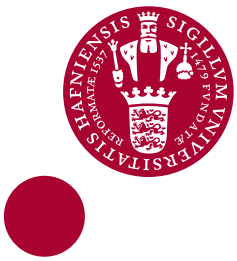

Identification and safety evaluation of Bacillus species occurring in high numbers during spontaneous fermentations to produce Gergoush, a traditional Sudanese bread snack

Thorsen, Line; Abdelgadir, W.S.; Rønsbo, Mie Hvillum; Abban, Stephen; Hamad, S.H.; Nielsen, Dennis Sandris; Jakobsen, Mogens

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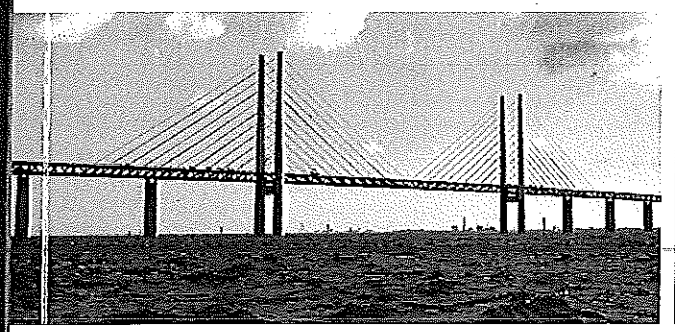

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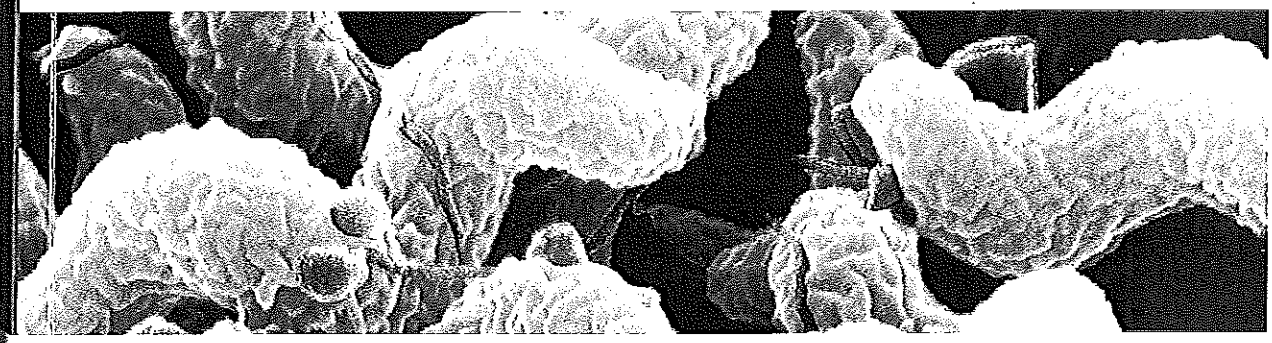

Final Programme & Abstract Book

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PEA1.69 **Rapid GC-MS-XCMS method for determination of *Gamma-aminobutyric acid* (GABA) produced by *Lactic acid bacteria* in various media.**

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A new method for a simultaneous determination of gamma-aminobutyric acid (GABA), other amino acids and precursors, produced by microorganisms, is proposed.
GABA is a ubiquitous non-protein amino acid, which can be produced during the bacterial fermentation by the alpha-decarboxylation of glutamic acid catalyzed by the enzyme glutamate decarboxylase. GABA is an important bioactive compound associated to functional and probiotic food products. The consumption of GABA-enriched foods such as yogurt, soybean, tempeh is reported to depress the elevation of systolic blood pressure in spontaneously hypertensive subjects. Conventional methods for the determination of GABA such as capillary electrophoresis, HPLC or amino acid analyzer are specific for other amino acids, expensive in terms of solvents and time consuming. With the method proposed it was possible to the detect all amino acids and GABA simultaneously. In this work a short time sample derivatization (30 seconds), with ethyl chloroformate, followed by a direct injection in the gas-chromatography coupled to a mass spectrometry (GC-MS) allowed a quantitative determination of GABA, glutamic acid, alpha-ketoglutaric acid and other amino acids in a minimal medium specific for lactic acid bacteria. In less 30 minutes it was possible to perform derivatization, chromatographic and data analysis by means of a new algorithm, XCMS. The method proposed, validated by Nuclear Magnetic Resonance (^{1}H-NMR), allowed a simultaneous determination of amino acids, in particular of GABA and glutamate at nanomole level. A screening of various strains of *Lactobacillus brevis* isolated from different food systems was performed. The strains, grown in MRS and in Skim Milk media added or not with sodium glutamate as a precursor, showed different performances in terms of GABA production. In particular one strain isolated from sourdough produced 3000 ppm in 24 hours, while another strain isolated from wine produced 5000 ppm after 144 hours in the presence of glutamate. The method proposed provides reliable and reproducible results and a methodological simplicity. Therefore it can contribute to a quick determination of GABA which is considered as an important functional molecule and target for selection of lactic acid bacteria used as starters in fermented foods.

PEA1.70 **Identification and safety evaluation of *Bacillus species* occurring in high numbers during spontaneous fermentations to produce Gergoush, a traditional Sudanese bread snack**

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Gergoush is a naturally fermented Sudanese Bread snack produced in three fermentation steps (primary starter, adapted starter and final dough), followed by three baking steps for a half to one hour at above 200°C. In the present study the microbiota of Gergoush produced with four different milk/legume based primary starters (faba beans, chick pea, lentils and white beans) was examined. Specific attention was on the identification of dominant *Bacillus* species and safety evaluation. During the fermentations lactic acid bacteria (LAB) and *Bacillus* spp. occurred in numbers of between 8.2-8.6 and 7.6-9.9 $\log_{10}$ CFU/g, respectively. Specifically the opportunistic pathogen *B. cereus* sensu lato occurred at between 6.1 and 7.8 $\log_{10}$ CFU/g. No bacteria were detected after baking. The pH ranged between 4.1 and 5.0. Two hundred *Bacillus* spp. isolates originating from the four different primary starters were further investigated. Species specific identifications was performed using internally transcribed 16-23S rRNA PCR, 16S rRNA gene sequencing, and selected phenotypic tests. Randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) with the PM13 primer was useful for grouping of the *Bacillus subtilis* group isolates at species level. Depending on the legume used, 40-68% of the isolates were identified as *B. cereus* sensu lato, 16-27% as *B. licheniformis*, 8-32 % as *B. subtilis* and 4-20% as *B. sonorensis*. One hundred and eighty *B. cereus* sensu lato isolates from the primary starter, adapted starter and final dough were identified as *B. cereus* sensu stricto (118 isolates) and *B. thuringiensis* (62 isolates) using the relevant phenotypic and genotypic tests. The safety of Gergoush was evaluated by use of PCR. None of the investigated *B. cereus* isolates were PCR positive with the EM1 primers specific for *B. cereus* strains producing the heat stable emetic toxin cereulide. *B. cereus* isolates from the primary starter were found to harbor at least one of the heat labile enterotoxin encoding genes *nhe(A,B or C)*, *hbl(A,D or C)* or *cytK* by PCR. Considering that no bacteria survived the baking process, and that the cereulide synthetase genes were not detected, the results indicate that Gergoush is a safe product. This study provides a novel method (PM13-PCR) for identifying *B. subtilis* group spp., and it is the first to identify the *Bacillus* of Gergoush to species level.

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PEA1.72 **Growth**

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