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

1. Struktuur

Toiduainete instituut (Department of food processing)

Direktor: Prof. Raivo Vokk

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 developments of systems biology, omics methods etc. have opened up new possibilities for studies of food production technologies and human nutrition. This new approach, foodomics, make possible integrated optimization of the food supply chains from farm to fork and even further taking into account also processes of human nutrition. Research in the Institute of Food Processing includes studies in cereal, yeast and dairy technologies with the emphasis on the description of metabolism and interactions of microorganisms in consortia and with different food matrices. Quantitative metagenomics and proteomics combined with the other "omics" methods are introduced to study the fermentation patterns in yeast and bacterial cells, rye bread sourdough and human gastrointestinal tract.

The research in 2014-2018 will be carried out in the framework of the project "Systems biology of food technology and science" (Project leader prof. Toomas Paalme) in collaboration with the Competence Centre of Food and Fermentation Technologies, Tallinn (Urmas Sannik) and Chair of Biotechnology of Institute of Chemistry, TUT (Prof. Raivo Vilu). The Institute of Food Technology is responsible for carrying out subprojects: Systems biology of lactic acid bacteria (Dr. Kaarel Adamberg); Yeast derived bioactive compounds (Ildar Nisamedtinov), Evolution of microbial consortia in food systems (Dr. Inga Sarand); Hydrolysis processes of proteins and B-complex vitamin bioavailability (Dr. Ildar Nisamedtinov); Studies on formation of flavor of model foods (Dr. Kristel Vene).

2.1 Struktuuriüksusesse kuuluvad uurimisgrupid

2.1.1 **Yeast derived bioactive compounds (**Dr. Ildar Nisamedtinov, PhD students Kaspar Kevvai, Kristel Hälvin, Allan Vilbaste, Mary-Liis Kütt)

Short description of the research topic
In addition to the classical applications, such as food fermentation processes, yeast has come to serve the needs of several other fields in food technology. The goal of this research project is to study the composition of yeast biomass with regards to the content of different bioactive compounds which can be used as supplements in food production processes, develop methods for enhancing their accumulation, purification and analytical determination.
The two major topics we were focusing on in 2013 were as follows:

(i) Analysis of the role of peptides in yeast extract in supporting the growth of lactic acid bacteria;

(ii) Uptake and accumulation of B-group vitamins by *Saccharomyces cerevisiae*

The main results achieved during the reporting period

(i) The role of peptides in yeast extract in supporting the growth of lactic acid bacteria

Lactococcus lactis subsp. *lactis* IL1403 was grown in medium containing unlabelled free amino acids and 15N-labelled yeast hydrolysate to gain insight into the role of peptides as a source of amino acids under conditions where free amino acids are abundant. We observed co-consumption of peptides and free amino acids and a considerable efflux of most free amino acids during growth. We did not observe significant differences between the peptide consumption patterns of essential and non-essential amino acids, which suggests that the incorporation of a particular amino acid is more dependent on its availability in a readily assimilated form than the organism's auxotrophy for it. For most amino acids the contribution of peptide-bound forms to the formation of biomass was initially between 30 and 60 % with the remainder originating from free amino acids. During the later stages of fermentation we observed a decrease in the utilization of peptide-bound amino acids, thus indicating that the more readily assimilated peptides are gradually exhausted from the medium during growth.

(ii) Uptake and accumulation of B-group vitamins by Saccharomyces cerevisiae

The uptake and accumulation of the B-group vitamins thiamine, riboflavin, nicotinamide, pantothenic acid and pyridoxine in *Saccharomyces cerevisiae* was studied by gradually increasing the specific dosage of vitamins in an ethanol-stat fed-batch culture. Thiamine, nicotinamide, pantothenic acid, and pyridoxine were almost completely taken up at low vitamin dosages. Thiamine was determined to be the major accumulating form of vitamin B_1 while most of the assimilated nicotinamide and pantothenic acid accumulated in cofactor forms. Despite the obvious uptake of pyridoxine, accumulation of B_6 vitamers was not observed. In contrast with the other vitamins studied, riboflavin began accumulating in the culture medium immediately after vitamin addition was initiated. By the end of the experiment, the apparent uptake of all vitamins exceeded their accumulation in the cells.

Publications

K. Kevvai, M.-L. Kütt, I. Nisamedtinov, and T. Paalme (2013) "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*, Jan. 2014.

K. Hälvin, T. Paalme, and I. Nisamedtinov, "Comparison of different extraction methods for simultaneous determination of B complex vitamins in nutritional yeast using LC/MS-TOF and stable isotope dilution assay." *Anal. Bioanal. Chem.*, vol. 405, no. 4, pp. 1213–22, Mar. 2013.

2.1.2 **"Sensory and aroma" research group. Dr.** Kristel Vene, Dr. Loreida Timberg, PhD students Rain Kuldjärv, Sirli Seisonen, Aleksandra Ošeka, Keili Kajava,

During the reporting period the effect of origin and processing on flavor and odor of basil, thyme and oregano (the quantitative descriptive sensory and instrumental analysis) was carried out in close cooperation with the Competence Center of Food and Fermentation Technologies (CCFFT). Different aroma extraction methods were tested on dried herbs, like supercritical CO_2 extraction in TUT, Chair of Analytical Chemistry; solvent-assisted flavor evaporation in CCFFT. The manuscript is under revision for resubmitting (Rain Kuldjärv, Keili Kajava and Mariliis Holm). Additionally, key aroma-active compounds in natural apple cider were determined using GC-Olfactometry and GC/MS (Mariliis Holm, bachelor's thesis).

Different statistical methods are being applied to correlate sensory data with instrumental analysis data. A review manuscript is being prepared on the topic (Sirli Seisonen). A group of sensory assessors have been gathered (total 25 assessors) to form and train a first professional sensory/olfactometry panel in Estonia. The trainings and further monitoring of the panel will be carried out in close cooperation with CCFFT.

Publications:

Vene, Kristel; Seisonen, Sirli; Koppel, Kadri; Leitner, Erich; Paalme, Toomas (2013). A Method for GC-Olfactometry Panel Training. Chemosensory Perception, 6(4), 179 - 189.
Timberg, L.; Koppel, K.; Kuldjärv, R.; Chambers IV, E.; Soontrunnarudrungsr, A.; Suwonsichon, S.; Paalme, T. (2013). Seasoned Sprat Products Acceptance in Estonia and in Thailand. Journal of Aquatic Food Product Technology, xx - xx. [ilmumas]
Loreida Timberg , Kadri Koppel , Rain Kuldjärv & Toomas Paalme (2014) Ripening and Sensory Properties of Spice-Cured Sprats and Sensory Properties Development, Journal of Aquatic
Food Product Technology, 23:2, 129-145, DOI: 10.1080/10498850.2012.700003

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

Research of this topic is focused on the impact of food compounds (oligo- and polysaccharides) on the growth and metabolism of probiotic bacteria as well as microflora of gastrointestinal tract. We will implement systems biology approach for complex microbial consortia of human gastrointestinal tract that is tightly linked to human health and wellbeing. Merging cultivation technologies with omics methods enables to understand metabolism and growth dynamics of bacteria in complex consortia. Aim of the studies is to elucidate quantitative peculiarities of metabolism of different undigestable (by human) saccharides by faecal bacteria using screening (eg Bioscreen, microcalorimetry) and different fermentation methods. Results of this work will be applied for prebiotic and synbiotic food production. In the year 2013 new fermentation system in cooperation with Department of Chemistry and Competence Center of Food and Fermentation Technologies (CCFFT) were established and first experiments with levan, inulin type fructans and human faecal consortia or *Bacteroides* spp were carried out. The work is continuing and currently first manuscript about the results of microcalorimeter experiments is under preparation.
The fructan studies are carried out in cooperation with scientists from University of Tartu within a project: Functional Food Ingredients (*European Regional Development Fund, project No. 3.2.0701.12-0041).*

2.1.4 **Evolution of microbial consortia in food systems** (Dr. Inga Sarand, PhD students Ene Viiard, Marianna Bessmeltseva)

The objective of the research group is development of the strategies to control the dynamics and stability of microbial consortia (e.g. starters, probiotic bacteria, spontaneous fermentations) during food processing chain. Close monitoring of the changes in microbial populations throughout the production process allows better comprehension and management of the microbial processes involved in food processing. We have conducted comparative metagenomic analysis of microbial consortia in the four industrial sourdough samples during prolonged backslopping processes and metagenomic analysis of bacterial consortia in spontaneous rye sourdoughs during two months of daily propagation using two most common bioinformatic tools, Mothur and QIIME. The obtained results were compared with the results obtained by traditional culture-independent method, denaturating gradient gel electrophoresis (DGGE). Dominant strains were isolated, their growth parameters determined and several strains used in composition of a multi strain starter potentially suitable for bakeries with different technological parameters. At the first stage the stability of this multi strain starter and chemical properties of prepared sourdoughs were tested in three parallel liquid sourdoughs (dough yield 400) which were fermented at 30 ºC for

12 hours during 55 back-slopping cycles. Also analysis of yeast communities in cereal and dairy products using contemporary culture-independent methods were performed. Yeast biodiversity in three different rye sourdoughs and three different kefir grains was evaluated using i) DGGE analysis followed by DNA fragment sequencing, ii) amplification of ITS region followed by fragments separation on agarose gel (ITS region length varies in different yeast species) and fragments sequencing and iii) metagenome analysis.

Publications

Viiard, E., Mihhalevski, A., Rühka, T., Paalme, T. and Sarand, I. (2013) Evaluation of the microbial community in industrial rye sourdough upon continuous back–slopping propagation revealed *Lactobacillus helveticus* as the dominant species. *J Appl Microbiol* 114, 404-12
Marianna Bessmeltseva, Ene Viiard, Jaak Simm, Toomas Paalme, Inga Sarand. Evolution of bacterial consortia in spontaneous rye sourdoughs during two months of daily propagation. *PLOS One*. Submitted

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

Stability is an important quality parameter of foods. There is 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. In food stability studies both instrumental and sensory methods are applied together.

Ice structuring proteins (ISP), also known as antifreeze proteins, are produced by various organisms live in cold environments to prevent ice crystal growth and re-crystallization. We have shown that Estonian climate with its cold winters (average temperature in winter is -4 °C) generates the production of ice structuring proteins in overwintering plants of winter rye (*Secale cereale*) and winter wheat (*Triticum aestivum*). The main goal of research group of Dr. Katrin Laos is to find a good natural (local) source of ISP-s to use them in frozen foods to improve their quality and shelf-life. The preliminary results showed that ISP extracts from winter wheat showed the re-crystallization inhibition activity at concentrations of 0.7 g protein/L and reduced ice crystal growth up to 90 % compared to a blank control after temperature cycling. As plant cultivars differ in their capability of ISP production, our goal is to select the most suitable cultivars, harvesting and ISP isolation conditions.

Publications

Klesment, Tiina; Stekolštšikova, Jelena; Laos, Katrin (2013). 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, xxx - xxx. [ilmumas]
Kivima, Evelin; Seiman, Andrus; Pall, Raili; Sarapuu, Evelyn; Martverk, Kaie; Laos, Katrin (2013). Characterization of Estonian honeys by botanical origin. Proceedings of the Estonian Academy of Sciences, xxx - xxx. [ilmumas]

2.2 Loetelu struktuuriüksuse töötajate rahvusvahelistest tunnustustest.

Prof. Toomas Paalme: Invited by the Faculty of Agriculture and Forestry of University of Helsinki to receive an Honorary Degree of Doctor of Science (Food Science),

2.3 T&A-ga seotud tunnustused ja hinnang oma teadustulemustele.

Tänukiri 2013. a. üliõpilaste konkursil doktoriõppe üliõpilaste astmes I preemia pälvinud Anna Traksmaa konkursitöö „Rukkileiva hapendamine ja leiva vananemine“ juhendamise eest (juhendaja Prof. Toomas Paalme).

Parim teadustöö, artikkel: K. Kevvai, M.-L. Kütt, I. Nisamedtinov, and T. Paalme “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*, Jan. 2014.