Spoilage microbiota of fresh fish: identification and characterisation of the spoilage potential

Bederfflora van verse vis: identificatie en karakterisatie naar bederfpotentieel

Flores d’altération du poisson frais : de l'identification et la caractérisation au potentiel d’altération

Katrien Broekaert, Bert Noseda & Frank Devlieghere

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Spoilage of fish

- Post mortem changes in fish:
  - Rigor mortis
  - Autolytic & bio-chemical changes

- Bacteriological growth and activity ➔ SPOILAGE OF FISH (95%)
  - Chemical changes (rancidity)
  - Physical changes

- Initial quality loss: autolytic ➔ makes nutrients available for bacterial growth.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Goal (cfu/g)</th>
<th>Tolerance (cfu/g)</th>
<th>End of shelf life (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total aerobic psychrotrophic count</td>
<td>$10^5$</td>
<td>$10^6$</td>
<td>$10^7$</td>
</tr>
<tr>
<td>Psychrotrophic lactic acid bacteria</td>
<td>$10^2$</td>
<td>$10^3$</td>
<td>$10^7$ (a)</td>
</tr>
<tr>
<td>Yeasts</td>
<td>$10^2$</td>
<td>$10^3$</td>
<td>$10^5$</td>
</tr>
<tr>
<td>Moulds</td>
<td>$10^2$</td>
<td>$10^3$</td>
<td>No visual growth</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>$10^2$</td>
<td>$10^3$</td>
<td>$10^3$</td>
</tr>
<tr>
<td>Coagulase positive staphylococci</td>
<td>$10^2$</td>
<td>$10^3$</td>
<td>$10^3$</td>
</tr>
<tr>
<td>Sulfite reducing bacteria</td>
<td>$10^2$</td>
<td>$10^3$</td>
<td>$10^5$</td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>Absent in 25g</td>
<td>Absent in 25g</td>
<td>Absent in 25g</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>Absent in 25g</td>
<td>Absent in x g</td>
<td>$10^2$</td>
</tr>
</tbody>
</table>
Total Viable Count analysis

TVC is defined as the number of bacteria (cfu/g) in a food product which develop into clearly visible colonies when the test is carried out under standard conditions.

TVC is only a measurement of the fraction of the microbiota able to produce colonies in the medium used under the conditions of incubation.

LIMITED INFORMATION

TVC: no measure of “total” bacterial population
no differentiation between types of bacteria
doubtful in the examination of frozen fish
the total number of microorganisms on fish ≠ microorganisms responsible for fish spoilage

microorganisms responsible for fish spoilage = rather only a small fraction of the microbiota

the “specific spoilage organisms” or SSOs (Dalgaard, 1995)

SSOs must be enumerated (and identified) for quality control or determination of the remaining shelf life of fish.

SSOs are specific to each fish species, and are still unknown for many fish species.
## TVC growth media

Growth media used for TVC of fish and fishery products

<table>
<thead>
<tr>
<th>Media</th>
<th>Temperature</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plate Count Agar (PCA) ISO – ICMSF</td>
<td>3d 21 °C</td>
<td></td>
</tr>
<tr>
<td>Iron Agar (IA)</td>
<td>5d 15°C</td>
<td></td>
</tr>
<tr>
<td>Total viable counts (TVC)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>and counts of H$_2$S-producers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marine Agar (MA)</td>
<td>3d 21 °C</td>
<td></td>
</tr>
<tr>
<td>Long and Hammer Agar (LH) - NMKL 184</td>
<td>5d 15°C</td>
<td></td>
</tr>
<tr>
<td>modification by Van Spreekens (1974)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>with an additional 1% w/v NaCl.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
EXAMPLE: DETECTION OF SPOILAGE MICROBIOTA

TVC > 7 log cfu/g ➔ spoilage organoleptically detectable ➔ no longer acceptable for consumption

LARGE DIFFERENCES BETWEEN GENERAL MEDIA!

• PCA lowest TVC, after storage on ice often > 1 log_{10} difference
• Results on MA and LH mostly alike (difference < 0.5 log_{10})
### EXAMPLE: DETECTION OF SPOILAGE MICROBIOTA

<table>
<thead>
<tr>
<th>Not growing on PCA</th>
<th>Only growing on PCA</th>
<th>Not growing on MA</th>
<th>Not growing on LH</th>
<th>Not growing on IA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photobacterium spp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shewanella spp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vibrio spp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudoalteromonas spp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psychrobacter spp.</td>
<td>Psb. cibarius</td>
<td>Psb. cibarius</td>
<td>Psychrobacter spp.</td>
<td>Psb. cibarius</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>Ps. fragi</td>
<td>Ps. fragi</td>
<td>Ps. fragi</td>
<td>Ps. fragi</td>
</tr>
<tr>
<td>Flavobacterium spp.</td>
<td></td>
<td></td>
<td></td>
<td>Brochotrix thermosphacta</td>
</tr>
</tbody>
</table>

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[www.foodscience.ugent.be](http://www.foodscience.ugent.be)
The shelf life of aerobically stored seafood depends on several factors (Jay, 1986):

1) the storage conditions,
2) the intrinsic factors of the seafood, and
3) the qualitative and quantitative composition of the initial microbiota, which is related to the environment, the water temperature, area of catch and the early handling and processing procedures.

Freshly caught seafood is naturally contaminated with a diversity of microbiota: e.g. *Aeromonas*, *Pseudomonas*, *Moraxella/Acinetobacter*, *Shewanella*, *Photobacterium* and *Flavobacterium* species (Liston, 1980).

These microbial populations may shift during storage (Shewan and Georgala, 1957).
<table>
<thead>
<tr>
<th>Type of product</th>
<th>Spoilage bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh chilled aerobic storage - high TMAO, pH&gt;6</td>
<td><em>Shewanella</em> spp.</td>
</tr>
<tr>
<td></td>
<td>- low TMAO, or low pH</td>
</tr>
<tr>
<td>Fresh chilled and modified atmosphere packaged</td>
<td></td>
</tr>
<tr>
<td>Marine products with TMAO</td>
<td><em>Photobacterium phosphoreum</em></td>
</tr>
<tr>
<td>Warm water low TMAO</td>
<td>Lactic acid bacteria; <em>Brochotrix thermosphacta</em></td>
</tr>
<tr>
<td>Tropical freshwater fish</td>
<td><em>Aeromonas</em> spp.</td>
</tr>
<tr>
<td>Fresh and lightly preserved seafood at ambient temp</td>
<td><em>Aeromonas</em> spp., <em>Vibrio</em> spp., <em>Photobacterium</em> spp.,</td>
</tr>
<tr>
<td>Lightly preserved and chilled products</td>
<td>Lactic acid bacteria; <em>B. thermosphacta</em>, <em>P. phosphoreum</em>, <em>Vibrio</em> spp., <em>Enterobacteriaceae</em></td>
</tr>
</tbody>
</table>
Dominant microbiota of shrimp during storage under several conditions/processing:

*Pseudoalteromonas* spp.  
*Psychrobacter* spp.

Fig: DGGE pattern from the microbiota of cooked shrimp swabbed from modified Long and Hammer medium (LH) under different storage and processing conditions.

m= marker, A= *Pseudoalteromonas* spp., B= *Psychrobacter* spp.
Dominant microbiota of ray during storage on ice:

Day 1: *Pseudomonas* spp.  
*Psychrobacter* spp.  
*Pseudoalteromonas* spp.  
*Flavobacterium* spp.

Day 3: + *Shewanella* spp.

Day 9: + *Arthrobacter* spp.

Fig: (GTG)₅ rep-fingerprint from the microbiota of fresh ray stored on ice at day 1.
# Bacterial spoilage of fish

## Freshwater fish

<table>
<thead>
<tr>
<th>Scientific grouping</th>
<th>Biological characteristics</th>
<th>Technological characteristics</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teleostei or bony fish</td>
<td>fatty fish (store lipids in body tissue)</td>
<td></td>
<td>trout, eel</td>
</tr>
<tr>
<td></td>
<td>lean (white) fish (store lipids in liver only)</td>
<td></td>
<td>Tilapia, perch, pike</td>
</tr>
</tbody>
</table>

## Marine fish

<table>
<thead>
<tr>
<th>Scientific grouping</th>
<th>Biological characteristics</th>
<th>Technological characteristics</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclostomes</td>
<td>jawless fish</td>
<td></td>
<td>lampreys, slime-eels</td>
</tr>
<tr>
<td>Chondrichthyes</td>
<td>cartilaginous fish</td>
<td>high urea content in muscle</td>
<td>sharks, skate, rays</td>
</tr>
<tr>
<td>Teleostei or bony fish</td>
<td>pelagic fish</td>
<td>fatty fish (store lipids in body tissue)</td>
<td>herring, mackerel, sardine, tuna, sprat</td>
</tr>
<tr>
<td></td>
<td>demersal fish</td>
<td>lean (white) fish (store lipids in liver only)</td>
<td>cod, haddock, hake, grouper, seabass</td>
</tr>
</tbody>
</table>
Bacterial spoilage of fish

- Initially (aerobic conditions): aerobic organisms use carbohydrates and lactate and produce CO$_2$ and H$_2$O $\rightarrow$ decrease in Eh (surface)

- In marine fish species, a decreased Eh favours facultative anaerobic bacteria (e.g. *Shewanella putrefaciens* and *Enterobacteriaceae*), reducing TMAO to TMA (unpleasant fishy odour):

\[
\text{TMAO-} \\
\text{CH}_3\text{CHOHCOOH} + (\text{CH}_3)_3\text{NO} \xrightarrow{\text{lactic acid reductase}} \text{CH}_3\text{COCOOH} + (\text{CH}_3)_3\text{N} + \text{H}_2\text{O} \\
\text{pyruvate TMAO pyruvate TMA}
\]

\[
\text{CH}_3\text{COCOOH} + (\text{CH})_3\text{NO} + \text{H}_2\text{O} \xrightarrow{\text{pyruvate TMAO acetic acid}} \text{CH}_3\text{COOH} + (\text{CH}_3)_3\text{N} + \text{CO}_2 + \text{H}_2\text{O} \\
\text{pyruvate TMAO acetic acid TMA}
\]
• Breakdown of amino acids:

\[
\begin{align*}
R - CH_2 - CH (NH_2) - COOH & \xrightarrow{\text{deaminase}} RCH_2 - CO - COOH + NH_3 \\
\text{R - CH}_2 - CH - NH_2 & \xrightarrow{\text{oxydase}} RCH_2 - COOH + NH_3
\end{align*}
\]
Bacterial spoilage of fish

- Breakdown of S-containing amino acids cysteine and methionine to $H_2S$, $CH_3SH$ (methanethiol) and $(CH_3)_2S_2$ (e.g. *Shewanella putrefaciens*)
  - detectable even at low ppb levels
  - low quantities have a considerable effect on the sensorial quality

- Decomposition of urea in *elasmobranchii* during storage:
  \[
  (NH_2)_2CO + H_2O \xrightarrow{\text{urease}} CO_2 + 2NH_3
  \]
Bacterial spoilage of grey shrimp

• Example:
  • Storage of MAP (50% CO₂-50% N₂) Grey shrimp (*Crangon crangon*) at 4°C:

![Graph showing bacterial spoilage over storage time]

Storage (Days)

log(kve)/g

0 1 2 3 4 5 6 7 8

Storage of MAP (50% CO₂-50% N₂) Grey shrimp (*Crangon crangon*) at 4°C:
Bacterial spoilage of grey shrimp

• Example:
  • Main metabolites originating from bacterial spoilage of MAP (50% CO₂-50% N₂) *Crangon crangon* stored at 4°C (determined with Thermal Desorption-GC-MS):
    • Trimethylamine
    • Acetaldehyde
    • Ethanol
    • Aceton
    • Isopropanol
    • Dimethylsulfide
    • Dimethyldisulfide
    • Carbodisulfide
Bacterial spoilage of grey shrimp

- Example: Why did spoilage occur so early?

- At a high product pH, amines tend to volatilize more =>

At pH ± 8.0, a minimal amine production might already cause sensorial deviations.

**Figure.** TMA headspace concentrations (µg/m³) of 5.0 mg spiked N in 50.0 g water (+) and in 50g mixed shrimp (●), in function of the pH. (determined with Selective Ion Flow Tube Mass Spectrometry SIFT-MS)
Bacterial spoilage of fish

Factors influencing bacterial growth and metabolism

Intrinsic factors = factors inherent to the fish species

pH, aw, Eh, nutrient content of fish, antimicrobial systems
Distribution of non-protein nitrogen in fish muscles of two marine bonyfish (A,B), an elasmobranch (C), and a freshwater fish (D) (Konosu and Yamaguchi, 1982; Suyama et al., 1977)
Bacterial spoilage of fish

Factors influencing bacterial growth and metabolism

Intrinsic factors = factors inherent to fish
- pH, aw, Eh, nutrient content of fish, antimicrobial systems

Extrinsic factors
- temperature, RH, change of atmosphere (MAP)

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THANK YOU FOR YOUR ATTENTION!

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