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Enzymes in Food Processing

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Recent Developments in Enzyme Biocatalysis for Food

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The increasing use of enzymes as biocatalyst in processing of food has been primarily aimed at obtaining easier processes, efficient raw material utilization and consistent product quality. The improved sustainability has been a side advantage. This is now changing, as the sustainability and health trends are becoming primary drivers for enzyme development.

Enzymes are used for mitigation of potential health risks. New developments enable for acrylamide prevention in coffee and new asparaginases with different levels of increased thermostability for breakfast cereals, coffee and French Fries. Glycidol ester formation in oils can be prevented by improved technology for lipase mediated triglyceride formation. Examples will also be given on how enzymes facilitate general health trends, including the use of xylanases to facilitate baking with whole wheat.

New enzymatic processes continue to widen the positive sustainability impact including improved CO_2 foot print and reduced use of chemicals. Examples will be given on new brewing solutions for brewing with barley and avoidance of cereal cooking.

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Enzymes in Dairy Technology

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While the study of the about 70 indigenous enzymes in milk has a long history and can be regarded as quite well known, the application of and research on exogenous enzymes in dairy technology is strongly increasing nowadays. The reasons therefore lay in the fact that enzymes with desired properties are discovered, produced and further improved faster and cheaper by the modern methods in biotechnology than ever before. Moreover, the properties of milk as a smooth liquid makes it very suitable for enzyme modifications also in large scale. The global milk production was about 762 x 106 metric tons per year in 2013, with a lion´s share of about 85% cow milk.

The principal components of milk are lactose (ca. 4.8%), lipids (ca. 3.6%) and proteins (ca. 3.5%). Thus, enzymes modifying these substrates are mainly used in dairy technology: β-galactosidases, lipases and peptidases. The application of these enzymes and the current research on enzymes in dairy technology will be considered in this lecture with a particular focus on lactose conversions and protein hydrolysis.

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Enzymes in Baking Technology

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The development of bread process was an important event in mankind. After the 19th century, with the agricultural mechanization, bread's quality was increased while its price was reduced; thereby white- and rye -bread became a commodity within almost everyone´s reach. An important aspect that contributed to evolution of the baking market was the introduction of industrial enzymes in the baking process, where bakery enzymes represent a relevant segment of the industry.

Baking comprises the use of enzymes from three sources: the endogenous enzymes in flour, enzymes associated with the metabolic activity of the dominant microorganisms and exogenous enzymes which are added in the dough.

The supplementation of flour and dough with enzyme improvers (technical enzymes) is a usual practice for flour standardization and also as baking aids. Enzymes are usually added to modify dough rheology, gas retention and crumb softness in bread manufacture, to modify dough rheology in the manufacture of pastry and biscuits, to change product softness in cake making and to reduce acrylamide formation in bakery products. The enzymes can be added individually or in complex mixtures, which may act in a synergistic way in the production of baked goods, and their levels are usually very low. Examples in context will be given. One actual topic of added technical enzymes can be described by problems how to make clear correlations to bake – ability as such and more?

Besides the demand for replacement of chemical additives by others from natural sources, there is an increasing concern among the consumers and consequently an increased demand for preservation and/or enrichment of foods with products that have beneficial effects on human health. Regarding baked goods, the use of enzymes to obtain dietary fiber enriched bread, for the development of gluten free products, to obtain products with increased contents of e.g. arabinoxylan oligosaccharides with prebiotic potential. Some novel examples to this background will be presented.

Modern baking technologies today are driven e.g. by novel flour -qualities, which are simplified characterized in comparison to former flours (around 20 years before): Rich in protein, containing strong proteins (e.g. elastic dough properties) and low enzyme activities (amylases etc.). For this underlying reason ,the actual baking-technology in Europe has to consider this novel requirements in case of mixing- , retarding -, freezing- or sour-dough /sponge –, baking -procedures etc. The reason and the need for novel technologies are e.g. understandable by the need to increase (optimize) the hydration of un-soluble biopolymers and more just to give endogenous enzymes of flours or ingredients time to work. In general the water-soluble properties of each enzyme on the one hand side and the complex –system of dough (batter) containing solid, liquid and gaseous phases on the other hand, limits the specific enzyme-attack in such systems. Because of the actual existing lower hydration properties of given flour-qualities, the specific hydration (and /or hydrolyzation) of e.g. un-soluble bio-polymers in dough or batters are one of future demands to optimize enzyme operations and applications in baking technology.

The presentation gives an overview of enzyme applications for baking purposes and will point out some actual problems given in baking technology together with some novel approaches in enzyme –application like enzyme supported fermentations (use of Phytases even for gluten free baked goods) or preservation aspects (cold adapted Chitinases) .

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Use of Lipases in Inter- and Trans-Esterification of Lipids

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Lipases are one of the most commonly-used enzyme groups in research and in industrial applications. The main application of these enzymes, together with phospholipases and esterases, is lipid modification. Recently, the application of lipases in biodiesel synthesis from renewable substrates has become an important task. There are promising possibilities to isolate new lipases from metagenomes and to improve the properties of enzymes through protein engineering methods.

This presentation will show the application of reaction medium engineering methods for the synthesis of structured triacylglycerols (sTAG), ascorbyloleate and flavor compounds. Some examples of the application of metagenome and directed evolution methods for discovering new enzymes and improving the properties of known lipases will be shown.

The application of one- and two-step methods for synthesis of MLM-type sTAG containing γ-linolenic acid (GLA) and conjugated linoleic acid isomers (CLA) will be presented. The reaction of transesterification was catalyzed by commercially-available lipases. Reactions were performed in organic solvents or in a solvent-free medium. Water activity in the reaction mixture was controlled by pre-equilibration over a saturated salt solution. In acidolysis of rapeseed oil with CLA, mainly isomers *c*9,*t*11 and *t*10,*c*12 were incorporated in triacylglycerols. The synthesis of CLA-rich triacylglycerols was influenced by the source of lipase and water activity. The aim of the study was to investigate the stability and selectivity of lipases during synthesis of sTAG by interesterification, acidolysis and two-step methods from selected bio-oils. Lipase stability and selectivity improvement methods were analyzed. Enzymes were immobilized on available carriers, including nanocarriers, to improve their stability. Furthermore, the use of medium engineering was analyzed, including the application of ionic liquids as media for the synthesis of ascorbyloleate. Selected examples of lipase application for biodiesel and ethyl butanoate catalyzed by Lipozyme TL IM and esterases from *Lactococcus* strains will be discussed.

Several examples of metagenome exploration for new lipase discovery and the application of protein engineering to improve lipase selectivity towards CLA will be presented. The future of lipid modification will also be highlighted.

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Application of Transglutaminases in Food Processing

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Transglutaminase (TGase) is an enzyme widely distributed in nature, which can be found in diverse animal tissues, fish and plants. The complete name of TGase is protein-glutamine γ-glutamyltransferase (EC 2.3.2.13), and it belongs to the group of acyltransferases. TGases catalyze the acyl-transfer reactions between a γ-carboxyamine group of a peptide-bound glutamyl residue (acyl-donor) and a primary amino group of a variety of substrates including the ε-amino group of lysine or lysyl residues (acyl-acceptor) in proteins. It crosslinks the protein molecules by the formation of covalent intra- and intermolecular isodipeptide bonds. This creates larger protein associations from smaller peptides and proteins.

Until the end of the 1980s, commercially available TGase was derived from the liver of guinea pigs. Its rare source and unacceptability, the extensive purification, as well as the Ca2+-dependence activity which leads to protein precipitation in some food systems (e.g. casein, soybean globulin or myosin) and the excessive high prices hindered commercialization. In 1989 scientists succeeded in the isolation of TGase of the microorganism *Streptoverticillium* S-8112, which has been identified as a variant of *Streptoverticillium mobaraense*, also known as *Streptomyces mobaraensis*. Compared with other TGases, the microbial isoform (mTGase) is Ca2+-independent and is stable over a wide range of temperatures and pH values. Due to the higher reaction rate, the broad substrate specificity and the favorable mass production by traditional fermentation, mTGase is particularly useful for industrial and biotechnological applications as a food additive. During the 90s, the use of TGase in the food industry started with the manufacture of *surimi* based products in Japan, which applies as a standard until today. In the last two decades mTGase had been used in several studies to modify the functional properties of food proteins. The main aim of these studies was to improve (i) solubility, water-holding capacity and thermal stability of food protein, (ii) texture and mechanical properties and (iii) emulsifying characteristics of food proteins.

Because of its unique attributes, mTGase is an enzyme widely used in the food industry. In the baking industry, mTGase can be used to modify wheat proteins aiming to improve the texture and volume of bread as well as the texture of pasta. In the meat and fish industry mTGase is used to manufacture restructured high-value products from lean trimmings, thus improving sustainability and profitability. In addition, it is used in the cooked ham and dry-cured ham production to eliminate cavities. Moreover, the use of mTGase in cooked sausages improves the texture of the final product, which results in an increase in breaking strength and hardness. In the dairy industry, mTGase can be used to modify casein. Yoghurts incubated with mTGase are predestined to prevent syneresis, which allows creating a creamy consistency and a smooth and dry surface. In the manufacture of cheese and curd yield is increased by using mTGase. In case of innovative and new products, mTGase can be used to modify food proteins to produce edible protein films applied to coat fresh fruits, vegetables and meat to extend the freshness as well as the shelf life.

The use of mTGase in the European food industry is allowed as processing aid according to the Directive 89/107/EC; its presence does not need to be labelled in the final product. It has been recognized as GRAS (Generally Recognized As Safe) by the US Food and Drug Administration. However, the German Federal Institute for Risk Assessment points out that food proteins modified with mTGase may lead to compounds that are structurally similar to gluten that causes the known immunological effects in celiac patients.

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Enzymatic Modification of Polysaccharides

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Carbohydrates provide the largest biomass on Earth and are central to many aspects of biotechnology with applications in biofuels, biomaterials, food and medicine. Carbohydrates are complex biomolecules and there is an urgent need to develop robust synthetic and analytical methodologies to fully exploit opportunities presented by glycobiotechnology.

We have developed a toolbox for the synthesis and analysis of complex carbohydrates and their function with a focus on chemoenzymatic synthesis of glycoconjugates such as glycopeptides and enzymatic modification of polysaccharides and glycoconjugates. The following biotransformations will be discussed in this talk: acylation, oxidation and glycosylation of polysaccharides and glycoconjugates.

Both et al Nature Chemistry. 2014; 6(1): 65; Noble et al J. Am. Chem. Soc. 2012; 134(31): 13010-13017; Šardzík et al J. Am. Chem. Soc. 2012; 134(10): 4521-4524; Castangia et al, Angewandte Chemie 2012; 51(52):13016-13018; Rannes et al. J. Am. Chem. Soc. 2011; 133(22): 8436-8439.

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Application of cold–adapted Enzymes in the Food Industry

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Cold temperatures are generally used to delay or even prevent microbiological spoilage of food and to avoid changes in nutritional value and flavour. The period of cold storage could be used for additional fermentation and optimization of foodstuff if organisms or enzymes are added that operate in the low temperature range. About 80% of the Earth's surface is permanently cold with temperatures below 5°C. Microorganisms as well as higher organized organisms populate habitats like ice, frozen soil, and cold water. Most of them function at these low temperatures often in combination with other extreme conditions like high salinity, low or high pH, high pressure. Cold-active enzymes from such organisms could be isolated and characterized, like α-amylases, proteases, lipases, cellulases, pectinases, ß-glucosidases, ß-galactosidases, peroxidases, chitinases, lysozyme, etc. The detergent and cleaning industriy already uses several of these enzymes thereby achieving a clear reduction in wash temperature and electricity used per wash. In contrast to the laundry industry, the food industry not only needs enzymes that operate at low temperatures but often requires enzymes working in sequential reactions. In this sense, cold-adapted enzymes have the additional advantage of being inactivated at quite low temperatures after having performed their job. The potential for using cold-adapted enzymes in the food industry is broad. Typical examples of use are, removal of lactose in refrigerated milk by means of ß-galactosidase and reduction of viscosity and turbidity in chilled juice by pectinase. Further, cold-active enzymes will be helpful in cheese processing and structural treatment of meat. However, the usage of cold-active enzymes is so far only in the beginning. But several projects are running and there are plenty of ideas focusing on different applications. Further, nowadays it is possible to modify the properties of cold-enzymes by means of genetic engineering as to enhance their performance as desired.

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Potential Application of Phytases in Food Processing

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Phytase [*myo*-inositol(1,2,3,4,5,6)hexakisphosphate phosphohydrolase], a phosphatase capable of initiating the stepwise dephosphorylation of phytate [*myo*-inositol(1,2,3,4,5,6)hexakisphosphate], is already used as a supplement in diets for monogastric animals to improve phosphate utilisation from phytate, the major storage form of phosphate in plant seeds. Recently, phytases have been found increasingly interesting for processing of food for human consumption, particularly because the decline in food phytate results in an enhancement of mineral bioavailability.

Different strategies could be applied to optimise phytate degradation during food processing and digestion in the human alimentary tract such as adjustment of more favourable conditions during food processing for the phytases naturally occurring in the raw material, addition of isolated phytases to the production process, and use of raw material with a high intrinsic phytase activity either naturally present or introduced by genetic engineering. Phytate hydrolysis during germination, soaking, cooking, and fermentation is a result of the phytase activity naturally present in plants and microorganisms. The capability to dephosphorylate phytate differs greatly between different plant and microbial species due to differences in their intrinsic phytase activities and properties. To optimise food processing in respect to phytate degradation, it is essential to know the properties of the natural occurring phytases and the properties of exogenous phytases added during food processing or heterologously expressed in plants and microorganisms. Furthermore, phytases may find application in the production of functional foods or food supplements with health benefits, because individual myo-inositol phosphates have been shown to have important physiological functions in man, such as prevention of diabetes complications, anti-inflammatory, antiangiogenic and antitumour effects. Phytases render production of defined myo-inositol phosphates in pure form and sufficient quantities possible. Phytases degrade phytate by sequentially removing phosphate from the *myo*-inositol ring in a regio- and stereospecific manner and the majority of phytases generates only one single *myo*-inositolpentakis-, tetrakis-, tris-, and bisphosphate isomer. Purification of the individual isomers from the reaction mixture could be achieved by ion-exchange-chromatography and different phytases pave the way for the production of different partially phosphorylated *myo*-inositol phosphates.

Last but not least, technological improvements are expected to occur due to phytate degradation during processing as shown for breadmaking, production of plant protein isolates, corn wet milling and the fractionation of cereal bran. However, but up to now, no phytase product for a relevant food application has found its way to the market.

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Enzymes from Basidomycetes for Food Applications

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Many basidiomycetes are edible and have a long history of save consumption (1). They grow well in surface or submerged culture including novel bag reactors (2). When triggered by appropriate supplements, such as renewable organic materials, silent genes are induced resulting in the formation and often secretion of an impres-sive diversity of hydrolases and oxidoreductases with unique properties. Ferulic acid esterases support the delignification of substrates in the natural habitats, but can also be used to improve the rheology of wheat dough and to liberate ferulic acid, an industrial flavour precursor (3). Amidases hydrolyse gluten, a recalcitrant wheat protein, and egg protein to salt taste enhancing dipeptides; they supply L-isoleucine to be converted to the beef broth flavour sotolone, and aspartic peptidases turn milk to fresh cheese (4). Cross-linking of proteins or phenol substituted polysaccharides using laccases is an elegant way to replace thickening additives. Peroxidases and dioxygenases bleach unwanted pigments, such as carotenoids or anthocyanins in foods which should not show a colour, such as whey or base wines. Sought-after flavour compounds, such as terpenoids, vanillin, piperonal or smoke flavour are amenable from side-streams of the food industry through enzymatic transformation (5). Flavours produced by biocatalysis fall under the legal definition of “natural flavours” in the EC and the US. Being now available as wild-type or recombinant, modified or evolved molecules, and combining selective catalysis with mild reaction conditions, enzymes are perfect tools to improve the composition of complex mixtures of natural chemicals, such as foods.

1. www.basidionet.de
2. Jonczyk P, Takenberg M, Hartwig S, Beutel S, Berger RG, Scheper T (2013) Cultivation of Shear Stress Sensitive Microorganisms in Disposable Bag Reactor Systems, J. Bio-technol. 167:370-376.
3. Nieter A, Haase-Aschoff P, Linke D, Nimtz M, Berger RG (2014) A halotolerant type A feruloyl esterase from Pleurotus eryngii, Fungal Biol. 118:348-357.
4. Giesler L, Linke D, Berger RG (2014) Factors limiting the enzymatic hydrolysis of wheat gluten. J. Agric. Food Chem. 62:4762-4768.
5. Kelle S, Zelena K, Krings U, Linke D, Berger RG (2014) Expression of soluble recombi-nant lipoxygenase from Pleurotus sapidus in Pichia pastoris, Protein Express. Purif. 95:233–239.

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Immobilized Enzyme Technology for Food Application

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There are very few examples of commercial food processing operations that use immobilized (IM) enzymes currently, despite their introduction in the 1970s. Although there are many advantages associated with immobilized enzymes including greater productivity with reuse of the enzyme, precise control over the automated and continuous reaction and no need to inactivate the enzyme downstream, there are of course disadvantages. Immobilization generally reduces the enzyme's activity and the enzymes are subject to mass transfer limitations. The cost of the matrix and support and regenerative capability of the biocatalyst also contribute to the cost of the IM enzyme process. In order for the immobilized process to be more economical than the soluble enzyme, the cost per unit of product must be less or there is a product improvement or biochemical advantage of producing the product with IM enzymes.

Two of the most successful examples of immobilized enzymes are the production of high-fructose corn syrup and the enzymatic modification of oils. The first commercial use of an immobilized enzyme for food constituent modification was introduced in 1970 by the Clinton Corning Processing Company for the enzymatic isomerization of glucose to fructose for the production of high fructose corn syrup. Improvements with the enzymatic method included improved product appearance and quality and the use of the IM enzyme allowed for a continuous process with a favorable economic advantage.

IM lipases are used as alternatives to hydrogenation and non-specific chemical esterification of oils to produce trans-fat free margarines and shortening, cocoa butter equivalents, medium chain triacylglycerols, diacylglycerols, fatty acid esters, and tailored fat products (Betapol®). The use of lipases in the immobilized form, versus sodium methylate, allows for oil modifications in solvent free systems, specificity in the modification and the products need minimal post treatment purification.

Previous commercial processes that used IM enzymes include IM beta-galactosidase for production of lactose hydrolyzed whey syrups and IM amylase for production of L-aspartic acid. Pilot scale processes have been developed including the production of 5'-ribonucleotides using IM 5'-phosphodisterase, production of isomaltulose using IM isomaltulose synthase, sucrose hydrolysis using IM invertase and aspartame synthesis using IM thermolysin. Examples of current research on IM enzyme technology are investigating the synthesis of functional oligosaccharides, tagatose production, pectin hydrolysis, reduction of bitter components in juices, and the synthesis of flavor and sugar esters.

Despite the numerous benefits associated with immobilized enzymes, the economics of the system still generally outweigh most other benefits. Advances in molecular biology, the use of multiple IM enzymes, genomics and microbial diversity may facilitate the use of IM by decreasing the cost associated with the purification and immobilization processes. Changes due to food regulations and the worldwide concern about chemical waste may spur the use of enzymes in general and perhaps the IM form.

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Improving Enzyme Performance in Food Applications

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As biological catalysts, enzymes are highly specific, they are biodegradable and able to perform the required chemical modifications under mild environments. Accordingly, it is easily understandable why their use of enzymes in food applications is deeply rooted. Enzymes, however, are often required to perform under conditions that are unlike those from their natural environment, namely when industrial processes are aimed at, where often high substrate concentrations have to be processed, under long time frames, at relatively high temperatures. Moreover, to comply with the evolving consumer demands and more strict regulatory issues, novel products and novel or improved processes are required. To cope with all these matters, enzymes or enzyme formulations displaying the required catalytic and stability features are looked after. To fulfil this quest, and therefore improve enzyme performance, different approaches can be considered, namely: screening the biota for the suitable enzymes; production of enzyme variants through protein engineering; and enzyme immobilization, typically for improved stability. Besides, these three methodologies are prone to be used in a complementary manner as to improve efficiency. The present work intends to summarize relevant exciting development in these methodologies that have occurred recently regarding enzyme use in the food area. Thus, the output conveyed by screening for natural enzymes has been significantly increased with the use of high throughput technology, by tapping environments relatively unexploited, viz. marine or semi-arid habitats, and with the introduction of metagenomic libraries. The production of improved enzymes variants, is possible either through a rational approach involving site-directed mutation, or through a random mutagenesis, directed evolution approach. Enhanced insight on enzyme structure and function relation has been gained, partly due to the merging of computational tools with the more traditional techniques of crystallography and nuclear magnetic resonance. Again, the increased knowledge of enzyme structure, coupled with a chemical, physical and structural perspective of the supports used for immobilizations, is contributing towards a more rational approach for enzyme immobilization, hence for the development of cost-effective, operationally stable immobilized biocatalysts. The implementation of these strategies within the scope of enzymatic applications in the food area has proved effective and further developments are therefore expected.

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Metagenome Approach for New Enzymes for Food Applications

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The food industry is using enzymes for a wide variety of processes under conditions that cover almost the complete pH-scale from alkaline to acidic and the temperature range of water in its liquid phase. Enzymes for the food industry are not chosen due to the catalysed reaction, but based on the effect that they cause during the preparation of the food. The starch-hydrolysis of α-amylases e.g. is used for the standardization of flour or as anti-staling enzyme in the baking industry, as liquefying agent during the production of high fructose corn syrup, or in the fruit juice industry for yield enhancement or anti-haze agent, to name a few. The conditions of use are consequently as diverse for a given enzyme functionality as is the effect that the user expects from the application of the enzyme. Access to wide enzyme diversity is consequently key to the successful discovery and development of any new enzyme due to the variability in the application conditions and the diversity of effects that are expected by the users.

The natural diversity of cultivable microorganisms has proven to be a good resource for new proteins. The accessibility of this resource, however, depends on the ability to cultivate organisms under laboratory conditions. It has been estimated that less than 1% of all microbial species can be cultivated by men (Torsvik et al. (2002)). Consequently the vast majority of the existing microbial biodiversity cannot be exploited with traditional approaches. Modern molecular biology has therefore developed methods to assess the vast amount of non-cultivable biodiversity. These technologies allow to screen or sequence the so-called metagenome (coined by Handelsman et al. (1998)) of various habitats. A metagenome is the collective genomic information of all microorganisms (Bacteria, Fungi, Algae, Protists and Archaea) indigenous in a given habitat at a given (sampling) time point. There are habitats with a wide genomic diversity like uncultivated forest soils or pasture land, which contain the genomic equivalent of 6000 to 8000 genomes of E. coli per cm3 of soil. The diversity in ecological niches with a high selective pressure in contrast, is generally much lower. E.g. in a salt-crystallizing pond only 7 E. coli genomic equivalents were found per cm3 of soil (Torsvik et al. (2002)). The genetic information of a given metagenome can be recovered by directly isolating DNA from environmental samples without the need of cultivation. The DNA can then be deposited in gene libraries that are subsequently screened for desired enzymatic activities. A compilation of enzymatic activities recovered by this approach can be found in Lorenz and Eck (2005).

A metagenome based approach to enzyme discovery is certainly not limited by the immense enzyme biodiversity. It is therefore important to use the right discovery strategy for a given application. A few approaches (e.g. Gabor et al. (2012)) will be presented and their advantages will be highlighted in view of possible applicability for enzyme discovery for the food industry.

Gabor, E., Niehaus, F., Aehle, W., Eck, J. (2012) Zooming in on metagenomics: molecular microdiversity of Subtilisin Carlsberg in soil, J Mol Biol. 418(1-2), 16-20
Handelsman, J., Rondon, M.R., Brady, S.F., Clardy, J., Goodman, R.M. (1998) Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products, Chem. Biol. 5, R245-R249
Lorenz, P., and Eck, J. (2005) Metagenomics and industrial applications, Nature reviews 3, 510-516
Torsvik, V., Ovreas, L., and Thingstad, T. F. (2002) Prokaryotic diversity--magnitude, dynamics, and controlling factors, Science 296, 1064-1066

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Production of Industrial Enzymes for Food Application

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Enzymes are used as key processing aids in the industrial production of safe, nutritious and healthy foods. Selling into the food industry with typically high raw material costs and little margin to spare, enzymes must be produced very economically.

This implies the use of highly efficient cell factories. These typically bacterial or fungal microorganisms are genetically tailored and fermented to secrete copious amounts of active enzyme protein. Additionally efficient downstream processing steps are required leading to the formulation of liquid or dried enzyme products. This presentation will illustrate some of the principles applied in today´s production of industrial enzymes for food application.

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Modern Downstream Processing for Protein Isolation and Fractionation

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The capture and isolation of proteins/enzymes from a cultivation broth or other complex mixtures of biomolecules is an essential step during biotechnological processes. Depending on the application, the purification can by far be the most cost demanding and time-consuming process step for the industry. Thus, it is of high interest to identify novel strategies for efficient and cost-saving downstreaming of polypeptides, especially from renewable resources.

Potato fruit juice (PFJ) is a protein-rich waste stream of the starch industry, primarily sold as low-value animal feed. PFJ is rich in patatin, a nutraceutical with applications in baby food and sports nutrition. In addition, protease inhibitors, as well as bioactive peptides can be found in high concentrations. By use of membrane adsorber technologies, these high value components can be captured from the raw waste stream. Direct adsorption to a membrane with large surface area (several m^2) enables high concentrations of the valuable products after desorption. By setting up a tandem purification mode with alternating adsorption, washing, and elution steps, continuous product recovery can be achieved [1].

Terpene synthases are enzymes of high interest for the flavor and fragrance industry. By cyclizing the universal terpene precursors like geranyl diphosphate or farnesyl diphosphate, these biocatalyst produce a huge variety of interesting compounds used in scent & care products, as well as certain foods. Terpene synthases from eukaryotic organisms (plants, fungi) are considered hard to express in recombinant prokaryotic hosts like E. coli. By employing reasonable fusion protein and fusion tag strategies, these synthases can be expressed in high amounts and purified to homogeneity for further use in enzyme bioreactors [2].

For applications where high purity of the product is an essential necessity, affinity chromatography is the method of choice to eliminate any contaminants from the product fraction. When searching for highly specific ligands with good binding affinity, aptamers can be a very cost-efficient alternative to monoclonal antibodies. Aptamers, single stranded DNA- or RNA-polynukleotides, bind specific to molecules through their complex three-dimensional fold [3].

[1] Schönbeck et al., J. Biotech. 168 (2013), 693-700.
[2] Hartwig et al., Prot. Expr. Purif. 97 (2014), 61-71.
[3] Walter et al., Eng. Life Sci. 12 (2012), 496-506.

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Risk Assessment of Food Enzymes by EFSA: First Experiences

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According to EU Regulations [1,2], all food enzymes currently on the EU market as well as all new food enzymes shall be subject to safety evaluation by the European Food Safety Authority (EFSA) and approval via a community list. With Regulation (EC) No 1056/2012 the deadline for submission of dossiers is set on 11 March 2015 [3].

To assist applicants in the preparation of dossiers EFSA's Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) has issued a guidance document with data requirements [4]. This document is complemented by an Explanatory Note for Guidance giving examples of scientific data. The Explanatory Note is likely to be updated if needed to incorporate new examples [5]. EFSA also has issued an "Administrative guidance document for the suitability check of applications for authorisation of food enzymes" together with a checklist to verify that all information required in the dossier is provided. [6]

EFSA has already started with the evaluation of the first dossiers and so far the CEF Panel has adopted opinions for 4 food enzymes.

The presentation aims at explaining the steps in the application process, presenting the elements of a scientific opinion on food enzymes and will give examples of critical issues related to data requirements and the risk assessment process.

1 OJ L 354, 31.12.2008, p. 7 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:354:0007:0015:en:PDF
2 OJ L 354, 31.12.2008, p. 1 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:354:0001:0006:EN:PDF
3 OJ L 313, 12.11.2012, p. 9 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:313:0009:0010:EN:PDF
4 http://www.efsa.europa.eu/en/efsajournal/pub/1305.htm
5 http://www.efsa.europa.eu/en/supporting/doc/579e.pdf
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Enzyme-induced Allergies and Strategies to Avoid Exposure in the Food Industry

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Exposure to airborne enzyme dust is a serious risk factor to develop allergy. Since the 1960s when allergies to enzymes in the detergent industries were firstly observed, an increasing number of different enzymes were reported to induce occupational allergy. Several case reports and cross-sectional studies give evidence for the allergenicity of the following enzymes in the food industry: alpha-amylase, glucoamylase, papain, bromelain, pepsin, chymosin, rennet, xylanase, cellulase, and phytase. Allergic sensitization can be diagnosed by in vitro measurement of specific IgE-antibodies in human serum samples or in vivo by skin tests. For proof of allergy, inhalant allergen challenge tests are the gold standard.

On the way to prevent sensitization and enzyme-induced allergy, methods for exposure assessment play a crucial role. Although enzymes can be measured by their catalytic function, usually immunoassays are the method of choice for exposure assessment for the following reasons: Firstly immunoassays have extremely low detection limits in accordance with the human immune system reacting also to very low allergen concentrations. Secondly, immunoassays are specific for their antigens while enzyme assays cannot discriminate between enzymes of different structure derived from different origins. Thirdly, the catalytic function can be increased, inhibited or abolished by factors greatly differing from the ones that influence antibody binding in immunoassays and reactivity of human immune system.

For some of the allergenic enzymes relevant in the food industry, immunoassays have been developed and were used to measure workplace exposure. In addition to sensitive immunoassays, optimized methods to sample and extract inhalant dust and a suitable exposure strategy and data analysis are necessary. Published studies on exposure assessment in relation to frequency of allergic sensitization were mostly performed in the bakery industry. To avoid inhalation of enzymes, particle sizes of baking additives can be increased by production of baking granulates, or moistening with fatty or watery liquids. Studies on airborne concentrations of enzymes like alpha-amylase and xylanase after different moistening procedures show a reduction of different particle size fractions by 50-90%.

Whereas exposure reduction in the case of many environmental allergens like e.g. grass or tree pollen is difficult, for enzyme allergens in an occupational setting exposure control and prevention strategies already exist or can be developed and should be used and improved consequently.

Besides the statutory assignment of IPA to prevent occupational diseases the author has no conflicts of interest.

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Tenderizing Enzymes in Meat Technology

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Meat tenderness is one of the most important attributes of meat quality. There is, however, considerable muscle-to-muscle variation influencing meat tenderness, especially in red meat such as beef. The main factors that determine the toughness of cooked meat are 1) the amount and quality of connective tissue; 2) muscle shortening early after slaughter leading to shortened sarcomeres in post-rigor meat; and 3) the extent to which the structural proteins in meat are degraded by proteases during chill storage leading to tenderization. In fresh meat this tenderization process is executed by proteolytic enzymes present in the muscle at time of slaughter. There is evidence that the cysteine protease µ-calpain is involved in this process. Another enzyme from the calpain family, m-calpain, has been shown to become activated under some conditions. Recent observations point to that the degradation process is influenced by several enzymes and thus is multi-enzymatic in nature. Fresh meat tenderization may thus be a result of the combined action of calpains, the proteasome, the caspase system and also the cathepsins. In meat products isolated enzymes from other sources can be added, however, challenges for successful application includes obtaining a uniform distribution of the added enzymes throughout the product and controlling the action of the enzymes to obtain an adequate level of proteolytic degradation – too little will not achieve the desired tenderization and too much will result in over-tenderization. Tenderizing effects of marinating meat with brine containing proteolytic enzymes from fruit extracts, such as ficin from fig, papain from papaya fruit and bromelain from pineapple have been shown. Actinidin is a cysteine protease found in kiwi fruit. Applying a brine solution with actinidin to a porcine ham muscle, *biceps femoris*, resulted in decreased WB shear force and improved sensory-assessed cooked pork tenderness without any effect on flavor and juiciness. The enzyme acted in the raw meat by a restricted degradation of proteins in both connective tissue and in myofibrils. Actinidin is therefore a good candidate enzyme for improving tenderness in processed meat.

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