Survival and growth of Salmonella and Vibrio in som-fak, a Thai low-salt garlic containing fermented fish product

Fermentation of raw fish is a common process in Asia for improvement of shelf life and safety, however, little is known about the survival of pathogenic bacteria in these products. Raw fish may be contaminated with Salmonella and Vibrio species. The purpose of this study was to determine survival and potential growth of Salmonella enterica serovar Weltevreden, S. enterica serovar Enteritidis, Vibrio cholerae and V. parahaemolyticus as influenced by the preservation parameters (sodium chloride, garlic and lactic acid) present in the Thai fermented fish product som-fak. The inhibitory effects of sodium chloride (0–4%), garlic (0–10%) and lactic acid (pH levels as in som-fak) were measured in modified brain heart infusion (BHI) broth at 30 °C. All bacteria were inhibited by 8–10% sodium chloride. Salmonella grew in all concentrations of garlic whereas Vibrio spp. were inhibited by 1.0–1.5%. Lactic acid was inhibitory at levels above 1.5%. The combinations of sodium chloride, lactic acid and garlic showed a distinct hurdle effect in the broth system. Neither S. Enteritidis, V. cholerae nor V. parahaemolyticus grew in garlic (0.5–1%), regardless of the level of sodium chloride (0.5–4% (w/v)), when lactic acid (0.5–2%) was present. S. Weltevreden was the least inhibited of the four bacteria and grew in the combination of 0.5% garlic and 0.5% lactic acid regardless of the NaCl level (0.5–4% (w/v)). Som-fak with 0 to 10% garlic or 2% glucose was inoculated with either (i) 103 CFU/g Salmonella Weltevreden, (ii) 106 CFU/g garlic fermenting Lactobacillus plantarum strain 509 or (iii) a combination of the two strains and stored at 30 °C. The Salmonella count increased to N108 CFU/g (N106 CFU/g for 10% garlic) in all types of som-fak inoculated with S. Weltevreden within the first day. Only a combination of at least 6% garlic and L. plantarum 509 was enough to prevent growth of the inoculated Salmonella whereas adding the Lactobacillus strain alone or in combination with glucose was insufficient to prevent growth. Our results show that Salmonella Weltevreden can grow in som-fak independently of the inhibitory substances normally present in this type of product, emphasising the importance of preventing contamination. However, our results also suggest that the use of garlic fermenting starter cultures in combination with garlic could improve safety of fermented fish products. © 2009
Fermentation and microflora of plaa-som, a Thai fermented fish product prepared with different salt concentrations

Plaa-som is a Thai fermented fish product prepared from snakehead fish, salt, palm syrup and sometimes roasted rice. We studied the effects of different salt concentrations on decrease in pH and on microflora composition during fermentation. Two low-salt batches were prepared, containing 6% and 7% salt (w/w) as well as two high-salt batches, containing 9% and 11% salt. pH decreased rapidly from 6 to 4.5 in low-salt batches, whereas in high-salt batches, a slow or no decrease in pH was found. Lactic acid bacteria (LAB) and yeasts were isolated as the dominant microorganisms during fermentation. LAB counts increased to 10^8-10^9 cfu g^-1 and yeast counts to 1-2 log lower. Phenotypic tests, ITS-PCR, carbohydrate fermentations and 16S rRNA gene sequencing identified LAB isolates as Pediococcus pentosaceus, Lactobacillus alimentarius/farciminis, Weisella confusa, L. plantarum and Lactococcus garviae. The latter species was only isolated from high-salt batches. Phenotypic characteristics, ITS-PCR and carbohydrate assimilation identified 95% of the yeasts as Zygosaccharomyces rouxii. It is concluded that the fermentation of plaa-som is delayed by a salt-level of 9% due to an
inhibition of LAB growth. The growth of Z. rouxii has no influence on the fermentation rate, but may contribute positively to the flavour development of the product.

**General information**
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Authors: Paludan-Müller, C. (Intern), Madsen, M. (Ekstern), Sophanodora, P. (Ekstern), Gram, L. (Intern), Møller, P. (Ekstern)
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Fermenteret fisk - tradition og fremtid

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Genotypic and phenotypic characterization of garlic-fermenting lactic acid bacteria isolated from som-fak, a Thai low-salt fermented fish product

AIMS: To evaluate the importance of garlic for fermentation of a Thai fish product, and to differentiate among garlic-/inulin-fermenting lactic acid bacteria (LAB) at strain level. METHODS AND RESULTS: Som-fak was prepared by fermentation of a mixture of fish, salt, rice, sucrose and garlic. pH decreased to 4.5 in 2 days, but omitting garlic resulted in a lack of acidification. LAB were predominant and approximately one third of 234 isolated strains fermented garlic and inulin (the carbohydrate reserve in garlic). These strains were identified as Lactobacillus pentosus and Lact. plantarum. Randomly Amplified Polymorphic DNA (RAPD) analysis revealed one major RAPD type (29 strains) isolated from all stages of fermentation. CONCLUSION: Garlic was essential for acidification of som-fak and garlic-fermenting strains constituted a significant, homogeneous part of the LAB flora. SIGNIFICANCE AND IMPACT OF THE STUDY: The present study indicates the role of fructans (garlic/inulin) as carbohydrate sources for LAB. Fructan fermenters may have several biotechnological applications, for example, as probiotics.

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Microbiology of fermented fish products: Significance of garlic and lactic acid bacteria with fructan hydrolase activity

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Purification and characterisation of an extracellular fructan beta-fructosidase from a Lactobacillus pentosus strain isolated from fermented fish

Lactobacillus pentosus B235, which was isolated as part of the dominant microflora from a garlic containing fermented fish product, was grown in a chemically defined medium with inulin as the sole carbohydrate source. An extracellular fructan beta-fructosidase was purified to homogeneity from the bacterial supernatant by ultrafiltration, anion exchange chromatography and hydrophobic interaction chromatography. The molecular weight of the enzyme was estimated to be approximately 126 kDa by gel filtration and by SDS-PAGE. The purified enzyme had the highest activity for levan (a beta(2→6)-linked fructan), but also hydrolysed garlic extract, (a beta(2→1)-linked fructan with beta(2→6)-linked fructosyl sidechains), 1,1,1-kestose, 1,1-kestose, 1-kestose, inulin (beta(2→1)-linked fructans) and Sucrose at 60, 45, 39, 12, 9 and 3%, respectively, of the activity observed for levan. Melezitose, raffinose and srachyose were not hydrolysed by the enzyme. The fructan P-fructosidase was inhibited by p-chloromercuribenzoate, EDTA, Fe2+, Cu2+, Zn2+ and Co2+, whereas Mn2+ and Cu2+ had no effect. The sequence of the first 20 N-terminal amino acids was: Ala-Thr-Ser-Ala-Ser-Ser-Ser-Gln-Ile-Ser-Gln-Asn-Thr-Gln-Thr-Ser-Asp-Val-Val. The enzyme had temperature and pH optima at 25 degreesC and 5.5, respectively. At concentrations of up to 12%, NaCl no adverse effect on the enzyme activity was observed.

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Characterization of lactic acid bacteria isolated from a Thai low-salt fermented fish product and the role of garlic as substrate for fermentation

Lactic acid bacteria (LAB) isolated from raw materials (fish, rice, garlic and banana leaves) and processed som-fak (a Thai low-salt fermented fish product) were characterized by API 50-CH and other phenotypic criteria. Lactococcus lactis subsp. lactis and Leuconostoc citreum were specifically associated with fish fillet and minced fish, Lactobacillus paracasei subsp. paracasei with boiled rice and Weisella confusa with garlic mix and banana leaves. In addition, Lactobacillus plantarum, Lactobacillus pentosus and Pediococcus pentosaceus were isolated from raw materials. A succession of aciduric, homofermentative lactobacillus species, dominated by Lb. plantarum/pentosus, was found during fermentation. In total, 9% of the strains fermented starch and 19% fermented garlic, the two main carbohydrate components in som-fak. The ability to ferment garlic was paralleled by a capacity to ferment inulin. An increased percentage of garlic fermenting strains was found during fermentation of som-fak, from 8% at day 1 to 40% at day 5. No starch fermenting strains were isolated during fermentation. Three mixed LAB cultures, composed of either starch fermenting Lc. lactis subsp. lactis and Lb. paracasei subsp. paracasei, or garlic fermenting Lb. plantarum and Pd. pentosaceus, or a combination of these strains were inoculated into laboratory prepared som-fak with or without garlic. In som-fak without garlic, pH was above 4.8 after three days, irrespective of addition of mixed LAB cultures. The starch fermenting LAB were unable to ferment som-fak and sensory spoilage occurred after three days. Fermentation with the combined mix of starch and garlic fermenting strains led to production of 2.5% acid and a decrease in pH to 4.5 in two days. The fermentation was slightly slower with the garlic fermenting strains alone. This is the first report describing the role of garlic as carbohydrate source for LAB in fermented fish products. (C) 1999 Elsevier Science B.V. All rights reserved.

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Evaluation of the role of Carnobacterium piscicola in spoilage of vacuum- and modified-atmosphere-packed cold-smoked salmon stored at 5 degrees C

The microflora on spoiled cold-smoked salmon often consists of a mixture of lactic acid bacteria (LAB) and Gram-negative bacteria. To elucidate the role of the different groups, a storage trial was carried out in which nisin and CO2 were used for the selective inhibition of the two bacterial groups. The shelf-life of vacuum-packed cold-smoked salmon, recorded by sensory evaluation, was four weeks at 5 degrees C and the microflora was composed of LAB \(10^6-10^7\) cfu/g with an associate Gram-negative flora in varying levels \(10^5-10^7\) cfu/g). The addition of nisin and/or a CO2-atmosphere increased the shelf-life to five or six weeks and limited the level of LAB to about \(10^4-10^6\), \(10^3-10^6\) and \(10^2-10^4\) cfu/g, respectively. CO2-atmosphere+/-nisin inhibited the growth of Gram-negative bacteria, whereas nisin had no effect on these in vacuum packages. The Gram-negative flora on vacuum-packed salmon was dominated by a Vibrio sp., resembling V. marinus, Enterobacteriaceae (Enterobacter agglomerans, Serratia liquefaciens and Rahnella aquatilis) and occasionally Aeromonas hydrophila. Irrespective of the addition of nisin and/or CO2-atmosphere, the LAB microflora was dominated by Carnobacterium piscicola, which was found to account for 87% of the 255 LAB isolates characterized. Whole-cell-protein patterns analysed by SDS-PAGE confirmed the Carnobacterium species identification. The spoilage potential of C. piscicola isolates was further studied by inoculation of approx. \(10^6\) cfu/g in cold-smoked salmon stored at 5 degrees C. The salmon did not spoil within 4 weeks of storage in vacuum-or CO2-atmosphere, and it is concluded that despite high levels (> \(10^7\) cfu/g) of C. piscicola, sensory rejection was caused by autolytic changes. This was supported by the development of soft texture and sour, rancid and bitter off-flavours at the point of spoilage, irrespective of the length of shelf-life and low or high total counts of LAB and Gram-negative bacteria. (C) 1998 Elsevier Science B.V
Projects:

**Improved utilization of low-value fish**

The specific objectives of this project were in three areas: 1. To examine and adapt traditional Asian preservation technologies for fish products. 2. To investigate and optimise the fermentation process used in traditional Asian fish products. 3. To study the composition and stability of lipids from low-value fish species. The results have identified potential new use of a large number of low-value fish species. The properties of Lactic Acid Bacteria (LAB) isolated from low salt fermented products have been studied and the capacity to ferment inulin from garlic was found to be an important criteria for selection of starter cultures, since garlic is added to most low-salt fermented products. The fatty acid profile from a great number of tropical fish has been determined. The stability of fish oil and the potential of spices as antioxidants has also been investigated.

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Period: 01/01/1994 → 31/10/1997
Number of participants: 4
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