KEEMIA- JA MATERJALITEHNOLOOGIA TEADUSKOND
TOIDUAINETE INSTITUUT
TEADUS- JA ARENDUSTEGEVUSE AASTAARUANNE 2014

1. Struktuur

Toiduainete instituut (Department of Food Processing)
Direktor: Dr. Loreida Timberg

- Toiduteaduse õppetool juh. Prof. Raivo Vokk
- Toiduainete tehnoloogia õppetool juh. Prof. Toomas Paalme

2. Teadus- ja arendustegevuse (edaspidi T&A) iseloomustus

Food, particularly its nutritional and sensory quality is becoming an increasingly important factor of human wellbeing. Recent development of systems biology and various *omics* methods have opened up new possibilities to study food production technologies and human nutrition. This new approach, termed foodomics, has made it possible to optimize food supply chains from the farm to the fork and even further taking into account internal processes that influence human nutrition. Research at the Institute of Food Processing focuses on cereal, yeast and nutrition with emphasis on the use of foodomics techniques to study the metabolism and interactions between microorganisms in consortia within various food matrices. Quantitative metagenomics and proteomics combined with other *omics* methods are used to study fermentation patterns of specific yeast, mammalian and bacterial cell cultures and various consortia within rye bread sourdough and the human gastrointestinal tract.

The IUT research project "Systems biology of food technology and science", led by Prof. Toomas Paalme, will be carried out from 2014-2018 in collaboration with the Competence Centre of Food and Fermentation Technologies (TFTAK) run by Urmas Sannik and the Chair of Biotechnology at the Institute of Chemistry, TUT, Prof. Raivo Vilu. The Institute of Food Processing is responsible for carrying out the subprojects: Systems biology of lactic acid bacteria, led by Dr. Kaarel Adamberg; Yeast derived bioactive compounds and fermentations, led by Ildar Nisamedtinov, Evolution of microbial consortia in food systems, led by Dr. Inga Sarand; B-complex vitamin analysis and bioavailability, led by Dr. Kristel Hälvin; Studies on the formation of flavor of model foods, led by Dr. Kristel Vene and Prebiotics and bacteria in food systems led by Dr. Signe Adamberg.

2.1 Struktuuriüksusesse kuuluvad uurimisgrupid

2.1.1 Yeast derived bioactive compounds and vitamins

(Dr. Ildar Nisamedtinov, Dr. Kristel Hälvin, PhD students: Kaspar Kevvai, Allan Vilbaste, Mary-Liis Kütt)

Short description of the research topic

In addition to the classical applications of yeast, such as food fermentation processes, strains of this organism now serve several other food technology needs. The goal of this research project is to study the content of various bioactive compounds within yeast biomass that can be used as food supplements and processing aids. In addition, we develop foodomics methods to enhance their accumulation, purification, and analytical determination.
In 2014 we focused on two major topics:

Analysis of the role of peptides in yeast extract that support the growth of *Saccharomyces cerevisiae*;
Development of methods to quantify bioavailable vitamins

The main results achieved during the reporting period

Saccharomyces cerevisiae S288c was grown in a synthetic grape juice medium containing ammonia, free amino acids, and yeast hydrolysate. Experiments with $^{15}NH_4Cl$ and ^{15}N-labeled yeast hydrolysate were carried out to gain insight into the consumption preferences of yeast cells towards assimilable nitrogen sources (ammonia, free amino acids, and peptides). We observed co-consumption of all three sources; approximately 40 % of the total nitrogen in the yeast protein fraction originated from yeast hydrolysate, while free amino acids and ammonia contributed 40 % and 20 %, respectively. Our results indicate that a number of amino acids are more readily obtained from peptides, particularly when the uptake of the free form is competitively inhibited and/or repressed. During the second half of fermentation we observed a temporary decrease in the incorporation of yeast hydrolysate-derived nitrogen, thus indicating that the more readily assimilated peptides are gradually exhausted from the medium during growth. Protease activity assays and size-exclusion chromatography analyses of the culture media indicated extracellular proteolysis during the later stages of fermentation. The results suggest that yeast extracts are superior natural fermentation aids in wine and cider production.

The efficiency of various sample pre-treatment processes was studied to determine the distribution of vitamers in quinoa seeds. Determining the distribution of different forms of vitamers in foods is important with respect to understanding the metabolism of vitamins and cofactors in the food chain. In quinoa seeds, we found that the most efficient method to determine the concentration of free vitamers was short cold extraction of finely ground seeds together with protease and phosphatase inhibitors. These inhibitors were also found to be effective in inhibiting the endogenous liberation of some of the free vitamers from their bound forms. Thus, the addition of phosphatase and protease inhibitors to the sample extract reduced the concentration of both free riboflavin and pyridoxal. In contrast, the concentration of free thiamine and pyridoxine did not decrease with the addition of enzyme inhibitors. Surprisingly, out of all treatments tested this treatment provided the highest concentration measurement for nicotinic acid. Notably, none of the extraction methods studied significantly influenced the recovery of free pantothenic acid. Ultrafiltration was found to be efficient in removing endogenous enzymes. Another benefit of ultrafiltration is that many compounds that interfere with the mass spectra of the compounds of interest are considerably reduced in the sample permeate. This effect is probably related to the removal of soluble proteins and other high molecular weight compounds, the hydrolysis products of which could interfere with the spectra of vitamers during LC-MS determination.

Publications 2014

K. Kevvai, M.-L., Kütt, I. Nisamedtinov, and T. Paalme (2014) "Utilization of ^{15}N-labelled yeast hydrolysate in *Lactococcus lactis* IL1403 culture indicates co-consumption of peptide-bound and free amino acids with simultaneous efflux of free amino acids.," *Antonie Van Leeuwenhoek*, 105, 511-522
Hälvin, K.; Nisamedtinov, I.; Paalme, T. (2014). Comparison of different extraction methods to determine free and bound forms of B-group vitamins in quinoa. Analytical and bioanalytical chemistry Volume: 406, 7355-66
Paalme, T..; Kevvai, K.; Vilbaste, A.; Nisamedtinov, I. (2014). Uptake and accumulation of B-group vitamers in *Saccharomyces cerevisiae* in ethanol-stat fed-batch culture WORLD JOURNAL OF MICROBIOLOGY & BIOTECHNOLOGY 30, 2351-2359
Hälvin, K (2014) „Determination of B-group Vitamins in Food Using an LC-MS Stable Dilution Assay". Supervisor Ildar Nisamedtinov, TUT Press, http://digi.lib.ttu.ee/i/?1818

2.1.2 Studies on the formation of flavor within model foods
(**Dr. Kristel Vene**, Dr. Loreida Timberg, PhD students: Rain Kuldjärv, Sirli Seisonen, Aleksandra Ošeka, MSc students Edgar Kelman, Julia Rosend, Keili Kajava)

Short description of the research topic
Food flavor analysis using both instrumental and sensory methods, is an important aspect of both product development and quality control. The main goals of this subproject are to develop and apply up-to-date sensory analysis methods and contribute to the field of flavor research.

In 2014 we focused on the following topics:
Determination of flavor changes and off-flavor development using instrumental and sensory analysis. Pickles and ice-cream as a model food
Sensory properties development and analysis of black pepper (*Piper nigrum*) and spice-cured sprats.
Characterization of the odor profiles using honeys and their corresponding flowers as well as home-made kvass as a model food.
The main results achieved during the reporting period

Thermo-shock only minimally affects overall flavor changes in ice cream. Regardless, we found that every application of thermo-shock increases both "rancid" and "aged" off-flavors in both cardboard and plastic packaging, particularly on the surface of the ice-cream. The most unacceptable flavour changes occurred in vanilla ice cream. From a chemical analysis, it became clear that during the thermo-shock the amount of esters, alcohols, acids, and aldehydes increase. "Rancid" off-flavor is caused by organic acids and "aged" off-flavor is caused by aldehydes. The "card-board"-like off-flavor present in cardboard packaging is caused by the migration of odor-active compounds such as 2-decenal, α-farnesene, and α-murolene.

We also aimed to identify the compound(s) responsible for a medicinal chalk-like off-odour in pickle brine. Sensory analysis confirmed the off-odour using comparative analysis with non-contaminated product. GC/MS and GC-O analysis led to the identification of 2-chloro-6-methylphenol as the primary compound that exhibits the medicinal odour note in pickle brine. The source of 6-chloro-2-methylphenol (RI = 1096) is most probably a chemical reaction between o-cresol, also found in the contaminated pickle brine, and the disinfectant sodium hypochlorite that most probably originated from rinsing water residue on the production line.
Manuscript accepted.

The aroma profiles of thirteen different honey samples from four botanical origins were investigated together with their blossoms to find if odour-active compounds specific to each blossom could act as markers for specific honeys. We tested heather (*Calluna vulgaris*), raspberry (*Rubus idaeus*), rape (*Brassica napus*), alder buckthorn (*Frangula alnus*) and the blossoms of their corresponding flowers. Gas-chromatography–mass spectrometry (GC–MS) and gas-chromatography–olfactometry were used to determine and identify odour-active compounds. The resulting data was analyzed using both agglomerative hierarchical clustering and correspondence analysis. Honeys from the same botanical origin clustered together; however, none of the identified compounds were exclusive to a particular honey/blossom combination. Heather honey was found to have a flavour profile that is most different to the others and both isophorone and 2-methylbutyric acid were found exclusively in heather honeys. Heather honey was characterized as having more "sweet" and "candy-like" notes, raspberry honeys had more "green" notes, while alder buckthorn had more "honey" and "floral" notes.
Article published

Odour-active compounds in homemade kvass were determined by GC-O using a modified frequency technique. First, the initial kvasswort was analyzed for odor-active compounds and

compared with the final product. The key aroma compounds originated from yeast fermentation and specifically the Ehrlich pathway, carotenoid degradation, lactone formation, degradation of amino acids, yeast cell lysis, and Maillard reactions that take place in bread or kvasswort production. Not all key aroma compounds could be identified and quantified, due to their low odour thresholds and response factors. Semi-quantification by multiple headspace extractions provided much higher concentrations compared with a single headspace extraction.

Publications 2014

Seisonen, S., Kivima, E., Vene K. (2014). Characterization of the aroma profile of different honeys and corresponding flowers using solid phase microextraction and gas chromatography-mass spectrometry/olfactometry. Food Chemistry, 169, 34–40

Timberg, L.; Koppel, K.; Kuldjärv, R.; Chambers IV, E.; Soontrunnarudrugsri, A.; Suwonsichon, S.; Paalme T. et al. (2014). Seasoned Sprat Products' Acceptance in Estonia and in Thailand JOURNAL OF AQUATIC FOOD PRODUCT TECHNOLOGY 23, 552-566
Timberg, L.; Koppel, K.; Kuldjärv, R.; Paalme T. (2014). Ripening and Sensory Properties of Spice-Cured Sprats and Sensory Properties Development JOURNAL OF AQUATIC FOOD PRODUCT TECHNOLOGY 23, 129-145

2.1.3 Prebiotics and bacteria in food systems (**Dr. Signe Adamberg**, Dr. Kaarel Adamberg, , Dr. Marju Puurand)

Short description of the research topic

The major topic we focused on in 2014 was: **"Design and application of novel levansucrase catalysts for the production of functional food ingredients".** The aim of this study is to elucidate quantitative peculiarities in the metabolism of various undigestable (by human) saccharides by faecal bacteria using screening (eg Bioscreen, microcalorimetry) and various fermentation methods. These studies are being carried out in cooperation with scientists from University of Tartu within the project: Functional Food Ingredients (*European Regional Development Fund, project No. 3.2.0701.12-0041)* in cooperation with the Competence Center of Food and Fermentation Technologies and National Institute of Chemical Physics and Biophysics and Premia Ltd.

The main results achieved during the reporting period

The metabolism of fructooligo and polysaccharides, including inulin and levan, in a widespread gut bacterium *Bacteroides thetaiotaomicron* was studied using different defined culture media. A shortage of amino acids was shown to stimulate the production of propionic acid by *B. thetaiotaomicron*. In addition, both poly-fructan and levan showed great potential for selective modification of gut microbiota. We also studied the metabolic potential of fecal microbial consortia in an environment containing levan using microcalorimetry. The rheological properties of levan-containing solutions were analyzed using a viscosimeter and compared with solutions of other polysaccharides. It was demonstrated that levan has a high potential as a diatary fiber source in ice cream supplement. In cooperation with Premia Ltd and CCFFT, we initiated inulin containing ice-cream trials that will be analyzed over the next 18 months.

Publications 2014

Adamberg, S.; Sumeri, I.; Uusna, R.; Ambalam, P.; Kondepudi, K. K.; Adamberg, K.; Wadström, T.; Ljungh, Å. (2014). Survival and synergistic growth of mixed cultures of

bifidobacteria and lactobacilli combined with prebiotic oligosaccharides in a gastrointestinal tract simulator. Microbial Ecology in Health and Disease, 25, 23062 -. http://dx.doi.org/10.3402/mehd.v25.23062

Adamberg, S.; Tomson K.; Vija H.; Puurand M., Kabanova N., Visnapuu T., Jõgi E., Alamäe T., Adamberg K. (2014) Degradation of fructans and production of propionic acid by *Bacteroides thetaiotaomicron* is enhanced by shortage of amino acids. Frontiers in Nutrition 1:21. *doi: 10.3389/fnut.2014.00021*

2.1.4 Evolution of microbial consortia in food systems

(**Dr. Inga Sarand**, Dr. Ene Viiard, PhD student Marianna Bessmeltseva)

Short description of the research topic:

Our research aims to develop strategies to control the quality of fermented food (mainly sourdough bread) by controlling both the composition of the microbial consortia and their dynamic growth. Two approaches has been used: i) tracking the changes in technological parameters resulting in the evolution of new microbial consortia *in vivo* or ii) composing new starters from preselected bacteria strains with certain technological/ functional traits. Both culture dependent (plating on selective media, fingerprinting and 16S rDNA sequencing) and culture independent (DGGE, high throughput sequencing) methods are routinely used for the analysis of microbial consortia within food.

The main results achieved during the reporting period

We followed the dynamic composition of the microbial community within an industrial sourdough after they installed a new controlled propagation system with different parameters. With a shift to a higher fermentation temperature, decreased inoculation rate, and prolonged fermentation time, the microbial community stabilized with an increased proportion of *L. helveticus* and *L. pontis*. Pyrosequencing did not reveal the emergence of new species within the sourdough community even after five months of back-slopping at the new fermentation conditions.

Performance of a "universal" nine strain starter composing from bacterial cultures selected from sourdoughs with different fermentation cycles was evaluated in a back-slopped liquid sourdough (T=30ºC, t_{cyc}=12h, water content 50%). *L. paralimentarius* M30I-3, *L. plantarum* M30I-1 and *L. brevis* M30I-2 were found to dominate after the first propagation cycle and the consortium remained stable for the next 13 back-slopping cycles. Anti-mold tests were performed both *in vitro* on agar plates and *in vivo* on the slices of rye sourdough breads fermented with single pure cultures of the tested various lactic acid bacteria. *L. brevis* M30I-2 showed the highest anti-pathogenic and anti-mould activity in this screening study of our sourdough starter collection.

Publications 2014

Bessmeltseva, M.; Viiard, E; Simm, J.; Paalme, T. (2014). Evolution of bacterial consortia in spontaneously started rye sourdoughs during two months of daily propagation PLOS ONE Issue: 4 Article Number: e95449

Viiard Ene (2014), PhD thesis "Diversity and Stability of Lactic Acid Bacteria During Rye Sourdough Propagation". Supervisor Inga Sarand, TUT Press: http://digi.lib.ttu.ee/i/?1824

2.1.5 Food Physics

(**Dr. Katrin Laos**, Dr. Anna Traksmaa, Dr. Tiina Lõugas, PhD students Aleksei Kaleda, Evelin Kivima, Tiina Klesment)

Short description of the research topic

Stability is an important food quality parameter. There is a need to ensure the stability of aroma, taste and structure, nutritional value and chemical composition, functionality of the products from "batch to batch" as well as during storage. Typically, food stability studies utilize both instrumental and sensory methods. Ice structuring proteins (ISP), also known as antifreeze proteins, are produced by various organisms that live in cold environments to prevent ice crystal growth and re-crystallization. The main goal of this research is to identify natural sources of ISPs that can be used within frozen foods to improve both their quality and shelf life.

The main results achieved during the reporting period

We have found that the Estonian climate, with its cold winters (average temperature in winter is -4 °C), has selected for the production of ice structuring proteins in overwintering winter rye (*Secale cereale*) and winter wheat (*Triticum aestivum*). Preliminary results indicate that ISP extracts from winter wheat display re-crystallization inhibition activity at concentrations of 0.7 g protein/L and reduced ice crystal growth by up to 90% compared with a blank control after temperature cycling. Because plant cultivars differ in their capability of producing ISPs, our goal is to select the most suitable cultivars and optimal harvesting and ISP isolation conditions. We found that local fish could also be another source of ice structuring proteins. Baltic herring (*Clupea harengus membras*) and sprat (*Sprattus sprattus balticus*) produce ISPs during winter and total soluble protein extracts can easily be made from either whole fish or fish waste. A crude extract was found to be able to inhibit ice recrystallization by 90% at a concentration of 0.3 g/L.
To isolate ISPs from total fish protein extracts, we applied a novel method of ice affinity purification (IAP). Compared with the original extract, one run of IAP is able to increase the activity of ice recrystallization inhibition by 10-fold. Additionally, this method is suitable for purification of any ISP source and can be easily scaled up for large-scale ISP production.
Publications 2014
Klesment, T.; Stekolštšikova, J; Laos, K. (2014). The influence of guar gum/furcellaran and guar gum/carrageenan stabilizer systems on the rheological and sensorial properties of ice cream during storage. Proceedings of the Estonian Academy of Sciences, 63 (2), 193-198
Kivima, E.; Seiman, A; Pall, R.; Sarapuu, E.; Martverk, K.; Laos, K. (2014). Characterization of Estonian honeys by botanical origin. Proceedings of the Estonian Academy of Sciences, 63 (2), 183-192

2.1.6 Systems biology study of lactic acid bacteria
(**Dr. Kaarel Adamberg** in cooperation with Department of Chemistry – Prof. Raivo Vilu, PhD students Kadri Aller and Karl Peebo)

Short description of the research topic

The aim of this subproject is to elucidate mechanisms of energy consumption and production pathways so that we can develop more effective (high yield) production processes for starter (lactic acid bacteria) cultivation. During the first year this work focused on determining the energetic costs of protein turnover and mechanisms of threonine metabolism in *Lactococcus lactis.*

The main results achieved during the reporting period

It has been proposed that protein turnover is the most important energy wasting process within the model lactic acid bacteria *Lactococcus lactis*. We studied the protein turnover of individual proteins (~ 800) within *L. lactis* in chemostat mode at 0.1 and 0.5 1/h and showed that almost half of the ATP produced was spent for resynthesis of proteins. In addition, protein turnover (on

average) increased by sevenfold with a fivefold increase in growth rate which was reflected by a 35 % increase of biomass yield at higher specific growth rate. This is an important finding to select or design strains having more effective proteome management.
We also studied threonine metabolism within *L. lactis* which is known to enhance flavors within dairy products as threonine is degraded to acetaldehyde and glycine. This pathway has not yet been fully described in *L. lactis*, however, our previous studies have found glycine production in defined amino acid media. Using a continuous cultivation approach with labelled threonine it was shown that threonine was cleaved mainly to glycine and acetaldehyde (over 90 %) which proves that a threonine aldolase pathway is present in this organism. This knowledge allows us to select potential dairy starter strains through the 'natural' modification of amino acid metabolism.

Publications 2014

Aller, K.; Adamberg, K.; Timarova, V.; Seiman, A.; Feštšenko, D; Vilu, R (2014). Nutritional requirements and media development for *Lactococcus lactis* IL1403. Applied Microbiology and Biotechnology, 98, 5871 - 5881.
Lahtvee, P-J.; Seiman, A.; Arike, Liisa; Adamberg, K.; Vilu, R. (2014). Protein turnover forms one of the highest maintenance costs in *Lactococcus lactis*. Microbiology-SGM, 160, 1501 - 1512.

2.2 Loetelu struktuuriüksuse töötajate rahvusvahelistest tunnustustest.
Prof. Toomas Paalme: Faculty of Agriculture and Forestry of University of Helsinki: Honorary Degree of Doctor of Science (Food Science), June 2014

2.4 Aruandeaastal saadud T&A-ga seotud tunnustused, ülevaade teaduskorralduslikust tegevusest, teadlasmobiilsusest ning hinnang oma teadustulemustele
Toimus oluline teaduspublikatsioonide kasv kui ka nende kvaliteet. Üldhinnang teadustööle „suurepärane".