PRICE: US $ 10

ISSN: 1114-6672

Numéro Double

إنفوسمك

INTERNATIONAL MAGAZINE

INFOSAMAK

Magazine Spécialisé dans la Pêche et les Industries de poisson

Numéro Double 2-3/2005 (Avril/Septembre)

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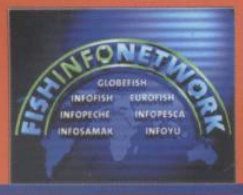

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FAST
a rapid test for determination of spoilage bacteria in fish

By COLIFAST AS

Spoilage of food caused by microorganisms is a concern worldwide. It is estimated that 10 % of global captured and cultured fish are lost post-harvest, mainly due to microbial spoilage.

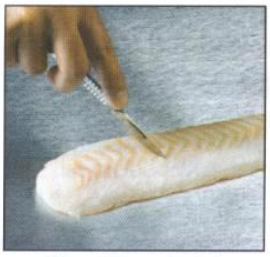
Since microflora adapt to the prevailing circumstances, the population composition changes from captured to final product. The growth and metabolism of microflora causing spoilage is determined by the raw material, food processing, and storage conditions. However, only a fraction of the spoilage microflora contributes to the changes in the sensory properties that make the product unsuitable for human consumption. The concept of specific spoilage organism (SSO), developed by the Danish Institute for Fishery Research Lyngby, has increased the knowledge and understanding of seafood spoilage. In newly captured seafood the SSOs are present in low numbers, they constitute a small fraction of the microflora, and the spoilage potential and activity are determined by their presence. The identification of SSOs facilitates the opportunity to predict the freshness and shelf life, which are important parameters in trading and processing of seafood.

In marine fish stored aerobically and chilled Shewanella putrefaciens is identified as the SSO.

S. putrefaciens is capable of producing H2S and reduction of TMAO to TMA, which during spoilage give the rotten egg and fishy off-odors, respectively. Remaining shelf life of the fish is closely correlated to the number of *S. putrefaciens* detected. Statistical models, like the seafood spoilage predictor (http://www.dfu.min.dk/micro/sssp/), have been developed, where the number of *S. putrefaciens* and storage temperatures are inserted, and the remaining shelf life is then determined.

Methodology

At present, detection and enumeration of S. putrefaciens and sulphide-producing bacteria (SPB) in seafood are performed by a spread plate method. Formation of black colonies on the agar, due to precipitation of iron sulphide (FeS), is a direct measure of the spoilage reaction. Since a 72 hour incubation period is required to maximize the count, and laboratory facilities and trained personal are needed, the method may be considered as time consuming and cumbersome. In 2003, Colifast AS together with the Norwegian Institute for Fisheries and Aquaculture Research developed a rapid test for estimation of the content of SPB in fish. The quantification is based on the formation rate of FeS which is measured as reduced background fluorescence in a liquid medium. This test, FAST, has been further developed and simplified, and require minimum of skills and laboratory facilities. Beside a vial containing the FAST medium, a sterile scalpel and an incubator is required to perform the analysis. The illustration below shows how the FAST test is performed. The measurement principle is based upon formation rate of FeS resulting from the growth of SPB in the liquefied FAST medium. During growth of SPB, ferric iron is converted to ferrous iron, and inorganic and organic sulphur compounds to sulphide, which precipitates as black FeS. The precipitation, which colours



1. Take a 1cm3 sample.

2. Place sample in FAST vial.

3. Incubate sample at 30°C.

4. Simple visual reading

the FAST medium black, is observed visually. The time to detect (TTD) the colour change from yellow to black in is correlated to the number of SPB found by the spread plate method. Levels of SPB are then estimated from the TTD as shown in Table 1.

The benefits of using the FAST test are summarized below:

Table 1. Interpretation of TTD and levels of SPB

Hours to colour change Yellow → Black	SPB/g	Quality Guideline*
3-6	> 5 000 000	Poor
7-10	500 000 - 5 000 000	Marginal
11-12	1000 - 500 000	Good
> 12	< 1000	Very good

* Recommendation given by the Norwegian Food Authority.

- Results are obtained within 3-12 hours.
- No special skills are required.
- An incubator and a sterile scalpel are needed.
- On site analyses and results.
- Handheld and portable.

Application areas

The demand for microbial testing is increasing and the food industry represents 49% of the total volume of industrial testing performed today. There is also an increasing awareness among consumers about food quality. To maintain satisfied customers who are loyal is important. In today's competitive market this means hard work, and the cost of losing a customer is high. Using the FAST test may ensure the customers, both the end-consumer and in the supply chain, that the quality is consistent with their requirements. Adverse publicity may be costly, and players that do not meet these requirements will lose in the long term.

The FAST test reveals whether the fish has been stored for a long time or/and if the chilled storage chain has been interrupted. Since the content of SPB determines the shelf life and freshness, it is of great interest to everyone handling fish that the content of SPB is low.

Since FAST requires minimal equipment and can be performed by persons without skills in microbiology, supermarkets are a target market. To our knowledge from Norway the turnover rate of employees at the fish-counter is high, meaning that persons with experience in fish quality and handling is lacking. This is a problem, since the fish-counter plays an essential part in attracting customers. Applying FAST as a tool in quality control and training of the employees, may yield a competitive advantage. Labels, marked "quality tested", are provided at the fish-counter and on the wrapping paper, to assure the customers.

One problem with microbial analyses is that they are retrospective, requiring days to provide results. Consequently, the tested food has either been consumed or at least released to the market. The FAST test provides the speed to result that many food companies need and require, to prevent unacceptable ingredients from entering the plant. Applying FAST gives the manufacturer itself the possibility to conduct in-process testing in the factory. This is a great benefit and is consistent with HACCP programmes. Using FAST may help the manufacturer to more quickly release product in the market with a high level of assurance that the quality is as prescribed. Use of FAST may work as an unbiased tool for complaint documentation, instead of sensory evaluation, which to some extent, is considered subjective.

Another application area for FAST is the sorting of raw material coming into the processing plants. Insurance as to whether the raw materials meet the company's specification for microbial integrity is of utmost importance. This will also give the producer an economic benefit, minimising wastage of raw material that can otherwise be used in lower value products.

Further perspective

S. putrefaciens is a bacterium capable of forming biofilms (Bagge, et al., 2001). Colifast has recently developed a swab test, FAST SWAB, based on the same principle as the FAST test. This gives the possibility to conduct rapid sanitary evaluation of target spoilage organisms like SPB on food preparation surfaces.

S. putrefaciens is also considered as a common and important spoilage bacterium in poultry, stored aerobically and chilled. Colifast has recently joined the largest poultry producer in Norway, in evaluating a spoilage test for SPB in poultry products.

FAST has international patents and patents pending.

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