Biotechnology for Natural Flavour Production

July 22 -23, 2013, Freising (Germany)

BOOK OF ABSTRACTS

Biotechnology of
Natural Products

BIOFLAVOUR
„4th EYFW, MC and WG Meeting“

- D A Y 1 -
Monday, July 22, 2013

Time	Speaker	Theme
10.00 – 10.30	***OPENING / GREETING***	
10:30 – 10:55	**Grucheattka,** Evamaria	Towards a platform organism for terpenoid production – *in silico* analysis of central pathways of *Saccharomyces cerevisiae* for pathway optimization
10:55 – 11:20	**Etschmann,** Marlene **/ Schrader,** Jens	Fungal 2-phenylethanol production beyond yeast
11:20 – 11:45	**Bolat**, Irina	Functional analysis and transcriptional regulation of two orthologs of *ARO10*, encoding broad-substrate-specificity 2-oxo-acid decarboxylases, in the brewing yeast *Saccharomyces pastorianus* CBS1483
11:45 – 12:10	**Gibson,** Brian	Influence of valine and other amino acids on total diacetyl and 2,3-pentanedione levels during fermentation of brewer's wort
12:10 – 12:35	**Querol,** Amparo	Correlation between wine aroma profile and gene expression in different *Saccharomyces* species ad their hybrids
12:35 – 13:50	**LUNCH**	
13:50 – 14:50	***POSTER SESSION)****page 3	
14:50 – 15:15	**Mantzouridou,** Fani	Potential for *in situ* production of food-grade formulations containing flavour-active compounds by immobilized *Saccharomyces cerevisiae* grown in orange peel hydrolysate
15:15 – 15:40	**Bugarski,** Branko **/ Nedovic,** Viktor	Raspberry wine fermentation by suspended and immobilized native microflora
15:40 – 16:05	**Safarik,** Ivo	Preparation and application of magnetically responsive yeast cells
16:05 – 16:30	**Smogrovicova,** Daniela	Flavour Stability of Beer Stored at Different Conditions
18:30 – 19:15	***Brewery Weihenstephan: guided Tour***	
19:30	**DINNER BRÄUSTÜBERL**	

- D A Y 2 -

Tuesday, July 23, 2013

Time	Speaker	Theme
09:00 – 10:30	***WORKING GROUPS MEETINGS***	
10:30 – 11.00	**COFFEE / TEA BREAK**	
11.00 – 11:25	**Weber,** Lutz	Automated Identification of Metabolic Pathways in Scientific Documents
11.25 – 11:50	**Sandell,** Mari	Complexity of Flavour properties and selected Nordic berries
11:50 – 12.15	**Schiller,** Doreen	Characterisation of a lipoxygenase involved in volatile formation during fruit ripening of *Malus* x *domestica*
12.15 – 12:40	**Bönisch,** Friedericke	Characterization of terpene glycosyltransferases from grapes (*Vitis vinifera*)
12:40 – 13:40	**LUNCH**	
13:40 – 14.05	**Laaksonen,** Oskar	Factors affecting taste of blackcurrant (*Ribes nigrum*) juice
14:05 – 14:30	**Breme,** Katharina	Flavour analysis in dairy products
14:30 – 14:55	**Yuceer,** Yonca **/ Togay,** Sine	Bioflavor Production from Agro-Wastes: Tomato and Red Pepper Pomaces
14:55 – 15:15	***Closing Remarks***	
15:15 – 15.30	**COFFEE / TEA BREAK**	
15:30 – 17:00	***Management Committee (MC) Meeting (reserved to MC Members)***	

Poster Session

Name	Poster Title
Borrull, Anna	Influence of the yeast strain and grape variety in the aromatic profile of sparkling wine
Breme, Katharina	Lactone formation ability of a chosen *Lactococcus lactis* subsp. *lactis* var. *diacetylactis* strain from the Agroscope Strain Collection during fermentation in cream
Čadež, Neža	Quorum sensing in *Saccharomyces cerevisiae* through production of aromatic alcohols
Djordjevic, Radovan	Raspberry wine fermentation by suspended and immobilized native microflora
Kregiel, Dorota	Monitoring of Pyruvate Decarboxylase Activity in Industrial Yeasts
Morrissey, John	Title Developing Kluyveromyces marxianus as a Cell Factory
Paraskevopoulou, Adamantini	Limonene microencapsulation by using *Acacia* gums of different chemical composition and its release characteristics
Romano, Patrizia**, Capece,** Angela	Influence of coinoculated fermentations with *Saccharomyces cerevisiae* strains on wine volatile composition
Rysell, Mia	Flavour Compound Production by *Debaryomyces hansenii, Yarrowia lipolytica* and *Saccharomyces cerevisiae*
Stribny, Jiri	The effect of *ARO10, ATF1* and *ATF2* genes from non-conventional *Saccharomyces* species on the wine aroma
Van Rijswijck, Irma	Non-conventional yeast species in novel sustainable food fermentation processes: Unravelling the eco-physiological implications of key metabolic pathways.
Yilmaztekin, Murat	Enhanced production of isoamyl acetate via biotransformation with *Lindnera saturnus* by in situ product removal with macroporous adsorption resins
Yuceer, Yonca**, Togay,** Sine	Bioflavour Production from Rice Bran by Using *Kluveromyces marxianus* and *Debaroymyces hansenii*
Yuceer, Yonca**, Togay,** Sine	Optimization of Bioflavor Production from Whey by Using *Kluyveromyces marxianus*: A Response Surface Approach

ORAL COMMUNICATIONS

Towards a platform organism for terpenoid production – *in silico* analysis of central pathways of *Saccharomyces cerevisiae* for pathway optimization

E. Gruchattka, O. Kayser, V. Schütz

Terpenoids (or isoprenoids) are structurally diverse natural products with important medicinal and industrial applications. Several of them (menthol, eucalyptol, linalool, limonene or patchoulol) are used in the fragrance or flavor industry [1]. Some terpenoids are rare as they are produced only in low amounts in plants; chemical synthesis of complex structures is often not possible or not eco-efficient. Moreover, the demand in 'natural' ingredients is increasing. The use of a microbial platform organism for the biosynthesis of plant terpenoids offers the possibility of large-scale, cost-effective and environmentally friendly industrial production ensuring a continuous 'natural' product supply independent from climate or cultivation risks. [2]

Today, *Saccharomyces cerevisiae* is one of the most widely used microorganism for heterologous plant terpenoid production. However, experimental terpenoid yields are still quite low [3, 4]. Thus, *S. cerevisiae* is analyzed *in silico* by means of elementary mode analysis [5] and compared to *E. coli* focusing on the yield of isopentenyl pyrophosphate, the general terpenoid precursor, to identify new metabolic engineering strategies for an enhanced terpenoid yield. The effect of heterologous enzymes or pathways is analyzed as well as the impact of different carbon sources on theoretical maximum terpenoid yield. Moreover, knockout strategies, using the constrained minimal cut sets approach [6], are identified to enforce a coupling of growth to a minimum terpenoid yield which is higher than any yield published in scientific literature so far.

[1] Caputi L and Aprea E (2011) *Recent Pat Food Nutr Agric* 3(1):9-16
[2] Chang MCY and Keasling JD (2006) *Nat Chem Biol* 2:674-681
[3] Westfall PJ *et al.* (2012) *Proc Natl Acad Sci USA* 109:E111-118
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[5] Trinh CT *et al.* (2009) *Appl Microbiol Biotechnol* 81:813-826
[6] Hädicke O and Klamt S (2011) *Metab eng* 13:204-213

Fungal 2-phenylethanol production beyond yeast

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The rose like flavour compound 2-phenylethanol and its microbial production has been the subject of intensive research in the last two decades. Yeasts from *Saccharomyces* [1] to *Kluyveromyces* [2] to *Yarrowia* [3] are microorganisms of choice for economically relevant processes. However, *Aspergillus niger* is also able to produce the aroma compound in noteworthy amounts.
This has been known for over a decade [4], however the topic was not investigated any further. One of the reasons for this reluctance may be rooted in the characteristic and often problematic morphology of fungi. In submerged culture they tend to form agglomerates which can grow up to a size of several centimeters in diameter. For most processes pellet formation is undesired, as the centre of the pellets is insufficiently supplied with oxygen and nutrients and therefore product formation is low.
The addition of talc microparticles leads to homogenous culture broths and increases enzyme production by several factors, as could be shown for chloroperoxidase in *Caldariomyces fumago* [5] and fructofuranosidase and glucoamylase in *Aspergillus niger* [6].
Here we show for the first time that microparticle enhanced cultivation (MPEC) is not only applicable to enzyme production but also works for secondary metabolites, *e.g.* 2-phenylethanol.

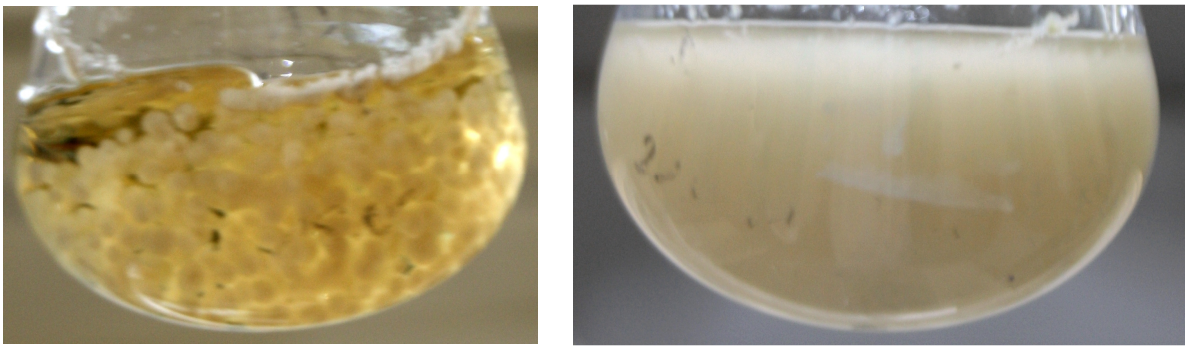

Fig 1: *Aspergillus niger* cultures without (left) and with (right) addition of 2% talc particles

Figure 1 shows that *Aspergillus niger* forms agglomerates of several millimeters in diameter in liquid culture which can be prevented if talc particles are added at the beginning of the cultivation.
Figure 2 shows an 80% increase of 2-phenylethanol concentration in the culture with talc particles compared to the control.
Due to the slow growth of *Aspergillus* fungal 2-phenylethanol production is not competitive with yeasts for the time being. However, possibilities for improving space-time-yield are legion. Just as yeasts *Aspergillus niger* is inhibited by 2-phenylethanol which calls for *in-situ* product removal by an organic phase, adsorber resin or organophilic pervaporation. With any of these techniques compatibility with microparticles has to be taken into account. Furthermore, *Aspergilli* can be easily modified genetically, therefore pathway engineering opens another route to higher product concentrations.

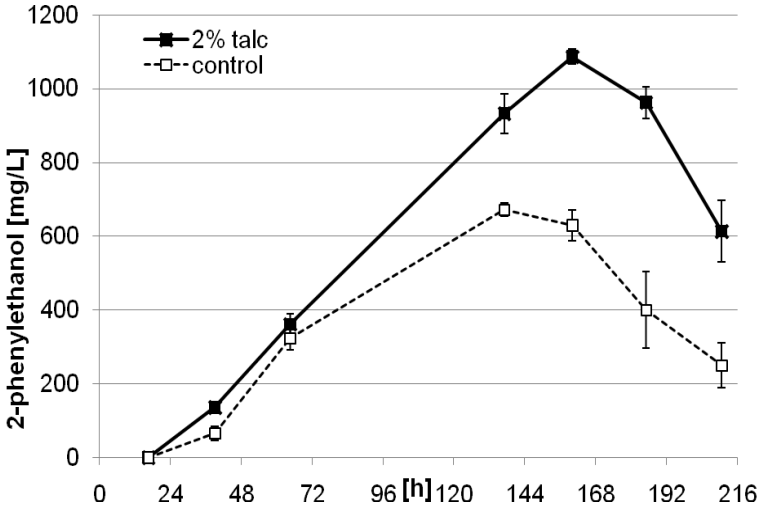

Fig 2: 2-phenylethanol formation kinetics of *Aspergillus niger* DSM 821 without and with the addition of 2% talc particles

References:

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[5] Kaup, B.A., Ehrich, K., Pescheck, M., Schrader, J. Biotechnol Bioeng, 99 (3), 491-498. 2008.
[6] Driouch, H., Sommer, B., Wittmann, C., Biotechnol Bioeng 3 (6), 1058-1068. 2010

Functional analysis and transcriptional regulation of two orthologs of *ARO10*, encoding broad-substrate-specificity 2-oxo-acid decarboxylases, in the brewing yeast *Saccharomyces pastorianus* CBS1483

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The hybrid aneuploid genomes of *Saccharomyces pastorianus* lager brewing strains consist of subgenomes similar to those of *S. cerevisiae* and *S. eubayanus*. The impact of this hybrid genome structure on flavour production and its regulation is incompletely understood. This study focuses on *ARO10*, which encodes a broad-substrate specificity oxo-acid decarboxylase involved in production of higher alcohols. In *S. pastorianus* CBS1483, four *ARO10* copies were identified on chromosome IV, of which three strongly resembled *S. cerevisiae ARO10* (`*ScARO10*') and one was highly similar to *S. eubayanus ARO10* (`*SeubARO10*'). Substrate specificities of LgScAro10 and LgSeubAro10 were compared by individually expressing them in a *pdc1Δ pdc5Δ pdc6Δ aro10Δ thi3Δ S. cerevisiae* strain. Both isoenzymes catalysed decarboxylation of the 2-oxo-acids derived from valine, leucine, isoleucine, methionine, with the highest affinity for phenylpyruvate. LgSeubAro10 showed a 2-fold higher activity with the isobutanol precursor ketoisovalerate than LgScArop10. In glucose-limited, anaerobic chemostats of *S. pastorianus* CBS1483, use of phenylalanine as nitrogen source induced transcription of both alleles. *LgSeubARO10* showed higher basal expression levels during growth with ammonia as the nitrogen source. In contrast to the situation in laboratory strains of *S. cerevisiae*, *LgScARO10* and *LgSeubARO10* in *S. pastorianus* were not induced by leucine. Like *ARO10*, *ARO80*, which encodes *ARO10* transcriptional activator, is located on CHRIV and counts three *S. cerevisiae*-like copies and one *S. eubayanus*-like copy. Deletion of *LgSeubARO80* did not affect phenylalanine induction of *LgSeubARO10*, revealing 'in trans' regulation across the two subgenomes. *ARO10* transcript levels showed a poor correlation with phenylpyruvate decarboxylase activities in cell extracts.
All together, these results provide new insights on flavour formation in lager brewing yeast and illustrate the complexity of functional characterization in aneuploid *S. pastorianus*.

Influence of valine and other amino acids on total diacetyl and 2,3-pentanedione levels during fermentation of brewer's wort

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Undesirable butter-tasting vicinal diketones are produced as by-products of valine and isoleucine biosynthesis during wort fermentation. One promising method of decreasing diacetyl production is through control of wort valine content since valine is involved in feedback inhibition of enzymes controlling the formation of diacetyl precursors. Here, the influence of valine supplementation, wort amino acid profile and free amino nitrogen content on diacetyl formation during wort fermentation with the lager yeast *Saccharomyces pastorianus* was investigated. Valine supplementation (100 to 300 $mg \cdot L^{-1}$) resulted in decreased maximum diacetyl concentrations (up to 37% lower) and diacetyl concentrations at the end of fermentation (up to 33% lower) in all trials. Composition of the amino acid spectrum of the wort also had an impact on diacetyl and 2,3-pentanedione production during fermentation. No direct correlation between the wort amino acid concentrations and diacetyl production was found, but rather a negative correlation between the uptake rate of valine (and also other branched-chain amino acids) and diacetyl production. Fermentation performance and yeast growth were unaffected by supplementations. Amino acid addition had a minor effect on higher alcohol and ester composition, suggesting that high levels of supplementation could affect the flavour profile of the beer. Modifying amino acid profile of wort, especially with respect to valine and the other branched-chain amino acids, may be an effective way of decreasing the amount of diacetyl formed during fermentation.

Correlation between wine aroma profile and gene expression in different *Saccharomyces* species ad their hybrids

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Members of the *Saccharomyces* genus are the main yeasts involved in wine fermentations. These yeasts also participate in the production and release of aromatic compounds. In addition, lowering fermentation temperature to achieve wines with improved aromatic profiles is desirable. In this background, it is interesting to find new yeast strains able to ferment at low temperatures efficiently, yielding wines with unique aromatic profiles. Recently isolated *S. cerevisiae* x *S. kudriavzevii* hybrids seem to have inherited beneficial traits from their parental species, like fermenting efficiently at low temperature or producing higher amounts of certain aromatic compounds. In this research work, we have analysed the expression of genes involved in flavour compounds production in three different cryotolerant Saccharomyces strains from the species *S. cerevisiae*, *S. uvarum* and *S. kudriavzevii* under low and moderate fermentation temperatures and also we have tested the ability of two *S. cerevisiae* x *S. kudriavzevii* hybrids to produce secondary aroma compounds and other metabolites influencing wine quality, such as ethanol, acetaldehyde or acetic acid, at chemical and molecular level employing microarray technology.
Agreement between flavour-related compounds concentration and transcriptome was investigated by DNA microarrays. Transcriptome analysis of the genes related to aroma production can give us an idea of the compounds that are going to be synthesised during the fermentation process but several factors affecting aroma synthesis must be taken also into consideration. The conclusions of this study are that acetate esters synthesis seems to be influenced by higher alcohol availability in a significant way, being this factor sometimes more important than acetyltransferase levels of expression. Furthermore, the higher importance of *ADH1* with respect to *ADH4*, and *EEB1* with respect to *EHT*1 was confirmed.
The hybrids used in this study showed different allele composition in several genes involved in aroma production, leading to different levels of expression and different oenological properties and aromatic profile in the resulting wines. Lalvin W27 pointed out in higher alcohol production at both temperatures, whereas VIN7 was the main acetate ester producer at both temperatures, and also yielded the higher ethyl ester amount at 12ºC. We did not find correlation between genome, transcriptome and chemical data in all cases. Temperature fermentation seems to have a crucial role in modulating aroma synthesis by yeasts, especially in hybrids because they may express the allele from one or other parental.
Taking in account these results we decide to analyses the genomic divergence among the three species of the gen involved in the aroma synthesis and the most different were *ARO1*, *ARO10*, *ATF1* and *ATF2* genes. The roles of the different alleles were tested by cloning in wine yeast strains (T73) and after microvinifications the aroma profile were analyzed. The present results indicate that the *S. kudriavzevii* and *S. uvarum* alleles introduced into T73 strain lead to higher production of some aroma compounds mainly those resulting from amino acids metabolism, such as isoamyl alcohol and isoamyl acetate. Together with the sparse dissimilarities of the amino acid sequences within the corresponding alleles, our results suggest that only a few amino acid substitutions could change the aroma production.

Potential for *in situ* production of food-grade formulations containing flavour-active compounds by immobilized *Saccharomyces cerevisiae* grown in orange peel hydrolysate

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During the last decades, the rising demand of modern consumers for natural food ingredients has stimulated wide interest in the development of alternative routes for the production of natural flavourings based on biotechnology. Nevertheless, industrial-scale application of microbial technology for bioflavour production has been hindered by high cost substrates and recovery methods due to low product yields.

The agro-industrial residues with negligible or even no-cost offer excellent possibilities to be used as substrates for flavour production by microorganisms. This trend is in line with our efforts to exploitate the whole residue after extraction of juice from orange fruit for the production of volatile esters of "fruity" aroma by using a commercial wine yeast strain [1]. Previously, it was demonstrated that the polysaccharide-rich fractions did not contribute to the *de novo* synthesis of bioflavour [1]. This finding was the driving force to carry out experiments for the hydrolysis of these carbohydrates into simple sugars and the full exploitation of the waste in the direction of flavour production. The main obstacle of orange peel hydrolysate use is the presence of D-limonene (found in the peel oil at a percentage higher than 90%, w/w), which along with the extremely toxic to yeast hydrolysis by-products (such as carboxylic compounds, furans, phenolic compounds), are expected to cause negative interference in the bioprocess performance [2, 3]. An effective way to address the substrate toxicity is the use of immobilized cells technology [2-4].

The aim of the present study was to examine an approach for producing bioflavour with the use of yeast cells entrapped within calcium alginate beads grown in dilute-acid hydrolysate of orange peel. According to the results, following incubation under limited oxygen supply immobilized cells synthesized *de novo* fruity flavour esters (i.e. ethyl hexanoate, octanoate, decanoate, dodecanoate and phenyl ethyl acetate). A trend of flavour molecules to be accumulated preferentially inside the micro-beads was observed. More specifically, when the highest amounts of volatiles production was achieved, percentages of 63.26 (1722 μg/L), 85.97 (1422 μg/L), 90.25 (1945 μg/L), 88.23 (4320 μg/L) and 81.0 % (2094 μg/L) for ethyl hexanoate, octanoate, decanoate, dodecanoate and phenyl ethyl acetate, respectively, were detected inside the beads. Limonene and α-terpineol were also detected inside the beads at levels of 18.1 and 3.7 mg/L, respectively. These results highlight the potential for *in situ* production of food-grade formulations containing a mixture of flavour-active compounds. These aroma-containing beads could be employed as flavour additives or enhancers in various food preparations without further processing.

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Raspberry wine fermentation by suspended and immobilized native microflora

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The objectives of this study were to identify native micro-flora in raspberry juice, to define the difference between spontaneous and controlled fermentations and monitor interactions between yeast cells in mixed fermentations. Fermentations were carried out at different conditions by immobilized and suspended *Saccharomyces cerevisiae* and *Lachancea thermotolerans* yeats strains isolated from raspberry juice. During the course of fermentation, glucose, fructose and sucrose utilization and ethanol, glycerol, malic acid, citric acid, acetic acid, tyrosol, tryptophol and phenylethanol level were monitored with HPLC. Also, monitoring of total number of yeast cells and relation between number of yeasts in mixed culture fermentation was performed. Rapid start of fermentation and faster fermentation was observed with immobilized cells in relation to suspended cells. The production of tyrosol proved to be temperature dependent and was significantly reduced at higher temperature of fermentation. The number of *L. thermotolerans* strain in mixed culture fermentation constantly decreased, and at 66 hours of fermentation there was not any left. Also, a higher amount of glycerol was detected in samples fermented by *L. thermotolerans* strain than by *S. cerevisiae* strain.

PREPARATION AND APPLICATION OF MAGNETICALLY RESPONSIVE YEAST CELLS

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Microbial cells are used in enormous number of industrial applications and biotechnologies, both in natural form or after their immobilization to an appropriate carrier. Cells immobilized to magnetically responsive carriers exhibit interesting properties, especially the possibility of their simple separation using an external magnetic field and repeated application.

Magnetic modification of microbial cells can be performed by various procedures. The most common methods used in many applications employ binding of magnetic nano- and microparticles on the cell surface, covalent immobilization of cells on magnetic carriers, entrapment of cells and magnetic particles into biocompatible polymers, cross-linking of cells or cell walls together with magnetic particles, binding of paramagnetic cations on the cell surface or biologically driven precipitation of paramagnetic compounds on the cell surface.

We have developed two extremely simple procedures for the preparation of magnetically responsive *Saccharomyces cerevisiae* (baker's yeast). The cells surface was modified in one step with magnetic iron oxide microparticles prepared by microwave assisted synthesis. Alternatively, magnetic chitosan microparticles were used for cells modification. Prepared magnetically responsive biocatalysts were applied for sucrose hydrolysis and hydrogen peroxide decomposition and also for organic xenobiotics removal. Further applications of these biocomposites are under study.

References:
Pospiskova,K., Prochazkova,G., Safarik,I.: One-step magnetic modification of yeasts cells by microwave synthesized iron oxides microparticles. Lett. Appl. Microbiol., in press

Flavour Stability of Beer Stored at Different Conditions

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Plastic bottles have become attractive alternative packaging for beer thanks to their weight and break-resistance. However, they have limited acceptance due to oxygen permeability, as beer is an oxygen sensitive beverage, and with an increase in access to oxygen its flavour reduces, affecting shelf life of beer. It is therefore critical that PET bottles offer sufficient barrier properties. The aim of our study was to compare beer stability, its physical-chemical and sensory properties, during storage in different PET bottles at the temperatures of 10, 20 and 30 °C. We used standard PET bottles, lightweight PET bottles with 2 % of oxygen scavenger (AMS) and PET bottles with 2 and 6 % of AMS. After three months of storage the highest concentration of oxygen (0,378 mg/l) was recorded in beer stored at 10 °C in the lightweight PET bottle with 2 % of AMS. Beers keeping in this type of container had the highest oxygen concentrations at all temperatures. There was not a significant difference in oxygen concentration in beers stored in the PET bottles containing 2 or 6 % of AMS. In these bottles the oxygen concentration was lower than 0.1 mg/l at all temperatures. The research confirmed that the oxygen concentration in beer is one of the most important parameters which influences stability of beer and its physical-chemical and sensory properties. In beers with the highest concentration of oxygen were the most significant aging changes. Samples stored at 30 °C reached generally the worst sensory results. Increase of acetaldehyde and diacetyl concentration during aging was directly proportional to storage temperature and oxygen concentration, however, increase of esters was similar in all beers, independently on storage temperature and bottle composition.

Acknowledgements
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Automated Identification of Metabolic Pathways in Scientific Documents

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OntoChem's uses its high-throughput text mining technology OCMiner to extract relevant data from scientific and general publications as well as patents. A knowledge extraction technology based on the semantic and syntactic analysis of natural language has been established to extract knowledge triples on chemical compounds such as their use, physicochemical, biological properties or metabolic information. These data points are extracted from millions of full text documents within days and provided to users via an open-access web-browser based search engine.

To facilitate a structured data extraction process, OntoChem has established a dedicated of ontologies focussing on plants, yeast and fungi, anatomy, physiological effects, diseases, as well as on food, flavors and fragrances. A chemical ontology has been built that focusses on natural products and can assign natural product classes automatically to chemical entities found in text documents. Thus, the chemistry knowledge used contains products and information about more than 15 million specific compounds. In particular, this information also includes economic uses of known compounds, or whether it a compound is a natural product, is toxic or being edible or may be used as a biomaterial.

In the work described here, an automated process was used to extract information about natural products, their metabolites and enzymes that are known to facilitate the metabolic transformation. Application examples will be given to show the utility for finding new uses of natural products in the cosmetics and nutrition industry.

References:

Bobach C, Böhme T, Laube U; Püschel A; Weber L. (2012) Automated compound classification using a chemical ontology. *Journal of Chemoinformatics*, 4(40):1-12.

Weber L; Böhme T, Irmer M. (2013) Ontology-based content analysis of US patent applications from 2001–2010. *Pharmaceutical Patent Analyst*, 2(1):39-54.

Factors affecting taste of blackcurrant (*Ribes nigrum*) juice

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Blackcurrant is a major berry crop in Europe. Juices form a major category of products of industrially processed blackcurrants. Press residues are often discarded, although they contain great deals of health beneficial phytochemicals. Enzymes increase the overall yield of juice and the contents of phenolic compounds. Untreated cold-pressed juice can be notably sour and sweet whereas enzyme-aided processing of berries increases astringency, bitterness and fermented flavour (Sandell et al. 2009; Laaksonen et al., 2012). Bitter, sour and astringent properties of berries may have negative impact on liking and thus consumption. However, sourness can be a desirable and expected feature to some extent in berry products (Laaksonen, Ahola, Sandell, 2013).

Juices from different blackcurrant cultivars vary in composition and sensory properties (Laaksonen et al., 2013). Enzyme-aided juice processing increases especially bitterness and mouth-drying astringency, which is contributed by flavonol glycosides. Pectins in juices without enzymatic processing can mask the mouth-drying astringency. Puckering astringency of the juices correlates positively with sourness and negatively with pH and is not affected by the pectins. The choice of cultivar and processing technologies affect the profiles and contents of chemical constituents that are significant contributors to the sensory properties of black currant juices (Laaksonen et al., 2013).

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Characterisation of a lipoxygenase involved in volatile formation during fruit ripening of *Malus* x *domestica*

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Flavour is a biochemically and genetically highly complex trait having a decisive impact on apple fruit quality. Due to limited information available on its molecular-genetic basis it has been more a random product of breeding than the result of targeted breeding strategies in the past. Biosynthesis of aroma compounds, such as alcohols and esters, is directly associated with the metabolism of fatty acids and lipids. Linoleic and α-linolenic acid are catabolized to precursors of fruit esters either by β-oxidation or the lipoxygenase (LOX) pathway. Recently, a map based QTL analysis for key aroma volatiles revealed a LOX candidate gene in close proximity to an important QTL cluster for ester-type volatiles (Dunemann *et al.* 2009). This gene is one out of 23 putative members of a LOX gene family of *Malus* x *domestica* and was designated as *MdLOX1a*. As expression of this gene is induced by ethylene it might play a role in the ripening process of apple fruit (Schaffer *et al.* 2007). Indeed, we observed increased expression levels in the cultivars Golden Delicious and McIntosh during fruit ripening and storage. Also, stereo- and regio-chemistry of hydroperoxy products produced by MdLOX1a with linoleic acid confirmed earlier results for the major products of LOX activity found in apple fruits after storage (Beuerle & Schwab 1999). MdLOX1a is a dual positional-specific enzyme producing both 13(*R*)- and 9(*S*)-hydroperoxy-octadecadienoic acid in a ratio of seven to one. Here, we demonstrate that site directed mutagenesis of one single amino acid in the substrate binding pocket leads to a change in both positional and stereo-selectivity of this enzyme.

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Characterization of terpene glycosyltransferases from grapes (*Vitis vinifera*)

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Monoterpenes are an important group of volatile components of grapes and wine, but the majority of terpenes are found as non-volatile glycosylated molecules. However, there are no glycosyltransferases (GT) known in grapes, which show specificity towards monoterpenes. Based on gene sequences of monoterpene-GT from *Arabidopsis thaliana*, we screened *in silico* for homologous sequences in the *Vitis vinifera* genome. About 70 putatively annotated *V. vinifera* genes were found. Additionally, we were interested in the alleles of the genes in the different cultivars. Alleles of several candidate genes were expressed in *E. coli* and analysed for their activity against six aroma-relevant monoterpenes. The recombinant UGT25 (UDP-dependent glycosyltransferase) protein was found to glycosylate the substrates nerol, citronellol and geraniol while terpineol, 8-hydroxylinalool and linalool were not converted. Interestingly, two of the ten identified UGT25-alleles showed no activity at all. By sequence alignment we identified three amino acids which distinguish the active from the inactive proteins. Site-directed mutagenesis of these positions was performed to verify the importance of these amino acids according to the enzyme activity. Additionally, we identified three other proteins which glycosylate aroma-relevant terpenes. These enzymes were also heterologously expressed and functionally characterized.

Complexity of Flavour properties and selected Nordic berries

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Perceived and natural berry flavour is a combination of odour, taste and chemesthesis and formed by various volatile and non-volatile chemical compounds. Moreover, many factors such as berry genus, variety, growing conditions, stage of ripeness and processing affect intensity and quality of flavour properties. Berries are very sensitive material to quality changes. Both analytical sensory evaluation studies in special sensory laboratory conditions and different instrumental analyses are needed to identify the actual flavour compounds. Multiregression methods should be applied to investigate the interaction between perceived flavour properties and chemical composition. This presentation demonstrates the complexity of berry flavour with studies carried out in our laboratory. Special focus will be in main properties of berry odour, taste and astringency. The berry samples include strawberry, black currant, bilberry, crowberry and sea buckthorn and they all have been growing in Finland.

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Flavour analysis in dairy products

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The flavour of dairy products is known to be influenced by numerous factors, such as the raw material, various treatments and process conditions applied during production, and by fermentation due to the presence of microorganisms. The careful choice of suitable analytical techniques allows to properly evaluate these flavours, but dairy matrices remain a challenge due to their inhomogeneous and complex nature and composition.
After a brief overview of flavour formation in selected milk products, the analytical procedure for dairy flavour analysis will be discussed. Traditional and state-of-the-art extraction techniques for the analysis of volatile compounds by gas chromatography (GC) will be presented. Whilst coupling GC to different physical detectors (e.g. mass spectrometry MS, sulphur specific detection by pulsed-flame photometric detection PFPD) can provide valuable information on the identity and quantity of target molecules, physiological detection by the human nose (olfactometry) is needed in order to evaluate odorant compounds and their impact on the overall odour of the product. Finally, sensory analysis allows to obtain a judgement of the overall flavour by trained panellists and to estimate consumer acceptance.

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Bioflavor Production from Agro-Wastes: Tomato and Red Pepper Pomaces

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Tomato and red pepper pomaces, byproducts from tomato and red pepper paste processing, contain seeds and peel residues. Both agricultural wastes have high amount of lipid derivative compounds such as carateonids. Specifically, norizoprenoids (eg. β-ionone, β-damascenone) were produced by thermal, enzymatic and microbial degradation of caroteneoids. In addition, pyrazines were naturally found in raw vegetables. For example, 2-isobutyl-3-methoxypyrazine is well known green pepper flavor compound. Recently, biotechnological processes have became a new approach to produce some flavor compounds from agricultural wastes by microbial cultures (Auguedo et al., 2004; Pinto, 2009; Harent and Colin, 1998; Galanakis, 2012).

The purpose of this study was to investigate the potential use of tomato and red pepper pomace for producing aroma compounds by using *Kluyveromyces marxianus* and.*Debaroymyces hansenii* For this purpose, liquid media was prepared with tomato and red pepper pomaces (10% w/v) and individually fermented by *K. marxianus* and *D. hansenii* during 72 hours at 30^{o}C. The growth of *K. marxianus* and *D. hansenii* were determined at 24, 48 and 72h of fermentation. Odor-active compounds were identified by gas chromatography-olfactometry (GC-O).

Biomass of *K. marxianus* increased 1.1 log (12.58 fold) and 1.2 (15.84 fold) in tomato and red pepper solutions during 72 h fermentation respectively, while *D. hansenii* increased 1.04 log (10.96 fold) and 2 (100 fold) in tomato and red pepper solutions respectively at the same fermentation conditions. Moreover, esters and alcohols including ethyl phenyl acetate (rose), phenyl ethyl alcohol (rose), isobutyl acetate (bubble gum) and ethyldimethylthiazole (earthy) were the aroma compounds determined at high intensities in cultured tomato and red pepper pomaces. Our future activities will include positive identification of aroma compounds generated by the growth of certain microorganisms by using gas chromatography- mass selective detector (GC-MS).

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POSTER

INFLUENCE OF THE YEAST STRAIN AND GRAPE VARIETY IN THE AROMATIC PROFILE OF SPARKLING WINE

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Cava (Certified Brand of Origin) is a Spanish sparkling wine elaborated by the traditional method (*champenoise* method). This method requires two successive fermentations. The first fermentation transforms grape must into base wine. The second fermentation is induced by adding the expedition liqueur solution (sucrose) and yeast. After this second alcoholic fermentation, the wine is aged on yeast lees at a temperature of 12ºC to 14°C. Ageing on lees lasts for at least nine months (the legally established time). During aging, yeast autolysis occurs, a slow process associated with cell death that involves hydrolytic enzymes that act to release cytoplasmic and cell wall compounds into the wine. During ageing on yeast lees, the organoleptic and foam properties of wine are modified, reflecting changes in wine composition.Therefore, the characteristics of the base wine, the yeast, and the aging time in contact with lees are the factors that contribute the most to the quality of cava, especially to its volatile composition.

The objective of this research is to investigate the influence of the grape variety and the second fermentation yeast strain on the aromatic profile of cava. For that, an experimental design was elaborated consisting on seven different base wines and two yeast strains. Some of the most important volatile compounds formed by fermentative activity , were quantified: higher alcohols, sulphur compounds and nonpolar esters. The analytical technique used for the detection and quantification of these compounds was gas chromatography. For each molecule a different method was used: direct injection for higher alcohols[1], head space in the case of low boiling point sulphur compounds[1], and the new method of head space-solid phase micro extraction (HS-SPME)[2] was used for non-polar esters determination. Furthermore, a sensory analysis was performed to study the organoleptic differences between grape varieties and yeast strains.

The results obtained indicate that, as expected, grape variety is important for the quality of the sparkling wine. The yeast strain plays the main role in the composition of the studied fermentative volatile compounds found in cava. For the yeast strains used in this study this is especially true in relation to the sulphur compounds, and for higher alcohols and higher alcohol acetates contents.

A discriminant analysis was performed to evaluate whether volatile composition allows to distinguish between the wines fermented by each yeast strain, and the different base wines (Fig. 1).

Wine samples were grouped according to strain type and to grape variety on the basis of their volatile composition. Figure 1 displays the plot of the discriminant analysis and shows that for 81% of the wines obtained from Y1 were correctly classified, and for Y2 76.2% were perfectly classified. The wines obtained from different grape varieties were correctly classified in 100% of the cases. The discriminant analysis allowed the cava base wines to be clearly distinguished by their chemical and volatile composition, as a function of the yeast strain which carried out the fermentation and the grape variety. On the basis of these data, conducting must fermentation with a specific yeast strain determines certain levels of some important volatile compounds, although this expected level could vary according to other oenological factors.

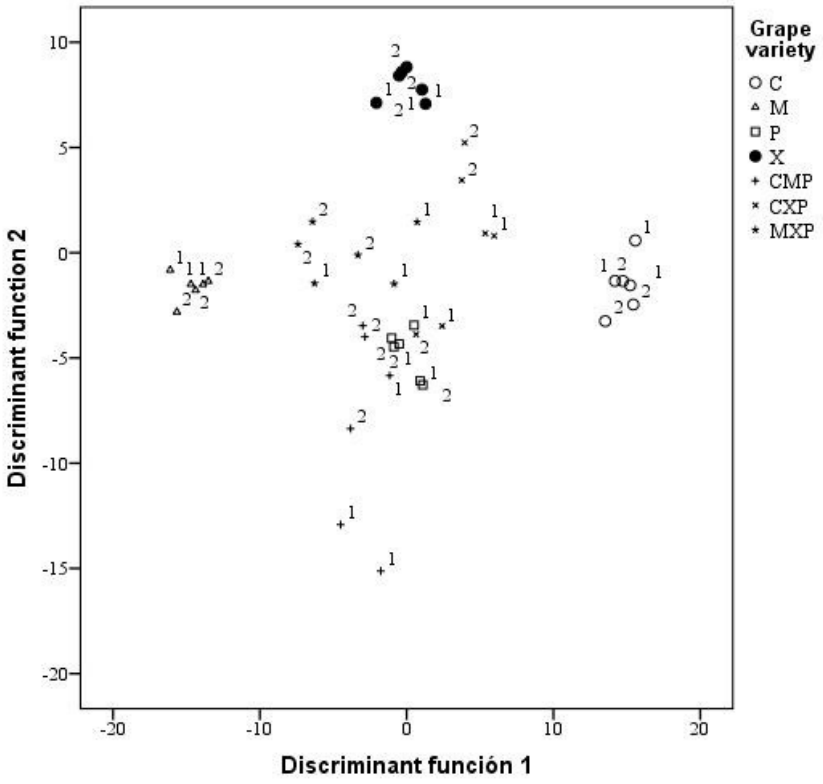

Figure 1. Discriminant analysis of *Cavas* and *Coupages* according to their volatile composition. C, Chardonnay; M, Macabeo; P, Parellada; X, Xarel·lo; 1 & 2, Y1 and Y2 yeast strains, respectively.

Key words: Cava, sparkling wine, yeast strain, grape variety, base wine, volatile compounds, gas chromatography, SPME.

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Lactone formation ability of a chosen *Lactococcus lactis* subsp. *lactis* var. *diacetylactis* strain from the Agroscope Strain Collection during fermentation in cream

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Lactones are potent flavour compounds that contribute to creamy, fruity and coconut-like notes in milk products. Although their chemical formation in milk products from hydroxy-fatty acid triglycerides is accepted, their production by lactic acid bacteria (LAB) and the metabolic pathways are to date still uncertain.
Here we report on the investigation of a potential microbial formation of lactones in cream with and without addition of hydroxy-fatty acids (HFA), supposed precursors of lactones. The strain of *Lc. lactis* subsp. *lactis* var. *diacetylactis* FAM18027 was selected out of 65 strains of different LAB species from the Agroscope Strain Collection for its ability to develop buttery and fruity aroma notes in cream during fermentation. Full-fat cream was fermented by FAM18027 at 30 °C for 24 h, with and without HFA supplementation. In addition, the same conditions were used to incubate cream without addition of bacteria. in presence or absence of HFA.
The formation of lactones was evaluated by head-space solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS).
δ-Octalactone and δ-decalactone were the main volatile lactones found in the samples. GC-MS analyses revealed an increase of the signals of these two lactones after 4 h of fermentation already. Addition of HFA in presence of FAM18027 seemed to slow down the fermentation, which led to a slower acidification and less pronounced lactone formation. Especially the formation of δ-octalactone was affected by HFA addition, resulting in a reduced formation and in an approximately three times lower signal after 24 h of fermentation.
The results clearly show that GC-MS signals of δ-octalactone and δ-decalactone in cream fermented with LAB were higher than in samples incubated without LAB. It can hence be concluded that FAM18027 formed lactones during fermentation at 30°C.
Thus, LAB strains such as FAM18027 may be used to increase the flavour of fermented cream and sour cream butter.

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Quorum sensing in *Saccharomyces cerevisiae* through production of aromatic alcohols

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As seen in bacteria, yeasts use a kind of communication or quorum sensing system as well. In this case, aromatic alcohols, such as phenylethanol, tryptophol and tyrosol were found to hold quorum sensing signaling function. In addition, higher aromatic alcohols produced by yeasts are one of the most important aromatic compounds in wine. One could satirically say that the aroma of wine depends on the efficiency of yeast communication. With intention to examine this phenomenon in more detail, we developed an experimental design for isolation, detection and evaluation of the three aromatic molecules during time course of *Saccharomyces cerevisiae* fermentations (Zupan et al., *submitted*). When compared to current published methods, our approach offers: i) a significant scale-down, a so called minifermentation, which is performed in 2 ml centrifuge tubes; ii) quick isolation and inexpensive HPLC-FLD-based detection and quantification of the aromatic molecules; iii) automatic, computer/image-based cell counting of multiple samples; and iv) a new perspective on quantification and data interpretation. The advantages make the method convenient for monitoring the release and for evaluating the dynamics of the aromatic alcohols during all stages of yeast fermentation. In this contribution we are presenting the principles of our methodological concept. The developed experimental design is currently used more intensively on examining the mechanisms of quorum sensing in yeasts of wine origin.

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Raspberry wine fermentation by suspended and immobilized native microflora

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The objectives of this study were to identify native micro-flora in raspberry juice, to define the difference between spontaneous and controlled fermentations and monitor interactions between yeast cells in mixed fermentations. Fermentations were carried out at different conditions by immobilized and suspended *Saccharomyces cerevisiae* and *Lachancea thermotolerans* yeats strains isolated from raspberry juice. During the course of fermentation, glucose, fructose and sucrose utilization and ethanol, glycerol, malic acid, citric acid, acetic acid, tyrosol, tryptophol and phenylethanol level were monitored with HPLC. Also, monitoring of total number of yeast cells and relation between number of yeasts in mixed culture fermentation was performed. Rapid start of fermentation and faster fermentation was observed with immobilized cells in relation to suspended cells. The production of tyrosol proved to be temperature dependent and was significantly reduced at higher temperature of fermentation. The number of *L. thermotolerans* strain in mixed culture fermentation constantly decreased, and at 66 hours of fermentation there was not any left. Also, a higher amount of glycerol was detected in samples fermented by *L. thermotolerans* strain than by *S. cerevisiae* strain.

Monitoring of Pyruvate Decarboxylase Activity in Industrial Yeasts

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The pyruvate decarboxylase (PDC, EC 4.1.1.1) is one of the key enzymes of yeast fermentative metabolism. PDC is the first enzyme which, under anaerobic conditions, leads to decarboxylation of pyruvate with acetaldehyde as the end product (Pronk et al., 1998). PDC activity was investigated in different industrial yeasts showing differences in ethanol productivity - the fermentative, Crabtree-positive distillery and brewery yeasts include the genera *Saccharomyces* (*S. cerevisiae, S. pastorianus*) and Crabtree-negative amylolytic strain *Debaryomyces occidentalis*. These strains were investigated as free and immobilized cells on chamotte carriers.
The fermentations were conducted under oxygen–limited conditions in mineral medium supplemented with 12% glucose. PDC activity was measured *in situ* after permeabilization with digitonin and incubation with substrate – sodium pyruvate at 30ºC for 20 min. Product of this reaction – acetaldehyde was detected chromatografically using GC technique. Fermentative activity of yeast strains was expressed in grams of carbon dioxide excreted during fermentation (Berlowska et al., 2009).
In Crabtree-positive strains the CO_2 production rates showed a clear positive correlation with the level of PDC activity. All Crabtree-positive yeasts contain higher levels of enzyme activity than the Crabtree-negative strain, both as free and immobilized cells. It was shown in all experiments that immobilization affected positively CO_2 productivity, while PDC activity was lowered, except bottom fermenting strain *S. pastorianus* 680 where PDC activity of immobilized cells was few times higher. In general, the behavior of Crabtree-positive, brewery top fermenting and distillery yeasts was very similar, for both free and immobilized state. However the Crabtree-negative strain and the bottom fermenting yeast have shown much higher fermentative activity after immobilization. Therefore we can conclude that the immobilization usually led to lower level of PDC activity, but the higher concentration of immobilized yeast cells in fermentation medium increases the rate of fermentation.

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Title Developing Kluyveromyces marxianus as a Cell Factory

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Kluyveromyces marxianus is a homothallic hemiascomycete yeast, frequently isolated from dairy environments, and widely used in biotechnology for applications that include production of ethanol, enzymes and biomass. The ability to produce ethanol by fermentation of sugars such as lactose and inulin is of particular interest because of the growing bioethanol sector. Certain traits, namely thermotolerance, high secretory capacity, GRAS/QPS status, and the fastest growth rate of any eukaryotic microbe render *K. marxianus* especially suitable for industrial exploitation. Industrially, and for research applications, a range of different wild-type strains is used and research from our group, and by others, has found that there is extensive phenotypic variation between strains. This variability includes parameters such as morphology, growth rate, capacity to produce ethanol, stress tolerance and level of enzyme production. One of our interests lies in understanding how this fungus responds to environmental stimuli that are relevant in industrial fermentations. Comparison of a bank of strains from European culture collections found that strains exhibited a wide variety of responses to thermo, osmotic and cell wall stress with some strains showing multi-stress resistance. These traits generally appeared unlinked indicating that, as with other yeasts, multiple resistance/adaption pathways are present in *K. marxianus*. The second focus of our research is on understanding carbon metabolism in this yeast. Biochemical pathways for using carbon and their regulation are important for applications such as ethanol production, biomass generation and enzyme production. Finally, there are currently limited genetic and molecular tools available for studying *K. marxianus* and our third focus is on development of these resources. An overall goal of our research on *K. marxianus* is to improve knowledge and develop tools to facilitate selection of improved strains for industrial applications.

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Limonene microencapsulation by using *Acacia* gums of different chemical composition and its release characteristics

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The incorporation of hydrophobic aroma compounds into powders by encapsulation is of great importance in the food and flavouring industries, since microencapsulation imparts protection against degradative reactions, prevents the loss of flavours during storage and provides controlled release. As wall materials have been used various biopolymers with emulsifying and stabilizing activity.

Acacia gum has been the encapsulating agent of choice for many years because it is an excellent emulsifier, has a bland flavour and provides very good volatiles retention during the drying process (Islam et al., 2002). It is described as a complex acidic branched polysaccharide, obtained as an exudate from *Acacia* trees, containing about 2% protein. Its emulsifying capacity is attributed to its ArabinoGalactanProtein fraction (AGP) where most of the protein is located. As most natural products, *Acacia* gum is subject to chemical variability that sharply affects its functional properties.

In this study, the effect of *Acacia* gum chemical composition on microencapsulation of limonene has been explored. For this reason, three *Acacia* gums, differing in their AGP content, were used, i.e. low (GA1), medium (GA2) and high (GA3), and their physicochemical characteristics were first examined. The microencapsulation of limonene was achieved by emulsification and subsequent water removal by applying the freeze drying technique. The initial emulsions were firstly compared in terms of their droplet size. All emulsions displayed surface-weighted mean ($d_{3,2}$) droplet sizes that fell within 1.061–1.122 μm, while the formation of limonene microcapsules was confirmed by Scanning Electron Microscope observation.

The microencapsulation process was monitored by encapsulation efficiency, encapsulation loading capacity and encapsulation yield. According to our results, all three *Acacia* gums showed only slight variations in their encapsulation parameters (p>0.05) as well as in their morphology. In general, the samples exhibited a porous structure as other lyophilised systems (Kaushik & Roos, 2007; Qian & Zhang, 2011). In the continuous mass of GA1 dry material scattered particles of limonene were found, while in case of GA2 and GA3, some aggregates were detected.

The stability of encapsulated *D*-limonene was also studied in view of the release characteristics. The powders were stored under the conditions of 0 and 50% relative humidity at 25 and 50 °C for two months. The rate of release of limonene was analysed using Avrami's equation (Soottitantawat et al., 2004). Retention of limonene during storage was dependent on the type of emulsifier, i.e. GA1, with the low AGP content, was found to be superior emulsifier over the other two GA samples to retain limonene during freeze drying. Furthermore, its release rate was closely related to the relative humidity and increased by increasing it. Finally, storage under different conditions also affected the morphology of the powdered samples, the effect being more obvious in the case of GA3.

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Influence of coinoculated fermentations with *Saccharomyces cerevisiae* strains on wine volatile composition

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One of the main characteristic widely influencing wine's quality and value is the aroma. The volatile fraction of wine can be made up of more than 800 different compounds with a wide concentration range varying from hundreds of mgL^{-1} to ·gL^{-1} or ng L^{-1} levels (Callejon et al., 2010). This great variety of volatile compounds is responsible for the complexity of the wine's bouquet and ensures its specificity and character. Some of the most important components of the wine aroma are already present in grapes, whereas a vast number of volatile compounds are formed and modulated by yeast during alcoholic fermentation. The volatile compounds synthesized by wine yeasts include higher alcohols, medium- and long-chain volatile acids, acetate esters, ethyl esters and aldehydes among others. The principal agent in winemaking is the yeast *Saccharomyces cerevisiae*. Different strains of *S. cerevisiae* can produce significantly different flavour profiles when fermenting the same must (Mauriello et al., 2009). This is a consequence of both the differential ability of wine yeast strains to release varietal volatile compounds from grape precursors and the differential ability to synthesise de novo yeast-derived volatile compounds. In order to improve the quality and stability of the wine, the practice of the inoculated fermentation, by starter cultures, is widely spread. Actual trend in winemaking is the use of mixed starter cultures as a tool to exploit the advantages of spontaneous fermentation in a situation, however, controlled. In this work, different wild *S. cerevisiae* strains were tested as mixed starter cultures during inoculated fermentations at laboratory scale. The same *S. cerevisiae* strains were tested also during monoculture fermentations. The experimental wines obtained from single and co-cultures fermentations were analyzed by gas-chromatography (GC and SPME-GC-MS) for the content of main secondary compounds, such as higher alcohols, aldehydes, esters, terpenic compounds, volatile fatty acids. The wines produced by mono-cultures were very different from the wines obtained by co-fermentations. Furthermore, the amounts of volatile components determined in mixed cultures wines differed significantly from those determined in blended wines. These results underline that, during the mixed fermentations, interactions between the different strains exert a strong influence on the yeast metabolism.

Tab. Metabolites (range) determined in wines by single and mixed *S. cerevisiae* cultures and in blending from monocultures wines

Compounds (mg/l)	Single	Mixed	Bledend wines
Acetaldehyde	15.8-57.3	26.7-42.0	21.9-45.7
Ethyl acetate	53.5-57.6	54.4-58.3	54.6-57.2
N-propanol	46.9-51.6	47-50.3	48.1-50
Isobutanol	21.9-35.6	25.1-34.4	28.4-33.6
D-amyl alcohol	46.3-55.1	47.5-55.4	51-57.3
Isoamyl alcohol	150-165.6	140.5-175	152.3-173
Acetic acid	135.6-323.3	261.5-331.5	206.7-277.5
∑ terpenes	10.2-32	31.8-49	20.4-33.7
∑ fatty acids	9.8-26.5	30.5-46.7	19.3-33.3

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Flavour Compound Production by *Debaryomyces hansenii, Yarrowia lipolytica* and *Saccharomyces cerevisiae*

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The yeasts *Debaryomyces hansenii*, *Yarrowia lipolytica* and *Saccharomyces cerevisiae* are all of importance for maturation and flavour development of surface ripened cheeses. However, the role in flavour development is so far only purely investigated for yeasts. Currently, an increasing proportion of cheeses are produced at larger production plants instead of as previously at smaller farmhouse-dairies. This process means that some of the original microbiota will be lost as well as special cheese types and flavour notes. In order to search for potential ripening-cultures, a cheese-surface model was set-up and the yeasts *Debaryomyces hansenii*, *Yarrowia lipolytica* and *Saccharomyces cerevisiae* were screened for their effect on volatile compound production. *D. hansenii* produced branched-chain aldehydes and alcohols when inoculated on a cheese agar surface, while *Y. lipolytica* resulted in production of sulphides as well as some furanes and short-chain ketones and *S. cerevisiae* resulted in production of esters. Even though major focus has been on bacterial flavour development, the results indicate that the yeasts themselves might be of importance for development of specific flavour compounds during cheese maturation. The results open for the possibility to select specific yeast cultures with potential to affect the flavour of surface ripened cheeses.

The effect of *ARO10*, *ATF1* and *ATF2* genes from non-conventional *Saccharomyces* species on the wine aroma

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The constantly developing wine market requires production of new wines with fresh and original qualities. In this context, the abilities of some non-conventional species from *Saccharomyces* genus have been recently described in winemaking. The species *S. kudriavzevii* and *S. uvarum* (*S. bayanus var. uvarum*) and their hybrids with *S. cerevisiae* have shown very interesting oenological properties leading to higher production of certain aromatic compounds suggesting that they take advantage of the alleles distinct from *S. cerevisiae*. Based on this information we decided to compare the homologous genes from *S. cerevisiae*, *S. kudriavzevii* and *S. uvarum* involved in the flavour compounds synthesis. The *in silico* analysis identified the most radical substitutions in *ARO10*, *ATF1* and *ATF2* genes. When expressed in winery *S. cerevisiae* T73 strain, the increased amount of several aromatic compounds was detected. A significant increase was observed above all in the production of isoamyl alcohol and isoamyl acetate. Our results and the fact of close phylogenetic relationship among the three species suggest that the modification of overall wine aroma could be reached only by substitution of a few nucleotides.

Non-conventional yeast species in novel sustainable food fermentation processes: Unravelling the eco-physiological implications of key metabolic pathways.

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The relatively diverse group of non-conventional yeast (NCY) species found in various natural food fermentation processes is scarcely studied since *Saccharomyces cerevisiae* has dominated yeast research. Nyanga et al. (2007) isolated many yeasts species from fermented *masau* fruits, among which *Saccharomyces cerevisiae* and the NCY species *Pichia fabianii* and *Pichia kudriavzevii* were found. The importance of exploring these NCY species is emphasized by their natural low ethanol production and relatively high aroma formation, which makes them interesting candidates for pure and/or mixed starter cultures. Understanding of their behaviour in food fermentation processes requires extensive knowledge of their physiology, metabolism and genomics and would facilitate their applicability as starter culture. Special focus on the activity and regulation of amino acid degradation pathways will reveal knowledge to control growth and aroma production under anaerobic conditions.

In this study, three isolates from the same niche (fermented *masau* fruits) have been selected; *Saccharomyces cerevisiae* 131, *Pichia fabianii* 65 and *Pichia kudriavzevii* 129 (Nyanga et al., 2007). Recent experiments showed the ability to steer the aroma production in these yeast species and demonstrated species specific aroma profiles.

Nyanga, L. K., M. J. Nout, T. H. Gadaga, B. Theelen, T. Boekhout, and M. H. Zwietering. 2007. Yeasts and lactic acid bacteria microbiota from masau (Ziziphus mauritiana) fruits and their fermented fruit pulp in Zimbabwe. International journal of food microbiology 120(1-2):159-166.

Enhanced production of isoamyl acetate via biotransformation with *Lindnera saturnus* by in situ product removal with macroporous adsorption resins

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The biotransformation of isoamyl alcohol obtained from fusel oil in a molasses based medium via in situ product removal (ISPR) with macroporous adsorption resin was carried out with *Lindnera saturnus* to produce isoamyl acetate. As a suitable adsorbent, the non-polar macroporous resin H103, selected from several resins tested showed high adsorption capacity for isoamyl acetate. Fed-batch biotransformation conducted with resin showed that the production of isoamyl acetate was increased 8 folds compared to trial carried out without using resin. At the end of the biotransformation carried out with macroporous adsorption resin H103, a total concentration of 308 mg/L isoamyl acetate (isoamyl acetate in aqueous phase plus adsorbed onto the resin) was produced. The contribution of in situ product removal with adsorption resins could be remarkable for making the process more feasible for industrial application. The authors gratefully acknowledge the financial support of The Scientific and Technological Research Council of Turkey (No. 111 O 369).

Bioflavour Production from Rice Bran by Using *Kluveromyces marxianus* and *Debaroymyces hansenii*

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Rice bran, a byproduct from milling process of the paddy rice, has approximately 34-62% starch, 15-22% oil, 11-15% protein, 24-29% dietary fiber and 6.6-9.9% mineral substances (Fuh and Chiang, 2001). Rice bran and its products (eg. rice bran oil) can be utilized as raw materials for manufacturing biotechnological products such as organic acids, enzymes, biosurfactants etc. Therefore, several studies were carried out on the production of high value products from rice bran by biotechnological processes (Oliveira et al., 2011; Tanaka et al., 2006; Zheng et al., 2007). Among the flavor compounds, a higher alcohols and esters can be produced by yeasts at high levels during fermentation. In this context, *D. hansenii* and *K. marxianus* have promising ability to produce fruity (eg. ethyl acetate) and rosey (eg. 2-phenylethanol) flavors (Etschmann et al., 2002; Breuer and Harms, 2006).

The aim of this study was to investigate the potential use of rice bran for producing flavor compounds by using *K. marxianus* and *D. hansenii*. For this purpose, 10 % (w/v) solution of rice bran was individually fermented by *K. marxianus* and *D. hansenii* during 72 hours at 30^{o}C at 120rpm in shake-flask condition. The microbial growth of *K. marxianus* and *D. hansenii* were determined at 24, 48 and 72h of fermentation. Odor-active compounds were identified by gas chromatography-olfactometry (GC-O).

As a result, it was determined that biomass of *K. marxianus* and *D. hansenii* increased 2 log (100 fold) in rice bran solution during 72 h fermentation. Esters including ethyl isobutyrate (sour), isobutyl acetate (bubble gum), phenyl ethyl acetate (rose), citronellyl acetate (rose) and ethyldimethylthiazole (earthy) were identified at high intensities in cultured rice bran solutions. Our future activities will include positive identification of aroma compounds generated by the growth of certain microorganisms by using gas chromatography- mass selective detector (GC-MS).

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Optimization of Bioflavor Production from Whey by Using *Kluyveromyces marxianus*: A Response Surface Approach

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Whey is a by-product of cheese industry. 140 million tons of whey was annually produced worldwide. Whey has aproximately 6-8 g/L of protein, 4.3-9.5 g/L of mineral substance and high amount of lactose (44-52g/L) (Athanasiadis et al., 2004). It was emphasized that whey and its products are valuable source for biotechnological applications. *Kluyveromyces marxianus* has many types of enzymes such as inulinase, β-galactosidase, β-glucosidase, endopolygalacturonases, protein phosphatases, carboxypeptidases and aminopeptidases. Specifically, flavor compounds and pectinolytic enzymes from *K. marxianus* have considerable economic advantages. It was emphasized that aroma compounds such as ethly esters can be (Fonseca et al., 2008; Löser et al., 2013) economically produced by biotechnological process by using *K. marxianus*.

The aim of this study was to investigate the production of bioflavor compounds by *K. marxianus* NRRL Y-6373. For this purpose, Response surface methodology was employed to optimize the production of some flavor compounds from whey cultured by *K. marxianus*. The effect of fermentation time, whey:water ratio and initial pH on microbial growth, the content of aroma compounds and lactose consumption were investigated in shake-flask culture condition. It was observed that fermentation time, initial pH and whey:water ratio were important factors for the growth of *K. marxianus.* Ethyl acetate (fruity), isoamyl alcohol (whiskey), isoamyl acetate (banana) and phenylethyl alcohol (rosey) can be produced from whey by using *K .marxianus.*.

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Biotechnology for natural flavour production

July 22 -23, 2013, Freising (Germany)

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	Name	Organization	Presentation	
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2	**Bönisch,** Friedericke	TU München, Germany	☒	☐
3	**Borrull,** Anna	University. Rovira i Virgili, Spain	☐	☒
4	**Breme,** Katharina	Agroscope, Switzerland	☒	☒
5	**Bugarski,** Branko	University of Belgrade, Serbia	☒	☐
6	**Cadez,** Neza	University of Ljubljana, Slowenia	☐	☒
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8	**de Billerbeck,** Gustavo M.	LISBP - INSA Toulouse	☐	☐
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10	**Djordjevic,** Radovan	University of Belgrade, Serbia	☐	☒
11	**Etschmann,** Marlene	Dechema, Germany	☒	☐
12	**Geertman,** Jan-Maarten A.	Heineken Supply Chain	☐	☐
13	**Gibson,** Brian	VTT, Finland	☒	☐
14	**Gruchattka,** Evamaria	TU Dortmund, Germany	☒	☐
15	**Kayser,** Oliver	TU Dortmund, Germany	☐	☐
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Waldinventur und nachhaltige Nutzung	4 2 77	Hans-Carl-von-Carlowitz-Platz 2	B5
Waldwachstumskunde	4 2 77	Hans-Carl-von-Carlowitz-Platz 2	B5
Wirtschaftslehre des Landbaus	4 1 06	Alte Akademie 14	E7
Zoologie	4 3 08	Liesel-Beckmann-Straße 4	D4/E4
Zoologie, AG Molekulare Zoologie	4 2 77	Hans-Carl-von-Carlowitz-Platz 2	B5
Weitere TUM-Einrichtungen			
Betriebsärztlicher Dienst	4 2 14	Maximus-von-Imhof-Forum 6	D5
Betriebshof	4 1 14	Weihenstephaner Steig 14	D7
Bibliothek Weihenstephan	4 2 20	Maximus-von-Imhof-Forum 1	D6
Bioanalytik (ZIEL)	4 1 08	Alte Akademie 10	E7
BLQ, Forschungszentrum für Brau- und Lebensmittelqualität	4 1 01	Alte Akademie 3	F7
Campus Office (Studiensekretariat)	4 1 02	Alte Akademie 8	F7
Campus Office/Allgemeiner Studienbetrieb/Prüfungsamt	4 1 01	Alte Akademie 1	F7/8
Dekanat	4 1 02	Alte Akademie 8	F7
Dr. Gudula-Wernekke-Rastetter Kindervilla	4 1 16	Weihenstephaner Steig 17	D7
Fachschaften	4 2 16	Maximus-von-Imhof-Forum 5	D5
Familienservice Weihenstephan	4 1 15	Weihenstephaner Steig 19	D7
Forschungsbrauerei	4 1 11	Weihenstephaner Steig 18	D7
Gewächshauslaborzentrum Dürnast	4 2 34	Dürnast 3	A4
Hans-Eisenmann-Zentrum, Geschäftsstelle	4 1 07	Alte Akademie 16	E7
Informationstechnologie Weihenstephan	4 2 20	Maximus-von-Imhof-Forum 3	D6
Krabbelstube Weihenstephan	4 2 39	Emil-Erlenmeyer-Forum 4	E5
Pressestelle Weihenstephan (CCC)	4 2 20	Maximus-von-Imhof-Forum 3	D6
Sprachenlabor	4 1 53	Weihenstephaner Berg 13	E6
Technische Zentrale	4 3 21	Lange Point 24	B2
Telefonzentrale	4 2 21	Emil-Erlenmeyer-Forum 7	D6
Veitshof	4 1 80	Veitsmüllerweg 4	H7
Versuchsstation Dürnast	4 2 30		A4
Verwaltungsstelle Weihenstephan	4 1 01	Alte Akademie 1	F7/8
Werkfeuerwehr Weihenstephan	4 2 38	Emil-Erlenmeyer-Forum 2	E5
Zentralbibliothek	4 2 20	Maximus-von-Imhof-Forum 1	D6
Zentralinstitut für Ernährungs- und Lebensmittelforschung (ZIEL)			
Geschäftsführung und Verwaltung	4 1 26	Weihenstephaner Berg 1	E6

Lehrräume / Lecture Rooms

Hörsäle			
HS 1-2	4 1 02	Alte Akademie 8	F/F7
HS 4	4 1 13	Weihenstephaner Steig 16	D7
HS 6	4 1 01	Alte Akademie 1	F7/8
HS 8-9	4 1 08	Alte Akademie 10	E7
HS 10-11	4 1 12	Weihenstephaner Steig 23	D/E7
HS 12	4 2 17	Emil-Ramann-Straße 2	C5
HS 14-16	4 2 14	Maximus-von-Imhof-Forum 6	D5
HS 17	4 3 17	Liesel-Beckmann-Straße 1	D4
HS 21-24	4 2 77	Hans-Carl-von-Carlowitz-Platz 2	B5
Allgemeine Seminarräume			
S 1-3	4 2 14	Maximus-von-Imhof-Forum 6	D5
S 4-6	4 2 77	Hans-Carl-von-Carlowitz-Platz 2	B5
S 7a,b	4 2 77	Hans-Carl-von-Carlowitz-Platz 2	B5
S 8-10	4 1 02	Alte Akademie 8	E/F7
Allgemeine Praktikumsräume			
P1-16	4 2 15	Maximus-von-Imhof-Forum 4	D/E5
CIP-Räume			
EGL 12	4 2 20	Maximus-von-Imhof-Forum 3	D6
DGL 01, 11	4 2 20	Maximus-von-Imhof-Forum 3	D6
PU	4 2 15	Maximus-von-Imhof-Forum 4	D/E5
HU 24A	4 2 14	Maximus-von-Imhof-Forum 6	D5
HU 34	4 2 14	Maximus-von-Imhof-Forum 6	D5
PC-Labor	4 2 77	Hans-Carl-von-Carlowitz-Platz 2	B5
CAD/GIS-Labor	4 1 53	Weihenstephaner Berg 13	E6

Sonstige Einrichtungen

Cafeteria	4 1 01	Alte Akademie 1	F8
Praktikantenamt	4 1 01	Alte Akademie 1	F8
Studentenwerk	4 1 01	Alte Akademie 1	F8
Deutscher Wetterdienst	4 1 07	Alte Akademie 16	E7
DFG Senatskommission	4 1 19	Hohenbachernstraße 15+17	C6
Mensa, Cafeteria	4 2 16	Maximus-von-Imhof-Forum 5	D5
UniBar	4 2 20	Maximus-von-Imhof-Forum 1	D6
Deutsche Forschungsanstalt für Lebensmittelchemie	4 2 25	Lise-Meitner-Straße 34	A6
Forst Cafeteria	4 2 77	Hans-Carl-von-Carlowitz-Platz 2	B5
Staatliche Fachschule für Blumenkunst	4 3 86	Am Staudengarten 6	F/G5
Staatliches Bauamt Freising	4 3 90	Am Staudengarten 2a	F5
Hochschulgemeinde		Hohenbachernstraße 9	C6
Gründerzentrum IZB		Lise-Meitner-Straße 30	A6

Hochschule Weihenstephan-Triesdorf University of Applied Sciences

Gebäudebezeichnung	Gebäude-Nr.	Anschrift	Übersichtsplan
C6	4 1 23	Weihenstephaner Berg 4, (1.OG)	D6
A3	4 1 25	Am Hofgarten 10	G7
Verbinder und Geräteschuppen	4 1 70	Weihenstephaner Berg 15	F/G6
A11	4 1 71	Weihenstephaner Berg 17	G7
A5+A6+A7	4 1 72	Am Hofgarten 4 + 6	F/G7
A4	4 1 73	Am Hofgarten 8	G7
A8	4 1 74	Am Hofgarten 2	F7/8
ehemalige Bienenhaus	4 1 75	ohne	G7
A1	4 1 76	Am Hofgarten 1	F/G7
A2	4 1 77	Am Hofgarten 3	G7
A9	4 1 78	Weihenstephaner Berg 16	F7
C4	4 1 79	Weihenstephaner Berg 5	E6
C5	4 1 99	Vöttinger Straße 27	G6
F9	4 2 76	Hans-Carl-von-Carlowitz-Platz 3	B/C5
Kohlscheune und Kühllager	4 3 70	ohne	E3
H6	4 3 72	Am Staudengarten 11	F4
Wohngebäude I, Infostelle	4 3 73	Am Staudengarten 9	F4
H7	4 3 74	Am Staudengarten 7	F4
H8	4 3 75	Am Staudengarten 14	F3
H9	4 3 76	Am Staudengarten 12	F/G4
H10	4 3 77	Am Staudengarten 10	F4
Heizzentrale/mechan.Labor/Schlosserei	4 3 78	Am Staudengarten 10	F4
H11	4 3 79	Am Staudengarten 8	G4
Wohngebäude II	4 3 80	Am Staudengarten 4	F4
Wirtschaftsgebäude Erberberg	4 3 81	ohne	C2
Erdlager-, Torflager- und Maschinenhalle	4 3 82	ohne	F4
H14	4 3 83	Lange Point 2	F3
H15	4 3 84	ohne	F4
Bienenhaus am Waldrand	4 3 85	ohne	C2
Informations-Pavillion	4 3 87	ohne	F4
Maschinenunterstellhalle, offene Fahrzeug-Gerätehalle	4 3 88	ohne	F4
Maschinenhalle	4 3 89	ohne	B2

Lehrräume / Lecture Rooms

FH Hörsäle			
FH 1 / 4 / 5	4 1 72	A5, Am Hofgarten 6	F/G7
FH 7	4 3 77	H10, Am Staudengarten 10	F4
FH 8	4 3 74	H7, Am Staudengarten 7	F4
FH 10 / 11 / 12	4 2 76	Hans-Carl-von-Carlowitz-Platz 3	B/C5
FH 14	4 1 25	A3, Am Hofgarten 10	G7
FH 15 / 16 / 17	4 1 76	A1, Am Hofgarten 1	F/G7
FH 20 / 21 / 22	4 1 79	C4, Weihenstephaner Berg 5	E6
FH Seminarräume			
FS 1 / 2 / 3	4 1 72	A5, Am Hofgarten 6	F/G7
FS 10 / 11 / 12	4 2 76	Hans-Carl-von-Carlowitz-Platz 3	B/C5
FS 14	4 3 83	H14, Lange Point 2	F3
FS 15 / 16 / 17 / 18 / 19 / 20 / 21 / 22 / 23	4 1 76	A1, Am Hofgarten 1	F/G7
FS 26	4 3 72	H6, Am Staudengarten 11	F4
FS 27	4 1 77	A2, Am Hofgarten 3	G7
FS 29	4 1 25	A3, Am Hofgarten 10	G7
FS 30 / 31 / 32	4 1 78	A9, Weihenstephaner Berg 16	F7
FS 35 / 36 / 37 / 38	4 1 99	C5, Vöttinger Straße 27	G6
FS 39	4 1 79	C4, Weihenstephaner Berg 5	E6
FS 40	4 1 79	C4, Weihenstephaner Berg 5	E6
FS 41	4 1 79	C4, Weihenstephaner Berg 5	E6
FS 42	4 1 79	C4, Weihenstephaner Berg 5	E6
FS 49	4 1 79	C4, Weihenstephaner Berg 5	E6
FS 43	4 1 79	C4, Weihenstephaner Berg 5	E6
FS 44	4 1 79	C4, Weihenstephaner Berg 5	E6
FS 45	4 1 79	C4, Weihenstephaner Berg 5	E6
FS 46	4 1 79	C4, Weihenstephaner Berg 5	E6
FS 47	4 1 79	C4, Weihenstephaner Berg 5	E6
FS 48	4 1 79	C4, Weihenstephaner Berg 5	E6
FH Praktikumsräume			
FP 11 / 12 / 13 / 14 / 15 / 16 / 17	4 1 76	A1, Am Hofgarten 1	F/G7
FP 20 / 21 / 22 / 23 / 24	4 1 99	C5, Vöttinger Straße 27	G6
FH EDV - Räume			
FEDV 1 / 2 / 3	4 1 79	C4, Weihenstephaner Berg 5	E6
FEDV 4 / 6 / 11	4 2 76	Hans-Carl-von-Carlowitz-Platz 3	B/C5
FEDV 5	4 1 76	A1, Am Hofgarten 1	F/G7
FEDV 8	4 1 25	A3, Am Hofgarten 10	G7
FEDV 9 / 10	4 1 79	C4, Weihenstephaner Berg 5	E6
FEDV 13 / 14	4 1 99	C5, Vöttinger Straße 27	G6
FEDV 15 / 16 / 17	4 1 79	C4, Weihenstephaner Berg 5	E6
FH Sprachenzentrum			
FSPZ 1	4 1 79	C4, Weihenstephaner Berg 5	E6
FSPZ 2	4 1 79	C4, Weihenstephaner Berg 5	E6

Bayerische Landesanstalt für Landwirtschaft

Büro- und Werkstattgeb. 13 mit Unterstellhalle	4 2 09	Am Staudengarten 3	F5
Bürogebäude 14	4 2 11	Vöttinger Straße 38	F6
Bürogebäude 1 (Präsidium)	4 2 50	Vöttinger Straße 38	E6
Nematodenlager	4 3 52	Am Gereuth	D1
Labor 1	4 3 53	Lange Point 4	E3
Labor 2	4 3 54	Lange Point 6	E3
Technologie 6	4 3 55	Kreuzstraße 2-4	D/E2
Betriebshof 1 BA	4 3 56	Am Gereuth 11	D1
Betriebshof 2 BA	4 3 57	Am Gereuth 11	D1
Hausmeisterwohnungen m. Garagen	4 3 58	Steinbreite 12; 14; 16;18	E2
Betriebswerkstätten m. Garagen	4 3 59		D2
Erdlager	4 3 60		E2
Technologie 5	4 3 61	Am Gereuth 2	D2
Mehrzweckgebäude 1	4 3 62	Lange Point 10	D3
Mehrzweckgebäude 2	4 3 63	Lange Point 12	D3
Technologie 4	4 3 66	Am Gereuth 4	D1/2
Großlager 1	4 3 67	Am Gereuth	D/E1
Großlager 2	4 3 67a	Am Gereuth	D1
Großlager 3	4 3 67b	Am Gereuth	D1
Technologie 3	4 3 68	Am Gereuth 6	D1/2
Technologie 2	4 3 69	Am Gereuth 8	D/E1/2

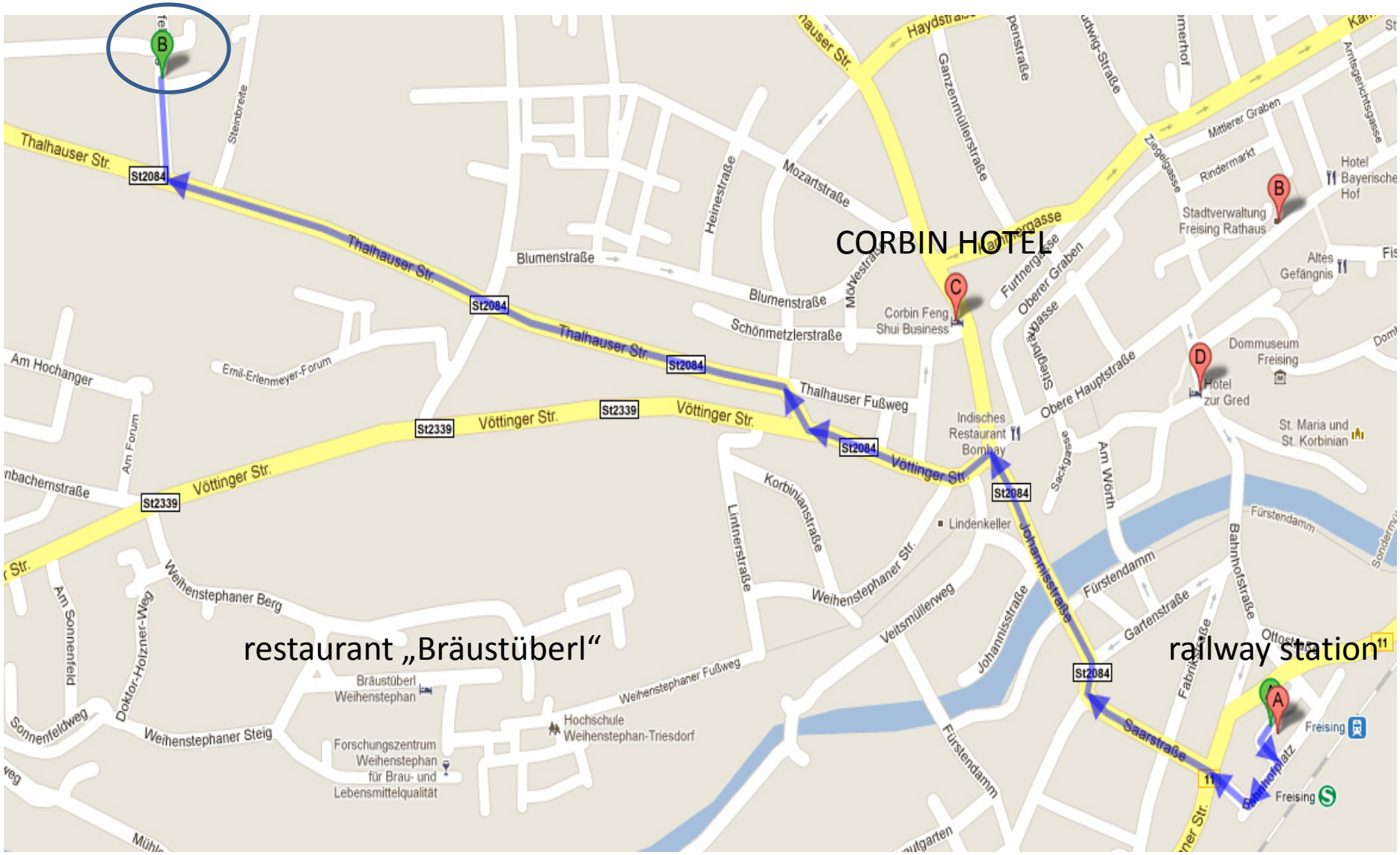
Bayerische Landesanstalt für Wald und Forstwirtschaft

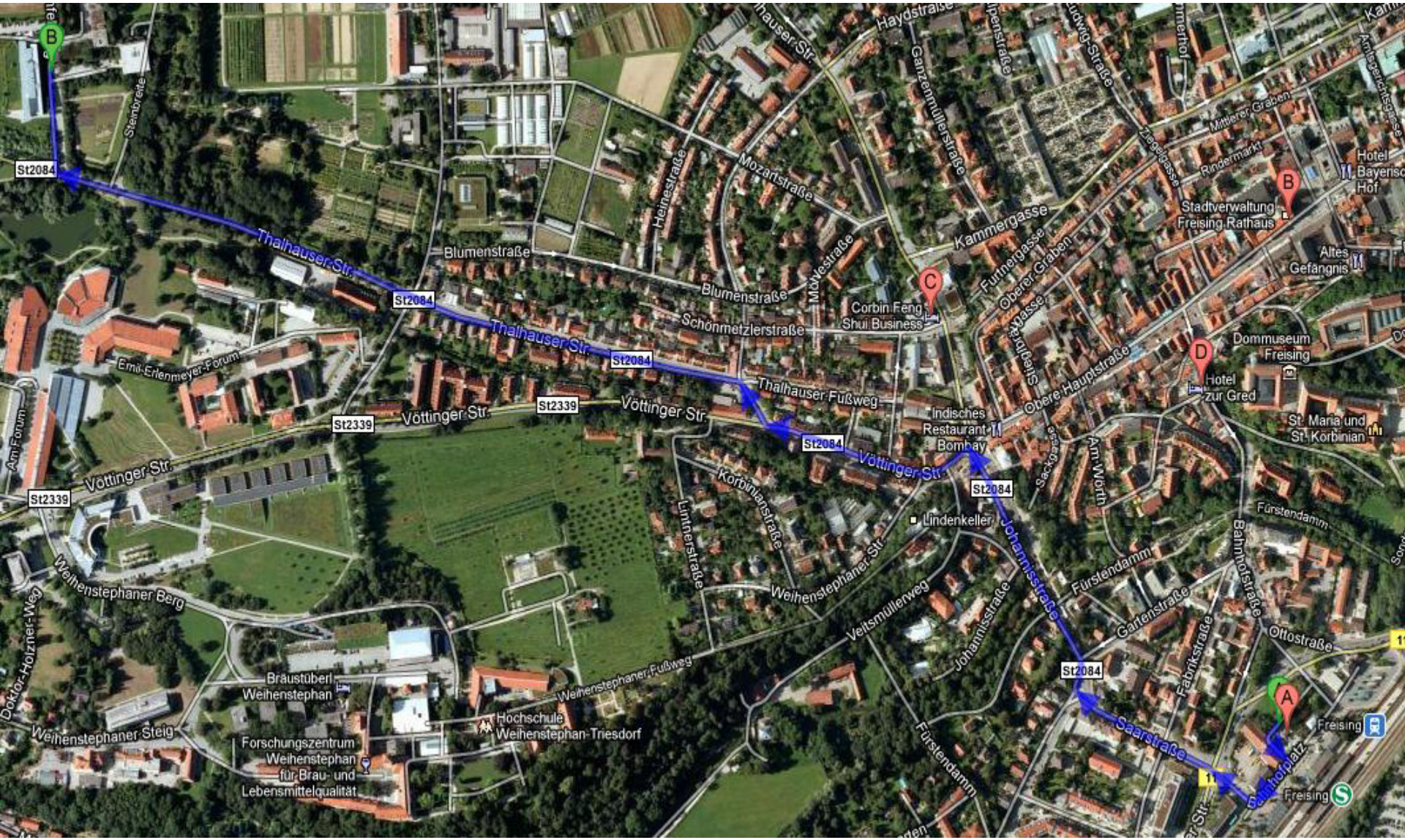
Bayer. Landesanstalt für Wald und Forstwirtschaft (LWF)	4 2 77a	Hans-Carl-von-Carlowitz-Platz 1	B5

Bayerische Staatsbrauerei Weihenstephan

Flaschenkeller	4 1 56	Weihenstephaner Berg 21	F7
Fassabfüllgebäude (Große Kustermannhalle)	4 1 57	Weihenstephaner Berg 14	F7
Bräustüberl	4 1 58	Weihenstephaner Berg 10	F7
Arbeiterwohngebäude	4 1 59	Alte Akademie 4	F7
Verwaltung d. Bayerischen Staatsbrauerei Weihenstephan	4 1 60	Alte Akademie 2	F7
Sud- und Maschinenhaus	4 1 61		F7
Flaschenfüllerei	4 1 62		F7
Stapelhalle Staatsbrauerei	4 1 64		F7

Venue of COST ACTION FA0907 BIOFLAVOUR
Biotechnology of Natural Products: 2 km from the railway stations (taxis are available)
Street (former name): Hochfeldweg 1
Street (new name): Liesel-Beckmann-Str. 1




B
St2084
Thalhauser Str.
Blumenstraße
Heinestraße
Mozartstraße
Haydstraße
Ganzenmüllerstraße
Ludwig-Straße
Kammergasse
Furtnergasse
Obere Graben
Mittlerer Graben
Rindermarkt
Amtsgerichtsgasse
Hotel Bayerischer Hof
Stadtverwaltung Freising Rathaus
Altes Gefängnis
C
Corbin Feng Shui Business
Schönmetzlerstraße
Moltkestraße
Thalhauser Fußweg
Emil-Erlenmeyer-Forum
Am Forum
St2339
Vöttinger Str.
Dommuseum Freising
D
Hotel zur Gred
Obere Hauptstraße
Indisches Restaurant Bombay
St. Maria und St. Korbinian
Sackgasse
Am Wörth
Lindenkeller
Korbinianstraße
Linnerstraße
Weihenstephaner Str.
Johannisstraße
Fürstendamm
Bahnhofstraße
Gartenstraße
Ottostraße
Fabrikstraße
Weihenstephaner Berg
Doktor-Holzner-Weg
Veitsmüllerweg
Weihenstephaner Fußweg
Braustüberl Weihenstephan
Weihenstephaner Steig
Hochschule Weihenstephan-Triesdorf
Forschungszentrum Weihenstephan für Brau- und Lebensmittelqualität
A
Saarstraße
Bahnhofplatz
Freising

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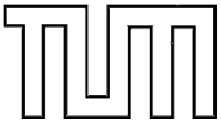

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