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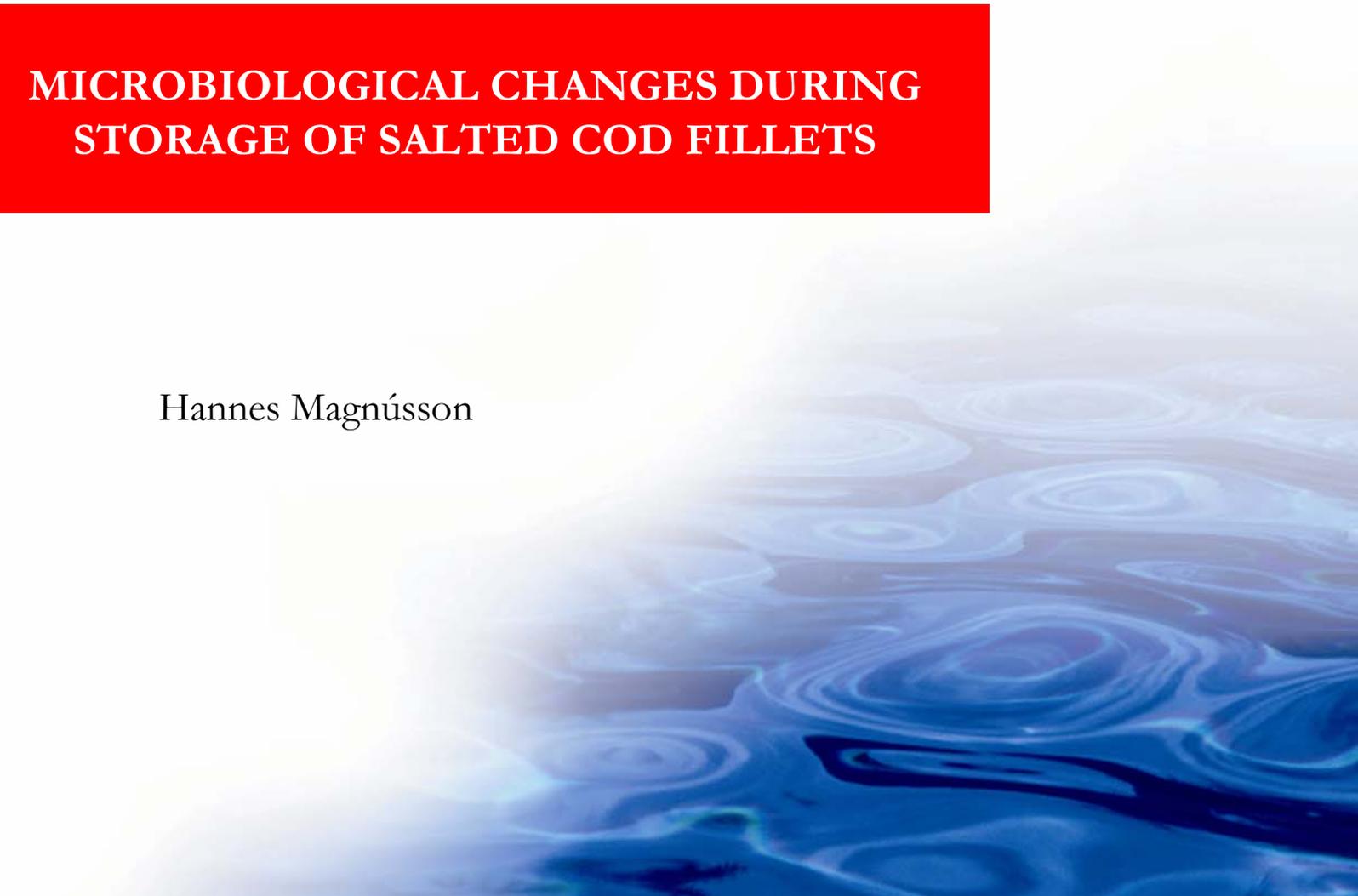


Rannsóknastofnun fiskiðnaðarins

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**MICROBIOLOGICAL CHANGES DURING
STORAGE OF SALTED COD FILLETS**

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<i>Titill / Title</i>	Microbiological changes during storage of salted cod fillets		
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<i>Ágrip á íslensku:</i>	<p>Tilraunir þessar voru gerðar á Rf árin 1992-1993 og hafa niðurstöður þeirra aldrei verið birtar. Söltuð þorskflök með um 20% saltinnihaldi voru geymd við 5°C í 330 daga og fylgst var með breytingum á örverufjölda og efnavísium yfir geymslutímann. Í ósöltuðum flökum fundust engir mjög saltkærir gerlar en við söltunina menguðust þau af slíkum gerlum. Eftir þækilsöltun fór gerlum fækkandi á öllum ætum sem notuð voru. Fyrstu 70 daga geymslunnar fundust svo til eingöngu roðagerlar á 20% söltu æti en eftir þann tíma náðu yfirhöndinni gerlar, sem mynduðu hvítar og gulleitar kóloníur á ætinu. Aldrei varð vart við roðaskemmdir á flökunum. Í lok geymslutímans var TVB ennþá undir 20mgN/100g og TMA fór aldrei yfir 1mgN/100g. Niðurstöðurnar sýna að söltuð þorskflök með um 20% saltinnihaldi sem geymd eru við 5°C eru mjög "stöðug" vara frá örverufræðilegu sjónarmiði.</p>		
<i>Lykilorð á íslensku:</i>	<i>Söltuð þorskflök, örverur, TVB, TMA, geymsla</i>		
<i>Summary in English:</i>	<p>The experiments presented here were carried out during the years 1992-1993 at IFL and have never been published.. The aim of these studies was to investigate microbiological and related chemical changes which take place during storage of salted cod fillets with about 20% salt content kept for up to 330 days at 5°C. Unsalted cod fillets did not contain any extreme halophilic bacteria but as soon as the fillets came into contact with the solar salt used, they were contaminated with such bacteria. After brining, a slight decrease in bacterial numbers occurred on all media tested over the storage period. During the first 70 days of storage, almost all bacteria on medium containing 20% salt were red-halophiles. As storage progressed numbers of such bacteria were overtaken by bacteria forming white and yellow colonies on this medium. The fillets never showed any signs of reddening ("pink"). At the end of storage on day 330, TVB was still under 20mgN/100g and TMA values never exceeded 1mgN/100g. Salted cod fillets containing about 20% salt and kept at 5°C are a very stable product from microbiological point of view.</p>		
<i>English keywords:</i>	<i>Salted cod fillets, microorganisms, TVB, TMA, storage</i>		

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1. INTRODUCTION

Salted fish has been exported from Iceland to some extent since the turn of the 20th century. It was in fact the main export product until World War II when frozen fish took over. In 2004, export of fishery products amounted to 60% of the total Icelandic export earnings. Salted fish, mainly cod (*Gadus morhua*), accounted for 6.6% of exported fishery products providing 17.4% of export value.

Salting of fish and fish products is a traditional preserving method. Many consumers, especially in southern Europe appreciate the special flavour and texture characteristics of desalted fish products. Therefore, salting is not only a method to prolong shelf life, but a method to produce fish products meeting the demands of selective consumers. During the salting process, chemical, flavour and textural changes occur. These changes are responsible for the ripening of salted cod. Fully salted cod contains about 20% salt and therefore has to be desalted prior to consumption. By far, the two main markets for salted cod are Portugal and Spain.

Very few studies have been published on the microbiological changes which take place during production and storage of salted cod. So far, microbiological studies have mainly been focused on red-halophilic bacteria which can lead to reddening of the fish if kept above 7°C ("pink") and osmophilic moulds forming brown or black spots ("dun"). The red-halophilic bacteria belong to two genera; *Halobacterium* which requires at least 10-15% salt for growth and *Halococcus* which needs at least 5-10%. Both groups grow best at around 20-25% salt. These bacteria are present in the solar salts (made from sea-water) used in the salting process (Shewan 1971, Huss and Valdimarsson 1990).

The experiments presented here took place in 1992-1993 at the Icelandic Fisheries Laboratories (IFL) and have never been published. The aim of these studies was to investigate microbiological and related chemical changes which take place during production and storage of salted cod fillets kept for up to 330 days at 5°C.

2. MATERIAL AND METHODS

2.1. Experimental design

Newly caught cod was used for the experiments. Cod fillets were brine-salted for 5 days and dry-salted for 7 days after which the fillets were packed in wax-coated

cardboard boxes and kept at 5°C for up to 330 days. For each kg of fillet, 1 kg of salt was used, thereof 1/3 for brining and 2/3 for dry-salting. Samples were collected from the raw material (day 0), from brined fillets on days 1, 2 and 5, after 7 and 9 days during dry-salting and on day 12 of newly packed fillets. Thereafter, samples were periodically taken during storage. Triplicate measurements were done on each sampling point.

2.2. Methods

2.2.1. Microbial counts

Total viable psychrotrophic counts (TVC) and counts of H₂S-producing bacteria were evaluated on iron agar (IA) containing 0.5% salt as described by Gram and others (1987). The pour-plate method was used and the plates incubated at 22°C for 3 days. IA with 10% salt added was also used. These plates were incubated at 22°C for 5 days. Bacteria forming black colonies on this medium produce H₂S from sodium thiosulphate and/or cysteine. Total viable mesophilic counts and counts of red-halophilic bacteria were done on DL-agar. Surface plating was used and plates incubated at 35°C for 14 days (Dussault and Lachance 1952). For all dilutions, 15% saltwater was used.

2.2.2. Chemical analysis

Trimethylamine (TMA) was measured according to AOAC (1990) with the change that KOH was used instead of K₂CO₃. Total volatile bases (TVB) were measured according to Antonacopoulos (1968) with a Struer automatic distillation unit. Measurement of salt content was done with the Volhard method according to AOAC 14th ed. 1985, method no. 937.09.

3. RESULTS AND DISCUSSION

3.1. Microbial counts

Before discussing the microbial results it might be helpful to look at how bacteria have been classified according to their salt requirements. Non-halophilic bacteria grow best at salt concentrations under 2%, slightly halophilic at 2-5%, moderate

halophiles at 5-20% and extreme halophiles over 20% (Jepsen and Skovgaard 1975). The red-halophilic bacteria ("pink") are extreme halophiles.

Results from microbial counts are shown in Figure 1 to 4. Changes in total viable counts on 3 different culture media during the first 3 weeks are shown in Figure 1. On day 0, unsalted cod fillets did not contain any extreme halophiles, very few moderate ones but about 10.000 non-halophilic bacteria per g (0.5% IA). As soon as the fillets came into contact with the solar salt used, they became contaminated with extreme halophilic bacteria originating from the salt (20% DL agar, day 1). At day 7, maximum numbers of these bacteria were obtained (ca. 1000/g). Minor changes were noticed in moderate halophiles on 10% IA over the 3 weeks period, their numbers being lowest from day 5 onwards or about 100/g. A slight decrease was observed in non-halophilic bacteria (0.5% IA) during that time but highest counts were obtained on this medium (4000-10.000/g). Figure 2 shows changes in total viable counts over the whole storage period. Slight decrease in bacterial numbers occurred on all media over the period. Lowest counts were always obtained on 10% IA, indicating that numbers of moderate halophilic bacteria were low. Red-halophilic bacteria and H₂S-producing bacteria were never seen on 0.5% or 10% IA over the whole storage period. In Figure 3 to 4 counts of "pink" bacteria are shown on 20% DL agar in comparison with total viable counts on the same medium. During the first 70 days of storage, almost all bacteria that survived on this medium were red-halophiles. On days 173 and 330 however, the numbers of "pink" bacteria were considerably lower than the total viable counts. Bacteria of unknown nature forming white and yellow colonies dominated on these sampling days and apparently were more salt-tolerant than the red halophilic bacteria. It should be mentioned that the fillets never showed any signs of "pink" colour.

3.2. Chemical analysis

Salt content of the cod fillets was 20.5% after brining and dry-salting (average salt of surface and thickest muscle). Results from TVB and TMA measurements are shown in Figure 5 to 6. During the first 3 weeks of storage, TVB increased slightly over the period or from about 14 to 17mgN/100g. A small drop in TVB was noticed on day 2 which might be due to leakage into the brine but during brining the salt draws water from the muscle and salt is taken up. At the end of storage on day 330, TVB was still under 20mgN/100g. TMA values never exceeded 1mgN/100g over the storage period.

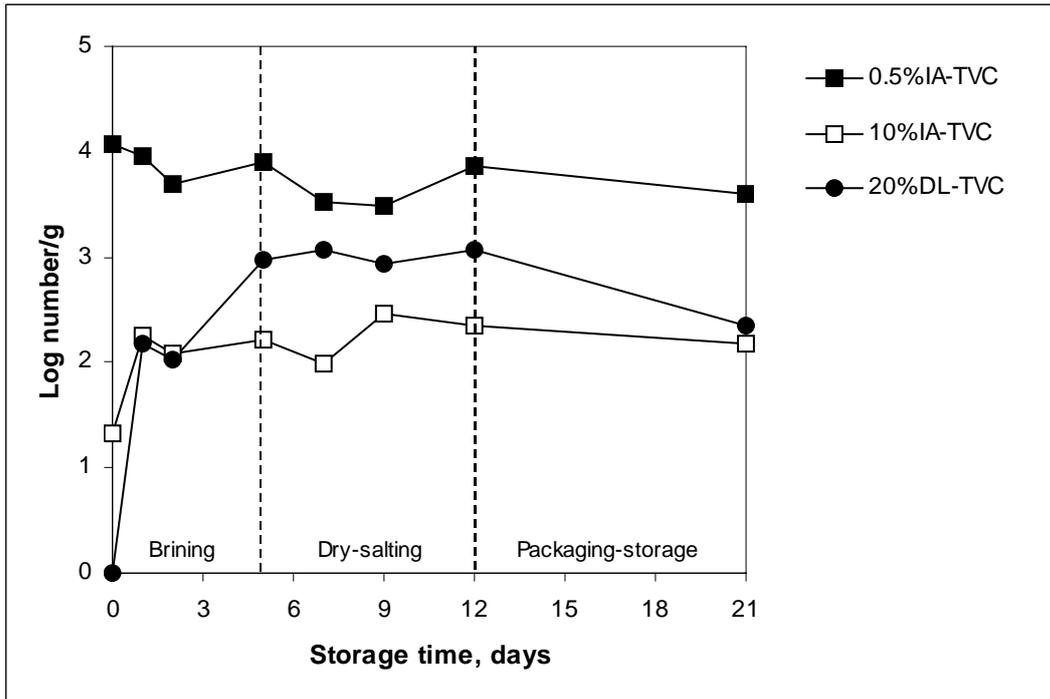


Figure 1. Total Viable Count (TVC) in salted cod fillets during the first 3 weeks.

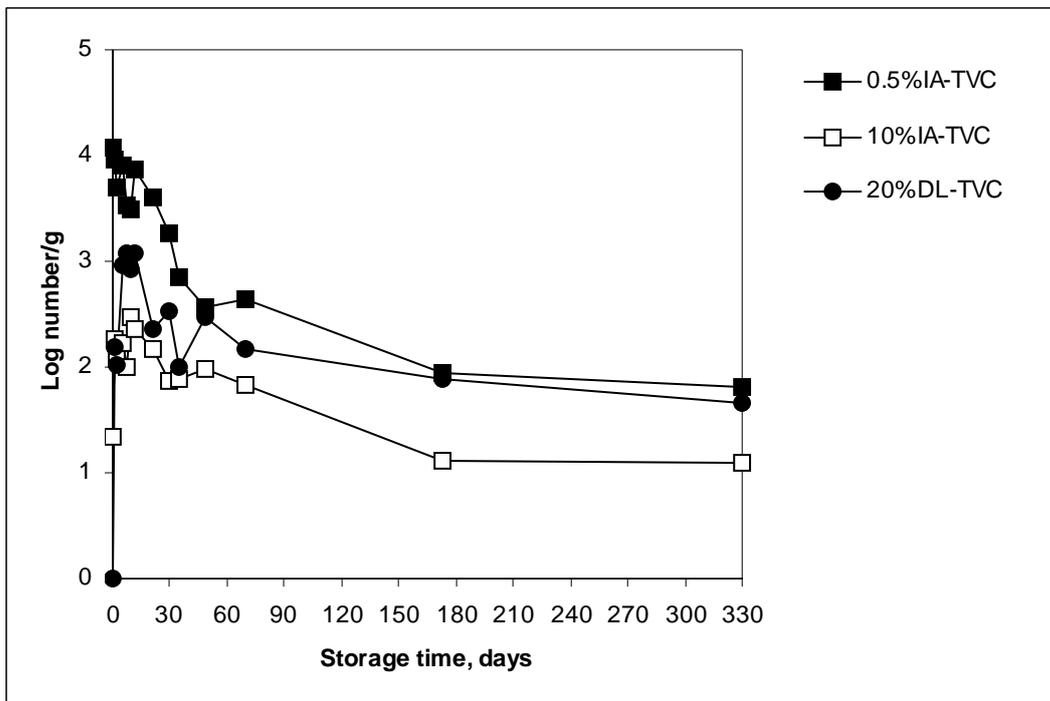


Figure 2. Total Viable Count (TVC) in salted cod fillets during storage at 5°C.

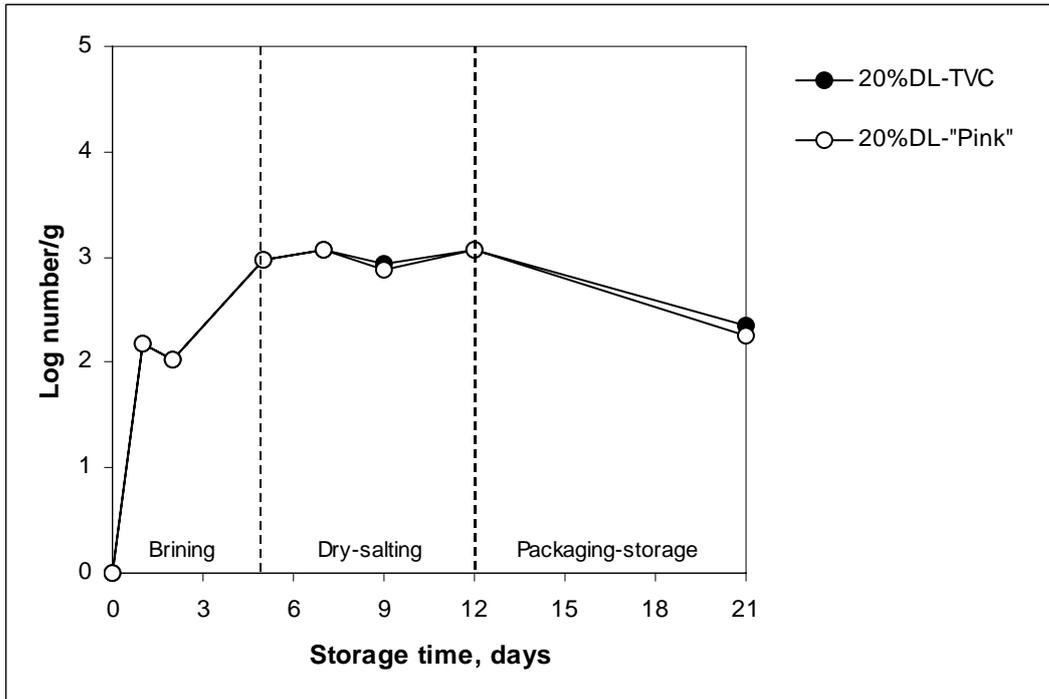


Figure 3. Total Viable Count (TVC) and counts of "pink" bacteria on DL agar in salted cod fillets during the first 3 weeks.

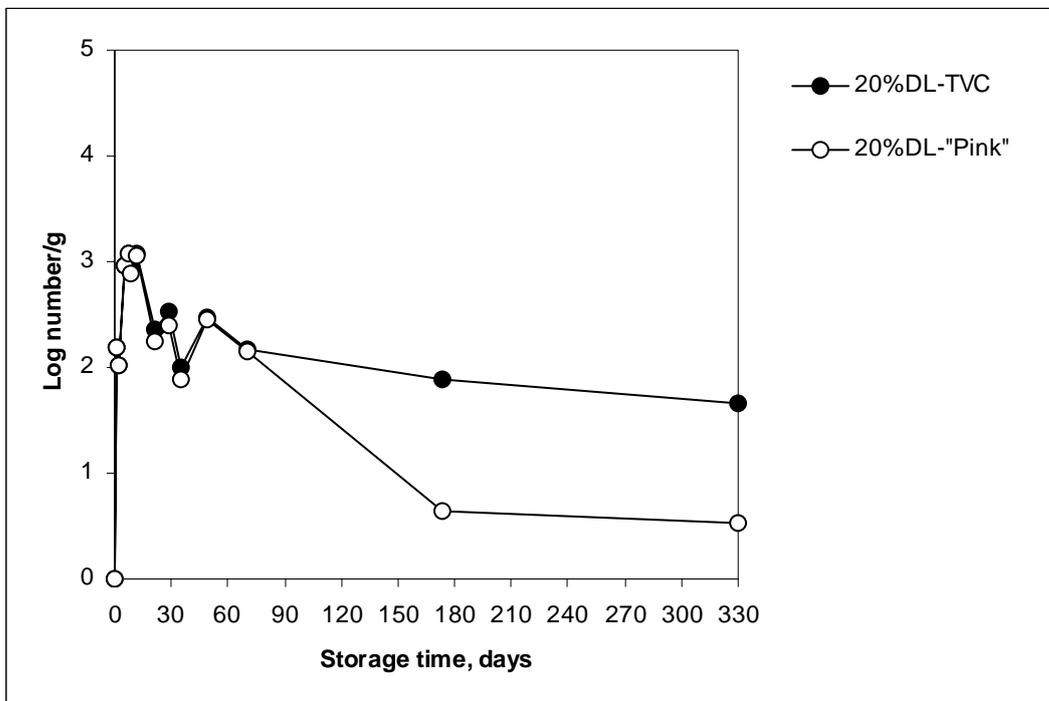


Figure 4. Total Viable Count (TVC) and counts of "pink" bacteria on DL agar in salted cod fillets during storage at 5°C.

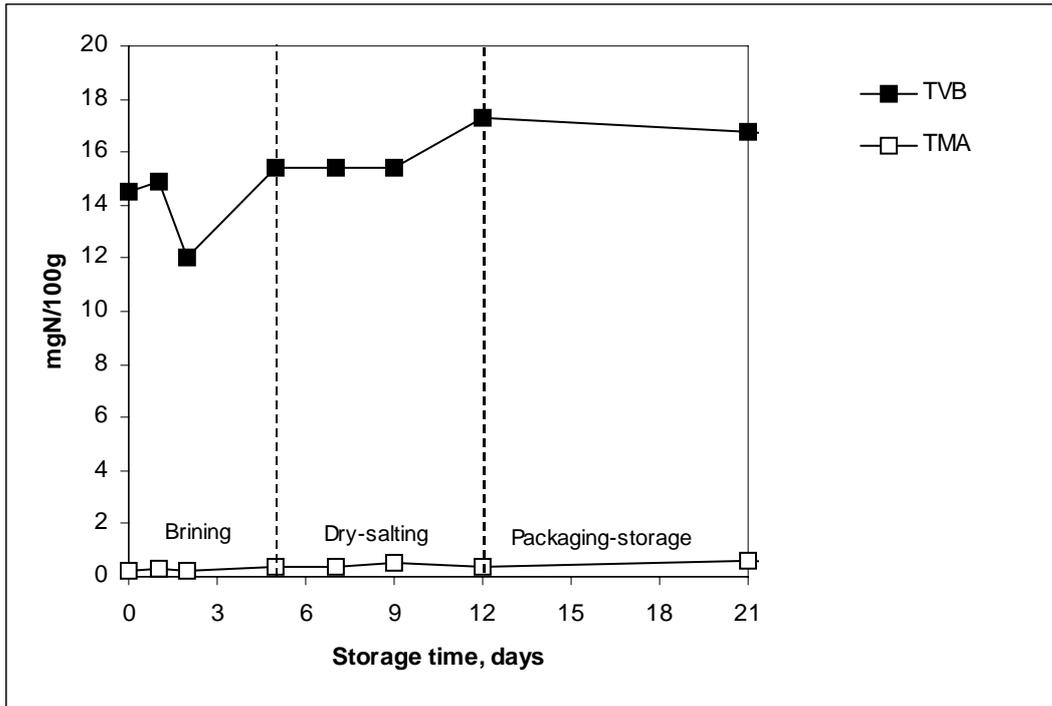


Figure 5. Total Volatile Bases (TVB) and trimethylamine (TMA) in salted cod fillets during the first 3 weeks.

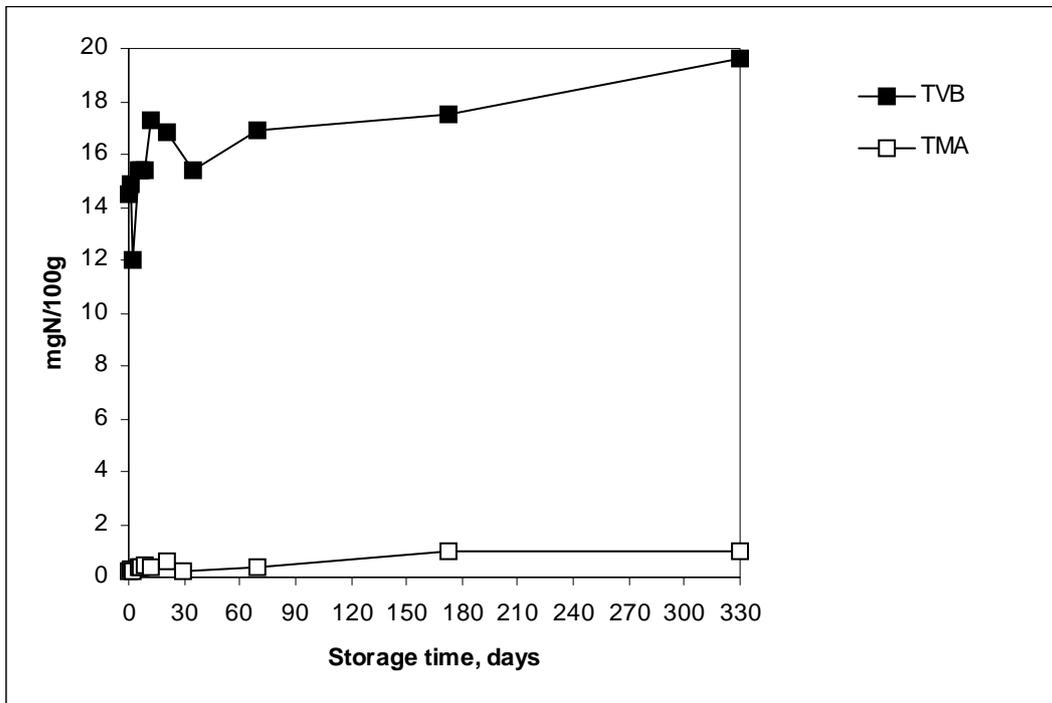


Figure 6. Total Volatile Bases (TVB) and trimethylamine (TMA) in salted cod fillets during storage at 5°C.

4. CONCLUSION

Unsalted cod fillets did not contain any extreme halophilic bacteria but as soon as the fillets came into contact with the solar salt used, they were contaminated with such bacteria. After brining, a slight decrease in bacterial numbers occurred on all media tested over the storage period. During the first 70 days of storage, almost all bacteria on medium containing 20% salt were red-halophiles. As storage progressed numbers of such bacteria were overtaken by bacteria forming white and yellow colonies on this medium. The fillets never showed any signs of reddening ("pink"). The results from these experiments confirm that salted cod fillets containing about 20% salt and kept at 5°C are a very stable product from microbiological point of view.

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