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50

V Symposium on Sourdough

Cereal Fermentation for Future Foods 2012

Helsinki, Finland

VTT

VTT TECHNOLOGY 50

V Symposium on Sourdough

Cereal Fermentation for Future Foods

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Kopijyvä Oy, Kuopio 2012

Welcome in the V Symposium on Sourdough in Helsinki!

Colleagues and friends all over the world, welcome to spend three days with the latest development in the science and technology of sourdough. The theme “Cereal fermentation for future foods” strives for an outlook on the potential of starter cultures in development of new foods for the demanding consumer. Building on both traditions and cutting-edge knowledge of lactic acid bacteria and the cereal biopolymer matrix, we believe that new cereal foods of high eating quality will be achieved.

To fully unlock the power of sourdough a multidisciplinary approach is needed – microbiology, chemistry, physics, nutrition and consumer sciences need to be combined with food and other technologies. Industrial production should be boosted by scientific findings. This all requests for active dialogue and good communication.

We hope that whether you are in the sourdough congress for the first or fifth time, you will find new results, new colleagues, and new ideas for your work with sourdough. With the 40 oral and 46 poster presentations we have a good program, but please also take the full advantage of the breaks and social program to to get stimulated by your colleagues.

Best wishes for a successful congress!

Kaisa Poutanen
Chair of scientific committee

Kati Katina
Chair of organizing committee

Organizing and Scientific Committees

The Scientific Advisory Committee
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ORAL PRESENTATIONS

Session 1

Microbial ecology of cereal fermentations

The potential of acetic acid bacteria for cereal fermentations

R. F. Vogel[1], S. Steger[1], M. Hermann[1], A. Pfaff[3], R. Novoa-Carballal[3], A. H.E. Müller[3], H. Rübsam[2], T. Becker[2] and F. Jakob[1]

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Strains of different *Gluconobacter* species are capable of incompletely oxidizing a wide range of carbohydrates, alcohols and sugars, whose end products can be used for various (food) biotechnological applications. In addition, some strains produce large amounts of exopolysaccharides (EPS) in the presence of sucrose. We have isolated EPSs from various acetic acid bacteria including *Gluconobacter* (*G.*) *frateurii*, *G. cerinus*, *Neoasaia chiangmaiensis* and *Kozakia baliensis* and demonstrated that these are levans, which are promising candidates for the improvement of volume, texture and shelf-life of breads. The structures of these EPSs were characterized with ^{1}H-NMR, ^{13}C-NMR, ^{1}H/^{13}C-NMR (HMQC), ^{1}H/^{1}H-NMR (COSY) and asymmetric field flow fractionation to reveal so far unmatched structural properties especially with respect to chain length. In baking experiments it was demonstrated, that these EPSs act as hydrocolloids resulting in an improved volume, texture and shelf life of wheat breads[1], and first insight into a structure/function relation of these EPSs was obtained.

Upon adaptation of fermentation conditions the investigated strains of *G. frateurii* and *G. albidus* were competitive and dominating in doughs with wheat, rye, sorghum and spelt, and *in situ* EPS production was up to 23 g/ kg flour base. Depending on fermentation conditions gluconate formation is favoured over acetate, resulting in sensorial and nutritional advantages.Taken together, the possibility is demonstrated to exploit acetic acid bacteria and their EPSs for sourdough fermentations as candidates for the development of a new generation of baking aids containing (prebiotic) fructans and gluconate.

This project was funded by BMELV through BLE in project no. 28-1-63.001-07.

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Artisan bakery or laboratory propagated sourdoughs: influence on the diversity of lactic acid bacterium and yeast microbiotas

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Seven mature sourdoughs type I were comparatively back slopped (80 days) at artisan bakeries and laboratory levels under constant technology parameters. The cell density of presumptive lactic acid bacteria and related biochemical features were not affected by the environment of propagation. On the contrary, the number of yeasts markedly decreased from artisan bakery to laboratory propagation. During late laboratory propagation, Denaturing Gradient Gel Electrophoresis (DGGE) showed that the DNA band corresponding to *Saccharomyces cerevisiae* was not more detectable in several sourdoughs. Twelve species of lactic acid bacteria were variously identified through a culture-dependent approach. All sourdoughs harboured a certain number of species and strains, which were dominant throughout time and, in several cases, varied depending on the environment of propagation. As shown by statistical permutation analysis, the lactic acid bacteria populations differed between sourdoughs propagated at artisan bakeries and laboratory levels. *Lactobacillus plantarum*, *Lactobacillus sakei* and *Weissella cibaria* only dominated in some sourdoughs back slopped at artisan bakeries, and *Leuconostoc citreum* seemed to be more persistent under laboratory conditions. Strains of *Lactobacillus sanfranciscensis* were indifferently found in some sourdoughs. Together with the other stable species and strains, other lactic acid bacteria temporarily contaminated the sourdoughs and largely differed between artisan bakery and laboratory levels. The environment of propagation has an undoubted influence on the composition of sourdough yeast and lactic acid bacterium microbiotas.

From a wheat sourdough to a gluten-free sourdough: adaptation and dynamic of the microflora

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The consumers and celiac patients request for higher gluten-free (GF) bread quality increases. Among some alternatives, the use of sourdough can represent a tool to improve the sensorial quality of GF breads (1). To obtain a Gluten Free Sourdough (GFSD), different strategies occurred: use of starters (2) or spontaneous fermentations from GF flours (3). In this study, a Wheat SourDough (WSD) was refreshed by regular back-slopping (every 12h) with GF flours (rice and buckwheat) to obtain a GF sourdough usable for breadmaking.

To characterize the adaptability and the dynamic of Lactic Acid Bacteria (LAB) and yeasts during regular sourdough back-slopping, two methods were used: (i) enumeration and molecular identification with 16S or 28S rDNA gene sequence analysis, (ii) PCR-TTGE to monitor the LAB dynamic during sourdough adaptation. All along the 5 months back-slopping, yeasts' counts stayed constant. *S. cerevisae* initially present in the WSD was not found in the GF sourdough were only *C.humilis* was recovered. Regarding LAB evolution, their overall number increased. Two phases were observed. In the first one, during the dilution of WSD, molecular identification showed the presence of *Lb. sanfranciscensis, Lb. plantarum* and *Lb. spicheri* which were initially present in the WSD. In the second phase, only *Lb.sakei* was found by cultural method even if by PCR-TTGE *Lb. sakei* and *Lb. sanfranciscensis* were detected. The presence of *Lb. sanfranciscensis* appeared to be underestimated by cultural-method. The *Lb.sanfranciscensis* BF, isolated from the WSD and found in GFSD, presented a specific PCR-TTGE profile compared to the reference strains of *Lb.sanfranciscensis.* For breadmaking, influence of GFSD/baker's yeast ratio has been studied. The GF bread analysis showed that the introduction of GFSD containing rice wholemeal flour, rice flour and buckwheat in a GF formulation could improve specific volume and crumb texture of GF bread.

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Process and microbial diversity of French traditional organic sourdough and sourdough breadmaking

C. Urien[1], E. Lhomme[2], X. Dousset[1,3], B. Onno[2] and D. Sicard[4]

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Sourdough (SD) still represents an attractive image and method for breadmaking process in France despite its technological constraints. However, bakers practices – from wheat culture to bread – and their effects on bread quality are not enough documented. Moreover, SD yeast and lactic acid bacteria (LAB), constitute a "reservoir" for inter-specific and intra-specific diversity studies[1],[2]). The aim of this project is (i) to investigate the diversity of the traditional SD breadmaking practices and (ii) to study the SD microbial dynamic and diversity during SD bread-making. This study is carried on with a network of French traditional organic bakers and farmers-bakers (RSP). Cultural diversity associated with bread-making was studied thanks to a semi-structured survey with 14 bakers. Microbial diversity was analyzed for five bakers with sampling and enumeration at different steps: "levain chef", "levain tout point", after kneading, before baking. Yeast and LAB isolates were identified by molecular methods (ARN 26S and ARN 16S gene sequencing, respectively). A TTGE approach had also been used to monitor the LAB dynamic.

A high diversity in bread-making methods was found. The origin, conservation and age of the starter, the way starter and dough are fed, sourdough and dough consistencies differed. Yeast diversity was also important and species changed between bakers and during the bread-making process. In some cases, stable kinetics with a major species (*Candida humilis* or *Torulaspora delbrueckii*) and minor species (*Saccharomyces cerevisiae*, *Candida parapsilosis* and *Pichia anomala*) were found. In other cases, the dominant species changed over time. Concerning LAB diversity, no major variation was observed during SD breadmaking steps. Low inter-specific diversity was detected between the different SD and *Lactobacillus sanfranciscensis* appeared as the major or dominant species in all SD. Future research prospects will be discussed concerning the metagenomic approach and the links between biodiversity *vs* bread-making practices.

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Microbial ecology of sourdough fermentations: diverse or uniform?

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Thanks to the fact that craftsmanship has determined bakery practices for a long time, a huge variety of bakery products, in particular those based on sourdough, exists. This may differ considerably from region to region. Most of these products, including breads and cakes, originate from very old traditions. As such, numerous bakery sourdoughs have been maintained alive for tens of years through backslopping procedures. It turned out that these traditional sourdoughs harbor a mixture of distinctive yeast and lactic acid bacteria species. These microorganisms may be hold responsible for the typical organoleptic quality of the concomitant bakery products, as backslopping results in the prevalence and metabolic activities of the best adapted strains. This diversity of natural sourdough starters likely accounts for the variety of artisan sourdoughs produced by bakeries, whether or not typical for a certain geographical region. Alternatively, sourdough starter cultures, comprised of one or more defined strains, are commercially available now, albeit that not all strains in use are competitive enough to dominate the sourdough fermentation processes that have to be started up. These starter cultures are mainly used for rapid acidification of the raw materials and for flavor formation upon fermentation. Also, industrial manufacturers produce dried sourdough powders that are used as non-living flavor ingredients in (industrial) bread production.

Whereas it has been initially thought that a relationship could be seen between the presence of certain lactic acid bacteria species and the geographical origin of a particular sourdough, it turned out through systematic and detailed taxonomic investigations that the species diversity of both lactic acid bacteria and yeasts of local sourdoughs has nothing to do with the geography of the sourdough production process. For instance, the typical sourdough lactic acid bacterium, *Lactobacillus sanfranciscensis*, has been found in various wheat sourdoughs throughout Europe. In contrast, its (unique) presence should be ascribed to other factors, which are mainly based on the fermentation technology and practical conditions applied. Single isolations of yeast and lactic acid bacteria species have caused former misinterpretations of their association with certain sourdough-producing regions, not only because of the random isolation itself but also regarding the single habitat (sourdough) explored. Instead, the dedicated use of basic raw materials as well as the technological procedures applied determine the stability and persistence of the lactic acid bacteria and yeast communities involved in sourdough fermentation processes. The nature and quality of the cereal flour as well as other ingredients, whether or not used traditionally, are a first determinant of the microbial communities of a sourdough fermentation ecosystem, although no straightforward relationship between cereal flours and microorganisms can be found. In addition, the type of technology applied, in particular with respect to backslopping practices (refreshment time and number of propagation steps), duration of incubation of the dough, temperature of incubation of the dough, pH of the dough, redox potential of the dough, dough yield, and/or microbial interactions determine the growth, survival, and persistence of the microbial communities (whether or not added) in the stable, ripe sourdough.

Evaluation of probiotic strains among lactic acid bacteria isolated from cereals for food applications

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Probiotic activities of lactic acid bacteria (LAB) represent an attractive field of research and a promising market for functional food or ingredients[1]. The aim of our study was i) to evaluate the probiotic potential of LAB isolated from cereals and ii) to test their fermentative capacity on an cereal beverage. Selected LAB strains isolated from cereals and mainly from sourdough were evaluated considering their resistance to gastric stress and their antimicrobial activities. Moreover, their abilities to modulate immune responses were also screened on *in vitro* HT-29 cell model. Finally, two strains were selected for further assessment of their anti-inflammatory capacity in a murine model of colitis. Our results showed that the tested cereal strains were rather sensitive to oxidative stress and bile salts and resistant to acid stress. Five strains (including *Lb.namurensis*) produced anti-*Listeria monocytogenes* bacteriocins. Using commercial probiotic strains as control, some cereal strains also modulate interleukin-8 secretion in HT-29 cells. The abilities to modulate immune response were strain-dependent, several strains showed anti-inflammatory effects, like *Lb.paralimentarius*, *Lb. plantarum*, or *Lb.frumenti*. Other strains of *Lb.sanfranciscensis* demonstrated pro-inflammatory effects. The most anti-inflammatory cereal strain *Lb paralimentarius* C14 and the commercial strain LGG were selected to evaluate their probiotic potential in a murine model of colitis. C14 and LGG showed high capacities to reduce pro-inflammatory cytokines production in colonic segment without alleviate colitis symptoms in mice. C14 and LGG strains were able to acidify cereal medium and to maintain high population level during storage. This study had shown *in vitro* potential probiotic activity of LAB strains isolated from cereals even if the results need to be confirmed *in vivo*. Thereby, cereal products could be both an alternative source for probiotic strains and a food medium to support them[2,3].

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Characterization of the microbial populations in atole agrio, a traditional Mexican fermented beverage

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In Latin America, fermented foods are an important part of the diet. These foods are made of plants or animal materials and they are based on a spontaneous fermentation which occurs because of the action of the developing microbiota naturally present in raw materials. Food fermentations are cost-effective ways to improve the microbiological safety, quality, nutritional value and organoleptic properties of perishable materials. However, these processes should be controlled and predictable. This could be done by the use of defined mixtures of microbes (starters) or together with the native spontaneous fermentation. Lactic acid bacteria (LAB) and yeasts are the most common microbial organisms found in both traditional and starter-guided industrial fermentations. The aim of this study was to characterize the microbial populations of a traditional fermented Mexican beverage, atole agrio, and its changes during the fermentation process. Atole agrio is prepared at ambient temperature through a 6–12 hour spontaneous fermentation from ground maize and water. After fermentation the product is filtered and boiled to achieve creamy texture. There are no previous publications on the fermentation of atole agrio and thus this research provides the first results. Total mesophilic microbes, LAB, yeasts, molds and Enterobacteriaceae were measured by plating technique from start materials, during fermentation and from end products, as well as the pH and temperature. The results showed relatively low levels of LAB (6.7 log cfu/ml) throughout the fermentation. The average level for total mesophilic bacteria was 8.3, yeasts and molds 6.9 and Enterobacteriaceae 5.8 log cfu/ml. The pH decreased from 7.5 to 4.5. Due to the short period of fermentation, the microbial community remains relatively stable. However, increasing the initial levels of LAB could lead to changes in the total microbial community and improve the organoleptic properties of the product. The research will continue by identification and characterization of all the isolated LAB strains and applying the LAB strain embossing the best growth properties as a starter to improve the quality and organoleptic properties of atole agrio.

Species diversity and community dynamics of lactic acid bacteria in spontaneous sourdough fermentations based on barley, oat, and teff flour

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Sourdough is a microbial ecosystem of lactic acid bacteria (LAB) and yeasts in a matrix of mainly cereal flour and water that undergoes a (spontaneous) fermentation. Culture-dependent and culture-independent microbiological analysis of different spontaneously fermented backslopped sourdoughs enabled to understand the ecology of this complex fermentation process. Spontaneous sourdough fermentations with barley, oat, and teff were performed by means of backslopping during a period of 10 days, both in the laboratory (high dough yield, 30°C) and in an industrial bakery environment (low dough yield, ambient temperature). Before each backslopping, samples were taken to determine pH, total titratable acidity, and concentrations of carbohydrates and organic acids, and to perform culture-dependent and culture-independent microbiological analyses. In general, a stable consortium was established after 4–6 days of backslopping. Whereas the pH values ranged from 3.7–4.6 in the laboratory sourdoughs and from 4.0–4.7 in the bakery sourdoughs, the TTA values were 8-15 ml for the laboratory sourdoughs and 7.5–21 ml for the bakery sourdoughs. A wide species diversity was found, including mainly *Lactobacillus fermentum, Lb plantarum,* and *Weissella confusa* for the laboratory sourdoughs and *Leuconostoc mesenteroides, Leuc. lactis,* and *Pediococcus acidilactici* for the bakery sourdoughs. However, it was difficult to link the species diversity of the sourdough microbiota with a particular flour used. Lactic acid (81–159 mM) and acetic acid (8–14 mM) concentrations in the laboratory sourdoughs were higher compared to the bakery sourdoughs. All carbohydrates were completely fermented after 2–6 days of backslopping in the laboratory sourdoughs, whereas in the bakery sourdoughs carbohydrates were found until the last day of backslopping. Breads produced from the teff sourdough displayed interesting properties regarding taste, flavour, and crumb texture and colour. To conclude, the fermentation conditions had a strong influence on the sourdough microbiota and the respective breads.

ORAL PRESENTATIONS

Session 2

Fermentation induced changes in the cereal matrix

Enzymatic and microbial conversions during sourdough fermentation

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Biochemical conversion of flour components during the bread process determines dough rheology, bread texture, and bread flavour. Metabolism of sourdough microbiota and the activity of cereal enzymes are interdependent. Microbial acidification, the removal of oxygen, and the accumulation of thiols by microbial metabolism modulate the activity of cereal enzymes. In turn, the provision of substrates for microbial growth is dependent on cereal enzymes and their activity contributes to the selection of fermentation microbiota. Sourdough lactic acid bacteria preferentially metabolise maltose and sucrose; starch and arabinoxylan degradation are dependent on cereal enzymes. The carbohydrate supply also determines the composition of microbial metabolites. Sucrose supports formation of acetate by heterofermentative lactobacilli, and the formation of exopolysaccharides by organisms exhibiting glucansucrase or fructansucrase activities. Maltose and glucose act as alternative glycosylacceptors for glucansucrases and fructansucrases and their concentration determines the ratio of exopolysaccharide to oligosaccharide formation by these enzymes.

Primary proteolysis is dependent on gluten-associated aspartic proteases with an optimum activity between pH 3.5 and 4.5. Peptidase activities of sourdough lactic acid bacteria determine the accumulation of (bioactive) peptides and amino acids. Sourdough LAB convert glutamine to glutamate. Strain-specific conversion of glutamate to γ-aminobutyrate allows enrichment of baked goods with this bioactive compound. The species-specific conversion of arginine to ornithine provides the precursor to 2-acetyl-1-pyroline, a key flavour compound of the wheat bread crust. Metabolism of phenylalanine through the transamination pathway generates phenyllactate, an antifungal compound.

Microbial metabolism of phenolic compounds is particularly relevant in sorghum and millet, which have a high content of phenolics when compared to wheat or rye. Decarboxylation or reduction of hydroxyl-cinnamic acids reduces their antibacterial activity; metabolism of phenolic acids was linked to resistance to these compounds, and to the competitiveness of lactobacilli in sorghum sourdoughs.

Effect of sourdough fermentation on fonio starch properties

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Fonio, a uniquely drought-tolerant millet with potential for development of nutrient-rich, gluten-free foods has been neglected by research. The few available studies on fonio do not really indicate the effect of fermentation on its starch, which is important for texture modification. Also, cereal-based foods in Africa are usually sourdough fermented. This study investigated the effects of sourdough fermentation on fonio starch properties. Fermented and unfermented fonio starches (two varieties each of white and black fonio compared with white tan-plant sorghum) were studied using the Rapid Visco Analyser, differential scanning calorimetry (DSC, for starch thermal properties), SDmatic (starch granule damage) and scanning electron microscopy (starch granule structure). Fermentation reduced the peak viscosity (LS mean of 195 RVU in fermented starches compared with LS mean of 208 RVU in unfermented starches) during pasting. Starch gel firmness also reduced significantly with sourdough fermentation (2.49 to 3.95 N versus 3.69 to 6.06 N). Starch thermal properties as determined by the DSC showed endothermic peaks in the range of 72.17 and 76.50^{o}C, onset of which was from 66.80 to 71.03^{o}C. A shift of endothermal transitions toward higher temperatures was generally observed in fermented fonio starches. Black fonio starches tended towards higher transitions than white fonio or sorghum starch. This can be attributed to the presence of more phenolic compounds in the black fonio than in the white, as seen in the darker color of the black fonio starches (LS mean 95.01–96.33 for L*) compared to the white fonio starches (97.24–97.83). Starch damage increased with sourdough fermentation (4.31 to 6.63 UCD in unfermented starches, 5.34 to 7.29 UCD in fermented starches). Sourdough fermentation also modified the structure of fonio starch granule causing slight swelling and rupturing of the granules. The findings indicate that the overall effect of sourdough fermentation on fonio starch could be due to partial hydrolysis by the microbial enzymes. Fermentation produced better viscoelastic dough indicating that sourdough fermentation is largely beneficial from a rheological and gluten-free food perspective.

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Investigation of the influence of process conditions on the microbiota of spontaneous sourdough fermentations reveals insights into the choice of an appropriate starter culture

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Ideal starter cultures applied during industrial sourdough production should in the first place outcompete the background microbiota of the flour. A focus of current research is the selection of flour-native cultures, as they are best adapted to the flour matrix. In the past, the influence of different cereal flours on the community dynamics of the microbiota upon backslopping of spontaneous sourdough fermentations was studied. In most cases, a stable sourdough microbiota was established after five days of backslopping, consisting of mainly *Lactobacillus fermentum* and *Lactobacillus plantarum* as sourdough lactic acid bacteria. A conducted meta-analysis of literature data showed that not only the flour, but also process parameters applied during sourdough production are of great influence on the final microbiota.

During the present study, a systematic approach of the influence of the process parameters temperature (23, 30, and 37°C), backslopping times (12, 24, 48, and 72 h), and dough yield (170, 270, and 400) during spontaneous wheat and barley sourdough fermentations was started. First, the influence of temperature and backslopping time on spontaneous wheat sourdoughs with a dough yield of 400 was shown. With a backslopping time of 24 h, low temperatures (23°C) led to the prevalence of *Leuconostoc citreum,* while high temperatures (30°C and 37°C) resulted in the prevalence of *Lb. fermentum*. Also, long backslopping times (48 h) at 30°C favored a co-prevalence of *Lb. fermentum* and *Lb. plantarum*. Second, an influence of the dough yield was found, whereby low dough yields (170 and 270 compared to 400) seemed to be disadvantageous for *Lb. fermentum* and *Lb. plantarum* but favourable for species of *Pediococcus* and *Weissella* during barley sourdough fermentations at 30°C with a backslopping time of 24 h.

Finally, wheat and barley sourdough fermentations, started with *Lb. fermentum* IMDO 130101, and bread production was carried out. This strain was able to prevail during these fermentations and delivered slightly sour-fermented breads with minimal loss of volume.

Partially germinated flour as a starting material for sour-dough

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Partial germination of grains and pulses is a natural means to improve the nutritional value of these raw materials and the produced flours. The process is traditionally established in Indian households and is of great interest for industrial application. Partial germination has been shown to strongly increase available nutrients in cereals (Rakčejeva, 2006). For instance, a threefold thiamine (vitamin B1) content and a sevenfold riboflavin (vitamin B2) content were found in wheat bread with 25% germinated grains. The process was also able to improve the digestibility of whole flours, e.g. by reduction of the phytic acid content. It furthermore significantly increased milling efficiency, loaf volume and shelf life of the bread (Morad and Rubenthaler, 1983, Seguchi et al., 2010). We also found promising results for partially germinated pulses. As compared to untreated material, the ROF flatulence factor was decreased by 70%, the fructose content was increased by 220%, and dialyzable minerals were markedly increased (iron +150%, zinc +60%, calcium +35%). We also found a fivefold higher thiamine content and a general increase in vitamins. Partially germinated pulses may therefore present a valuable additive to sour-dough leavened breads.

The complete process can be run in a single, easily transportable unit with a capacity of max. 1100 tons per year. The processing steps of raw material soaking, partial germination and drying are successively performed batch-wise. In each batch, about 10 tons of grains or pulses may be processed for a duration of 3–4 days, depending on the desired degree of germination. Our state-of-the-art technology guarantees a homogeneous germination process, as well as a product that continuously meets high quality and safety standards. The partial germination process presents a complementary method to sour-dough fermentation in the production of nutrient rich and easily digestible products.

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Influence of bioprocessing on structure and properties of rye bran, subsequent *in vitro* conversions of phenolic compounds and *in vivo* bioavailability

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Influence of bioprocessing on the structure and chemical properties of rye bran, *in vitro* microbial release and conversion of phenolic acids of bran and *in vivo* bioavailability of phenolic compounds of breads enriched with bran were studied. Rye bran was bioprocessed (BP) with hydrolytic enzymes (Grindamyl Max Life and Depol 740) and with *S. cerevisiae* for 24 hours at 20 °C. BP bran and native bran were introduced to wheat baking to obtain 10–11 % DF content of bread. Chemical composition (DF, AX, protein, starch, fat, phenolic acids), acidification, microbial growth (CFU) and microstructure of brans, bran enriched breads and digested breads were analyzed. Bran breads were subjected to *in vitro* enzymatic digestion and colon models and time course of metabolite formation was followed with gas chromatography. Fifteen subjects were served the bran breads and control wholegrain rye bread in random order after overnight fasting. Urine samples were collected over12 and 24 hour periods from the subjects, and the content of phenolic compounds was analysed by gas chromatography.

Fermented bran had pH of 6.5 and TTA-value 9.2 and contained lactic acid 0.33 g/kg and acetic acid 0.24 g/kg. CFU's were 1.6x10E7 for lactic acid bacteria and 1.6x10E8 for yeasts. BP bran had significantly reduced total DF content (-22%, $p<0.5$), fructan and beta-glucan content (-46% and -36%, $P<0.05$) and significantly increased soluble fibre content (+22%, $P>0.05$), soluble AX (+346%, $P>0.05$) and free ferulic acid (+3600%, $P>0.05$) content. Microstructural analysis of bran revealed clear degradation of aleurone cell wall structure. BP bran had higher *in vitro* short chain fatty acid formation as compared to native rye bran. *In vivo,* bioprocessing of rye bran clearly increased the excretion of ferulic and sinapic acids in urine. Increased amounts of the hepatic metabolites vanillic acid and 3,4-hydroxybenzoic acid were also observed in urine.

The use of sourdough lactic acid bacteria as a cell factory for delivering functional biomolecules and food ingredients in gluten free bread

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The incidences of celiac disease (CD) or other allergic reactions / intolerances to gluten are increasing largely due to improved diagnostic procedures and changes in eating habits. CD is a permanent autoimmune enteropathy, triggered in genetically susceptible individuals by ingesting gluten from wheat, rye, barley, and other closely related cereal grains. Currently, the estimated prevalence of CD is around 1 % of the population in the western world and medical nutritional therapy (MNT) is the only accepted treatment for CD. To date, the replacement of gluten in bread presents a significant technological challenge for the cereal scientist due to the low baking performance of gluten free products (GF).

The increasing demand by the consumer for high quality GF bread, clean labels and natural products is rising. Microbial fermentation by means of lactic acid bacteria and yeast is one of the most ecological/economical methods of producing and preserving food. Sourdough has been used since ancient times for the production of rye and wheat bread, its universal usage can be attributed to the improved quality, nutritional properties and shelf life of sourdough based breads.Consequently, the exploitation of sourdough for the production of GF breads appears tempting. This presentation will highlight how sourdough lactic acid bacteria can be an efficient cell factory for delivering functional biomolecules and food ingredients to enhance the quality of GF bread.

Molecular characterisation of a *Weissella confusa* dextransucrase gene

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The use of homopolysaccharide-producing strains of lactic acid bacteria has been shown to improve technological properties and shelf-life of sourdough wheat bread. The positive effects are partly assigned to dextrans which act as hydrocolloids and thickening agents. Dextran is produced by an extracellular dextransucrase which catalyses the breakdown of sucrose and polymerisation of the released D-glucosyl units into dextran. As lactic acid bacteria can be used to produce dextrans and other homopolysaccharides *in situ*, the use of suitable starter cultures in sourdough baking can eliminate the need for several additives. *Weissella confusa* VTT E-90392 is an efficient dextran-producing strain that has performed well in such baking applications [1, 2].

The aim of this study was to identify the dextransucrase encoding gene or genes in *W. confusa* VTT E-90392. By using primers targeting dextransucrase (glucosyltransferase) consensus sequences a gene fragment was amplified and the full coding sequence was subsequently determined. Also, complete coding sequences for dextransucrases were acquired from two *Weissella cibaria* strains by initially using primers targeting known *Weissella* dextransucrases. All of the genes coded a secretional protein that was similar to known *W. cibaria* dextransucrases (74–75 % amino acid identity for the *W. confusa* and 98–99 % for the *W. cibaria* strains). The *W. confusa* dextransucrase gene was cloned to and expressed heterologously in *Lactococcus lactis.*

There has been no previously reported full sequence data from *W. confusa* genes involved in dextran synthesis. The gene information allows for example mutant construction and opens the way for better understanding of the dynamics of homopolysaccharide production in sourdough environments.

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Challenges in understanding the functionality of dextrans in sourdough applications: ramified or linear structure, high molar mass molecules or aggregates

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Several studies have highlighted the potential of dextran-enriched sourdough in improving the technological properties of sourdough wheat bread. Essentially, dextrans act as hydrocolloids in bread and because they are produced *in-situ* during sourdough fermentation, they are not considered as additional food additives. The structure-function relationship of dextrans in sourdough bread still requires further research. The current hypothesis is that high molar mass linear dextrans are more efficient in improving bread volume than high molar mass highly branched dextrans.

We have studied the structural and physicochemical properties of dextrans from *Weissella confusa* VTT E-90392 and *Leuconostoc citreum* VTT E-93497 with a potential application in sourdough. The results indicated that despite their simple monosaccharide composition, dextrans have a complex ramified structure even in the case of *W. confusa* which only contained few (3%) branch linkages. The molar mass of the dextran in aqueous solution was significantly higher (10^7 g/mol) compared to the value obtained in dimethylsulfoxide (DMSO) (10^6 g/mol). The lower values in DMSO originated from individual chains while the values in aqueous solution were skewed by the presence of compact aggregates. The hydrodynamic properties and intrinsic viscosity of the dextrans were consistent with their compact ramified structure. Quantitative and qualitative analysis showed that *W. confusa* E392 produced ~1.5% polymeric dextran and a significant amount of oligosaccharides (4.1%) within 17 or 24 h sourdough fermentation from an initial 10% sucrose.

A thorough evaluation of the structural properties of dextrans and the challenges involved in determining their structural and physicochemical properties will be presented. As a conclusion, taking into consideration the hydrodynamic properties of dextrans in addition to their structural features and molar mass, when comparing the functionality of dextrans produced by different strains is proposed [1]. The technological impact of the significant amount of oligosaccharides produced during sourdough fermentation should also be taken into account.

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Wheat germ stabilisation: heat treatment or sourdough fermentation? An industrial case

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Wheat germ is strongly susceptible to oxidation, being characterized by a high content of unsaturated oil (Sjövall et al., 2000). In order to be used in various food products, wheat germ is conventionally stabilized by heat treatments (Sudha et al., 2007), even if they often cause a decrease in the nutritional value of germ. This work was aimed at investigating the suitability of sourdough fermentation for stabilizing germ from common wheat (*Triticum aestivum sp.*) on industrial scale. For this purpose, the raw germ (WG) was fermented with a sourdough and dried at low temperature (WGF). WGF was compared to wheat germ stabilized by using the heat treatment commonly applied on industrial scale (60s @120°C) (WGH). All the treatments were carried out in collaboration with *Molino Quaglia S.p.A*. Physico-chemical characteristics (a_w, pH and TTA), enzymatic activities (alpha-amylase, lipase and lipoxygenase), development of rancidity (headspace analysis) during storage (90 days @ 25°C) of wheat germ samples, and rheological properties of germ-enriched dough by MVAG, Farinograph, and Rheofermentometer were evaluated. Sourdough fermentation seemed not to affect the pH (6.8±0.01 for WG, 6.7±0.13 for WGF), and consequently the TTA (14.1±3.4 for WG, 17.5±0.7 for WGF), of wheat germ, probably due to the industrial conditions used for fermentation. Nevertheless, sourdough fermentation on industrial scale was effective for decreasing the enzymatic activity of wheat germ. Indeed, in the case of WGF the lipase activity was 2.6-fold lower than that found in WG, according to Rizzello et al. (2010), and about 1.5-fold lower than that found in WGH. With regard to the hexanal in headspace during wheat germ storage, both WGH and WGF samples showed a strong decrease in its concentration (4÷7-fold lower than that found in WG), confirming the good stabilization action of sourdough fermentation. Furthermore, the sourdough fermentation seems to have a similar effect on rheological properties of enriched dough as the conventional heat treatment. In conclusion, the industrial sourdough fermentation followed by low temperature drying could be considered as a useful process for making a stabilized product better than that one obtained with conventional heat treatment.

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Exploring sourdough yeasts for leavening capacity of the sour rye bread dough

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Sourdough yeast has been utilized to produce light-pored bread for centuries. Most multi-stage sourdough processes aiming at both souring and leavening, has been reduced to one-stage fermentations aiming only at souring. Today bread leavened solely by sourdough is once again in demand, due to consumers' interest for natural foods.

Leavening by baker's sourdough differs from that of baker's yeast. Sourdough is a daily-made and intermediate, not a ready-to-use product. Its leavening capacity is defined by fermentation process applied, prevailing lactic acid bacteria and yeast strain present. The method used for assessing leavening capacity affected the validation of the sourdough yeast with best leavening capacity. Therefore a laboratory sourdough started from fresh pure cultures of yeast and lactic acid bacteria was backslopped once prior assessment. The leavening capacity of the sourdough yeast was studied in the subsequent bread dough containing sourdough as an ingredient.

The Finnish *Candida milleri* showed superior leavening capacity of bread dough compared to strain from culture collections of *C. milleri*, *Kazachstania exigua* and of sourdough related *Saccharomyces cerevisiae.* The leavening capacity was related to strain and origin thereof. The fermentation rate of the sourdough affected the leavening capacity of the yeast in the subsequent bread dough. Slower acidification of the sourdough resulted in a higher leavening rate of the yeast in the subsequent bread dough. By carefully choosing a test method that resembled the one-stage baker's sourdough, it was possible to evaluate and enhance the leavening capacity of sourdough yeasts.

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ORAL PRESENTATIONS

Session 3

Applications of microbes in cereal based foods

Starter cultures for cereal based foods – a challenge between tradition and innovation

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Fermented cereals play a significant role in human nutrition in all parts of the world were cereals grow. This fermentation are started spontaneously ore there has been traditional techniques developed in order to keep starter cultures for this processes alive. With the growing impact of industrial microbiology during 20th century this traditional starter culture propagation was replaced often, especially in the dairy industry, by the use of pure, frozen or freeze-dried cultures grown on microbial media.

In contrast to the production of ethanol from cereals, in sourdough a pasteurisation step before inoculation is avoided due to gelatinisation of starch and inactivation of endogenous enzymes. Therefore cultures must be competitive to the relatively high microbial load of the cereal raw materials and well adapted to the specific ecology determined by the kind of cereal and the process conditions. Less adapted cultures could be used, but then the process of back-slopping of cultures is limited. Main quality criterion for a starter culture in sourdough production is its activity – either for acidification or leavening, respectively.

Nowadays starter cultures have not only to fulfil the requirements of artisanal bakeries. They shall also deliver sourdoughs with constant composition as they are required in industrial baking with its continuous bread production lines. On the other hand, traditional fermentation processes in bread production are more and applied to industrial baking, aiming on improved freshness and clean labels.

Multifunctional bakery bio-ingredients and nutritional supplements by fermentation of co-cultures of lactic acid bacteria and proprionibacteria

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Microorganisms and their applications in biopreservation offer much potential for delivering novel solutions to the increasing demand for food products that are naturally-produced, nutritious and safe without addition of chemical additives. Furthermore these solutions might also alleviate nutritional problems, such as inadequate intake of folate and B12 in the general population or in specific groups. In the present research mixed cultures of lactic acid bacteria (LAB) and propionibacteria (PAB) were used for their complementary carbohydrate metabolism; LAB mainly produce lactate and PAB metabolize lactate to acetate and propionate. The overall goal of this project was to produce multifunctional ingredients by fermentation of cereal based medium for the bakery sector: organic acids for antimicrobial effects; folate and vitamin B12 for nutrition; and exopolysaccharides (EPS) for improved texture and shelf life.

A broad screening of high potential LAB and PAB cultures was first carried out for the studied characteristics using high-throughput micro tests in cereal-based medium. *Lb. plantarum* SM39 (high folate producer) *P. freudenreichii* DF16 and three *Weissella* strains producing EPS with different characteristics were selected. Two-step coculture fermentation processes (3 d anaerobic/4 d aerobic) were optimized aiming for high vitamins, EPS and antimicrobial activity. At optimal conditions, high and simultaneous production of natural folate (5-formyltetrahydrofolic acid) and vitamin B12 were reached, about 6 and 1 mg/l (c.a. 2 and 30% RDA in 1 ml), respectively, and about 20 g/l EPS was produced. Strong antimicrobial effects of the bioingredients (added to dough at 0.1 to 0.3% equivalent propionate levels) were shown in bread-making tests, spiked with different mold or bacilli spores. A high stability of natural vitamins (ca 60% folate retention), acetate and propionate during baking was observed with minimal losses during storage; and EPS addition led to improved texture of breads with a dose-dependent response. Indeed, the novel multifunctional bioingredients developed in this project offer new possibilities for adding value to natural bread and bakery products, i.e. high and stable vitamins and extended shelf life.

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Practical aspects of industrial rye sourdough baking

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Finland has a long history of baking whole grain all rye sourdough bread. Traditionally sourdough was maintained easily as dry dough in walls of wooden dough bins. Even today most of rye bread produced industrially in Finland is made by all rye sourdough but keeping the sourdough is not as easy needing special care. Industrial sourdoughs are, mostly, in-house selection of different sourdoughs of varying lactic acid bacteria (LAB) and yeasts, both traditional and new sourdoughs according to consumer taste and changing market demand. They all have their own carefully formulated procedures to keep them stable in backslopping process. Usually the sourdough is made batch wise and it should be stable during the usage in the bakery process cycle. Sourdough is analysed routinely using methods such as titratable acidity and pH. Nowadays also the LABs can be identified. The combinations of strains in the sourdough are different in every bakery although some major species are usually detected in most of them. Today following the change and development of the strains is considerably easier than even 10 years ago. New DNA fingerprint techniques make are available with price level and easiness where also at least larger bakeries can use these when needed.

Rye itself offers good basis for culture media including nutrients needed for survival of yeasts and LAB specific to sourdough. However quality parameters of the flour have to be well balanced to guarantee that there are no changes in sourdoughs. Ingredients with special characteristics are used at different stages of dough preparation. Rye raw material changes annually. This natural phenomenon affects baker's work every year when the new harvest flours arrive and the process parameters are re-evaluated. To keep the consumer quality constant and appealing to the consumer bread improvers are used in production. Rye sourdough baking is totally different compared to standard wheat baking processes. This makes the final product different but also brings variety to consumer choices. Therefore also the improvers have to be different, they need to be compatible with differing grain constituents and their functions as well as production pH. Therefore special improvers are offered to these products.

Sourdough lactic acid bacteria: exploration of non-wheat cereal based fermentation

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Cereal-based foods are not only the major contributors to staple diet all over the world, but also a very important source of biological as well as of cultural diversity, as testified by the wide range of cereal-based fermented foods (Guyot, 2012). A trend that is increasingly attracting bakery industries as well as consumers is the use of non-conventional flours for the production of novel products, characterised by peculiar flavour and better nutritional value. Lactic acid bacteria microbiota of several non-wheat cereals (emmer, spelt, fonio, amaranth, buckwheat, teff) has been recently deeply investigated with the aim of studying the biodiversity and finding cultures for sourdough fermentation. In this perspective, ancient grains are nowadays of big interest, mainly as niche products often having healthier and more natural features compared to modern wheat (Coda et al., 2010a; 2010b;). Moreover, research on ethnic cereals and cereal based foods especially from extra-European Countries should be encouraged in order to improve nutritional and sensory quality of the products and to establish piloted sourdough fermentations. As the consequence, an important economic benefit for developing countries and a more diversified market for bakery goods could be at reach. Currently, the use of these ancient or ethnic grains is mainly limited to traditional typical foods and the bread making is not well standardized with consequent negative effects on the final properties. The challenge in fermenting such cereals is mainly based on the capacity to combine good technology and sensory properties with demonstrated nutritional and health benefits. One of the requisites for the obtainment of higher quality products is the employ of autochthonous starter cultures, which are more suitable for the application as functional starter strains, since they have less competition during the fermentation (Minervini et al., 2010). Thus, the choice of the starter mixture has a critical impact on the final quality of the bread, and only those strains that dominate and outcompete contaminants should be applied for specific sourdough fermentations (De Vuyst et al., 2009). In this sense, screening and characterization of lactic acid bacteria is very useful in the improvement of a peculiar flour, from both the nutritional and technological point of view.

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Improving bread quality of high protease activity flour by using sourdough and liquid rye dough

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Pre-harvest damage to wheat caused by some Heteroterean insects (True bugs) such as Suni bug (*Eurygaster* spp.) is widespread in Europe, Middle East, North Africa. Similar damage has been associated by *Nysius huttoni* in New Zealand[1]. Suni bug damaged flour has high protease activity and it causes the quality losses of the bread. Degradation of the gluten network due to protease activity, leading to a decrease of dough viscoelasticity and producing sticky dough resulted in low volume of bread with poor crumb and crust characteristics. Suni bug proteinases have a high specificity for gluten proteins and particularly for the high molecular weight (HMW) subunits of glutenin which are the major determinants of gluten quality[2,3]. The protease is an endopeptidase and active within a wide range of pH 3-10 at (35°C)[4,5]. Sourdough fermentation has been used for years in order to improve bread texture, shelf life and aroma by developing the acidity.

In this study, the effect of two different sourdough level (20% and 40%) and liquid rye dough (1% and 2%) on the high protease activity flour (HPAF) was investigated. The sourdough was prepared with lactic acid bacteria (*L. plantarum* and *L. sanfrancissensis*) and commercially liquid rye dough. Dough samples were prepared by addition of 20% and 40% sourdough and the liquid rye dough at %1 and %2 levels. The doughs were divided into two portions and one of them is fermented for two hours another one is not fermented. After freeze drying of the dough samples, proteolytic activity was monitored by using SDS-PAGE[2]. The results showed that high level of sourdough (40%) prepared with *L. plantarum* and low level of (20%) of sourdough prepared with *L. sanfrancissensis* affected high molecular weigh (HMW) glutenin subunits of HPAF at 50% damage level positively. Addition of 2% liquid rye dough had also positive effect on 50% of high protease activity flour (HPAF). In conclusion, the proteolytic activity of bug damaged flours can be decreased by using lactic acid bacteria and liquid rye dough.

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Substitution of calcium propionate by fermented flour in bakery products

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The fermentation of flour is in essence a biochemical conversion of available carbohydrates in the substrate into organic acids, through the classical metabolic pathways of lacto bacilli. During this process propionates are formed.

In principle it should be possible to differentiate between propionates from fossil origin and propionates formed during the fermentation of flour using isotopes measuring equipment. Our research, in collaboration with the University of Pavia, has shown that it is possible to make a distinction between the propionates in function of their origin.

Our research also shows that fermented flour cannot be regarded as a simple alternative to calcium propionate from fossil origin. In reality the numerous and various metabolites present in the fermented flour, reinforce each other and contribute to the extension of the microbiological shelf life of bread. Consequently a 1:1 exchange of calcium propionate with fermented flour is not always necessary.

Wheat bran sourdough as a functional ingredient

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Several studies indicate that high-content whole grains diets work as a protective factor to chronic diseases. This decreased risk is related to the high content of fiber and bioactive compounds found mainly in the bran, which is usually removed during milling because of its fast rancidity. The increasing demand for functional foods and the pressure to ensure the exploitation of agro-industrial by-products have attracted great interest in bran-enriched foods/flours. The Healthgrain European Project recently emphasized the possibility to increase the amount of bioactive compounds in cereals by-products through biotechnological processes. Bran fermentation has been shown an efficient pre-treatment in order to enhance technological and nutritional properties of high fiber products (Katina *et al.* 2007). From a nutritional point of view, the fermentation effect on water-extractable arabinoxylans (WEAX) deserve particular attention, because of the positive effects on glycaemic and insulinaemic responses (Lu *et al.* 2000). Moreover, microbial xylan-degrading activity positively affects the bioavailability of functional compounds commonly found in the bran. This study aims to develop an innovative biotechnological process of wheat bran stabilization by microbial acidification. Briefly, bran sourdoughs were produced at 18 °C through continuous propagation by back-slopping of ripe dough (10% inoculum) until a stable microbiota was established. At each refreshment step (every 24h), analysis of the bacterial content and the acidity of the dough, measured as pH and total titratable acidity (TTA), were performed on the ripe sourdough. Furthermore, the amounts of fiber and bioactive compounds, such as WEAX, ferulic and phytic acids, were determined before and after bran fermentation. Lactic acid bacteria (LAB) rapidly increased after the first day of bran fermentation and reached high amounts (10^9 CFU g^{-1}). Yeasts population fluctuated during propagation, but after 8 refreshments it stabilized at the level of 10^7 CFU g^{-1}. The TTA and pH developments followed the LAB growth with the pH rapidly decreasing from 6.5 to 4.1. Results suggest that wheat bran sourdough is a "stable" functional ingredient for bakery products that can be used to improve their nutritional and technological properties.

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ORAL PRESENTATIONS

Session 4

Use of fermentation to improve safety and shelf-life

Lactic acid bacteria producing anti-fungal compounds: from plant protection to cereal product

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Fungal food spoilage plays a pivotal role in the deterioration of food and feed systems and some of them are also able to produce toxic compounds for humans and animals. The mycotoxins produced by fungi can cause serious health hazards, including cancerogenic, immunotoxic, teratogenic, neurotoxic, nephrotoxic and hepatotoxic effects and Kashin-Beck disease. In addition to this, fungal spoilage/pathogens are causing losses of marketable quality and hygiene of foodstuffs, resulting in major economic problem throughout the world. Nowadays, food spoilage can be prevented using physical and chemical methods, but no efficient strategy has been proposed so far to reduce the microbial growth ensuring public health. Therefore, lactic acid bacteria (LAB) can play an important role as natural preservatives. The protection of food products using LAB is mainly due to the production of antifungal compounds such as carboxylic acids, fatty acids, ethanol, carbon dioxide, hydrogen peroxide and bacteriocins. In addition to this, LAB can also positively contribute to the flavour, texture and nutritional value of food products. This presentation focuses on the use of LAB for food preservation given their extensive industrial application in a wide range of foods and feeds.

Fermentation aided control of antinutritive compounds in pulses and cereals

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Sourdough-type fermentation by lactic acid bacteria provides safe and beneficial circumstances for modification and control of certain antinutritive compounds found in some food materials. As for cereals, the enzymatic degradation of phytate in sourdough process is well known. Prolamins present in barley can be eliminated enzymatically in malting and brewing to make gluten-free beer, suitable for those having gluten intolerance. Elimination of the prolamins of wheat and rye in sourdough has been studied but the task is much more challenging, and so far no industrial applications are known. As for pulses, there are more and different antinutritive compounds to be eliminated, such as trypsin inhibitors, lectins, tannins and alpha-galactosides. Many pulses, such as faba bean, lentils, chickpea, common bean and soybean need pretreatments for removal or elimination of the harmful components. Surprisingly, fermentation has not been much used in traditional treatments for antinutrient elimination. We have recently started a project on food applications of faba bean *(Vicia faba).* While the contents of trypsin inhibitors and lectins are low in faba bean, this bean is characterised by glycosides called vicine and convicine, which cause hemolytic anemia (favism) in genetically susceptible individuals. Breeding for vicine-free and convicine-free faba bean cultivars is in progress, but processing methods for their elimination are also of interest. Experiments with an autoclaved faba bean suspension fermented 2d with *L. plantarum* have shown that vicine can be eliminated and convicine greatly reduced by certain lactic acid bacteria (McKay 1992). The paper aims to give an overview on the potential of sourdough type in elimination of antinutritive compounds in cereals and pulses.

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Application of a novel bioingredient based on co-fermentation of *Weissella confusa* and *Propionibacterium freudenreichii* to improve shelf-life and quality of bakery products

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High specific volume, crumb elasticity, moisture content and low staling rates are characteristics attributed to high quality bakery goods. Besides, microbial spoilage must be prevented during the shelf life period which may require addition of preservatives, primarily propionic and acetic acid and their salts for antifungal activity. However dough structural properties and yeast activity can be adversely affected by these compounds leading to decreased final bread quality. This project aimed to develop multifunctional bioingredients acting concomitantly as natural preservative and bread improver. For this cocultures, lactic acid bacteria (LAB) and propionibacteria (PAB) were selected for their complementary metabolism on sugars. Exopolysaccharide (EPS) producing LAB and PAB were selected by a phenotypic screening. In a preliminary baking test, twenty-two strains belonging to *W. confusa*, *P. freudenreichii* and *jensenii*, exhibiting different phenotypic EPS characteristics, were tested for their functionality in bread. The three best performing strains representing different EPS-producing *W. confusa* (glucan, fructan, cell bound polysaccharides) were co-cultivated with *P. freudenreichii* JS 15, selected for high EPS production, in a sucrose supplemented cereal medium. The fermented ingredients contained 18.5±1.3 g/l EPS, 10.8±0.2 g/l propionate and 5.9±0.4 g/l acetate, and were used in a baking test at three concentration levels (7.5; 15 and 30% of flour weight giving ca. 0.5, 0.9 and 1.9 g propionate and 0.8, 1.6 and 3.2 g EPS per kg dough, respectively). Dough and bread characteristics were compared to non-supplemented and chemically acidified controls containing the same acetic and propionic acid concentrations.

Important bread quality parameters such as specific volume and crumb firmness were found to be negatively affected with increasing acid concentrations in a dose-dependent way. We observed strong corrective effects of the bioingredient with EPS. Addition of the bioingredient from the most promising co-culture, *W. confusa* 11GU-1 and *P. freudenreichii* JS15, led to increased specific volume by ca. 20% for each supplementation level compared with bread produced with same acid addition and crumb firmness was decreased up to 2.5 fold after 72h of storage. Our study showed that the novel multifunctional bioingredients can be used to produce high quality natural formulated bread with antifungal active propionate concentration to increase shelf life and high EPS concentration to replace chemical preservatives and texturizers.

Improving commercial gluten-free breads by decreasing staling and hardness as verified by instrumental and sensory evaluations

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A gluten free diet is essetial for celiac patients. Improved screening for celiac disease and increased awareness resulted in an rising demand for gluten-free products in recent years. The food industry has responded by development of gluten-free products that are comparable to gluten containing counterparts (*1*). Baked goods are a major contributor to the overall market of gluten-free products, however, most gluten free breads on the market have reduced flavor, dry texture and have poorer quality compared to traditional wheat flour bread (*2*). This study aimed to perform a consumer sensory evaluation of commercial gluten-free breads available in Edmonton. Results obtained from the sensory evalutation of fresh and 72 h staled commercial GFBs were correlated with the instrumental analysis. Sensory and instrumental analyses demonstrated that texure defects are a major problem in the quality of commercial GFB. Based on these evaluations three GFB formulations were developed to improve sensory and textural properties: A standard dairy GFB, non-dairy high starch GFB and fermented flaxseed GFB. Flaxseed was fermented overnight with a commercially available gluten-free sourdough starter culture. Consumer and expert sensory evaluations in combination with instrumental texture analysis were performed on the experimental GFB. The non-dairy high-starch GFB exhibited the hardest crumb, staled fastest, and was the least preferred in sensory evaluations. In comparison, fermented flax GFB and dairy GFB showed improved texture properties, and delayed staling. Congruently, sensory evaluation revealed that the fermented flax GFB was the most preferred. To identify components of fermented flaxseed that improve the texture of GFB, chemical analysis of the carbohydrate composition of fermented flaxseed was carried out. The presence of arabinoxylans in flaxseed and their solubilization during fermentation likely contributed to the improved texture of the flaxseed GFB. Overall, instrumental texture analysis were in good correlation with sensory evalutaion and are therefore a suitable tool for quality analysis of GFB. Consumer evaluation faciliated the setting up for improved GFB formulations. Baking with fermented flaxseed has functional attributes that may provide consumers with higher qualtiy GFB.

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Microbial ecology of sorghum sourdoughs: selection of competitive microbiota by substrate supply and antimicrobial phenolic compounds

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In Africa, sourdough is exploited mainly as an intermediate product for the production of fermented cereal products such as beverages, gruels, porridges and breads (1). Cereal fermentations in many parts of Africa are mainly based on sorghum and millet. Their use selects for fermentation microbiota that differ from wheat and rye sourdoughs (2, 3). However, it is still unclear which ecological factors select for specific microbiota in sorghum fermentations. This study compared the competitiveness of *Lactobacillus sanfranciscensis* and isolates from ting, a Botswana fermented sorghum product (3), in wheat and sorghum dough. Fermentations were characterised by determination of cell counts, pH, and quantification of metabolites. Lactobacilli in sourdough were quantified by plating and quantitative PCR.

Sorghum and wheat sourdoughs offered glucose and maltose as major carbon source, respectively, but no appreciable differences in the metabolites produced by *L. sanfranciscensis* or ting isolates were observed. *L. sanfranciscensis* grew in wheat but not in sorghum sourdoughs, or sorghum sourdoughs supplemented with 2% maltose, 1% tryptone, 0.1 % L-cysteine and 2 % sucrose. In wheat and sorghum sourdoughs that were inoculated with equal cells counts of *L. sanfranciscensis*, *L. parabuchneri*, and *L. casei,* fermented at 28°C or 34°C and propagated by back-slopping every 24h, cell counts of *L. sanfranciscensis* decreased progressively during propagation of sorghum sourdoughs. In contrast, ting isolates were overgrown by *L. sanfranciscensis* after three propagations in wheat sourdoughs independent of the incubation temperature. The anti-microbial activity of extracts of four different sorghum flours were evaluated and *L. sanfranciscensis* but not *L. parabuchneri* and *L. casei* was inhibited by phenolic extracts from sorghum.

Microbiota of sorghum sourdough differ from wheat and rye because sorghum contains active concentrations of antimicrobial phenolic compounds, and offers glucose as major carbon source.

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ORAL PRESENTATIONS

Session 5

Nutritional and sensory properties of fermented foods

How the sourdough may affect the functional features of leavened baked goods

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Sourdough fermentation is one of the oldest food biotechnologies, which has been studied for its effect on the sensory, structural, nutritional and shelf life properties of related leavened baked goods. Acidification, proteolysis and activation of a number of enzymes as well as the synthesis of microbial metabolites cause several changes during sourdough fermentation that affect the dough and baked good matrix, and influence the nutritional/functional quality. Indeed, the reference literature is very rich of results showing how the sourdough fermentation may affect the functional features of leavened baked goods. In the form of pre-treating raw materials, fermentation through sourdough may stabilize or to increase the functional value of wheat germ and bran fractions. Sourdough fermentation may improve the properties of the dietary fibre complex, reduce the glycaemic response of bread and increase the uptake of minerals. Microbial metabolism during sourdough fermentation may also produce new nutritionally active compounds, such as peptides and amino acid derivatives (e.g., γ-amino butyric acid) with various functionalities, and potentially prebiotic exo-polysaccharides. The wheat flour digested via fungal proteases and selected sourdough lactobacilli has been demonstrated to be probably safe for celiac patients.

LC-MS/MS Quantification of ACE-Inhibitory peptides in sourdough bread

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Angiotensin-converting enzyme (ACE) inhibitory peptides can be accumulated in rye malt sourdoughs to concentrations exceeding their in vivo inhibitory levels (1). This study analysed the concentration of ACE inhibitory peptides throughout the bread-making process to achieve the production of bread with active concentrations of ACE inhibitors. IPP, LPP, VPP, LQP, IQP, LIP, LLP and IIP were quantified by LC/MS/MS in MRM mode as described (1). Steamed bread, bread, and soda crackers were produced to assess products differing in their thermal treatment. Tripeptides were quantified in sourdoughs, bread doughs, and in the final products.

Peptide concentrations were compared in products prepared by addition of 10% whole wheat sourdough, or 3% of rye malt sourdough supplemented with gluten and fungal protease. Products made with rye malt sourdough exhibited higher peptide levels than products made with wheat sourdough. IPP was the predominant tripeptide, followed by LQP, IQP, LPP. Other peptides were found in low quantities only. The concentrations of IPP, LPP and VPP remained stable during kneading and proofing but decreased about 50% during baking. IQP and LQP decreased 10–20% after kneading and proofing and 10-30% after baking. Steamed bread exhibited higher peptide concentration than soda crackers prepared from the same sourdough. In steamed bread, concentrations of IPP, LQP, IQP and LPP were 19, 12, 8, and 8µmol/kg, respectively. The corresponding concentrations in soda crackers produced from the same dough were 4 µmol/kg (IPP), 0 µmol/kg (LQP), 0 µmol/kg (IQP) and 3 µmol/kg (LPP), demonstrating that baking decreased peptide levels.

The use of sourdough enriched in ACE-inhibitory peptides allowed the production of baked goods with active peptide concentrations. The behavior of peptides during dough processing and baking differed. IPP, LPP and VPP remained stable during kneading and proofing but decreased subsequently. The concentration of other tripeptides was reduced predominantly at the dough stage. In keeping with the different thermal treatment during processing, the concentration of tripeptides in steam bread was higher than in soda crackers.

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Intestinal fermentation of plant polysaccharides – what microbe does what?

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Since early life, intestinal microbes dominate our body and outnumber our own cells by one or more orders of magnitude. In the intestinal tract they constitute the largest microbial ecosystem that is close to our heart: our microbes inside that play an important role in health and disease. The collective genome of these microbes has been determined and includes over 3 M genes, thus contributing considerably to the coding capacity of our system. However, unlike our own genome, the intestinal microbiome is not strictly vertically inherited. Moreover, this personalized organ can be modified by a variety of food and pharma treatments that target its composition, stability and activity. These are instrumental in providing cause-effect relations to complement the abundance of associations that are presently being reported.

This contribution will provide an overview of the present state of the art in the human microbiome and focus on the capacity of colonic microbiota to degrade plat-derived polysaccharides, such as starch, inulin, or more complex ones. The role of these microbes will be highlighted together with their capacity to produce short chain fatty acids, including acetate, propionate and butyrate. Moreover, a series of human interventions will be discussed that illustrate the role of the microbes inside.

Folate enhancement by bioprocessing

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Cereals are notable dietary sources of folate. Beyond the traditional vitamin activity – prevention of megaloblastic anaemia and neural tube defects – other health benefits are being intensively studied. Folate intake often falls below recommendation (300–400 µg/d).

Many countries have adopted mandatory folic acid fortification of cereal products to ensure adequate folate intake. However, also non-fortified cereal products are good folate sources and their folate contents can be enhanced by bioprocessing. In Finland cereal products contribute to one third of the daily folate intake. Thus, even a moderate increase in folate content can significantly influence folate status.

Folate synthesis by microbes during fermentation results in marked increase in folate content (1), and bread may contain more folate than the flour it is made of. Fermentation of germinated grains may lead up to 7-fold increase in folate levels (2). Interestingly, it is possible to produce also vitamin B12 in plant-based foods that naturally lack this vitamin. Results on folate production by both endogenous and added yeasts and bacteria in different cereal matrices will be presented. Best combinations of folate-rich matrices with folate producers resulted in folate contents of ca 90 µg/100 g.

Sourdoughs can be utilised in gluten-free baking to improve the nutritional profile. GF cereals are usually not fortified, and especially starch-based products and refined flours are poor folate sources (< 10 µg/100 g) compared to their gluten-containing counterparts. On the other hand, pseudocereals such as amaranth and buckwheat have similar folate levels to wheat and rye, for example. In our experiments, folate content of commercial GF bread with buckwheat was up to 70 µg/100 g. Prolonged yeast fermentation combined with the use of quinoa and rice sourdoughs in oat baking resulted in folate contents of ca 42–66 µg/100 g (3). Germination and fermentation offer feasible approaches to the development of folate-rich ingredients.

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Influence of lactic acid fermentation on iron-gallic acid complexation

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Gallic acid (GA) is a phenolic acid which can be found in legumes, fruits, nuts and beverages. These products are also rich in minerals like iron and zinc. GA contains a galloyl group leading to complexation with iron, and thus decreasing the bio-availability of iron. It is known that complexation of GA-iron is pH-dependent. Therefore, lactic acid fermentation could be a technique to improve the bio-availibilty of iron. On the other hand different lactobacillus strains are able to metabolise GA into pyrogallol (PYR).

In this study, it was aimed to investigate the influence of pH and microbial conversion of GA on iron complexation during fermentation with *Lactobacillus plantarum* and *Lactobacillus collinoides*. Different concentrations GA (0, 0.5, 1.5 and 3 mM) were added to a modified MRS broth (2g/l galactose as carbon source), combined with 10 µg/ml Fe^{2+}. Incubations were done for 14 days at 30 °C.

PYR was detected as the only conversion product of GA. At the end of the incubations, a decrease in pH to pH 5 and 5.7 for *L. plantarum* and *L. collinoides* respectively were measured. During fermentation, a shift from a green/black colour towards brown-red was observed for both bacteria in the fermentation medium, which could give an indication of complex formation. From the results obtained by LC-MS and UV-spectrophotometry, it could be concluded that the effect of pH on complexation was more pronounced compared to the bacterial conversion of GA to PYR.

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Fermentation of cereal malts with single microbial strains – A biotechnological opportunity to enhance key aroma compounds in bakery products

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Flavour is considered to be the most important attribute of baked goods. However, bread available on the market often shows a rather bland aroma [1]. It is well known that extended dough fermentation times as well as pre-fermentation of doughs are useful means to significantly improve bread flavour. However, it has been shown in previous studies that, due to the reducing power of enzymes present in baker's yeast, bread flavours produced by a straight dough procedure can hardly be modified. One approach to produce baked goods with new, attractive aromas is a fermentation of cereals outside the bread dough, e.g., using different cereal varieties. This material can then be added as flavouring material to the dough prior to baking.

An interesting odorant for bread is the clove-like smelling 2-methoxy-4-vinylphenol, which can be generated from free ferulic acid by various microorganisms [2]. The availability of free ferulic acid in cereals is, however, a limiting factor. A release of ferulic acid from the arabinoxylans in cereals can be achieved for example by alkaline treatment, but this is not permitted in food production. A further idea is the use of cereal malts containing higher amounts of aroma precursors.

The aim of this study was, therefore, to screen different yeasts with respect to their ability of generating a clove-like aroma from different malts. A corn malt dough fermented with *Pichia fermentas* for seven days showed the most intense and interesting aroma. The characteristic aroma active compounds were identified by means of the molecular sensory science concept. Indeed, 2-methoxy-4-vinylphenol showed a very high odour activity value (OAV) and could be identified as the odorant responsible for the clove-like overall aroma. Furthermore it was shown, that application of the fermented material to a bread dough significantly improved the aroma.

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Salt reduction in bread – Sourdough as a promising solution

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The dietary intake of sodium chloride (NaCl) has increased considerably over the last few decades due to changes in the human diet leading to an increase in a number of diseases including hypertension and other cardiovascular health problems [1]. Numerous international health agencies, as well as the food industry, have now recommended a NaCl intake level of about 5–6 g daily, approximately half the average current daily consumption level. Cereal products, and in particular bread, are a major source of NaCl in the human diet of industrialised countries [2]. Therefore, any reduction in the concentration of NaCl in bread would have a major impact on global health. However, NaCl is a critical ingredient in bread production, and its decrease can have a deleterious effect on the production process. This includes an impact on dough handling as well as final bread quality characteristics, such as shelf-life, bread volume and sensory characteristics. In the present study, the impact of NaCl reduction on the dough characteristics was assessed using fundamental and empirical rheological analyses as well as the influence of salt reduction on yeast regarding the aroma profile during proofing. Furthermore, the quality characteristics of the breads were assessed as well as the analyses of the major bread aroma compounds using GC^2-MS.

Decreasing NaCl addition from current usage levels (1.2 %) 0.6 %, 0.3 % and 0.0 % reduced the dough resistance to extension, extensibility and complex modulus without affecting the ratio of liquid to solid behaviour. An increasing amount of NaCl of 2.0 %, 3.0 % and 4.0 % Changes in gas holding capabilities of doughs with NaCl concentrations between 0.0% and 4.0 % were observed, however affecting the final bread quality for NaCl levels > 0.6 % only. The incorporation of sourdough fermented with *Weisella cibaria* MG1 and *Lactobacillus reuterii* FF2 at levels of 10–20 % compensated changes in bread volume and structure. Decreasing amounts of NaCl resulted in major changes regarding microbial shelf-life as well as the analysed aroma compounds. Depending on the amount of NaCl the shelf-life was shortened by 1 to 2 days (NaCl < 1.2 %) and prolonged by 1 to 2 days (NaCl > 1.2 %) respectively. The addition of sourdough fermented with the specific *Lactobacillus amylovorus* DCM 19280 producing antifungal compounds at a level of 5 % could compensate the lack of salt whereas the addition of 20 % prolonged the shelf-life up to 14 days independent of the NaCl concentration. Differences of aroma profiles could be determined using GC^2-MS analyses. The analysis was focused on the compounds which are synthesised through the Ehrlich pathway by yeast cells. With increasing amounts of NaCl in the dough less aroma compounds could be detected. Overall, breads produced with reduced amounts of NaCl (0.3 % and 0.6 %) were found to be comparable to the control (1.2 % NaCl) in terms of dough and bread characteristics and baking performance whereas sensory attributes and aroma profiles showed clear differences between the NaCl concentrations. Sourdough was assessed

as a promising option to overcome the shelf-life problem improving texture and flavour [3] at the same time.

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Set up of a biotechnological protocol for the production of mild-gluten wheat flour bread by sourdough fermentation

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Beyond celiac disease, the scientific community has recently defined as gluten "sensitivity" all the disorders attributed to gluten ingestion that do not meet diagnostic criteria for celiac disease and are probably involved in several pathologies, such as the irritable bowel syndrome (1). Although the involvement of gluten ingestion is clear, very little is known about the quantity and the mechanisms that can trigger diseases and digestive problems. It has been shown that selected lactic acid bacteria and fungal proteases, routinely used in bakeries, degraded gluten to less than 10 ppm during sourdough fermentation (2). Two independent clinical challenges (3, 4), carried out administrating baked goods on a daily base, made with rendered gluten-free flour, showed that a wheat flour derived product is not toxic after administration for 60 days to celiac patients. Nevertheless, baking of the treated flour is possible only by the use of structuring agents (2).

With the aim of reducing only in part gluten concentration of wheat flour, the parameters of sourdough fermentation previously proposed for the complete gluten hydrolysis were modified, setting up a biotechnological process preserving the sensory acceptability and the technological properties of the flour. The degree of proteolysis, corresponding to a reduction of ca. 30% of the initial gluten, was investigated by R5-ELISA, two-dimensional electrophoresis, RP-FPLC, and free amino acid analyses. The mild-gluten flour was used for bread making at pilot plant scale, and compared to a wheat flour bread control. Chemical, structural and sensory features were studied, showing the technological suitability of the mild-gluten flour and the good overall taste of the bread. The *in vitro* protein digestibility of the mild-gluten bread was higher than the control (83 vs. 79%), as well as the nutritional quality, estimated by the calculation of chemical and protein scores, essential amino acids index, protein efficiency ratio, biological value, and nutritional index. In response to the evolution of consumer demand, the protocol for obtaining wheat mild-gluten flour by sourdough fermentation can be considered as a useful tool for the production of innovative and healthy foods.

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The effect of oat utilization on antioxidant activity, dietary fiber and β-glucan contents of tarhana: traditional Turkish fermented cereal food

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Tarhana is a traditional Turkish fermented cereal-based food and can be simply defined as a mixture of yoghurt, cereal flours, bakers' yeast, different vegetables, herbs, and spices. After mixing process, tarhana dough is fermented for 3 to 5 days and immediately dried. Both lactic acid bacteria (*Steptococcus thermophilus and Lactobacillus bulgaricus)* in yoghurt and bakers' yeast fermentations occur simultaneously during tarhana production. In this study, yogurt 50 % (wt/wt, flour base) and baker's yeast 2 % (wt/wt, flour base) were used in the formulation. Oat flour and steel cut oats were used to replace wheat flour in the formulation at the levels of 10, 20, 30% and 40% (wt/wt) for tarhana preparation. Control samples included no oat products. Oat contains high amounts of dietary fiber. Especially β-glucan is the major soluble fiber in oats. β-glucan and other soluble fibers have hypocholesterolemic effects.

Tarhana with 40% steel cut oats had the highest IDF, SDF and TDF values, followed by tarhana with 30, 20% steel cut oats. There were significant ($p < 0.05$) differences in IDF, SDF and TDF levels between control and tarhana samples supplemented with oat products. β-glucan contents of tarhana samples with oat products were in the range of 1.02–1.50%. Statistically differences between β-glucan levels of control and oat supplemented tarhana samples were also found significantly ($p < 0.05$). Control sample had the lowest β -glucan content (0.13%) while tarhana with 40% steel cut oats had the highest value (1.50%). As the levels of oat flour and steel cut oats increased in formulations, β-glucan contents increased. In general, antioxidant activities determined in the samples supplemented with steel cut oats were lower compared to those obtained in the samples supplemented with oat flour. There were also significant ($p < 0.05$) differences in antioxidant activities between control and samples with oat products. Results showed that supplementation of tarhana with oat products improved the nutritional quality of tarhana by causing significant increases in dietary fiber and β-glucan contents and antioxidant activity.

Potential of sourdough in delivering more of the grain in palatable foods

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The consumption of foods rich in whole grain and grain fibre is known to reduce the risk of chronic diseases such as type 2 diabetes and cardiovascular diseases. The current intake is, however, below recommendations. Among the actions to improve the situation are technologies for improvement of palatability of the recommended type of foods. Sourdough fermentation as a processing step offers one option for designing the flavour and texture of foods high in outer layers of the grain. The presence of endogenous enzymes in bran and wholegrain flour are activated during sourdough fermentation, causing hydrolysis of cell wall components and other dough biopolymers. These assist in improvement of bread volume and thus crumb softness in baking, and reduce the staling rate of high-fibre bread. Bioprocessing of both rye (Katina et al. 2007) and wheat (Katina et al. 2012) brans has been shown to improve their use as a baking ingredient. Sourdough also has potential in mixed-flour baking such as using oat (Flander et al. 2011) and barley flours, adding both nutritional benefits and taste variability in the white-type bread basket.

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List of posters

	Wednesday 10th - Thursday 11th October Authors are present on Thursday 11th October at 10.20-11.10
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S1.02	Biodiversity and Stability of Lactic Acid Bacterial Community at Tarhana Dough Fermentation; a Sourdough Included Fermented Food Ö. Şimsek, S. Özel and A. H. Çon, University of Pamukkale, Turkey
S1.03	Biodiversity of Lactic Acid Bacteria involved in the fermentation of *DOKLU*, a traditional corn food in Ivory Coast N.M. Assohoun, S. Messaoudi[1], X. Dousset[1] and B. Onno[1], [1]LUNAM Université ONIRIS, France
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S1.05	Microbial community analysis during preparation and propagation of type i sourdough through pyrosequencing F. Minervini, R. Di Cagno, M. De Angelis, A. Lattanzi, E. Pontonio and M. Gobbetti, Universita degli Studi di Bari, Italy
S1.06	Draft Genome Sequence of *Lactobacillus rossiae* DSM 15814^T M. De Angelis, R. Di Cagno, F. Minervini, C. G. Rizzello, M. Calasso and M. Gobbetti, University of Bari, Italy
S1.07	Evaluation of yeast populations in spontaneously fermented rye sourdoughs using different culture-independent methods M. Bessmeltseva, E. Viiard and I. Sarand, Tallinn University of Technology, Estonia
S1.08	High-throughput sequence-based analysis of the bacterial composition of four industrial rye sourdoughs in Estonia E. Viiard, M. Bessmeltseva, T. Paalme and I. Sarand, Tallinn University of Technology, Estonia
S1.09	Interactions between EV3 phage and its host strain *L. sanfranciscensis* H_2A Claudia Picozzi[1], Matthias Ehrmann, Ileana Vigentini[1], Roberto Foschino[1] and Rudi Vogel, [1]Università degli Studi di Milano, Italy
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S1.13 Phylogenetic, genetic, and physiological analysis of sourdough isolates of *Lactobacillus reuteri*: food fermenting strains are of intestinal origin

M.Shu-Wei Su[1], P. L. Oh, J. Walter, and M. Gänzle, [1]University of Alberta, Canada

S1.14 Secretome of *Lactobacillus plantarum* LPL400 changes as response to quorum sensing

M. Calasso, R. Di Cagno, C. Daniela, M. Gobbetti and M. De Angelis, University of Bari, Italy

S1.15 Transcriptome analysis of *L. sanfranciscensis* TMW 1.1304 in response to electron acceptors and the presence of *Candida humilis*

M. Stetina[1], J. Behr[1], S. Sieuwerts, E. J. Smid and R. F. Vogel[1], [1]Technische Universität München, Germany

S1.16 Cultivation of selected Gluconobacter-Strains on cereal substrates and in-situ production of exopolysaccharides

M. Hermann and R. F. Vogel, Technische Universität München, Germany

Session 2. Fermentation induced changes in the cereal matrix

S2.01 Analysis of dextrans *in situ* produced by prospective lactic acid bacteria strains in vegetable-based model food

Q. Shi[1], R. Juvonen, K. Honkapää, N. H. Maina[1], L. Virkki[1], R. Lantto and M. Tenkanen[1], [1]University of Helsinki, Finland

S2.02 Chemical, Rheological and Sensory Evaluation of Pate Stuffed with Broccoli (Brassica oleraceae L.)

E. A. Hassan A. M. S. Hussein and A. A. A. Hussein, National Research Centre, Egypt

S2.03 Dextran from *Weissella cibaria* with potential application in gluten free cereal products

T. Jagan Mohan Rao and A. Goyal, Indian Institute of Technology Guwahati, India

S2.04 Potentials of dextran hyper-producing Weissella confusa Cab3 isolated from fermented cabbage for sourdough

S. Shukla and A. Goyal, Indian Institute of Technology Guwahati, India

S2.05 Influence of lactic acid bacteria on the oxidation reduction potential of buckwheat sourdoughs

A. Capuani, J. Behr and R. F. Vogel, Technische Universität München, Germany

Session 3. Applications of microbes in cereal based foods

S3.01 Cereals sourdough fermented by means of *Lactobacillus* and *Propionibacterium* and its effect on properties of toast bread

M.Slukova, P. Zidova and J. Prihoda, Institute of Chemical Technology Prague, Czech Republic

S3.02 Establishing a standardised mixed starter for Korean rice sourdough

J. Lee and W. Holzapfel, Handong Global University, Korea

S3.03 Glucose – galactose syrup effect on the qualities of wheat dough and bread

D. Kunkulberga, D. Klava and G. Benkite, Latvia University of Agriculture, Latvia

S3.04 Lactic fermentation of legume extracts - the effect on sensory and technofunctional properties and their application in different food matrices

J. Bez[1], S.Toelstede[1] W. Back, B. Klotzbücher and H.G. Berger, [1]Fraunhofer Institute for Process Engineering and Packaging, Germany

S3.05 *Lb. paracasei* in sourdough: growth, acidifying capability, and VOCs production in different acidity values and temperatures.

F. Rossi, L. Iacumin[1], F. Siano, C. Raffaele and A. Reale, [1]Università degli Studi di Udine, Italy

S3.06 Potential of brewer's spent grain-sourdough and enzymes for high fibre breads
A. Ktenioudaki and E. Gallagher, Teagasc Food Research Centre Ashtown, Ireland

S3.07 Sourdough bread disappearance and revival in some countries consuming white breads
H. Chiron, INRA - (National Institute of Research and Agronomy) BIA, France

S3.08 Sourdoughs for bread-making with barley flour: sensory and technological evaluation of barley breads
C. Garofalo[1], M. Mariotti, L. Aquilanti[1], A. Osimani[1], L. Fongaro, A.-S. Hager, E. Zannini, E. K. Arendt and F. Clementi[1], [1]Università Politecnica delle Marche, Italy

S3.09 In situ synthesized exopolysaccharides in liquid sourdough influence textural and sensory profiles of brioche
Y. Laurent[1], F. Bredon, M. Panouillé[1], C. Béal[1] and M. Bouix[1], [1]INRA-Agroparistech, CBAI, France

S3.10 Analysing a shipwreck beer
R. Juvonen[1], E. Storgårds[1], M. Raulio[1],T. Hofmann, A. Mikkelson[1],A. Wilhelmson[1], M. Dresel, B. Gibson[1] and J. Londesborough1, [1]VTT, Finland

Session 4. Use of fermentation to improve safety and shelf-life

S4.01 Antifungal activity of selected starters during sourdough fermentation and long-term effect on storage of bread
R. Coda, C. G. Rizzello and M. Gobbetti, Universita degli Studi di Bari, Italy

S4.02 Influence of bioprocessed bran on rheological properties of wheat dough and on bread quality
K. Hartikainen, N. Sozer, K. Poutanen and K. Katina, VTT, Finland

S4.03 Production of antimicrobial compounds *in situ* by sourdough consortium fermentation
D.I. Serrazanetti, C. Montanari, L. Vannini, R. Lanciotti, A. Gianotti and M.E. Guerzoni, University of Bologna, Italy

S4.04 Thermal Treatment Influence on the Quality of Fine Rye-Bread Packaged in Different Polymer Films
T. Rakcejeva, L. Dukalska, I. Murniece, D. Klava, O. Petrova and M. Sabovics, Latvia University of Agriculture, Latvia

S4.05 Influence of bioprocessing and particle size of wheat bran on texture and sensory properties of high fibre wheat bread
R. Coda, I. Kärki, R.-L. Heiniö, K. Poutanen, K. Katina, VTT, Finland

S4.06 Characteristics of Chinese Steamed Bun made from Thai Traditional Fermentation Starter (Loog-Pang)
N. Luangsakul and K. Jaikwangi, King Mongkut's Institute of Technology Ladkrabang,Thailand

Session 5. Nutritional and sensory properties of fermented foods

S5.01 Volatile Compounds of a Commercial Chinese Steamed Bun and Chinese Steamed Bun made from a Thai Traditional Fermentation Starter (Loog-Pang)
N. Luangsakul and T. Puttongsiri, King Mongkut's Institute of Technology Ladkrabang, Thailand

S5.02 Alleviation of the adverse effect of cooking on sorghum protein digestibility and protein fractions through malt pretreatment and fermentation
A.H. Wedad, Industrial Research and Consultance centre, Sudan

S5.03 Aromatic and sensory characterization of dry sourdough
C. Petel[1], C. Marzin[1], E. Charrie, J. Rouille, B. Onno, C[1]. Prost[1], [1]LUNAM Université ONIRIS, France

S5.04 Artisanal Lactic Acid Bacteria Utilization for the Development of Gluten-Free Sourdough
G. Komen and S. Harsa, Izmir Institute of Technology, Turkey

S5.05 Enhancing iron bioavailability from tef-injera by improving the fermentation process
M. Fischer, I. Egli, L. Meile, R. Hurrell, ETH-Zurich, Switzerland

S5.06 Fibre, protein and mineral fortification of wheat bread through incorporation of both milled and fermented malt rootlet and brewer's spent grain
E. Zannini, D. M. Waters and E. K. Arendt, University College Cork, Ireland

S5.07 Gut metabolome modulation in healthy volunteers subjected to two different cereal-based food diets
A. Gianotti , D.I. Serrazanetti and D. Taneyo Saa, University of Bologna, Italy

S5.08 Phytase-active yeast and lactic acid bacteria isolated from grain-based food
L. Nuobariene, N. Arneborg, F. K. Vogensen and Å. S. Hansen, University of Copenhagen, Denmark

S5.09 Propionibacteria produce active vitamin B12 in cereal matrices
B. Chamlagain, M. Edelmann, S. Kariluoto, V. Ollilainen, P. Varmanen and V. Piironen, University of Helsinki, Finland

POSTER PRESENTATIONS

Session 1

Microbial ecology of cereal fermentations

Aerobic cultures of *Lactobacillus plantarum* and *Weissella cibaria* as potential starter in sourdough production

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Sourdough fermentation is a traditional process used to improve quality of baked goods because of interactions and metabolic activities of yeasts and lactic acid bacteria (LAB). In the modern bakery biotechnology, type III sourdoughs are the most convenient way to supply souring and aromatic compounds to obtain stable and standardized end products. Since several studies demonstrated that aerobic/respiratory pathway in some LAB offers helpful traits for industrial applications, we investigated the effect of aerobiosis (air, with hemin and menaquinone) on the growth and metabolic pathway of the well characterized (Zotta *et al*., 2007, 2008; Ricciardi *et al*., 2009) sourdough strains *Lb. plantarum* DBPZ1015 and *W. cibaria* DBPZ1006, in order to find suitable liquid aroma carriers for bread making. Strains were cultivated in shaken flasks and then in a bioreactor using liquid malt wort as substrate and cultures were directly used as starter for dough fermentation and bread production. Acidification, leavening and sensory properties of bread were evaluated. Doughs made with commercial yeast (Y) were used as control.

W. cibaria was more capable of using maltose and produced 6.6 g lactic acid/L/g biomass and 7.3 g acetic acid/L/g biomass under controlled growth, than *Lb. plantarum*, confirming its competitiveness in sourdough fermentation and suggesting its potential use as type III sourdough starter. Even if, unlike *Lb. plantarum*, *W. cibaria* did not synthesize an heme-dependent catalase, the heterofermentative strain exhibited a significant O_2 uptake (82 µmol O_2/min/g biomass) that could be an useful trait for the production of aerated sourdoughs.

As expected, pH values were slightly higher in control doughs, while Y/LAB association resulted in more leavened products, especially when *W. cibaria* was added. Consequently, the produced bread had more appreciable sensory properties and palatability due to acetate production and malted flavour notes were recognizable. An increased shelf-life was also observed in Y/LAB based bread. This work suggests that aerobic metabolism of LAB could be useful to control both artisanal and industrial fermentations and further investigations on this way is needed to improve sourdough technology.

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Biodiversity and stability of lactic acid bacterial community at tarhana dough fermentation; a sourdough included fermented food

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Tarhana is a traditional fermented food prepared with mixing wheat flour, sourdough, yogurt and a variety of vegetables (tomatoes, onions, green pepper), salt, and spices (mint, paprika) followed by fermentation for consuming it as a soup. The main microbiota of tarhana is consisted with lactic acid bacteria and yeasts. Therefore in our study the objective is to screen the diversity of lactic acid bacteria and their stability during the fermentation days. Polyphasic approach including culture dependent and culture independent methods were used at determination of both diversity and stability. LAB isolates were classified into 43 different groups with $(GTG)_5$ fingerprint analysis and these groups were identified as *Lactobacillus plantarum* (16), *Lactobacillus brevis* (7), *Leuconostoc mesenteriodes* (2), *Leuconostoc pseudomesenteriodes* (1), *Pediococcus acidilactici* (1), *Lactococcus lactis* (3), *Lactobacillus fobifermentas* (1), *Lactobacillus mindensis* (1), *Lactobacillus paralimentarius* (1), *Lactobacillus alimentarius* (1), *Lactobacillus namurensis* (3), *Lactobacillus casei* (1), *Lactobacillus pentosus* (1), *Lactobacillus farciminis* (3), *Leuconostoc citreum* (1). DGGE analysis substantially supported the diversity of tarhana incoporating *L. sanfranciscensis*, *S. thermophilus, L. delbrueckii* subsp*. bulgaricus.* Additionally DGGE analysis showed that *L. sanfrancisccensis, L. farciminis* and *L. alimentarius* were able to be established at more tarhana doughs with later fermentation days that other LAB strains only maintained their existence. Here we conclude that i) sourdough lactic acid bacteria accompany to the fermentation of tarhana, ii) Tarhana dough has complex ecosystem due to its wide ingredients, iii) *L. sanfranciscensis, L. farciminis* and *L. alimentarius* showed cell growth at later fermentation days indicating that these strains could prevail in this competitive ecosystem.

Biodiversity of Lactic Acid Bacteria involved in the fermentation of *DOKLU*, a traditional corn food in Ivory Coast

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Many traditional food are produced from cereal or plant and represent a basic part of the daily diet of many people on this planet (1). Lactic Acid Bacteria (LAB) fermentation is often predominant in these products (2). *Doklu* belonged to African traditional food (1) and is mainly consumed in Ivory Coast. *Doklu* is produced after spontaneous fermentation of corn dough. During the preparation of *doklu*, after cleaning and washing, whole maize grains are soaked in water for 2 or 3 days, milled, mixed in water and left to undergo spontaneous fermentation for 24 to 72 h. The dough obtained is precooked, shaped into balls, wrapped in maize husks and boiled for 2 h. During fermentation, a succession of endogenous micro-organisms results in a population dominated by LAB. In this study, fermenting corn dough was collected from *doklu* producers at different stages of fermentation (0h, 24h, 48h and 72h). After enumeration and isolation, representative LAB isolates were identified by combining morphological, biochemical and molecular methods (16S rDNA amplification and sequencing). During fermentation, a succession of LAB species was observed. Thus, at the beginning of fermentation, *P. pentosaceus* (50%) and *P. acidilactici* (33%) were the predominant LAB species in corn dough with *L. fermentum* (17%). After 24h*, L. plantarum* (64%) was in highest number while *W. cibaria* (22%), *P. acidilactici* (7%) and *L. fermentum* (7%). *L. plantarum* (56%) and *P. pentosaceus* (39%) were the predominant LAB after 48h of fermentation and then *L. fermentum* (5%). At the end of fermentation, *Lb. fermentum* (100%) was predominant in corn dough. The microflora dynamic was also analysed using temporal temperature gel electrophoresis (TTGE) technique. The LAB strains isolated from doklu have also been tested for their antimicrobial activities.

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Building up the Italian sourdough library

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Sourdough has been, is and probably will be more and more the unique traditional starter to obtain otherwise irreproducible quality of leavened baked goods (1). More than one hundred traditional and/or typical Italian leavened baked goods are produced through sourdough biotechnology (2). The dominant microbiota, the practises of propagation, and use of a given sourdough represent a heritage, from a biodiversity and cultural points of view. In order to preserve and to exploit such a heritage, the idea of a sourdough "library" was conceived. The sourdough library hosts dominant microbiota (in form of frozen pure cultures) and practices (in form of written protocols) of several sourdoughs and is the foundation for reconstituting, in case of need, a natural starter culture which cannot be otherwise obtained.

The Italian Sourdough Library was therefore built up by sampling, throughout Italy, 37 sourdoughs used for manufacturing traditional and/or typical bread (e.g., Pane di Altamura PDO, Pane Casareccio di Genzano) and sweet baked products (e.g., Panettone, Nadalin). In addition, flour used for sourdough propagation and information regarding the sourdough-related protocols were collected. All sourdoughs were biochemically characterized (concentrations of organic acids, ethanol, and free amino acids) and their lactic acid bacteria and yeasts were studied through culture-dependent and -independent approaches. Concentrations of carbohydrates and free amino acids in the flour were also determined in order to establish the influence of flour nutrients on the sourdough microbial diversity.

Beyond its cultural and practical usefulness, the project on the Italian Sourdough Library allowed to highlight some interesting features of sourdough ecosystems. For instance, *Triticum durum* flours, characterized by the high level of nutrients, correlated with the sole or main presence of obligately heterofermentative strains, and the low cell density of yeasts in the mature sourdough.

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Microbial community analysis during preparation and propagation of type i sourdough through pyrosequencing

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Traditional (type I) sourdough is a complex microbial ecosystem, because it harbours, at different cell densities, many microbial groups, even taxonomically very distant (1). Numerical ratios and interactions between sourdough microbiota affect the performance of this natural starter culture. The complexity of the traditional sourdough ecosystem may change depending on factors such as flour, bakery environment and technological parameters. Combination of culture-dependent and -independent (e.g. PCR-DGGE) methods are time consuming and expensive approaches, and do not capture unidentified microorganisms or offer comprehensive analysis of microbial community structures within multiple samples at one time (2). High throughput sequencing technologies (e.g., pyrosequencing) are powerful tools with potential for resolving microbial diversity of highly complex food environments (3). To our knowledge, no study has been carried out applying high throughput sequencing technologies for monitoring microbial ecology of sourdough.

The microbiota of *Triticum aestivum*, *Triticum durum* and *Secale cereale* flours, its evolution during the preparation of the related sourdoughs and the microbial stability of the mature sourdoughs was studied mainly through pyrosequencing. Besides, microbial counts with selective media and concentrations of main metabolites will be useful as complementary information to the findings from pyrosequencing. Many different bacteria and fungi, several of which are usually associated with soil rather than sourdough, were identified in flour samples; however, Firmicutes (e.g., lactic acid bacteria) and yeasts (e.g., *Saccharomyces* sp.) dominated since the late phases of preparation of sourdoughs.

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Draft genome sequence of *Lactobacillus rossiae* DSM 15814^T

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Lactobacillus rossiae is an obligate heterofermentative lactic acid bacterium that is frequently isolated in sourdoughs, spelt flour, pineapple fruits and in the gastro-intestinal tract of animals and humans. Genotypic and phenotypic diversity of *L. rossiae* strains isolated from sourdough was described (1). Some strains were selected for their antifungal activity (2) and applied in sourdough biotechnology for glutamate production (3) and for wheat germ fermentation (4). Genomes of several lactobacilli species typically (e.g., *Lactobacillus brevis* and *Lactobacillus plantarum*) or solely (*Lactobacillus sanfranciscensis*) isolated from sourdoughs have been sequenced and annotated so far (5). The genome sequence of the type strain of *L. rossiae* DSM 15814^T (=CS1^T=ATCC BAA-822^T) will be useful to explore its biotechnological properties. A total of 30,544,098 whole-genome shotgun 100bp pair-end reads were generated by using Illumina sequencing technology. Library preparation was performed using TruSeq DNA-seq sample preparation protocol. Reads were assembled to a 2,9 Mb draft version (N50 of 150 kb) with CLC Genomics Workbench assembler. The annotation was done by merging the results obtained from the RAST (Rapid Annotation using Subsystem Technology) server and checked by BLAST analysis when needed. In addition, the scaffolds were searched against the KEGG, UniProt, and Cluster of Orthologous Groups (COG) databases to annotate the gene descriptions. RAST genome annotation evidenced 2,722 predicted coding sequences (CDSs). Comparative genomic analysis indicates that the closest genome is that of *L. brevis* (Genome ID: 387344.13) and *L. plantarum* WCFS1 (Genome ID: 220668.1). There are many carbohydrate, amino acid and derivatives subsystem features, including genes involved in central carbohydrate, monosaccharide, and fermentation metabolism. There are also many protein and DNA metabolism subsystem features. The genome sequence provides new avenues to further explore gene-based functional and technological applications of *L. rossiae*. In addition, comparative genomics analysis and functional genomics analysis could also be carried out to trace the origin and evolution of *L. rossiae*.

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Evaluation of yeast populations in spontaneously fermented rye sourdoughs using different culture – independent methods

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Microbial consortia of rye sourdoughs usually contain the homo- and heterofermentative species of lactic acid bacteria (LAB) and yeast. LAB play crucial role in formation of right rheological and organoleptical properties of rye dough, whereas the main function of yeasts is the dough leavening. In stable sourdough microbial consortia LAB and yeast are often in symbiotic relationships. Thus evaluation of sourdough ecosystem in addition to detection of LAB also requires fast and sensitive methods for detection of yeasts.

In the present study yeast population of six spontaneously fermented rye sourdoughs were studied using three culture-independent methods: denaturing gradient gel electrophoresis (DGGE), amplification of internal transcribed spacer regions 1 and 2 followed by agarose gel electrophoresis (PCR-AGE) and PCR-based 454 pyrosequencing. The rye sourdoughs were propagated in triplets at 20 and 30^{o}C during 56 days.

Both DGGE and PCR-AGE analysis detected similar composition of yeast population in studied sourdough samples, which consisted from representatives of *Saccharomyces unisporus, Saccharomyces cerevisiae, Candida krusei* and *Candida glabrata* species. Metagenomic analysis of the same DNA samples additionally detected the presence of yeasts belonging to species *Candida utilis* and *Candida catenulata*, who's DNA compose less than one percent from the DNA of whole population. However, no yeasts belonging to the *C. glabrata* specie were detected by this method. Thus the yeast composition of sourdoughs obtained by PCR-based 454 pyrosequencing, which is highly recommended for identification of bacterial populations (Jung et al. 2011, Leite et al. 2012), correlated only partly with the results obtained by DGGE and PCR-AGE methods.

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High-throughput sequence-based analysis of the bacterial composition of four industrial rye sourdoughs in Estonia

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Culture dependent techniques and traditional molecular methods, such as denaturing gradient gel electrophoresis (DGGE) and Sanger sequencing used so far for the characterization of the sourdough microbial populations may provide inaccurate results. The sourdough lactic acid bacteria (LAB) can grow poorly on laboratory media due to their adaptation to the sourdough environment and complex nutritional requirements. DGGE may also give indefinite results, since some LAB give multiple bands and species present at low concentrations cannot be detected. In addition amplified fragments purified from the DGGE are often too short to obtain reliable sequencing results. These limitations make studying the composition and dynamics of LAB populations challenging.

In this work we implemented the 16S rDNA pyrosequencing approach to determine the composition of LAB in industrial rye sourdoughs used for bread making in four Estonian bakeries with different process parameters. The Roche 454 GS FLX System was chosen for the analysis, for it gives longer amplicons compared to other pyrosequencing platforms. This type of analysis is relatively new in the study of food related consortia (Dobson *et al.*, 2011).

Our results showed that the microbial composition of the rye sourdoughs differed remarkably in all four bakeries. The species *Lactobacillus helveticus, Lactobacillus amylovorus, Lactobacillus pontis, Lactobacillus panis, Lactobacillus gallinarum, Lactobacillus sanfransiscensis, Lactobacillus vaginalis* and *Lactobacillus panis* were found dominating in various combinations in different bakeries. In addition bacteria that were present at low concentrations (forming 1–10% of the population) were detected. The microbial composition of the sourdoughs varied in samples taken from the same bakeries at different time intervals. Hence, it can be claimed that industrial sourdoughs are dynamic systems and changes in the consortia do occur. This is likely affected by the consistency of process parameters during the propagation of sourdough.

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Interactions between EV3 phage and its host strain *L. sanfranciscensis* H_2A

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Lactobacillus sanfranciscensis is the predominant bacterium in traditionally fermented sourdoughs. Previous studies on ϕEV3, a bacteriophage active on this species, highlighted the presence of a gene coding for a protein having a homology of 33% with dextranase I of *Paenibacillus* species. Through lysogeny experiments the transduction of *dex* gene from the viral particle to a bacterial strain (H_2A) was demonstrated.

Furthermore, it was also shown that the enzyme is constitutively expressed and extracellular active for at least 120 generations. To understand which kind of advantage the presence of a dextranase could have on *Lb. sanfranciscensis*, growth curves of strain with (H_2Adex+) or without (H_2A) the gene were compared. Both strains did not grow well (OD_{600} < 0.3) when dextran was the only carbon source, and an increase of maximum growth rate as a result of enzyme expression was not observed. However, H_2Adex+ reached cell concentration values (OD_{600} = 1.4–1.5) higher than the ones of H_2A strain (OD_{600} = 0.4–0.9) when glucose and/or maltose were present in the medium, suggesting a positive contribution of the enzyme. Thus, the dextranase is supposed to be a lysogenic conversion gene located in the phage lysis module.

Sequence analysis of the prophage surrounding regions revealed that the EV3 integration site is in the $tRNA_{Leu}$ gene locus of the *L. sanfrancicensis* genome.

Influence of scale-up on sourdough lactobacilli diversity, physico-chemical characteristics and sensory properties

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Sourdough microbiota diversity and activity are influenced by numerous factors related to the substrate (flour type), the process conditions (temperature) and the microbiota (microbial interactions). So the fermentation handling leads to the implantation of a specific LAB and yeasts ecosystem. Results of laboratory scale studies are frequently difficult to be generalized at industrial scale. In this work, we studied the influence of scale-up on the lactobacilli diversity, physico-chemical characteristics and sensory properties of a French wheat sourdough, propagated by backslopping at pilot and industrial scales over 10 days.

During the 10 consecutive days of the study, sourdoughs were daily refreshed, except during week-end. The initial fermentation step was started with the same mother dough. Each refreshment step and sampling was performed simultaneously. Volume ratios were strictly controlled as well as the temperature (adjusted every 2h). Analyses covered pH, TTA and dry matter measurements, LAB counts and sensory analysis. Lactobacilli diversity was evaluated by morphological classification, REP-PCR genotyping and 16S rRNA gene sequence analysis identification.

Fermentation temperatures and dry matters ranged between 28 and 36°C and between 43.5 to 45% in the two systems. If the pH and TTA curves followed a similar evolution, a significant and constant difference of +0.13 pH unit was noted in the industrial sourdough. Both sourdoughs were composed of 4 dominant *Lactobacillus* species – *Lb. panis*, *Lb. frumenti*, *Lb. amylolyticus*, *Lb. acetotolerans* – with different ratios following the scale level considered. The homofermententative and strictly heterofermentative species represented respectively 90 and 7% of the total cell counts for the industrial sourdough and 76 and 21% for the pilot sourdough. Breads made with the two sourdoughs were identical during the first five days but differed significantly thereafter, the industrial sourdough breads showing a darker crust color.

Finally, this work enabled to mimic the evolution of an industrial sourdough at the laboratory scale. Influence of scale-up was particularly controlled during the first five days. An original lactobacilli consortium was observed in these sourdoughs.

Characterization of dextransucrase in *Weissella confusa* and *Weissella cibaria* strains isolated from sourdough

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Several studies have shown that *Weissella cibaria/confusa* are suitable starter cultures for sorghum or wheat sourdough fermentation and are of great interest for exopolysaccharide (EPS) production. Some strains were shown to produce from sucrose homopolysaccharides (glucan, dextran) and derived oligosaccharides and could thus generate functional metabolites such hydrocolloids and prebiotics in cereal fermentations. Dextran production from *Weissella* strains remained relatively unexplored compared with the one from *Leuconostoc, Lactobacillus* and *Streptococcus* genera. Dextran synthesis is catalyzed from sucrose by extracellular, soluble or cell-associated, dextransucrases (E.C. 2.4.1.5; also referred as glucosyltransferases) that belong to the glycoside hydrolase family 70 (http://www.cazy.org). Only one gene encoding dextransucrase from *W. cibaria* has been characterized so far.

We previously demonstrated that several *W. cibaria* and *W. confusa* isolated from traditional wheat sourdoughs synthesize linear dextrans containing α-(1→6) glucose residues with few α-(1→3) linkages from sucrose (1). In addition, these strains were shown as suitable starter in wheat sourdough fermentation without strong acid production .We were thus interested in the characterization of the dextransucrase activity of two of these strains: *W. cibaria* LBAE K39 and *W. confusa* LBAE C39-2. We have shown that *Weissella* dextransucrase activity is constitutive and is assigned to a unique 180 kDa protein, mainly soluble. Availability of draft genome sequence from *W. cibaria* and *W. confusa* has allowed retrieving in each species complete dextransucrase encoding sequence. Deduced amino acid sequence analysis revealed common structural domains of glucansucrases. However, sequence comparison with other glucansucrases clearly supports that *Weissella* dextransucrases are phylogenetically related and form a distinct group within LAB glucansucrases. The dextransucrase DSRC39-2 was cloned, expressed on *E. coli* His-Tag system and purified. Subsequently, biochemical characterization of the recombinant enzyme has been performed. Overall, this work provide basis for future applications of these attractive species for cereal applications.

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Optimization and lactic acid bacteria (LAB) characterization of a spelt sourdough for organic bread production

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Sourdoughs have been used for centuries as the only starter used to obtain craft products. In the last years, many studies focused to various aspects of sourdoughs production, such as mapping of lactic and yeast microflora, factors that influence fermentative activity of microorganisms, type of products resulting from fermentation of doughs, compounds used by the microbial species and their interactions, microflora evolution during dough's production and performance of some microrganisms within this particular ecosystem.

Purpose of this study was the optimization and LAB characterization of a sourdough obtained using spelt flour for the production of organic bread. The best conditions of time of fermentation, temperature, pH and the amounts of water and flour to obtain a sourdough suitable for baking were choosen.

Microbiological analysis were carried out using classical and molecular techniques to have a view of the biodiversity of the lactic acid bacteria during the natural fermentation. Sourdough were collected at different times of fermentation and subjected to traditional and molecular microbiological analysis. After the counts, the plates were used for bulk formation. DNA was extracted directly from the bulks and amplified by PCR and emulsion-PCR (ePCR), using universal primers. DGGE analysis and sequencing of the resulted bands allowed fingerprinting of the microbial populations present in the fermentations.

Chemical analysis showed that, in order to obtain a sensorially acceptable bread, pH value of sourdough had to be kept around 4.0. Quantitative determination showed that lactic acid bacteria concentration was higher than the yeasts count values (100:1 ratio), according to what reported in literature. LAB ranged from 10^3 to 10^9 cfu/g. Using PCR-DGGE, *Lactobacillus sanfranciscensis* and *Weissella cibaria* resulted as the main species involved in the natural fermentation. Conversely, a more complex picture of the involved LAB species was obtained using ePCR-DGGE. For the first time this technique associated with DGGE analysis was applied. The obtained results confirmed the efficiency and usefulness of e-PCR, due to its capacity to avoid preferential amplification in terms of concentration and type of DNA.

Phylogenetic, genetic, and physiological analysis of sourdough isolates of *Lactobacillus reuteri*: food fermenting strains are of intestinal origin

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Lactobacillus reuteri colonizes the intestinal tract of animals and humans (3), and also is a stable member of sourdough microbiota (2). The evolutionary relationships of food-associated lactobacilli to intestinal representatives of the same species remain unknown. *L. reuteri* lineages have evolved to become host-specific; this species is thus an excellent model organism to characterize the evolutionary trajectory of a species that is both host- and food-associated (1). This study aimed to determine whether sourdough isolates of *L. reuteri* are derived from intestinal ecosystems.

Multi-locus sequence analysis assigned three rye sourdough isolates of *L. reuteri* to lineages which are almost exclusively composed of rodent isolates. Sourdough isolates *L. reuteri* FUA3400 and FUA3401 cluster closely with human isolates in the Lineage II; two sorghum (ting) isolates form an outgroup of the Lineage II together with two rodent and two human isolates, and one swine isolate. MSLA analyses were confirmed by characterization of the host-specific traits through biochemical and genetic methods. Comparative genome hybridization revealed that the sourdough isolate *L. reuteri* LTH2584 had very similar genome content when compared to the model rodent isolate 100-23.

L. reuteri isolates investigated in this study were all isolated from sourdoughs maintained by continuous propagation. The unambiguous assignment of 5 out of 7 sourdough isolates to human or rodent origins does thus not represent contamination but demonstrates that intestinal *L. reuteri* are capable of long-term persistence in food fermentations. The observation that strains autochthonous to the human gut are competitive starter cultures for use in food fermentations provides new opportunities for the development of probiotic foods.

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Secretome of *Lactobacillus plantarum* LPL400 changes as response to quorum sensing

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Extra-cellular and cell surface-associated proteins (secretome or exoproteome) have a key role in the adaptation of a bacterium to changing environmental conditions (1–3). The exoproteome of lactic acid bacteria is involved in various processes such as cell wall metabolism, degradation and uptake of nutrients, quorum sensing (QS) and binding to substrates or hosts (3). A proteome-scale comparative study of the secretome may provide information towards the understanding of diversity, function and adaptation of lactic acid bacteria to their specific environmental niches. The present study aimed at investigating the secretome of *Lactobacillus plantarum* LPL400 grown in mono-culture or in co-culture with *Lactobacillus sanfranciscensis* DPPMA174 or *L. plantarum* DPPMA20. All strains were isolated from sourdoughs and previously identified by partial sequencing of the 16S rRNA gene. Based on the role of pheromone plantaricin A (PlnA) and AI2 in QS (4), the exoproteome of LPL400 was also determined for cells grown in the presence of 2.4 µg/ml of chemically synthesized PlnA or 1 µg/ml of the AI-2 precursor molecule 4,5-dihydroxy-2,3-pentanedione (DPD). Compared to mono-culture, *L. plantarum* LPL400 did not show growth inhibition when co-cultured with *L. sanfranciscensis* DPPMA174, *L. plantarum* DPPMA20, PlnA or in the DPD containing medium. Compared to mono-culture, the amount of extra-cellular and cell surface-associated proteins of *L. plantarum* LPL400 was affected by culture conditions. Protein spots showing decreased (13 spots) or increased (30 spot) amounts during growth on co-culture with *L. sanfranciscensis* DPPMA174, *L. plantarum* DPPMA20, PlnA or DPD containing medium were identified by MALDI-TOF-MS/MS or LC-nano-ESI-MS/MS. Over-synthesized proteins were involved in biofilm formation, adhesion, stress response and DNA repair. Under-synthesized proteins were mainly extracellular proteins with unknown function.

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Transcriptome analysis of *L. sanfranciscensis* TMW 1.1304 in response to electron acceptors and the presence of *Candida humilis*

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Genome sequencing of *L. sanfranciscensis* TMW 1.1304 originally isolated from Type I sourdough revealed insight into its high adaptation to rye and wheat sourdoughs [1]. In this work we investigated transcriptional responses to environmental conditions including the presence of oxygen and fructose as electron acceptors, and the presence of *Candida humilis* (formerly *milleri*), which is part of the autochtonous microbiota of traditional sourdoughs.

Using microarrays, gene expression was determined of exponentially growing *L. sanfranciscensis* TMW 1.1304 cells under five different culturing conditions : (i) anaerobic (reference condition), (ii) aerobic , (iii) in presence of fructose, and in the presence *Candida humilis* in the ratios of (iv) 1/100 and (v) 1/10 in MRS media.

Generally, all observed expression changes of annotated genes were low and most of the stronger regulated genes encode small hypothetical proteins and pseudogenes. The cultivation in media with fructose led to a higher expression of ribosomal proteins, and genes, involved in carbohydrate metabolism, that indicate a switch to acetate formation. Interestingly, the response to oxygen has some overlap with the fructose response, and resulted in a similar change of gene expression as compared to the cultures mixed with *Candida*. In both conditions genes for fatty acid synthesis are lower expressed whereas genes coding for specific membrane transporters and amino acid metabolism are upregulated. The findings suggest that *C. humilis* affects the redox milieu similar to oxygen and fructose in a way that the *Lactobacillus* benefits from enhanced ATP formation as well as the availability of nutrients.

The limited regulation observed is in accordance with the fact that this strain has only 2 two-component regulatory systems and 38 transcriptional regulators and rather follows a “wait and hit” strategy than regulation to be competitive in its favourite nutrient-rich environment.

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Cultivation of selected *Gluconobacter*-Strains on cereal substrates and *in-situ* production of exopolysaccharides

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Resulting from their ability to incompletely oxidize a wide range of carbohydrates, sugars and alcohols, *Gluconobacter* species are applied in various food biotechnological processes. We have recently demonstrated that some strains produce large amounts of fructans in sucrose-containing media, which have a positive effect on bread volume and texture and are able to retard bread staling (Jakob et al., 2012). As the isolation of pure EPS is a multistep, energy- and time-consuming procedure and clean label products are desireable, *in situ* production of fructans by *Gluconobacter* strains on cereal substrates was investigated.

The growth and competitiveness of *Gluconobacter frateurii* (TMW 2.767) and *Gluconobacter albidus* (TMW 2.1191) were tested in wheat, rye, oat, millet and spelt doughs under aerobic and anaerobic conditions at a dough yield of 500. Fermentations in which *G. frateurii* and *G. albidus* became dominant (<10 % contaminants) were repeated upon supplementation of sucrose (50, 75 and 100 g/L), and growth, EPS-yield and fermentation metabolites were studied. *G. frateurii* as well as *G. albidus* became dominant and produced maximum EPS-amounts in aerobic, sucrose-enriched spelt (22–23 g/kg flour after 24 h and 8–18 g/kg flour after 48 h, respectively) and wheat doughs (6–9 g/kg flour after 48 h and 4–11 g/kg flour after 24 h, respectively). In aerobic rye dough with *G. albidus*, 6–12 g EPS/kg flour was detected after 24 h.

The lowest remaining sugar contents were recovered in spelt doughs. Here, sucrose and glucose concentrations of ~186 and 88 g/kg flour were found, respectively, for *G. frateurii* after 24 h. For *G. albidus*, ~118 g/kg flour sucrose and no glucose were detected after 48 h. These results show for the first time that *in-situ* production of EPS on cereal substrates by selected *Gluconobacter*-strains is possible and may enable exploitation of novel strains and EPSs for baking applications.

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POSTER PRESENTATIONS

Session 2

Fermentation induced changes in the cereal matrix

Analysis of dextrans *in situ* produced by prospective lactic acid bacteria strains in vegetable-based model food

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Nowadays consumer trend goes after healthy and natural foods. The prospect of some lactic acid bacteria (LAB) introducing natural thickeners during sourdough fermentation has attracted growing interest. These LAB utilize sucrose to produce exopolysaccharides, such as dextrans, which impart positive technological properties on the bread and could replace hydrocolloid additives. Dextran producing LAB could be exploited for the production of additive-free foods.

In this study, four prospective dextran producers, namely *Weissella confusa* E392, *Leuconostoc lactis* E2298, *Leuconostoc mesenteroides* E461 and *Leuconostoc citreum* E497, were selected for fermentation of vegetable model foods based on viscosity increment. The structure of the dextran produced by each strain was analyzed with NMR spectroscopy after purification from cell mass on MRS-sucrose agar. All four dextrans were mainly composed of α-(1→6)-linked D-glucosyl units; dextrans synthesized by *W. confusa*, *Lc.mesenteroides* and *Lc. lactis* had less than 4% α-(1→3) branch linkages; while *Lc. citreum* dextran contained 11% α-(1→2) and 4% α-(1→3) branch linkages [1].

The relatively simple structure of *W. confusa*, *Lc.mesenteroides* and *Lc. lactis* dextrans enables their direct enzyme-assisted *in situ* quantification in food matrixes [2]. Moreover, the enzyme-resistant oligosaccharides can be used for fingerprinting the type and amount of branch linkages in dextrans produced in different media and conditions. *W. confusa* and *Lc. lactis* were able to introduce approximately 1.5% dextran from 5% sucrose in the carrot model food. The size of dextran present in model food was evaluated without isolation by the lately developed DOSY NMR method [Maina et al, submitted]. All dextrans produced by the strains studied possessed very high molecular masses (10^7–10^8). The knowledge on the structure and molar mass of dextrans produced *in situ* in food matrixes will contribute to the understanding of the structure-function relationship and behavior of dextrans in different foods.

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Chemical, rheological and sensory evaluation of pate stuffed with broccoli (*Brassica oleraceae L.*)

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This study aimed to produce a baked dietary product (pate) enriched with broccoli florets that sprayed in the field with methionine. Treated broccoli florets were separately stuffed in pate at three levels (5, 10 and 15% w:w) in the form of minced, steamed blanched and fried broccoli florets. A Significant increase in reduced glutathione content was observed in the methionine-treated broccoli florets compared with methionine untreated broccoli florets during processed to minced, steam blanched or as fried with butter. The pate stuffed with methionine – treated broccoli florets at different levels possessed higher fiber and protein contents if compared to control sample (Not stuffed pate). Stuffing the processed pate had no effect on the estimated rheological properties, color attributes, baking tests and organoleptic properties. Increasing the ratio of stuffed methionine treated broccoli florets increased loaf weight and decreased the crumb moisture. The results revealed that, stuffing pate with methionine treated broccoli florets had enriched the nutritive value and baking quality. Generally, Pate stuffed with methionine treated broccoli florets (5:15%) did not significantly affect technological, rheological, sensory quality of pate and improved its nutritional values.

Dextran from *Weissella cibaria* with potential application in gluten free cereal products

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Lactic acid bacteria common to sourdoughs include members of *Leuconostoc*, *Pediococcus*, *Weissella* and other genera. *Weissella cibaria* is a gram-positive, catalase-negative, short rods, heterofermentative lactic acid bacteria with GenBank Accession Number: AB621969.1 was isolated from peel of apple. Celiac disease (CD) is the most common food-induced enteropathy in humans caused by intolerance to wheat gluten and similar proteins originating from barley and rye in genetically susceptible individuals (1). Recently, the applicability of the EPS-producing *Weissella cibaria* was investigated in gluten-free sourdoughs (2). Gluten-free breads containing sourdough fermented by *W. cibaria* were softer than the ones containing no EPS because during the fermentation it was able to produce dextran (2).The incorporation of dextran in bakery products improves gelatinization of starch, softness, crumb and loaf volume. In the present study *Weissella cibaria* produced significant high amount of dextran (38 mg/ml) in fermented broth. The ^{1}H and ^{13}C NMR spectral analysis of dextran from *Weissella cibaria* confirmed the presence of D-glucose monomer with 93.06% of α(1→6) linear and 6.94% of α(1→4) branching. Scanning electron microscopic study revealed the porous web like nature of the dextran which aid in the water holding capacity of the dextran thus giving it potential application as stabilizer and texturising agent in food and baking industry. From the rheological study it was observed that dextran followed a typical non Newtonian pseudoplastic behavior. Long-chain, high-molecular-weight polymers that dissolve or disperse in water improves rheological (gelling, thickening) or physico-chemical (emulsion, stabilisation, particle suspension etc) properties, are important tools for food product formulation. Thus, the high production, low degree of branching and pseudoplastic behavior make the dextran of *Weissella cibaria* a burgeoning candidate for the production of high quality gluten-free cereal products.

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Potentials of dextran hyper-producing *Weissella confusa* Cab3 isolated from fermented cabbage for sourdough

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Weissella species are gram-positive, catalase-negative, short rods or coccobacilli, obligately heterofermentative lactic acid bacteria (LAB). Recently *Weissella* sp. has drawn attention for its high dextran production capacity and linearity in its dextran. In our earlier report *Weissella confusa* Cab3 (GenBank Accession Number: GU138518.1) was isolated from fermented cabbage [1]. Important features of dextran for its use in sourdough bread are its high yield, low degree of branching and high molecular weight. *Weissella confusa* Cab3 produced significant high yield (98%) of dextran which could be explored for its impact on bread quality. The ^{1}H and ^{13}C NMR spectral analysis of dextran from *Weissella confusa* Cab3 confirmed the presence of polysaccharide with linear α(1→6) linkages with almost no branching. Scanning electron microscopic study revealed the hydrocolloid nature of the dextran which aid in the water holding capacity of the dextran thus giving it potential application in sourdough. Dextran improves dough stability and gas retention through an interaction with gluten network. From the rheological study it was observed that dextran followed a typical non Newtonian pseudoplastic behavior. Molecular weight of dextran is being analyzed by gel filtration. Gel filtration of dextran from *Weissella confusa* Cab3 by Sephadex G-100 showed a single peak suggesting that it was a mono-dispersed polymer. Thus, the high yield, high molecular weight, linear structure, no polydispersity, pseudoplastic behavior make the dextran of *Weissella confusa* Cab3 a potent candidate for sourdough baking.

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Influence of lactic acid bacteria on the oxidation reduction potential of buckwheat sourdoughs

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Sourdough fermentations are widely used for the production of bread and other bakery products. Until now the pH value is the most used control parameter taken into account during sourdough technology. While the redox status of cereal proteins decisively influences dough rheology and bread structure (Vermeulen et al., 2005) no information is available about the influence of sourdough microbiota on the oxidation reduction potential (ORP) or the use of redox potential (Eh_7) measurements as a fermentation control parameter.

The influence of growth and metabolism of lactobacilli on the oxidation reduction potential of buckwheat sourdoughs was investigated. Fermentations were carried out using buckwheat doughs with a DY (dough yield) of 350 at 30°C using a stirring speed of 600 rpm in a 500 mL fermenter. pH, ORP and pO2 measurements were performed using Mettler Toledo (pH), Schott (Pt vs Ag/AgCl) and Ingold oxygen electrodes. The Eh_7 was calculated against standard hydrogen electrode at pH 7.

Upon inoculation with lactic acid bacteria (LAB) ORP changes were observed. Each strain exhibits a different ORP time course curve. The Eh_7 after 8 hours using *Lactobacillus plantarum*, *Lactobacillus sakei*, *Weissella cibaria* and *Pediococcus pentosaceus* reached 89.2 ± 4, 104.5 ± 15.3, 30.2 ± 4 and 181.5 ± 2.6 mV, respectively. *W. cibaria* showed the highest reducing activity and *P. pentosaceus* the lowest one. The maximal reduction rate was not correlated with the highest acidification rate nor with the maximal growth rate. Instead, the ORP changes occurred concomitantly with the acidification, and the acid production decreased after 2 hours upon the reducing step. These results showed the possibility to carry out ORP measurements in dough system. As strains exhibit very characteristic ORP curves this parameter can be used as real time control tool to monitor the time course of fermentation and metabolic activity. This enables an evidence-based determination of fermentation end and/or (intermediate) harvesting point.

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POSTER PRESENTATIONS

Session 3

Applications of microbes in cereal based foods

Cereals sourdough fermented by means of *Lactobacillus* and *Propionibacterium* and its effect on properties of toast bread

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Sourdough (SD) based on spontaneous fermentation of rye flour has been used in traditional way of bread production. The standard mixture of Lactobacilli is currently used too for wheat or rye breads to reach a typical bread taste. However, an addition of fermented products – SD can also improve the shelf life of bread. In this work, SD fermented using both *Lactobacillus plantarum* and *Lactobacillus sanfranciscensis* and *Propionibacterium freudenreichii* subsp. *freudenreichii* was used to find out their ability to inhibit growth of common bread spoilage molds.

SD made from wheat or rye or barley flour, and from rye or barley bran, with the addition of lactic acid and propionic acid was added to wheat toast bread formula. Their inhibition ability was compared to the ability of natural organic acids formed during fermentation of the same types of sourdough. Inhibition activity of natural SD and synergic effect of SD with added acids were determined by means of growth of molds artificially inoculated on bread crumb.

The bread crumb with different added SD was evaluated on penetrometer (crumb toughness) and sensorially. Fermented types of cereal SD affect sensorial and nutritional properties of bread, inhibit growth of molds, and prolongate shelf life of product.

Establishing a standardised mixed starter for Korean rice sourdough

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Sourdoughs are complex ecosystems which are widely used to produce a variety of wholesome baked goods. However, the consumption of cereals (*Triticae* such as wheat, barley and rye) containing gluten, may cause digestion problems and also induce a strong auto-immune response (coeliac disease) in the small intestine of sensitive persons. With an increasing part of the population suffering from typical but also asymptomatic gluten related disorders, there is a growing need for gluten-free cereals and bakery products. A major objective of this study was to develop gluten-free sourdough bread based on rice flour. Two traditional fermented Korean foods, *Kimchi* and *Doenjang*, served as a potential source of strains which were adapted to rice flour. Consecutive sub-culturing was repeated ten times at 26 ℃, when a pH of 3.9–4.0 and TTA of 10–12 ml (0.1N NaOH/g) was reached. Subsequently, lactic acid bacteria (LAB) and yeasts were isolated after plating on MRS5C and YGC agar plates, respectively. The identity of the 20 strains obtained was determined by biochemical procedures and 16S rRNA sequencing. All yeasts were identified as *Candida* species, while 5 different LAB species were obtained. The 5 selected LAB strains comprised *Leuconostoc citreum* GLHH1, *Lactobacillus plantarum* subsp. *plantarum* GLHH3, *Leuconostoc mesenteroides* subsp. *mesenteroides* GLHH4, *Lactobacillus brevis* GLHH10, *Lactobacillus sakei* subsp. *sakei* GLHH26. Each strain was adapted to rice flour, and cell numbers and pH values determined after 24 hours fermentation. Combining 1.0×10^7CFU/g per strain, dynamics of a mixed starter was studied during 24 hours fermentation. By sampling every 3 hours, pH, TTA, and plate counts (MRS agar) were determined. The pH values ranged between 3.9 and 4.0 after 24 hours. It was shown possible the isolated strains were adapted to rice flour and fermented. DNA from the dough was extracted by the xanthogenate nucleic acid isolation method. The DNA was amplified with L1GC and HDA2 primers for DGGE, showing different fermentation patterns based on the small DNA fragments. Our study suggests the suitability of the test strains as rice sourdough starters, with particularly *Leuconostoc mesenteroides* strains producing sufficient amounts of CO_2, in combination with the yeast, for gluten-free sourdough of Korean rice flour.

Glucose – galactose syrup effect on the qualities of wheat dough and bread

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Glucose – galactose syrup is produced from cheese whey. It has a sweet taste and it can be used instead of sugar to produce different products. Glucose – galactose syrup is a brand new product in Latvia market and there is a need to find more possibilities for its usage. Dry matter of glucose – galactose syrup is about 70%, it contains 31% glucose and 20% galactose. When using this syrup it should be noted that it contains about 15.4 g of lactose per 100g of syrup.

The aim of this reserch is to investigate the glucose – galactose syrup effects on the quality of wheat dough and production process. The bread for the research were prepared using a standard recipe containing wheat flour, fresh yeast, salt, and 4 and 12% sugar and glucose-galactose syrup. Dough samples were tested using qualitative and quantitative analysis of yeast. The qualities of wheat dough were analyzed by capacity of yeast and the volume changes in the dough. Bread samples were tested to find out their porosity, volume and organoleptic characteristics. During the five day storage time we tested the level of moist and the hardness of crumb.

The results showed that addition of glucose – galactose syrup to wheat dough has an influence on the activity of yeast cells. Were determined that during the yeast process the amount of live cells are decresing by 23% and the amount glycogen-containing cells are decrising by 28% in the dough with glucose – galactose syrup. Whyle the amount of dead cells increase by 66%. Yeast capacity to lift was reduced if the added sugar and syrup is highly concentrated. Results indicated that addition of the glucose – galactose syrup lowers the wheat dough fermentation rate and reduces the volume of bread.

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Lactic fermentation of legume extracts – the effect on sensory and technofunctional properties and their application in different food matrices

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Legume protein extracts have highly promising technofunctional properties for many foods and could be used as baking aids to improve sensorial and nutritional properties, but their application in food is often restricted due to their green and beany off-flavours. In this research study pea (*Pisum sativum* Santana) and lupin (*Lupinus angustifolius* Vitabor) protein extracts were fermented with several lactic acid bacteria in order to improve the sensory profile and to evaluate the influence of the fermentation on the technofunctional properties of the extracts.

Defatted lupin flakes or pea flour, respectively were extracted with optimized parameters (variables: temperature, s:l ratio, pH) followed by a submerged and optimized fermentation (variables: temperature, inoculum). Five different strains of lactic acid bacteria were studied (*Lactobacillus plantarum, Lactobacillus perolens, Lactococcus paracasei, Pediococcus pentosaceus)* with respect to their growth behavior, ability to degrade oligosaccharides and to improve the sensory profile of the protein extracts. Precipitated and spray-dried protein extracts were further evaluated regarding the aroma active marker compounds, protein solubility, emulsifying and water binding capacity.

Best results were obtained with *Lactobacillus plantarum* which showed good oligosaccharide degradation, stable fermentation behavior and which delivered sensory highly appreciable protein extracts. Fermented protein extracts could be successfully applied in different food applications and suggest themselves as protein and flavor source for mayonnaise, muffins, gluten free bakery or milk drinks as a substitute for milk protein.

Lb. paracasei in sourdough: growth, acidifying capability, and VOCs production at different acidity values and temperatures

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Sourdough features are strongly influenced by fermentation temperature, pH, leavening time and microbial composition. The major flavour-active compounds are produced by lactic acid bacteria (LAB) and yeasts individually or in association. The role of LAB species most frequently occurring and able to dominate sourdough fermentation, i.e. *Lb. sanfranciscensis, Lb. plantarum* and *Lb. brevis*, has been well defined. However, little is known about a specific role of *Lb. paracasei* in sourdough fermentation. The natural presence of this species in typical bread dough samples suggests that its possible involvement in fermentation and flavour formation deserves to be clarified. For this reason in this study a LAB belonging to *Lb. paracasei* species (strain I1), isolated by a traditional ripe sourdough, was characterized with respect to growth capability, acidifying capacity and production of volatile compounds (VOCs). The strain was inoculated in sterilised flour suspensions at 6 log CFU/g at three different temperatures (25, 29 and 37 °C) and at different pH values (4.0, 5.0 and 5.8) and the above parameters were followed for 43h. A sterilised flour suspension without inoculum was used as control sample.

Faster microbial growth occurred at 37°C at 5.8 initial pH. In these conditions a maximum of 8.5 log CFU/ml were reached after 20 h, then a decline was observed. At the other temperatures maximum growth values were lower, however a decrease did not occur until the end of the trial. Also in acidic conditions active growth was observed and the strain increased again up to 6.3 log CFU/ml at pH 4.0 at all temperatures after a slight initial decrease. *L. paracasei* I1 exhibited a strong acidifying capacity with final pHs between 3.17 and 3.52. The pH decrease at the end of the experiment was comparable at the different temperatures and pHs tested, however a faster pH drop and a higher total titratable acidity (TTA) level was reached at 37°C and pH 5.8. A further pH decrease was observed also in samples with an initial pH of 4.0, demonstrating that the bacterial strain tested tolerates well the harsh physicochemical conditions of sourdough.

Preliminary results obtained by VOCs evaluation showed that this species can contribute to flavour definition of sourdough. This study indicated that the species *Lb. paracasei* comprises strains that perform well in sourdough definition. Therefore, a better explanation of the role of this microorganism in bread production is considered opportune.

Potential of brewer's spent grain-sourdough and enzymes for high fibre breads

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Novel sources of dietary fibre, such as those generated from by-products of food processing have received much attention recently. Such a by-product is brewer's spent grain (BSG), which is generated during the brewing process. It has predominantly been used for animal feed, however there is currently strong argument for its use for human consumption as it is rich in dietary fibre and protein. This study presents information on the use of BSG-sourdough for the formulation of high fibre breads. BSG sourdoughs were prepared by fermenting milled BSG flour with a commercial starter culture for 24 h at 25 ^{o}C. In addition, a commercial bread improver and xylanase were used in combination with the sourdough. The resulting breads were examined for their baking quality, staling kinetics and nutritional properties. Wheat flour was substituted at a level of 15 % and pup-loaves were prepared and studied over a period of 15 days. The baking quality of the breads (volume, texture, crumb structure) was greatly improved by the addition of the improver and the xylanase but remained unaffected by the use of sourdough. The staling of the breads was monitored by Texture Profile Analysis (TPA), moisture content measurements, freezable water content and amylopectin recrystallization. The use of sourdough although delayed the appearance of mould on the breads, however it did not affect the crumb texture during the 15 days storage period. The flavour of breads was enhanced with the use of sourdough; sensory analysis was carried out to investigate the differences in aroma and flavour as perceived by panellists. The nutritional properties of the breads are also being investigated. Trials to establish the effect of sourdough fermentation and enzymes on fibre, mineral composition, antioxidant properties and polyphenol composition are ongoing.

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Sourdough bread disappearance and revival in some countries consuming white breads

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Largely dominant in the domestic manufacture of bread before the advent of commercial bakeries, sourdough bread production has declined steadily throughout the nineteenth and twentieth century in countries consuming mainly white bread[1]. Besides the desire to eat after all, the same bread as affluent people, the main technological explanatory factors appear to have been the craze for refined flour from roller mill and the arrival on the market of very pure baker's yeast, allowing a shorter , more consistent and simpler breadmaking process[2]. From the year 1860, methods based on Viennese bread skill with yeasted preferments have known a very large audience. Later, english and american authors [3] of bakers books, associate the sour aromatic note of bread to a major fault. In addition, making sourdough bread was incompatible with the emerging industrialization of white bread.

The continued decline in bread consumption, the success of the standard white bread (with or without crust) and the trend whereby he was eaten fresh (even oven-hot) has been fatal to sourdough bread. Its aromatic richness , its propensity to keep well and its other benefits could not counteract the feeling of urban consumers to associate its relative crumb firmness with stale bread even bread of the past. In France, the loss of expertise in sourdough bread make up was narrowly avoided thanks to some bakers likely to support the production of organic foods but also by provincial master bakers defenders of local know how[2].

From the 1970s a shy movement of renewed interest in the sourdough bread is emerging in somme communities 4 and microbiologists started to publish on the subject. Some bakeries specialize in making sourdough bread. Around 1990s a revived interest in artisan-type breads in countries were mass produced (highly yeasted) white bread occupy over 90% of the market share, is interestingly associated with a re-appropriation of the conduct of sourdough fermentation. This is supported not only by elite craftsmen but also by amateurs who report their experiments on internet 4. The traditional three refreshments method used in France 5 could be revisited, modernized, in order to produce sourdough bread with a mild acidity 6.

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Sourdoughs for bread-making with barley flour: sensory and technological evaluation of barley breads

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A renewed interest in barley based food has been observed throughout the world because of the high nutritional value of this cereal. Barley is rich in antioxidants, lysine, essential vitamins, minerals, and γ-glucans (the latter was shown to lower blood cholesterol levels and glycemic index). Consumption of wholegrain barley foods seem to be even associated with increased satiety and weight loss. In spite of these nutritional/functional properties, up to now barley has been exploited only marginally by the baking industry, due to its low ability to form a gluten network. Moreover, the use of sourdough to improve baking and sensory properties of barley bread has only been partially explored. In a previous study, three sourdoughs (S_W, S_B, S_{WB}) were produced by introducing a defined lactic acid bacteria (LAB) and yeast multi-strain starter culture into three doughs made of different percentages of wheat and barley flours (100% wheat, 100% barley, 50% wheat plus 50% barley).The composition of the microbiota of the three stable sourdoughs was investigated through a polyphasic approach including PCR-DGGE and LAB viable counting, followed by wild strains isolation, identification, and molecular typing (Zannini *et al.*, 2009).

In the present study, the same sourdoughs were characterized from a technological point of view, and the corresponding breads (B_W, B_B, and B_{WB}) were compared on the basis of their physico-chemical and sensory properties, just after baking and during shelf life (6 days). In addition, flours, sourdoughs, doughs and breads were evaluated for their phytate and β-glucan content to follow their break down during processing. Some significant differences were seen between the two barley breads (B_B and B_{WB}) and B_W, with B_B exhibiting the lowest specific volume and the highest moisture. Interestingly, no significant differences among samples for crumb density, elasticity, dryness, stickiness, sweetness, and overall acceptability were perceived until the end of shelf life thus confirming the possibility of a successful exploitation of barley sourdoughs in baking industry.

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In situ synthesized exopolysaccharides in liquid sourdough influence textural and sensory profiles of brioche

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Leuconostoc spp. are traditionally used to produce homopolysaccharides from sucrose, which increase the viscosity and could improve dough rheology, texture, volume and sensory attributes of bread (1, 2). The aim of this study was to compare the influence of sourdough containing *in situ* formed exopolysaccharides (EPS) through two starter cultures of *Leuconostoc* spp. on the textural and sensory profile of butter brioche. Five different sourdoughs were produced in 7 L bioreactors with addition of sucrose to wheat fraction flour and water according to the experimental design. The mixture of flour, sucrose and water was inoculated with 400 ml of starter cultures to obtain about 6.5 log CFU/g. Two levels of pH was controlled by automatic addition of sodium hydroxide solution (10 g.L-1). pH and NaOH consumption were measured by WCIDUS software (INRA, France) to determine tmax (fermentation time with highest acidification speed in h), Vmax (highest speed of acidification in upH.min-1) and MNaOH (quantity of NaOH consumed after 25h of fermentation in g). The fermentation was carried out for 25 h at 20°C under moderate agitation (300 rpm) to keep homogeneous aspect of the liquid suspension. Zero-shear rate viscosity was obtained from flow curves fitted to Cross equation for shear-thinning liquid sample. Butter brioches were prepared with 0, 8 or 16 g of sourdough per 100 g of flour. To keep a constant water/flour ratio, flour and water present in the sourdough was calculated to replace the equal amount of flour and water in control brioche. For crumb texture analysis, a slice of 25 mm thickness and 50 mm per 50 mm were removed from the center of each brioche. Texture profile analysis (TPA) was performed in triplicate with a compression equal to 25% of the original height of the sample to determinate the instrumental attributes (hardness, springiness, cohesiveness). Sensory analysis of fresh brioche was performed with a panel of 20 judges using semistructured scales, scored 1-5, in which extremes were described. Evaluated attributes were grouped into textural or organoleptic characteristics. The results and discussions will be focused on the influence of sourdough containing *in situ* formed EPS through two starter cultures of *Leuconostoc* spp. on the textural and sensory profile of butter brioche.

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Analysing a shipwreck beer

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In the summer of 2010 the wreck of a schooner was discovered in the Baltic Sea off the Åland Isles at a depth of about 50 m. Archaeological evidence suggests that the schooner occurred during the 1840´s. The cargo consisted of luxury items, including more than 150 bottles of champagne. Five bottles assumed to contain beer were found. Two intact bottles were analysed by VTT Technical Research Centre of Finland and Technische Universität München. The aim of the study was to find out what the raw materials of the beer could have been and isolate and identify any living yeast cells and other microbes from the beer.

The liquid was identified as beer due to the presence of hop residues, malt sugars, aromatic compounds and amino acids typical of beer. Various types of microbes differing in size and morphology were observed by microscopy. Dead yeast cells and DNA were detected in both beers, but no viable yeast cells were recovered. However, remarkably stable lactic acid bacteria (LABs) were discovered alive in both beers. To our knowledge they are the longest survived bacteria yet discovered in beer. Four different species and several genotypes were isolated and identified. The ability for long survival was therefore not linked to a single species or genus, but appears to represent a more wide-spread ability. The two beers had different compositions of live LABs and some chemical compounds and thus they probably represent two different beers. The isolated bacteria are of high scientific interest and have potential industrial applications. They provide interesting models to study mechanisms of long-term survival in non-spore forming bacteria. Their long-term survival in beer also implies that the strains are very stress tolerant and potentially stable in various applications

POSTER PRESENTATIONS

Session 4

Use of fermentation to improve safety and shelf-life

Antifungal activity of selected starters during sourdough fermentation and long-term effect on storage of bread

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In recent years, bio-preservation (the use of microorganisms and/or their metabolites to prevent spoilage and to extend the shelf life of foods) has gained interest due to the increasing demand for more natural and preservative-free foods. Lactic acid bacteria are considered useful bio-preservative organisms because of their capacity of synthesizing or releasing various antimicrobial and antifungal molecules. Synergistic activities between different compounds synthesized during sourdough fermentation can be responsible for the overall antifungal effect. Peptides with antifungal activity were identified in the water-soluble extract of sourdough fermented with *Lactobacillus brevis* AM7 (1) and *Lactobacillus plantarum* 1A7. Besides lactic acid bacteria, yeasts can have antifungal activity due to the synthesis of cell wall-degrading enzymes, killer toxins and antibiotic metabolites, and ethanol and ethyl-acetate. The association of yeasts and lactic bacteria has been suggested as an alternative for the preservation of bread during storage (2). When *Wickeramomyces anomalus* LCF1695 or *Meyerozyma guilliermondiii* LCF1353 were used in combination with *L. plantarum* 1A7 for sourdough fermentation, fungal growth in bread was delayed during long-term storage (2). A synergic activity of peptides produced by *Lb*. *plantarum* 1A7, and ethanol and ethyl acetate synthesized by *W. anomalus* LCF1695 was hypothesized. Ethyl acetate and ethanol were the main antifungal metabolites also released by *M. guilliermondii* LCF1353; moreover, the capacity to synthesize an exo-*β*-1,3-glucanase was shown. The mechanism of action and the inhibitory spectra were investigated. Fungal growth was delayed at least until 14 days of storage of bread at room temperature, under conditions of high artificial inoculum.

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Influence of bioprocessed bran on rheological properties of wheat dough and on bread quality

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A diet abundant in dietary fibre has been shown to decrease the risk of obesity, type II diabetes and coronary heart disease. Wheat bran, which has a fibre content of about 50%, is a suitable source of insoluble fibre for cereal foods. Bran addition affects the rheological properties of dough and the volume of bread, though, if added in sufficient amounts to enable EFSA nutrition claim. Bioprocessing of bran with yeast or cell wall degrading enzymes (xylanase, cellulase) prior to baking is known to increase the volume and soften the bread crumb. The aim was to assess the changes due to bran addition along bread-making chain in relation to the rheological properties of dough. Two types of bioprocessed bran (yeast fermented with and without enzymes) were studied in bread making at substitution levels 10% and 20%. Also, untreated bran and long-chain inulin were used for comparison. Rheological properties of the bran dough (substitution level 20%) were assessed with Kieffer extensibility test for uniaxial extension and Chopin Rheofermentometer for CO_2 retention and dough development. Viscosity of freeze dried dough was measured by RVA to assess the state of starch. Bread texture was analysed by measuring specific volume, density and instrumental texture by TPA test. Chemical analyses for native and bioprocessed bran were accomplished. The results were analysed by Pearson correlation and PLS.

The specific volume of bread decreased up to 30%, and hardness increased up to 130% by the addition of 20% untreated bran. Addition of 10% untreated bran didn't have a significant effect on bread volume. Treatment of bran with yeast and enzymes affected positively specific volume and crumb hardness of bread, and modified the properties of dough: extensibility decreased 40% but resistance to extension remained on the level of wheat dough, and CO_2 production accelerated 40% but CO_2 retention ability was reduced 11%. Bioprocessing of the bran increased the amount of soluble arabinoxylans 3–7-fold depending on the bioprocessing type and the total amount of dietary fibre in bran was decreased up to 7 percentage units. This study is financially supported by the European Commission in the communities 7th Framework Programme, Project DREAM (222654-2), in which the aim is to design food models and to develop methods that help to better assess food processing for improved quality.

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Production of antimicrobial compounds *in situ* by sourdough consortium fermentation

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Strains belonging to *L.sanfranciscensis*, *S.cerevisiae* and *C.milleri* are involved in the production of specific metabolites in sourdough such as volatiles and organic acids. Some of those compounds, phenyllactic, lactic, acetic acids and medium chain fatty acids (MCFAs), can contribute to imrpove sensorial properties and shelf life, due to their antimicrobial activity. The inhibition of moulds in bakery products and the need to reduce ethanol or other antifungals make the generation *in situ* of antimicrobial metabolites important for long shelf life bakery products. A method to produce lactones and MCFAs *in situ* has been set up. A two-step fermentation of a dough (DY 220) was developed: the first step (17 hrs), in a 7 litres fermenter, was performed using *L.sanfranciscensis*. The variables considered were oxygen and osmotic stress. In all the fermentation starch was added as “trapping” compound (Vernocchi et al., 2008). In the second step the conditioned media (CM), obtained by the centrifugation of the fermented dough, was inoculated with different combinations of *L.sanfranciscensis*, *C.milleri*, *S.cerevisiae*, *L.rossiae* and *L.amylovorus* and incubated for 4 hrs. The volatile molecules profiles were obtained by GC-MS-SPME. Regarding the compounds endowed with antimicrobial activity, the more frequent detected were lactones, furanones, acetic acid, and MCFAs. However their concentration depended on the strain and the condition tested. The strain inoculated in the second step that exhibited the higher lactones production was *C.milleri.* Also *L.rossiae* showed a good attitude to produce lactones when inoculated in the CM of *L.sanfranciscensis*. Moreover in all the combinations the release of signalling and aromatic compounds was observed. These results suggest that the exposure of fresh cells of yeast and LAB to CM of *L.sanfranciscensis* generates molecules able to significantly contribute to final product stability. Preliminary results suggest that such interaction between species and metabolites involves communication phenomena as well as conversion (e.g., cyclization of hydroxyacids to lactones) of precursors produced during the first step.

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Thermal treatment influence on the quality of fine rye-bread packaged in different polymer films

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Latvia is a country with wide large bread making traditions especially in rye and fine rye-bread production. Traditionally, fine rye-bread prepares using rye fine rye-flour (type 700), parboiled flour and sourdough. Fine rye-bread fermentation time range from 20 to 24h; shelf-life − from 5 to 10 days. The main problem for Latvia bread enterprises is to prolong bread shelf-life mainly for export. One of the possible ways could be ready-packaged bread secondary thermal treatment, mainly for microbiological safety. However pasteurization process cannot to stop stalling. Bread staling is a process of chemical and physical changes including moisture redistribution, drying, starch retrogradation, increased firmness, as well as loss of aroma and flavors. Bread molding and staling results in the decrease of consumer acceptance for bakery products and also increase in economic losses. Baking destroys most moulds but surface recontamination can occur during packaging.

The main purpose of this study was to investigate the changes in quality parameters of fine rye-bread packaged in different polymer films during thermal treatment process. Whole loafs of bread were placed in polymer pouches, which were sealed in reduced pressure air ambiance, bread was thermally treated in at + (130; 140; and 150) ± 5 ºC within 40min, as long as the core temperature of the samples have reached accordingly +80±1 ºC. For bread packaging pouches were used: anti-fog Mylar®OL12AF and thermo resistant combined polymer material. Main quality parameters was analysed using standard methods: temperature in bread core, bread crumb and crust firmness value, starch granules volume. In the current research it was proved, that polymer films significantly influence fine rye-bread quality parameters changes during thermal treatment. Thermo resistant combined polymer material film could be recommendable for packaged fine rye-bread thermal treatment, for maximal bread quality parameter keeping.

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Influence of bioprocessing and particle size of wheat bran on texture and sensory properties of high fibre wheat bread

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Wheat bran in bread and breakfast cereals are one of the major fibre source in the Western diet (FAO, 1996). The high content of fibre and bioactive compounds of wheat bran provide great nutritional potential and a reason to promote use of bran as source of fibre. Despite the nutritional advantages, the use of native bran can have drawbacks on the taste of cereal foods as well as on texture of breads (Noort et al., 2010). The best way to stimulate the consumption of whole grain products is by improving their perceived attractiveness both in terms of sensory and texture quality. Particle size reduction of bran has been shown to be a useful strategy to improve the technological, nutritional and sensory potential (Hemery et al., 2011; Rizzello et al., 2012), even if negative interactions of bran on gluten network formation have been reported (Noort et al., 2010). The development of innovative processing techniques such as the use of enzymes or fermentation with selected starters have been shown to be a promising approach in overcoming the detrimental effect of bran on the texture of high fibre wheat bread (Katina et al. 2012). Micronization and sourdough fermentation of durum wheat bran has been proved to improve textural and sensory features of bread (Rizzello et al., 2012). In the current work, micronized fractions of wheat bran having different particle size (450, 160, 50 µm) were fermented 8 h or 24 hours with *Lactobacillus brevis* E95612 and *Kazachstania exiguaa* C81116 with or without the addition of commercial enzyme preparations containing α-amylase and β-glucanase and xylanase activities. Breads containing 15% of bioprocessed or native bran were prepared and specific volume, instrumental hardness, acidity level and sensory profile of breads were determined. Kinetics of growth and acidification showed that a faster growth of the starters was allowed in sourdoughs made with 160 and 50 µm bran. When enzymes were added, the growth and acidification were further enhanced. In general, the technological functionality of bran improved significantly due to fermentation both with and without enzymes. Wheat bread containing 8 h fermented and enzyme treated bran with particle size of 160 µm had better volume and shelf-life if compared to wheat control bread and to the other fermented brans having different particle size. Reduction of particle size increased smoothness of mouthfeel and provided darker colour in bran-containing breads. Researches into clarifying the influence of particle size and different fermentation types on the nutritional properties of bran are in progress.

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Characteristics of Chinese steamed bun made from Thai traditional fermentation starter (Loog-Pang)

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Chinese steamed bun (CSB) made from starter dough was studied the constitution of starter dough by using different sources of Thai traditional fermentation starter (Loog-Pang) from different rural areas. In the study, four steps of fermentation were utilized to reconstitute the CSB starter doughs. The starter doughs from each step of fermentation and Chinese steamed bun were characterized. The results showed that the pH values of the mixture of Loog-Pang, wheat flour and water (day zero of fermentation) were in the range of 4.9–5.8. The pH values of all starter dough decreased continuously until the fourth day. The pH values of all starter doughs from the fourth-day fermentation ranged between 3.4–3.8. TTA values of all starter doughs were dramatically increased by the first 24 h of fermentation. The total count of LAB and yeasts increased continuously until the fourth-day fermentation whereby slight increase was found in all starter doughs since the second-day fermentation. The total counts of LAB and yeasts of all starter doughs were in the range between log 9.4–10.8 cfu/g and log 9.3–11.2 cfu/g, respectively. The specific volume of the CSBs made from the Lopburi and Nakornprathom Loog-Pang starter doughs gained higher specific volume (3.80–3.96) than others. The spread ratio of the CSBs made from the Lopburi and Nakornprathom Loog-Pang starter doughs at the fourth-day fermentation achieved the spread ratio values of 1.71 and 1.68, respectively. Regarding the firmness of CSB, it was found that CSB made from the Lopburi, Suphanburi and Nakornprathom Loog-Pang starter doughs at the fourth-day fermentation obtained less firmness (438, 818 and 982 g, respectively) than others. For sensory properties of CSBs, CSBs made from the Lopburi and Nakornprathom Loog-Pang starter dough (the fourth-day fermentation) achieved the highest scores of all attributes except for the appearance of the CSB made from the Lopburi Loog-Pang starter dough.

POSTER PRESENTATIONS

Session 5

Nutritional and sensory properties of fermented foods

Volatile compounds of a commercial Chinese steamed bun and Chinese steamed bun made from a Thai traditional fermentation starter (Loog-Pang)

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The purpose of this study is to survey qualitatively volatile compounds of Chinese steamed bun made from starter dough. The survey was conducted in samples from a commercial producer: starter dough (cSD), Chinese steamed bun dough (cD) and Chinese steamed bun (cCSB) and samples from laboratory-made (prepared from a Thai traditional fermentation starter, Loog-Pang): starter dough fermented 3 days (lpSD3), starter dough fermented 4 days (lpSD4), Chinese steamed bun dough (lpD) and Chinese steamed bun (lpCSB). A total of thirty-three volatile compounds were detected. Volatile compounds was detected from this research were alcohols, aldehyle, esters, ketones, acids, terpenes. Alcohols included ethanol, 1-Hexanol, isoamyl alcohol and ethyl hexanol. The 1-Hexanol was found in lpSD3 and lpSD4. Isoamyl alcohol were found in cSD and cCSB whereas ethyl hexanol was detected from only cCSB. Octadecanal was the only aldehyde detected from this study and found in lpSD3. Esters included ethyl acetate, benzyl acetate, linalyl anthranilate, 2-tertiobutylcyclohexyl acetate, methyl palmitate, methyl stearate, ethyl linoleate and methyl oleate. Ethyl acetate was detected from all samples except Chinese steamed bun dough from both sources. For Benzyl acetate, linalyl anthranilate, 2-tertiobutylcyclohexyl acetate were detected from only lpD. Methyl palmitate was found in lpSD3, lpCSB and cSD. Methyl stearate and methyl oleate were detected from lpSD3 and lpCSB, respectively. Ethyl linoleate was detected from lpSD3 and lpSD4. The 2-Heptadecanone was only ketone detected from cSD and lpSD3. In addition, hexane, dodecane, tridecane, hexadecane, ecosane, butylhydroxytoluene, aromadendrene and limonene were detected from one to two samples per each. Limonene, which is a type of terpene compounds was detected only from Chinese steamed bun from both sources. Regarding acids, myristic acid, palmitic acid and stearic acid were found in test samples.

Alleviation of the adverse effect of cooking on sorghum protein digestibility and protein fractions through malt pretreatment and fermentation

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The rural Sudanese traditionally eat sorghum, which makes up about 80% of their staple food and covering more than 60% of the total cultivated cereal area. Fermented sorghum products stand out as the most sophisticated foods, prepared by complicated procedures. The Sudanese make about 30 different fermented foods and drinks from sorghum. These products seem to be unique in several ways when compared with those of the other African countries. This fact interpreted by that about 12 types of sorghum bread are prepared in rural Sudan, while Africa is not famous for breads. Secondly, a number of foods and drinks are made from malted sorghum grain, including not only opaque but also clear beers; both traditional beverages are not common in Africa. Moreover, energy-rich and easily transportable and prepared food for travelers is made from sorghum malt. Fermentation and germination has been cited as good options for increasing digestibility and improving nutritional quality of sorghum proteins. In this poster session, simple and suitable domestic processing methods (malting and fermentation) with special reference to the combinations of fermentation and malt as an option for increasing digestibility and improving the nutritional quality of sorghum has been conducted. For this, two sorghum cultivars, Mugud (low tannin) obtained from the agricultural Research and Technology Corporation and Karamaka (high tannin) obtained from Western Sudan as a local cultivar were used. The flour of both seeds cultivars was mixed with 5% malt. Then the flour with or without malt was fermented for 16 h. Samples were taken every 4 h during fermentation to study changes in pH, titratable acidity, protein content, in vitro protein digestibility and protein fractions. Fermentation of the flour with or without malt resulted in an increase in crude protein content and titratable acidity with a decrease in pH for both cultivars. Moreover, the fermented flour with or without malt was cooked to study changes in *in vitro* protein digestibility and in protein fractions of the cultivars. For both cultivars, a highly significant ($P \leq 0.05$) increase in (globulin + albumin) fraction was observed during fermentation of the flour while other fractions were fluctuated. Cooking of the fermented dough significantly ($P \leq 0.05$) reduced the fractions of both cultivars except G3-glutelin and the insoluble proteins which were significantly ($P \leq 0.05$) increased. Malting followed by fermentation had a slight effect on the fractions except G3-glutelin and the insoluble proteins which were significantly ($P \leq 0.05$) reduced for both cultivars. Cooking significantly ($P \leq 0.05$) reduces the *in vitro* protein digestibility of the treated cultivars but the extent of the reduction is lower in malted samples. Results obtained revealed that addition of malt followed by fermentation is a useful method to improve the nutritional value of sorghum even after cooking.

Aromatic and sensory characterization of dry sourdough

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Bread consumption had decreased since the beginning of the 20st century and, in this context, use of sourdough could be a way to improve and diversify bread range, regarding its aromatic contribution (1,2). However, considering the sourdough process constraints, an alternative could be the use of Dry SourDough (DSD) for bread sensory properties enhancement. The aims of this study were i) to characterize DSD and DSD bread volatiles compounds and ii) to study the effect of sourdough process parameters on DSD aromatic profile. The factors studied were the flour type (ash content), the homofermentative / heterofermentative lactic acid bacteria ratio and the SD fermentation time. At the end of the fermentation phase, liquid sourdough was dried with a drum dryer. Volatile compounds of the subsequent DSD and the DSD bread samples were extracted by Headspace Solid Phase MicroExtraction, and then identified and quantified by GC-MS (3). Finally, a sensory analysis was performed to evaluate the effect of DSD on bread flavor perception.

The different DSD parameters tested influenced volatiles compounds and DSD aromatic profile. Major variations of aldehydes, acids and alcohols proportions were observed. Moreover, changes were noticed on aldehydic compounds considering their synthesis pathway. Addition of DSD in bread recipes enlarged the diversity of bread volatiles *vs* the control. Differences were observed between volatile compounds of breads obtained with the different DSD studied. These observations were confirmed by sensory analysis.

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Artisanal lactic acid bacteria utilization for the development of gluten-free sourdough

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Celiac disease (CD), gluten sensitive enteropathy, is a chronic inflammatory disease characterized by atrophy of the villi on the small intestine mucosa, and is induced in genetically susceptible people by the ingestion of proteins present in gliadins in gluten, secalins in rye and hordeins in barley (Di Sabatino and Corazza, 2009). The strict withdrawal of gluten from the diet leads to a mucosal recovery and healthy small intestine. Although gluten-free product market is growing, quality deficiencies make the development of new solutions necessary. Since gluten is responsible for extensibility and elasticity of dough, absence of gluten may lead to insufficient structure, especially in bread production. The enhanced quality of bread can be accomplished by the utilization of sourdough fermentation.

In our experiments, several artisanal lactic acid bacteria (LAB) cultures have been used to develop wheat sourdough fermentation. During the process, dough acidity profile and LAB counts were recorded during 48 h of fermentation. Also, the electrophoretic patterns of gliadins were investigated by SDS-PAGE and 2-D electrophoresis. As fermentation progressed acidity was increased by the microbial activity. Electrophoresis results pointed out there have been some modifications related to gliadin hydrolysis. Acidity increased with the activity of LAB and that probably caused the activation of acidic proteinases of wheat such as aspartic and serine proteinases (Bleukx et al., 1997;1998). Additionally, studies related to incorporation of naturally gluten-free flours and starches in gluten-free sourdough formulations are underway. The enhancement of quality characteristics (texture, sensory, nutritional value etc.) of dough and bread is aimed with the usage of functional ingredients in sourdough technology.

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Enhancing iron bioavailability from tef-injera by improving the fermentation process

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In Ethiopia anemia and iron deficiency are highly prevalent in young children and are a major public health concern. Children are introduced early to injera, the Ethiopian staple food prepared by fermenting and baking of tef flour. Injera could potentially be a good source of minerals, such as iron, but the bioavailability of the minerals is limited by the absorption inhibitor phytic acid (PA) present in tef. PA can be degraded by the enzyme phytase present in microorganisms and certain cereals.

The objective of this study was to potentially increase the bioavailability of minerals from injera by improved fermentation using selected lactic acid bacteria (LAB) and partly replacing tef by other cereals.

Lactic acid bacteria, exhibiting PA degrading activity were isolated from injera-dough and used as starter cultures in small-scale fermentation. After 48 hours, the pH was measured and PA degradation was calculated following determination of inositol phosphate (IP6+IP5-level). The selected strains were characterised by 16S rDNA sequencing and their pre-dominance after 48 hours was assessed by $(GTG)_5$ foot printing. To assess the quality of the resulting product, the fermentation was upscaled and the injera pancake was baked in a hot pan.

The pH of inoculated samples decreased to 3.4–3.9 as observed in Ethiopian tef-injera samples. In tef-flour samples, inoculated with strains of *Lactobacillus fermentum*, *L. crustorum*, *L. casei* or *Pediococcus pentosaceus*, PA-reduction did not exceed the PA reduction observed in the non-inoculated control. With selected *L. buchneri* strains, PA was reduced > 60% to around 0.4 g/ 100 g flour. Lower PA levels, down to 0.1 g/ 100 g, were achieved when tef flour was partly replaced by whole grain wheat or barley flour containing native phytase activity. *L. buchneri MF58* and a 75/25 - tef/wheat-variant were selected with traditional tef-injera for baking. The remaining PA in the respective injera pancakes was 0.3, 0.1 and 0.6 g/ 100 g dry matter. Due to the baking process, the final PA-levels were slightly lower than observed in small-scale experiments.

Further mixtures of LAB, yeasts and cereals are under investigation. The most promising combination will be chosen to prepare injera low in PA. The impact of PA reduction on iron bioavailability will be investigated in a human study.

Fibre, protein and mineral fortification of wheat bread through incorporation of both milled and fermented malt rootlet and brewer's spent grain

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Barley ranks fourth amongst all cereals in total world production and cultivation area with its primary use in malting and brewing applications. The two major bulky by-products of beer production are brewer's spent grain (BSG) and rootlets. Currently, these barley derivatives are predominantly used as animal fodder. The aim of our research was to exploit these nutrient-rich raw materials as functional ingredients in wheat bread, up to a level of 20%, thus offering consumers a high nutrition alternative to wholemeal breads with improved technological attributes. BSG and rootlets were milled and then incorporated into bread formulations. Additionally, they were processed by traditional lactic acid bacteria (LAB) fermentation, using *Lactobacillus plantarum* FST 1.7, before use as ingredients.

The four materials (BSG, rootlets, BSG sourdough (SD) and rootlet SD) were characterised from a nutritional perspective to ascertain their potential benefits as functional food ingredients. BSG contained (% w/w); 22.13 % protein including an exceptionally high levels of essential amino acids, 1.13 % minerals, 131 mg/L polyphenols, 50.2 % dietary fibre and 51.1 % (total fats) essential fatty acids. Analyses showed that rootlets contributed natural nutrients and bioactive compounds such as (% w/w); 36.75 % protein, 2.88 % minerals, 102 mg/L polyphenols, 43.0 % dietary fibre and 67.2 % (total fat) essential fatty acids. Rootlets are particularly rich in essential amino acids, particularly lysine (6.6 %, total protein).

Additionally, BSG and rootlet sourdough addition to the bread, at certain levels, resulted in significantly softer breads with increased springiness. Rheological measurements showed a positively correlated increase in resistance of the dough in line with by-product and SD incorporation. Additionally, supplemented breads were acceptable up to levels of 10 % for each ingredient resulting in products which compared favourably with wholemeal breads from a nutrient, technological and textural perspective. Furthermore they were well accepted by sensory panellists. Using these by-products/ SD as a mainstream food ingredient would have the additional benefit of increasing the market value of these brewing by-products.

Gut metabolome modulation in healthy volunteers subjected to two different cereal-based food diets

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The influence of diet on the health of host may be mediated by changes that occur in the composition and metabolism of the gut microbiota. Indeed, the nutritional, trophic and protective activities of this complex ecosystem have been demonstrated. Two groups (15 people each one) of healthy volunteers having a nutritionally equivalent diet were studied. One group consumed only cereal based foods (bakery foods and pasta) obtained by wheat the other one obtained by khorasan wheat an ancient grain. The same intervention study has been repeated in a different period of year. About 110 different gut microflora metabolites were analysed in stools. The main classes of compounds such as alcohols, ketones, aldehydes, hydrocarbons, acids, sulphur compounds, nitrogen compounds and short chain fatty acids (SCFA) were detected in faeces by means of GC-MS/SPME analysis. Results were characterised by a very high individual variability. Moreover the metabolite changes occurring in the two intervention periods (spring/summer and autumn/winter) were different probably due to the interaction of the cereal based foods with the other foods or to other aspects regarding the different lifestyle of the two periods (sport, work or holiday period, psychological condition etc.). On the other hand SCFA and sulphur compounds increased in khorasan wheat fed volunteers in both the intervention periods. The other groups of compounds varied not homogeneously in terms of quali/quantitative metabolite changes. We may conclude that gut metabolome "*per se*" showed a weak efficacy in discriminating the effect these two cereals in healthy volunteers. Anyway SCFA and sulphur compounds in khorasan wheat fed volunteers seemed a suitable marker of diet intervention.

Phytase-active yeast and lactic acid bacteria isolated from grain-based food

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The consumption of whole-grain-based products has been recommended because of their high content of dietary fiber, B-vitamins, vitamin E and several minerals of which P, Mg, Fe, Cu, Ca and Zn are the most important. However, the main part of minerals in cereals are complexly bound to phytic acid as phytate, a phosphor-rich component used by all cereal grains as mineral store. Phytate are insoluble at psychological pH, and, therefore, minerals are unavailable for absorption in the human intestine. Reduction of phytate in grain-based food by cutting off the phosphate groups causes an increased absorption of the minerals. Reduction of phytate can be achieved by enzymatic degradation during food processing activity of phytases from the cereals or by addition of phytase-active lactic acid bacteria (LAB) or yeasts. However, there seems to be no high phytase-active yeasts or LAB available for bread industry today.

Identification of high phytase-active microorganisms is necessary in order to find prominent candidates for the production of wholemeal bread with high content of bioavailable minerals. High phytase-active yeasts and/or LAB adapted in sourdough matrixes might be a good choice for the bread industry. Therefore, the goal was to identify phytase producing yeasts and LAB from different sourdoughs and to measure the activities.

In total, 221 yeast and 168 LAB colonies were isolated from Danish and Lithuanian sourdoughs. To investigate the phytase activity of yeast and LAB isolates growth test was done by using media with phytic acid dipotassium salt as only phosphorus source. Due to the fact that some microorganisms may grow on solid medium but not in liquid medium and *vice versa*, growth tests were performed both on solid and in liquid medium. Furthermore, volumetric and specific activities were calculated for both yeast and LAB intra- and extracellular phytases. The testing of phytase-positive isolates were carried out at conditions optimal for leavening of bread dough (pH 5.5 and 30 °C).

High extracellular phytase activity was found in isolates of *S. cerevisiae*, followed by *C, humilis* and *P. kudriavzevii*[1]. *Candida humilis* has, to the best of our knowledge, never been described as phytase positive. Within LAB isolates, *L. frumenti* had the highest extracellular volumetric activity.

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Propionibacteria produce active vitamin B12 in cereal matrices

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In situ production of vitamin B12 in cereal matrices using food grade microbes (such as propionibacteria) offers a novel application of fermentation for natural enrichment of plant-based foods with vitamin B12. In addition, it is essential to verify that the synthesized B12 vitamers in cereal matrices are active forms of vitamin B12. Vitamin B12 synthesis in this study was carried out in barley and rye malt matrices by fermenting sterilized malt matrices (33% w/v) using a strain of *Propionibacterium freudenreichii* subsp. *shermanii* at 30°C for 24 h. The vitamin B12 in fermented matrices was extracted as cyanocobalamin, and analyzed by both microbiological assay (MBA) and by our new UPLC/UV method with immunoaffinity purification. A UPLC-MS (Quadrupole ion trap) was used to confirm that the synthesized vitamers were active vitamin B12.

Average vitamin B12 production in barley and rye malt matrices (n = 3) by *P. shermanii* was 2.5 µg/100 g and 2.7 µg/100 g, respectively, according to the MBA. The results obtained by the UPLC/UV method were 60–70% of MBA results. The UPLC-MS revealed the presence of 5,6-dimethylbenzimidazole (DMBI) as lower ligand in the structure of the vitamin B12 form in sample extracts, thus confirming that the synthesized vitamer was active vitamin B12. The difference between MBA and UPLC/UV results could be due to vitamin B12 analogues and other substances produced by microbes that give a positive response in the MBA or immunoaffinity purification associated variations in UPLC analysis. Nevertheless, the synthesized amount is significant when the dietary intake level is considered (recommended intake 2.5 µg/d). We are further investigating other potential propionibacteria and factors related to food matrices affecting B12 synthesis to maximize vitamin B12 production in cereal and other plant-based matrices.

This preliminary study showed that propionibacteria can produce relevant amount of active vitamin B12 in cereal models that fermentation of cereal-based foods can be of significant relevance for the natural enrichment of foods with vitamin B12.

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Title	**V Symposium on Sourdough** **Cereal Fermentation for Future Foods 2012**
Author(s)	Kati Katina, Katri Hartikainen, Kaisa Poutanen & Annemari Kuokka-Ihalainen
Abstract	Throughout the world different kinds of fermented cereal foods constitute a fundamental part of the diet. These foods also give their flavour to identity of local food cultures. Having roots in artesanal traditions, modern use of cereal fermentation relies on science-based understanding of microbial metabolism and its impact on the biologically active cereal matrix. The diversification of industrial raw materials and products and awareness of healthy nutrition opens new applications to fermentative processing steps. The 2012 symposium in Helsinki follows a successful series of international sourdough symposia held in Verona, Brussels, Bari and Freising. The aim is to feature the latest scientific progress in the interplay of the fermentation microbes and the cereal raw material, ranging from microbiological, biochemical, molecular and biological aspects to technological, nutritional and consumer properties of the products.
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VTT employees publish their research results in Finnish and foreign scientific journals, trade periodicals and publication series, in books, in conference papers, in patents and in VTT's own publication series. The VTT publication series are VTT Visions, VTT Science, VTT Technology and VTT Research Highlights. About 100 high-quality scientific and professional publications are released in these series each year. All the publications are released in electronic format and most of them also in print.

VTT Visions

This series contains future visions and foresights on technological, societal and business topics that VTT considers important. It is aimed primarily at decision-makers and experts in companies and in public administration.

VTT Science

This series showcases VTT's scientific expertise and features doctoral dissertations and other peer-reviewed publications. It is aimed primarily at researchers and the scientific community.

VTT Technology

This series features the outcomes of public research projects, technology and market reviews, literature reviews, manuals and papers from conferences organised by VTT. It is aimed at professionals, developers and practical users.

VTT Research Highlights

This series presents summaries of recent research results, solutions and impacts in selected VTT research areas. Its target group consists of customers, decision-makers and collaborators.

Cereal Fermentation for Future Foods 2012

Throughout the world different kinds of fermented cereal foods constitute a fundamental part of the diet. These foods also give their flavour to identity of local food cultures. Having roots in artesanal traditions, modern use of cereal fermentation relies on science-based understanding of microbial metabolism and its impact on the biologically active cereal matrix. The diversification of industrial raw materials and products and awareness of healthy nutrition opens new applications to fermentative processing steps.

The 2012 symposium in Helsinki follows a successful series of international sourdough symposia held in Verona, Brussels, Bari and Freising. The aim is to feature the latest scientific progress in the interplay of the fermentation microbes and the cereal raw material, ranging from microbiological, biochemical, molecular and biological aspects to technological, nutritional and consumer properties of the products.

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