Mass spectra were processed in MetAlign using parameters optimized for the spectra recorded on the Thermo Finnigan ultrafast GC/TOF-MS. Four replicate measurements were performed per sample condition (combination of bacteria). The resulting data sets, which consisted of ~10,000 aligned time-mass peaks and their intensities in each sample, were reduced by analysis of variance (ANOVA) filtering. A one-way ANOVA test was applied to select only those time-mass peaks that displayed a statistically significant different signal in at least one of the sample conditions. The stringency of this filter was kept very low by using a P value cutoff of 0.5. Filtered data sets consisted of approximately 3,600 time-mass peaks. The Random Forest algorithm was applied on these data sets using an implementation of the algorithm in R (http://www.r-project.org/) written by A. Liaw and M. Wiener. A unique culture label for each of the mixtures was used as the response variable, whereas the 3,600 selected time-mass peak intensities were used as predictor variables. The mtry parameter (number of variables randomly sampled as candidates at each split) was set at the default value (square root of the number of variables), and the number of trees grown was set at 5,000. Sets of approximately 100 time-mass peaks with high importance in the resulting classifier were selected for further manual inspection. Using the Xcalibur software (Thermo Finnigan) and AMDIS (27), most of these time-mass peaks could be associated with high confidence to volatile compounds present in the NIST mass spectral library (http://www.nist.gov/srd/nist1a.htm). Subsequently, individual time-mass peak intensities were summed by compound.

### RESULTS

**Differential profiling of mono- and mixed-culture fermentations.** To assess whether the approach can be used for discrim-
ination of cultures at the species and strain level based on volatile compounds produced, 11 single and mixed-culture fermentations were prepared. Batches containing 250 ml of preheated milk were inoculated with *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. plantarum*, three strains of *S. thermophilus*, and mixtures of these (Fig. 1). The batches, including a control batch containing uninoculated milk, were incubated at 37°C in a water bath, and acidification of the cultures was recorded using the CINAC system. After 26 h, fermentations were stopped by cooling on ice and samples were taken to determine species-specific viable counts and to prepare for headspace analysis. The final pHs of the cultures varied between 4.0 for two of the cultures containing both yoghurt bacteria and 6.4 for the milk inoculated with *Lb. plantarum* only (Fig. 1). The culture containing *S. thermophilus* strain LMD9 and all mixed cultures including both *S. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus* showed the highest acidification rates. The volatile compounds present in each culture after fermentation were analyzed in quadruplicate using ultrafast GC/TOF-MS followed by alignment of the mass spectra and further processing of the resulting peak lists using Random Forest. To determine which mass peaks most significantly contributed to the distinction of the 12 different groups, the peak lists generated by MetAlign were first filtered by ANOVA, and the remaining peaks were used to build a Random Forest predictor. This allowed accurate prediction of nearly all samples. Only discrimination of the *S. thermophilus* CNRZ1066 culture from the mixed culture with *Lb. plantarum* seemed to be limited (Fig. 1). To identify the compounds corresponding to discriminatory mass peaks, they were traced back in the original spectra using the mass spectrometer software. Whenever possible, compounds were identified using the NIST library. Finally, a pair plot of the summed total ion count signals of relevant compounds was generated to visualize which compounds allowed discrimination of the different groups as well as to reveal possible relations between the levels of different compounds produced (Fig. 2). From this plot, compounds responsible for distinction of the flavor profiles of the different combinations of bacteria can be identified. Distinction of the cultures containing *Lb. delbrueckii* subsp. *bulgaricus* only or together with *Lb. plantarum* was mainly due to the higher levels of 2-heptanone and 2-propanone produced and the low level of diacetyl produced compared to the other cultures (Fig. 2, I). The presence of *Lb. plantarum* led to high levels of 2-methylpropanal, 3-methylbutanal, and 2-pentanone in milk but only in the absence of other lactic acid bacteria (Fig. 2, II). The level of 2-methylpropanal was also relatively high in the milk fermented with *Lb. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* strain LMG18311 (Fig. 2, III). Separation of the three different strains of *S. thermophilus* was mainly due to differences in the levels of diacetyl produced (Fig. 2, IV, blue, lilac, and pink diamonds).

**FIG. 1.** Hierarchical clustering using Random Forest sample proximities of mass peak profiles generated by ultrafast GC/TOF-MS analysis of volatile compounds in headspace samples. The tree represents 48 mono- and mixed cultures of lactic acid bacteria in milk, with uninoculated milk as a control, after 26 h incubation at 37°C. Each condition (12 conditions in total) was tested in quadruplicate to illustrate that differences in volatile compounds present after incubation are big enough to allow reproducible discrimination. ANOVA-filtered GC/TOF-MS peak data were used to construct a Random Forest classifier (see Materials and Methods). From the classifier, sample proximities were calculated, which are a measure of the similarity of samples. To display the results, a hierarchical tree based on these sample proximities was built, so that samples appearing close in the tree are those that had a close proximity in the Random Forest classifier. The tree shows that Random Forest is able to discriminate most sample types based on GC-MS peak data. With one exception (indicated with an arrow), all replicates cluster together. Acidification properties and species-specific CFU counts (CFU) of the cultures are presented in the right panel. St, *S. thermophilus*; Lp, *Lb. plantarum* WCFS1; Lb, *Lb. delbrueckii* subsp. *bulgaricus* ATCC BAA365.

Differential profiling of a *Lactococcus lactis* strain collection.

To examine whether the approach outlined here can also be used to distinguish strains in a collection of a single species, 40 *Lc. lactis* strains (Table 1) were cultured in triplicate in 10-ml crimp-cap vials containing 5 ml milk with additionally 2% glucose and 0.5% Casitone to ensure good growth of all strains,
including lactose-deficient and nonproteolytic ones. An approach similar to that described above was chosen, except that we first performed an outlier identification. Before analyzing the aligned mass peaks with Random Forest, the mean absolute deviation for all peaks and all sample triplicates were calculated. Subsequently those outliers that were very far off the median value of the triplicates and for which the median signal value was \( \frac{1}{n} \sum_{i=1}^{n} x_i \) were repaired. By analyzing the resulting data set, Random Forest accurately predicted approximately 55% of the strain combinations. Hierarchical clustering based on Random Forest sample proximities revealed a significant correlation between the volatile compound profiles and strain origin (dairy/nondairy) as well as subspecies level (Fig. 3). A pair plot generated as described above, in which the samples were classified into 5 main groups (I to V) based on the results of the cluster analyses, showed that nearly all compounds contributed to the separation of the volatile compound profiles into two main groups (Fig. 3 and 4). In groups III to V, with predominantly nondairy strains, the strains produced relatively high levels of these compounds, while the levels were relatively low in groups I and II. Within the latter group, separation of \( L.c. lactis \) subsp. cremoris (into group I) was...
mainly due to the production of acetoin, but aldehydes and diacetyl also contributed significantly.

To illustrate that flavor production levels can also be linked to gene content when whole-genome hybridization (CGH) data for the strains are available, we focused on 3-methylbutanal, which is a key flavor compound in many food products (25) and whose production by Lc. lactis is known to be strain dependent (26). Production of this compound and related aldehydes requires alpha-keto acid decarboxylase, encoded by either kdcA or kivd (6, 26). The presence or absence of these genes in each of the individual strains was evaluated using pangenomic microarray hybridization data (1). For all strains that produced high levels of 3-methylbutanal, the averaged hybridization signals of oligonucleotide probes on the microarray that targeted kdcA, kivd, or both were high enough to confirm the presence of the alpha-keto acid decarboxylase coding capacity (Fig. 3). Also, some of the strains that did not produce high levels of 3-methylbutanal were positive for the presence of kivd but for these, with only one exception, signals of probes that targeted the 3' terminus of the gene thought to be essential to produce a functional alpha-keto acid decarboxylase (26) were low (Fig. 3).

DISCUSSION

Metabolomics of biological samples using mass spectrometry is a challenging field (7, 8, 14). In particular, GC-MS and LC-MS are increasingly utilized for differential profiling of biological samples because of their inherent robustness, sensitivity, and large dynamic range (23). As a result of recent advances in these technology platforms and development of tools for high-throughput processing of the data, many new applications are conceivable. One of these is volatile compound profiling of food products, for instance to assess the effects of altered process conditions or formulation on the organoleptic properties. We evaluated a nontargeted GC-MS-based approach to screen for differences in volatile compounds present in milk samples fermented by mono- and mixed cultures of lactic acid bacteria. The approach can for instance be used to assess the effects of the addition of adjunct cultures to well-characterized mixed-culture fermentations (18, 22), but it may serve many other applications where differential profiling of volatile compounds in food products may yield valuable information. We used ultrafast GC/TOF-MS to separate and...
detect volatile compounds, MetAlign to enable comparison of the data, and Random Forest to detect mass peaks that allowed discrimination of the samples.

To increase the sensitivity, volatile compounds in food products are usually first concentrated by solid-phase microextraction (SPME) or related techniques prior to GC-MS analysis (12, 17). We omitted concentration steps, since they are relatively time-consuming, and we were able to detect many compounds known to contribute to the flavor characteristics of fermented food without it, by using ultrafast GC/TOF-MS. Ultimately, after optimizing the GC-MS configuration, the time required to record one GC-MS chromatogram was approximately 7 min. When the CO₂ cooling step, which was required for separation of some highly volatile compounds such as acetaldehyde and methanethiol, was omitted, this time could be reduced to 4 min. MetAlign has already been successfully applied in several studies, including a GC-MS-based screening of volatile compounds (29), and a new version of this metabolomics tool was launched recently (16). Since mass peaks that belong to one compound are separated in individual peaks with unique identifiers by this application, a tool for multivariate mass spectral reconstruction (MMSR) was also developed (29). However, the number of peaks that proved to be relevant for discrimination of our samples was relatively low, and therefore we performed this operation manually.

We showed that the approach outlined here is strong enough to discriminate different species and mixtures of lactic acid bacteria based on volatile compounds produced in milk and even different strains in a collection. The sensitivity of the approach largely depends on the volatile compounds produced or removed by individual species or strains and the levels of these compounds. For instance, the influence of Lb. delbrueckii subsp. bulgaricus on the volatile compound profile of milk fermented by a proteolytic S. thermophilus strain was still detectable while it was outnumbered by more than 4 orders of magnitude (Fig. 1). Remarkably, the levels of the methylated sulfides that allowed discrimination of these samples, and dimethyl trisulfide (DMTS) in particular, were relatively low for the monocultures of Lb. delbrueckii subsp. bulgaricus and S. thermophilus LMD9 (Fig. 2), which suggests that the increased levels in the mixed culture are the result of interaction between the two species. In line with this observation, high levels of aldehydes and 2-pentanone were observed only in the milk fermented with Lb. plantarum, not in mixed cultures containing this species. Clearly, the other species directly or indirectly affected the levels of these compounds by, for instance, limiting

FIG. 4. Pair plot showing the differences in levels and correlations of volatile compounds detected in fermented milk cultures of 40 Lactococcus lactis strains. Groups correspond to those obtained from the hierarchical clustering in Fig. 3. For an explanation of pair plots, see the legend to Fig. 2.
their production by \textit{Lb. plantarum} or by reduction to the corre-
spending alcohols, which are less volatile. Interactions between
strains were also observed for the formation of 2-heptanone by
\textit{Lb. delbrueckii} subsp. \textit{bulgaricus}. Formation of this compound by
this species has been reported \((28)\), but unfortunately there is no
information on how it is produced in lactic acid bacteria. All
together, these data reinforce the increased awareness that bac-
teria could employ certain volatiles to influence other microor-
ganisms \((13)\).

We also showed that differences in flavor production levels
may be linked to strain-specific genetic markers when genomic
data of one or more of the strains is available. Production of
high levels of 3-methylbutanal requires alpha-keto-acid deca-
boxylase, which is encoded by the \textit{kdcA} gene and is unique to
some strains of \textit{Lc. lactis} \((26)\). When we focused on this en-
zyme alone, we could not find a correlation between the
3-methylbutanal production levels of the strains and signal
intensities of probes that target the \textit{kdcA} gene on a pan-
genomic \textit{Lc. lactis} microarray \((\text{data not shown})\). However,
when we included \textit{kivd} in these analyses, a gene that also has
been associated with production of aldehydes in \textit{Lc. lactis} \((6)\),
we found that all 3-methylbutanal-producing strains harbor an
alpha-keto-acid decarboxylase gene, of which the gene se-
quence similarity for most strains was highest to \textit{kivd}. Since
some of the strains that produced low levels of 3-methylbutanal
also seemed to be positive for \textit{kivd}, the hybridization signals for
probes that target the 3’ terminus of this gene were investigated
separately. The gene sequence of \textit{kivd} appeared to be highly
similar to a putative indole pyruvate decarboxylase in \textit{Lc. lactis}
\text{IL1403}, and Smit et al. hypothesized that a 270-bp deletion at
the 3’ terminus of this gene \((\text{with respect to } \text{kdcA})\) is essential for
a functional alpha-keto-acid decarboxylase \((26)\). When the
signals of the probes that target this part of the gene were
considered, only one strain in the upper branch of the tree \((\text{low
levels of 3-methylbutanal}) \text{[Fig. 3]}\) appeared to be positive for
the full \textit{kivd} gene. A possible explanation could be that the
enzyme in this strain is not functional or not expressed under
the conditions tested.

The development of tools to facilitate differential profiling
of biological samples using hyphenated mass spectrometry has
widened the application window of these platforms toward
high-throughput screening. To extract relevant information
from large mass spectral data sets, alignment and data analysis
are crucial steps that are still prone to improvement. We
showed that with little effort a combination of a dedicated
alignment package and the Random Forest algorithm can be
applied to screen for relevant differences in volatile compo-
ounds present in fermented foods. The approach is universal
and can be used for many applications where differences in
volatile compounds need to be assessed.

ACKNOWLEDGMENTS

We acknowledge Jan van Riel for technical assistance, Herwig Bach-
mann for useful suggestions, and Kasper Hedinga for critical reading of
the manuscript.

REFERENCES


6. de la Plaza, M., C. Pelaez, and T. Requena. 2009. Regulation of alpha-
ketosolavurate decarboxylase expression in \textit{Lactococcus lactis} IFPL730. J.

modelling to understand metabolic networks. Comp. Funct. Genomics
\textbf{2}:155–168.

8. Fiehn, O. 2008. Extending the breadth of metabolite profiling by gas chroma-

activity of lactic acid starters during freezing and frozen storage. J.
Dairy Res. \textbf{67}:83–90.

ciation with diet and blood pressure. \textit{Nature} \textbf{453}:396–400.

produced by thermophilic and mesophilic single strain dairy starter cultures.

headspace solid-phase microextraction for the analysis of specific flavors in
enzyme modified and natural Cheddar cheese using factorial design and


14. Katajamaa, M., and M. Oresic. 2007. Data processing for mass spectrometry-

\textbf{38}:642–649.

for hyphenated full-scan mass spectrometry data preprocessing. Anal.
Chem. \textbf{81}:3079–3086.

purge and trap and solid phase microextraction techniques for studying the
volatile aroma compounds of three European PDO hard cheeses. Int.
Dairy J. \textbf{15}:741–758.

using \textit{Lactobacillus} strains with probiotic potential as starter adjuncts. Int.
Dairy J. \textbf{16}:52–60.


proaches for optimal flavour production in dairy fermentation. Int. Dairy J.
\textbf{18}:781–789.

Effect of wild strains used as starter cultures and adjunct cultures on the
volatile compounds of the Pecorino Siciliano cheese. Int. J. Food Microbiol.
\textbf{122}:269–278.

23. Robinson, M. D., et al. 2007. A dynamic programming approach for the
alignment of signal peaks in multiple gas chromatography-mass spectrometry
experiments. BMC Bioinformatics \textbf{8}:419.

with fast feature-generating capabilities of anaerobic micro-organisms. J.

production and breakdown pathways and relevance for flavour in foods.

\textit{Lactococcus lactis} branched-chain alpha-keto-acid decarboxylase involved in

27. Stein, S. E. 1999. An integrated method for spectrum extraction and com-
pound identification from gas chromatography/mass spectrometry data.


metabolomics. Large-scale profiling of tomato fruit volatiles. Plant Physiol.
\textbf{139}:1125–1137.