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MICROBIOLOGICAL CHANGES DURING STORAGE OF LUMPFISH CAVIAR

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Titill / Title	**Microbiological changes during storage of lumpfish caviar**		
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Ágrip á íslensku:

Tilraunir þessar voru gerðar á Rf 1989-1991 og hafa niðurstöður þeirra aldrei verið birtar. Könnuð voru áhrif gerilsneyðingar, saltstyrks, geymsluhita og bensóats á vöxt ýmissa örveruhópa í grásleppuhrognakavíar. Tilraunahópar voru 12 og var kavíarinn geymdur við 4 og 22°C í alls 2 ár. Engin örverufjölgun varð yfir geymslutímann í gerilsneyddu hópunum (alls 7 hópar). Fjöldi mjólkursýrugerla óx hratt í þremur af ógerilsneyddu hópunum fyrstu 2 mánuði geymslutímans. Gersveppir náðu hins vegar yfirhöndinni í hinum tveimur hópunum. Gersveppir reyndust vera þolnari en mjólkursýrugerlar gegn samverkandi áhrifum lágs geymsluhita og mikils saltstyrks. Aðrir örveruhópar voru ætíð í mjög litlu magni í öllum tilraunahópum, ef þeir á annað borð fundust. Í lok geymslutímans voru 5 af 7 gerilsneyddu hópunum enn án áberandi skemmdareinkenna og 2 af 5 ógerilsneyddu hópunum (báðir við 4°C).

Lykilorð á íslensku: *Grásleppuhrogn, kavíar, örverur, gerilsneyðing, geymsluþol*

Summary in English:

The experiments presented here were carried out during the years 1989-1991 at IFL and have never been published. The effects of pasteurization, salt concentration, storage temperature and use of sodium benzoate on various microbial groups in lumpfish caviar were studied. Twelve experimental groups were kept at 4 and 22°C for 2 years. No increase of microbes was observed in any of the seven pasteurized groups during the 2 year storage. The number of lactic acid bacteria (LAB) in unpasteurized caviar increased rapidly during the first 2 months of storage in three of the groups. In the other two groups, yeasts however increased rapidly. The yeasts were more tolerant towards combined effect of high salt and low storage temperature than LAB. Other microbial groups were always in very low numbers, if detected at all, in all experimental groups. At the end of storage, five of the seven pasteurized groups had not developed any obvious signs of spoilage. In unpasteurized caviar, two groups of five (both kept at 4°C) were still unspoiled at the end of storage.

English keywords: *Lumpfish roes, caviar, microorganisms, pasteurization, storage time*

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1. INTRODUCTION

Caviar made from salted lumpfish (*Cyclopterus lumpus*) roes has been exported from Iceland since the beginning of nineteen sixties. Europe has been the main market for this product.

Very few studies have been published on the microbiological changes during storage of lumpfish caviar. Danish studies on lightly salted lumpfish roes stored at 5°C led to the conclusion that *Morganella morgannii* was a significant spoilage organism of the roes (Basby and others 1998). With regard to pathogenic bacteria, an interesting study was published in 1979 on the effect of salt content and pH on toxigenesis by *Clostridium botulinum* in lumpfish caviar (Hauschild and Hilsheimer). Spores from types A, B and E were inoculated into caviar jars which were pasteurized at 62°C and then incubated at 30°C for upto 4 weeks. *C. botulinum* was able to grow and produce toxin at a combination of ≤4.67% salt (water-phase) and pH ≥5.6. No toxin was however formed at salt concentrations of ≥5.56% or at pH ≤5.0. Spores of the most heat-sensitive type of *C. botulinum*, type E, should be destroyed at 82.2°C for 30 min (Huss 1981). Commercial heat pasteurization alone (72-80°C for a few min) is therefore not sufficient to destroy type E spores in contaminated seafood.

The experiments presented here were carried out during the years 1989-1991 at the Icelandic Fisheries Laboratories (IFL) and have never been published. The effects of pasteurization, salt concentration, storage temperature and sodium benzoate on different microbial groups were studied.

2. MATERIAL AND METHODS

2.1. Experimental design

The caviar samples were prepared from salted lumpfish roes at the food processing plant ORA in Kópavogur, Iceland. The salt content of the roes was 12.3% (15.3% water-phase salt). The caviar was made according to the factory´s recipe and vacuum-packed in 100g glass jars with a metal lid. The caviar was dyed with black food colourant during the production. A part of the caviar was pasteurized at 78°C for 30 min. Three temperature sensors were used during the process. The core temperature of

the samples was higher than 70°C for 11 min. and the highest recorded core temperature was 74°C (3-4 min). Caviar samples were kept at 4°C and 22°C for 24 months and examined after 0-2-4-6-8-10-12-16-20-24 months.

Experimental groups are shown in Table 1.

Table 1. Experimental groups.

Group description	% NaCl	Na-benzoate	Pasteurized	Storage temp
3.5 % salt-B-P-4°C	3.5	+	+	4°C
3.5 % salt-B-NP-4°C	3.5	+	-	4°C
4.7 % salt-B-P-4°C	4.7	+	+	4°C
4.7 % salt-B-P-22°C	4.7	+	+	22°C
4.7 % salt-B-NP-4°C	4.7	+	-	4°C
4.7 % salt-B-NP-22°C	4.7	+	-	22°C
4.7 % salt-P-4°C	4.7	-	+	4°C
7.1% salt-B-P-4°C	7.1	+	+	4°C
7.1% salt-B-P-22°C	7.1	+	+	22°C
7.1% salt-B-NP-4°C	7.1	+	-	4°C
7.1% salt-B-NP-22°C	7.1	+	-	22°C
7.1% salt-P-4°C	7.1	-	+	4°C

P: Pasteurized, NP: Not Pasteurized, B: Benzoate

2.2. Methods

2.2.1. Microbial counts

Basic methodology for microbial counts was according to Speck, 1976 unless otherwise stated. Butterfield´s buffer was used for all dilutions.

Total viable psychrotrophic counts (TVC) were done on Plate Count Agar (PCA) containing 0.5% NaCl with the pour-plate method. Plates were incubated at 22°C for 3 days.

The medium deMan, Rogosa and Sharpe agar with sorbic acid (MRS-S), prepared according to a description in International Journal of Food Microbiology, 5, (1987), was used for counting lactic acid bacteria (LAB). Surface-plating was used and plates incubated at 22°C for 3 days under microaerophilic conditions.

Potato Dextrose Agar (PDA) acidified to pH 3.5 was used for counting yeasts and moulds. Surface-plating was used and plates incubated at 22°C for 5 days.

Counts of coliform bacteria were done by the 3-tube MPN-method. LST broth was used for pre-enrichment and BGLB broth for total coliforms and EC broth for faecal coliforms.

Bacillus spore counts were done on PCA containing 0.5% NaCl with the pour-plate method. Plates were incubated at 35°C for 2 days. Diluted samples were heated at 80°C for 15 min to kill vegetative cells.

Counts of sulphite-reducing clostridia were done by the 3-tube MPN-method in Differential Reinforced Clostridial Medium (DRCM) according to Freame and Fitzpatrick (1971). Diluted samples were heated at 80°C for 15 min prior to inoculation.

2.2.2. Chemical analysis and sensory examination of samples

Measurement of salt content was done with the Volhard method according to AOAC 14th ed. 1985, method no. 937.09 and moisture content according to the same reference, method no. 930.15 using oven temperature of 103 ± 2°C.

Measurement of benzoic acid was done with a quantitative determination by gas chromatography (NMKL method no.103, 1984). In this method the benzoic acid is isolated from the sample by extraction with ether and successive partitionings into sodium hydroxide solution and chloroform. Acids are converted to trimethylsiliyl (TMS) esters and determined by gas chromatography. Phenylacetic acid is used as an internal standard for benzoic acid.

The pH was measured in a mixture of 5 g caviar and 5 ml deionized water using the Radiometer PHM 80.

During the storage time, the caviar samples were examined by 3-4 members of IFL´s trained sensory panel with regard to discoloration (loss of black colour), swelling, vacuum, surface moulds and smell. The vacuum was measured with a gauge meter in 3 samples from each experimental group.

3. RESULTS AND DISCUSSION

3.1. Microbial counts

Results of total viable counts (TVC) are shown in Fig. 1-3. TVC in all experimental groups are shown in Fig. 1. No increase was observed in any of the seven pasteurized groups during the 2 year storage time; the TVC ranging from log 2.0-2.5/g in most of the groups. The use of sodium benzoate did not have any noticeable effect on TVC in pasteurized caviar (Fig. 2). The effect of salt concentration and storage temperature on

TVC in unpasteurized caviar is shown in Fig. 3. The growth rate during the first 2 months of storage was very rapid in all groups. Highest numbers were reached in groups with 3.5% salt kept at 4°C, exceeding log 7/g after 2 months and 4.7% salt at 22°C, reaching log 8/g after 4 months storage. TVC in groups with 4.7% salt kept at 4°C and 7.1% salt at 22°C were similar. Lowest counts were found in the group with 7.1% salt kept at 4°C.

Counts of lactic acid bacteria (LAB) in unpasteurized caviar are shown in Fig. 4. Highest counts were obtained in the group with 3.5% salt kept at 4°C, exceeding log 7/g after 2 months. At that time LAB counts in groups 4.7% and 7.1% salt at 22°C were between log 5-6/g. Combined effect of low temperature storage (4°C) and high salt content (4.7% and 7.1%) had drastic effect on LAB growth. Thus, no such bacteria were isolated during the first 16 months of storage in the 4.7% salt group at 4°C and never in 7.1% salt at 4°C. It is interesting to compare the growth curves for LAB and TVC in the group 3.5% salt at 4°C. These growth curves were very similar, strongly suggesting that the LAB were the dominant flora in this group. Growth curves for LAB and TVB in the group 7.1% salt at 22°C were also similar after 6-10 months storage but LAB counts were lower during the first 4 months. In group 4.7% salt at 22°C were TVC reached log 8/g after 4 months storage, the LAB were only log 5.4/g, indicating that some other microorganisms were dominant at that time. No LAB were found in any of the pasteurized caviar groups.

Counts of yeasts in unpasteurized caviar are shown in Fig. 5. In the 3 groups were LAB grew well, i.e. 3.5% salt at 4°C and 4.7% and 7.1% salt at 22°C, the number of yeasts increased during the first 2 months but decreased rapidly after that. From month 6 onwards, yeasts were never found in these groups. It appears that LAB and other microorganisms outgrew yeasts in these groups. However, yeasts were more tolerant towards high salt and low temperature storage than LAB, outgrowing them in groups 4.7% and 7.1% salt at 4°C. The growth curves for yeasts and TVC were very similar all the storage time in the group 7.1% salt (4°C) and during the first 16 months in the group 4.7% salt (4°C), indicating that yeasts were the dominant flora in these groups. Moulds were only found in the group 3.5% salt at 4°C and then in very low numbers. No yeasts or moulds were detected in the pasteurized caviar.

Total coliforms were only occasionally found and always in very low numbers (<MPN 1/g). Faecal coliforms were never found in any groups.

Bacillus spores were found in all experimental groups during the 2 year storage time but always in very low numbers. Highest count (log 2.9/g) was obtained in the unpasteurized group 3.5% salt at 4°C after 6 months storage. In all other groups the count was always less than log 2.3/g.

Sulphite-reducing clostridia were only detected in one experimental group (3.5% salt, 4°C, unpasteurized), the highest number being log 2.3/g after 6 months storage.

3.2. Chemical analysis and sensory examination of samples

The average salt concentration of the experimental groups was 3.5, 4.7 and 7.1%. The average moisture content was 78.9, 77.3 and 74.6%. The water-phase salt content was calculated and found to be 4.2, 5.7 and 8.7%, respectively. The average value of sodium benzoate was 1100mg/kg (0.11%).

Results from vacuum measurements in unpasteurized caviar are shown in Fig. 6. An inverse correlation was between vacuum versus TVC and LAB counts in the groups with 3.5% salt kept at 4°C and the groups with 4.7 and 7.1% salt at 22°C. It is therefore evident that some of the microorganisms have been gas-producing, resulting in swelling of jars and eventually loosening of the lids. In the groups 4.7% and 7.1% salt at 4°C in which yeasts dominated, full vacuum was maintained throughout the storage time strongly indicating that the yeasts in these groups were not gas-producing. Full vacuum was maintained in all pasteurized groups.

Results from pH measurements are shown in Fig. 7-8. The pH was in the range of 5.0-5.5 in all experimental groups over the storage period. There was a slight tendence for a drop in pH with increasing storage time in the unpasteurized caviar (Fig. 7). The pH values were generally slighty higher in the pasteurized caviar apart from the initial values in the groups with the highest salt content (Fig. 8).

Four experimental groups were judged defected due to discoloration (loss of black colour) over the storage period. These groups were: 4.7% salt/unpasteurized/22°C after 2 months, 7.1% salt/unpasteurized/22°C after 10 months, 4.7% and 7.1% salt/ pasteurized/22°C after 20 months. In addition, the group 3.5% salt/unpasteurized/4°C was defected after 4 months due to surface moulds. After 8-10 months, a distinct sour smell had developed, probably due to the high numbers of lactic acid bacteria in this group. Other experimental groups did not show obvious signs of spoilage over the storage period.

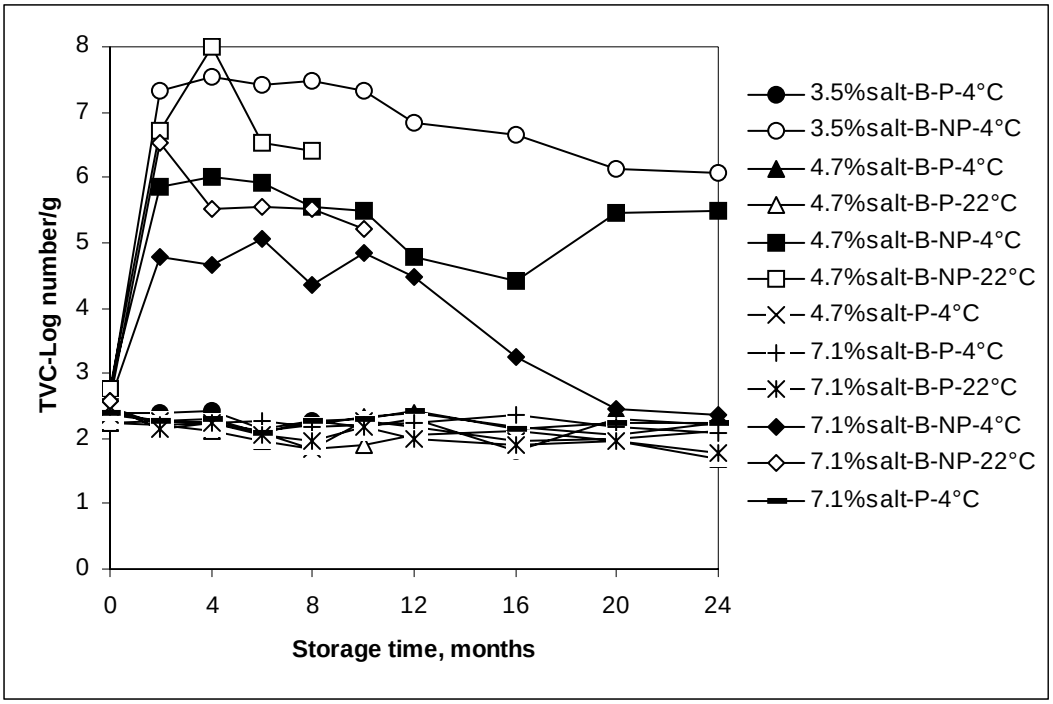

Figure 1. Total Viable Count (TVC) in caviar. All experimental groups. Effect of pasteurization. (B=Benzoate, P=Pasteurized, NP=Not Pasteurized).

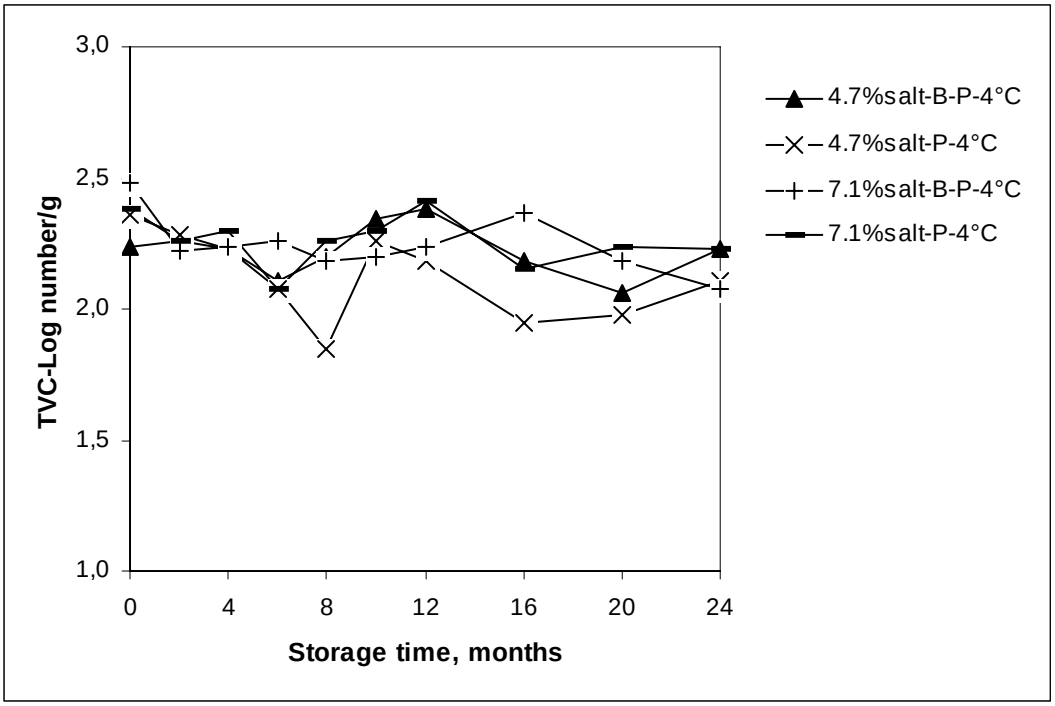

Figure 2. Total Viable Count (TVC) in pasteurized caviar. Effect of sodium benzoate. (B: Benzoate, P: Pasteurized).

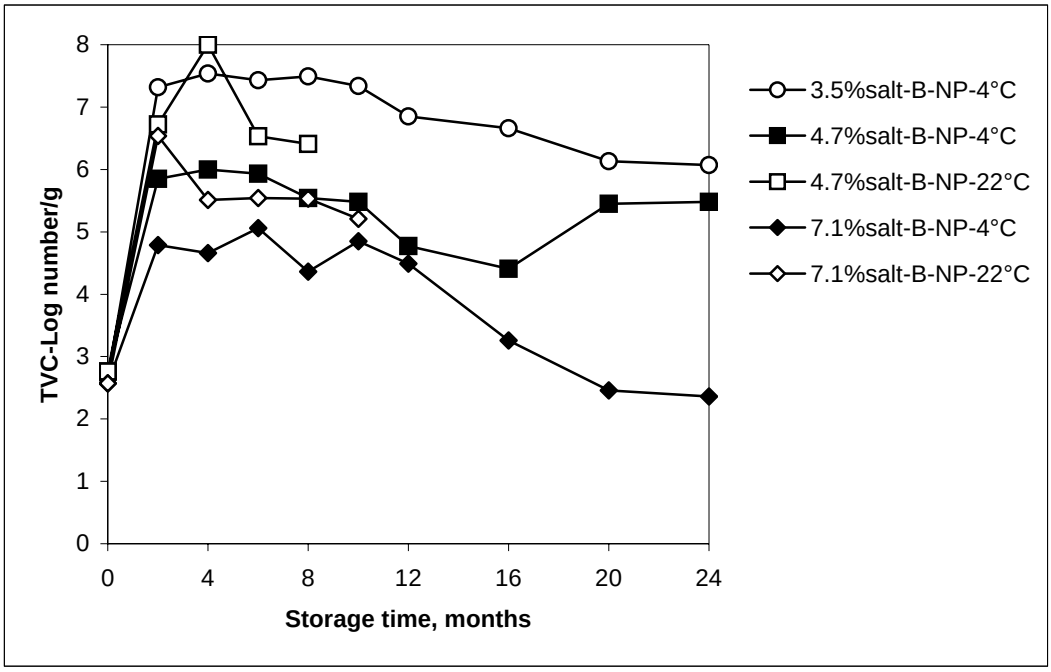

Figure 3. Total Viable Count (TVC) in unpasteurized caviar. Effect of salt concentration and storage temperature. (B=Benzoate, NP=Not Pasteurized).

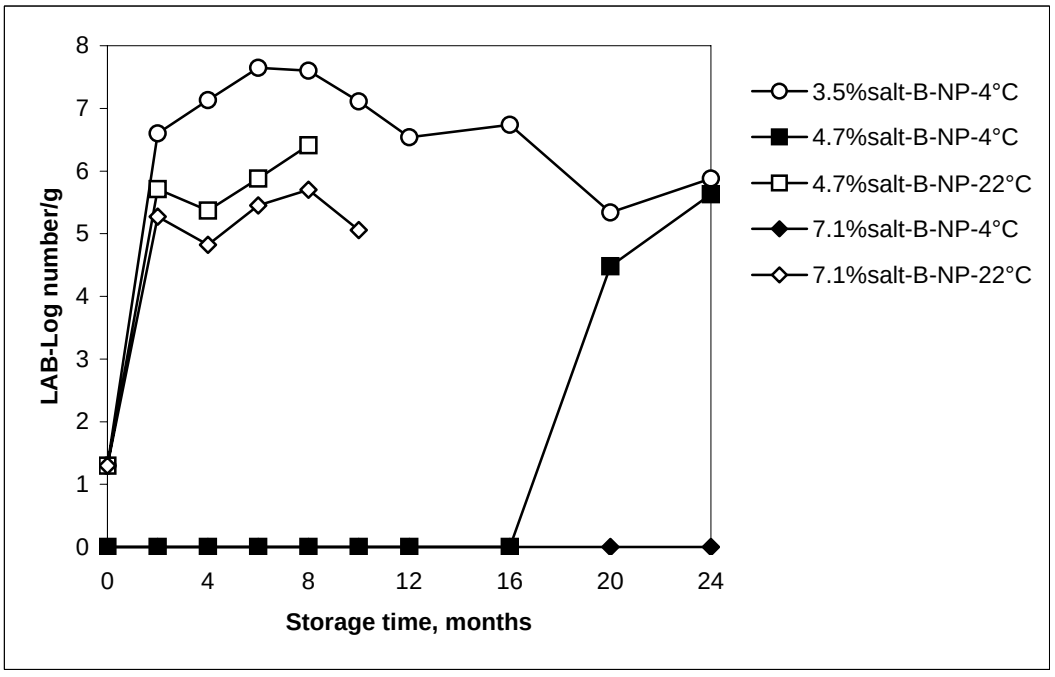

Figure 4. Lactic Acid Bacteria (LAB) in unpasteurized caviar. Effect of salt concentration and storage temperature. (B=Benzoate, NP=Not Pasteurized).

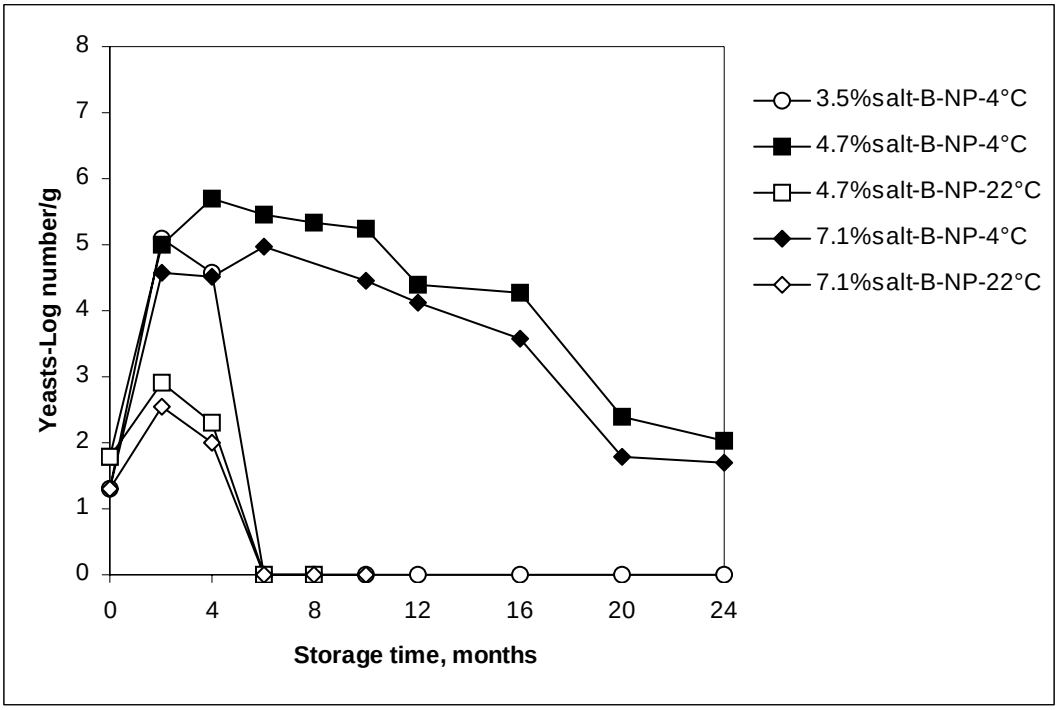

Figure 5. Yeasts in unpasteurized caviar. Effect of salt concentration and storage temperature. (B=Benzoate, NP=Not Pasteurized).

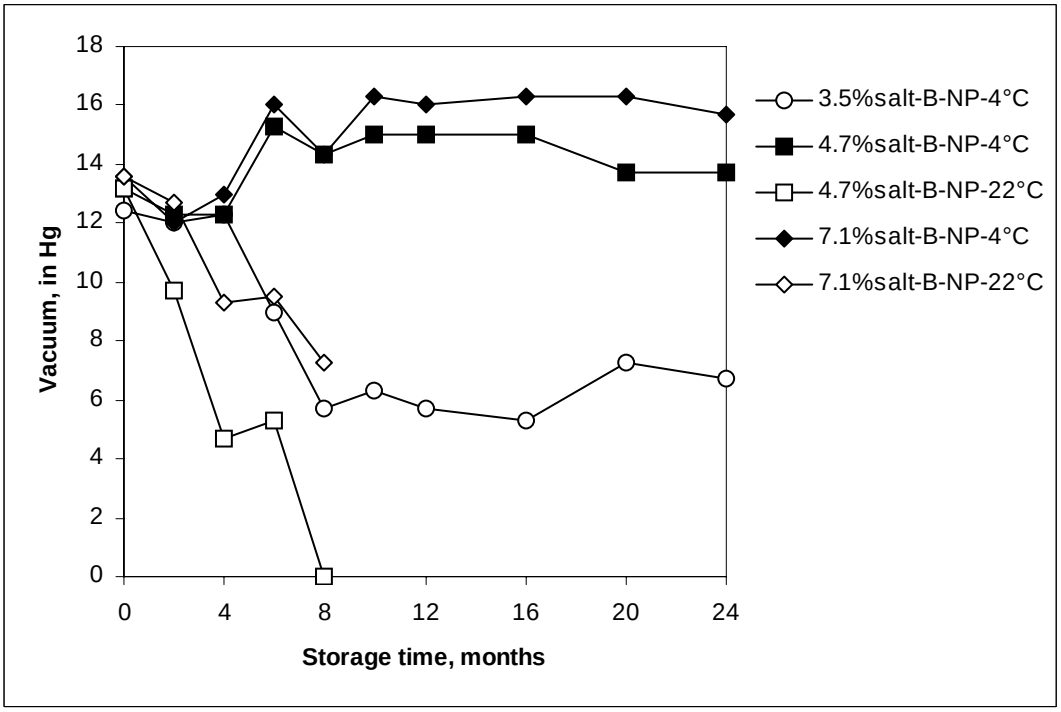

Figure 6. Vacuum in unpasteurized caviar. Effect of salt concentration and storage temperature. (B=Benzoate, NP=Not Pasteurized).

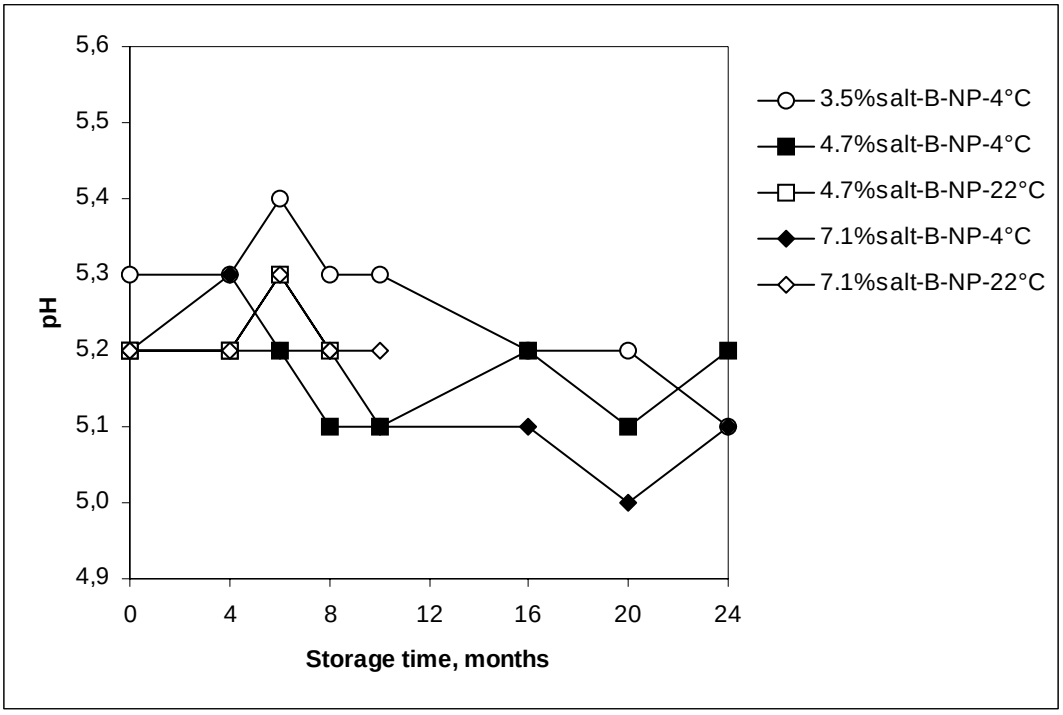

Figure 7. pH in unpasteurized caviar. Effect of salt concentration and storage temperature. (B=Benzoate, NP=Not Pasteurized).

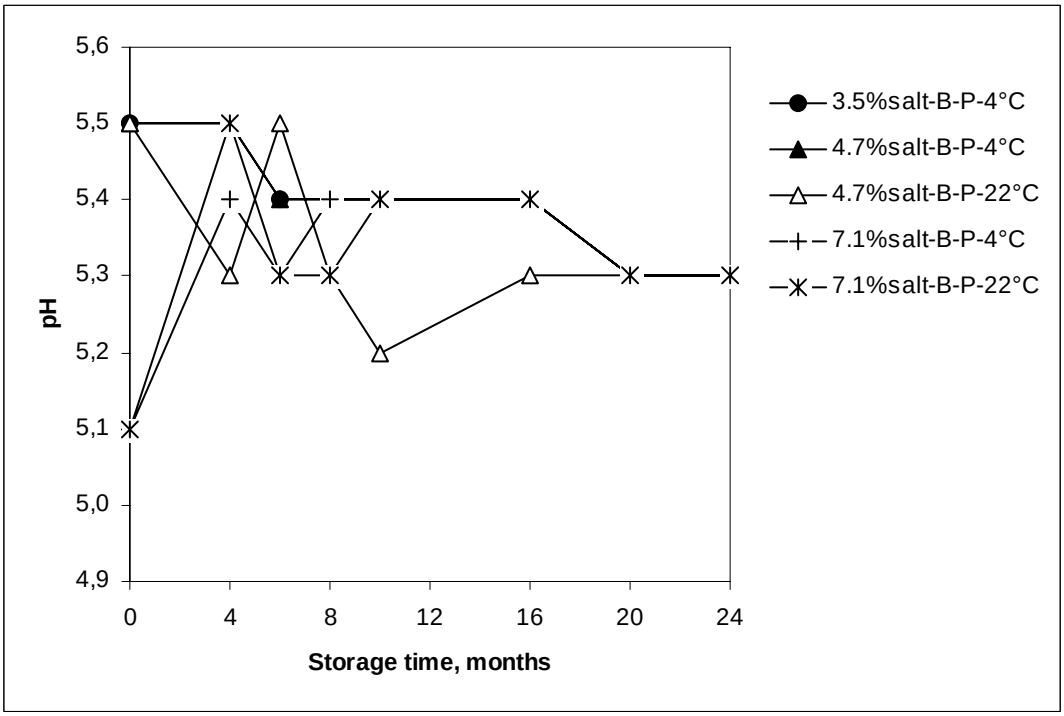

Figure 8. pH in pasteurized caviar. Effect of salt concentration and storage temperature. (B=Benzoate, P=Pasteurized).

4. CONCLUSION

The pasteurization process of the caviar seems to have initially killed all the microbes which otherwise might have grown in this product, since no growth was observed in any of the pasteurized groups throughout the two year storage time. Lactic acid bacteria and yeasts were the most dominant microflora in unpasteurized caviar. The yeasts were more tolerant towards combined effect of high salt and low storage temperature than lactic acid bacteria. At the end of storage, five of the seven pasteurized groups had not developed any obvious signs of spoilage. In unpasteurized caviar, two groups of five (both kept at 4°C) were still unspoiled at the end of storage.

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