FISHERIES TRAINING PROGRAMME
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Final Project 2002

Estimation of microbiological and chemical variations in minced fish processing of Atlantic pollock (*Pollachius vireos*)

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Abstract

The objective of this study is to look into the microbiological and chemical variation of minced fish processing from Atlantic Pollock (*Pollachius vireos. L*). Samples were collected from raw material on a processing line and also from whole fish prior to mincing. Measurements on microbial load (total and H_2S producing bacteria, total and faecal coliform) were performed and also chemical variation was measured (protein, fat, TVB – N, TMA, pH and moisture). The effect of storage was studied and samples taken from mince product stored frozen at – 30 °C.

Lean fish is suitable for mince production. Minced fish is more susceptible for microbial spoilage that whole fish due to greater surface are of minced fish. Strict hygiene standards must therefore be applied in mince production. Storage conditions of -30 degree seem to be good for minced fish to maintain high quality. The quality evaluation (microbiological and chemical variation) for mince fish after frozen stored showed after 50 days was acceptable.

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1. INTRODUCTION

Fisheries are an important industry in Iran. The initial efforts are directed towards the development of valuable seafood products, in northern Iran (Caspian Sea), southern Iran (Persian Gulf and Sea of Oman) and inland natural and semi-natural water bodies. Iranian fisheries co-operation (Shilat) tries to increase the production of commercially valuable species. The utilisation of lean and less valuable fish is a big problem in Iranian fisheries. Species of the family *Clupeidae* in the Caspian Sea and Persian Gulf are commercially very important. These species are quite suitable for the production of minced fish. Minced fish is the raw material for the production of fish burgers, crackers, balls, sausages, chips etc. In some countries, these are the major products, while in others it is merely a by- product. Minced fish is mostly produced from low-value fish species such as menhaden, herring, capelin, sardine, anchovy, pollock and other *Clupeidae*, although some companies use high-value species such as cod. Washing is required to remove the fat from fattier fish in preparation of minced fish. The aim of this project is to study the processing of minced fish, its quality after thawing, shelf-life, and microbiological and chemical variations in minced fish.

The most common way a food loses its acceptable quality is from the growth of micro-organisms or from non-microbial causes like lipid oxidation. Food that seems acceptable can be unsafe for consumption because of the growth or presence of pathogenic micro-organisms or because of toxic chemicals. Micro-organisms, which include bacteria, yeasts, and moulds, gain access to food through various sources. The types vary greatly depending on their source, the effectiveness of sanitation during all phases of handling and the method used in the processing and storage. Different physical, chemical and biological preservation methods are applied to make food safer and to extend shelf-life by destroying and retarding or preventing the growth of pathogenic and spoilage micro-organisms that might be present in the food. Many marine products are frozen for longer shelf-life and can be distributed in frozen condition with the development of freezer chains. Seafood is processed and frozen on vessel immediately after catch or frozen on land at the factory after cleaning, gutting, removing shells, filleting and cutting. It is frozen in plate freezers or individually quick frozen (IQF) by contact freezing. Frozen seafood is characterised by its hardness and irregularity of shape, especially with the protrusion of fins, spines and sharp edges. To maintain quality under this condition, freezing resistance and antifreezing strength is required from the package. Seafood is also susceptible to freezer burn. In a cold and dry atmosphere, ice sublimes. The surface of frozen product is easily dehydrated and rancidity occurs from such adverse influence. (Martin 1992). Fish mince, as compared with intact fish muscle, is unstable and is prone to rapid deteriorate change during storage. Mechanical deboning causes disruption of the tissue and exposure of the meat to air, which accelerates several deteriorating processes, such as lipid oxidation, bacterial proliferation and resulting in protein changes. The higher temperature associated with deboning also affects the stability of the mince. The mince contains about 20% soluble components belonging to the sarcoplasmic fraction and about 2 - 3% connective tissue (although this may be as high as 10% in the case of elasmobranch species). The rest is composed of myofibrillar protein. Washing of the mince in chilled water removes soluble compounds, significant amounts of lipids, blood and pigments to give concentrated myofibrillar protein, having a bland taste. Fatty fish like mackerel or sardine may require washing with bicarbonate to remove oil. (Martin 1994). Pollock is a lean fish (0.2 – 0.3% fat) with high protein content in fillets (17.5%) and minced fish from Atlantic pollock is well suited to colouring after processing. Experience shows that lean fish is the best species for mince fish processing.

2. LITERATURE REVIEW

2.1 Minced fish production in some countries

In some countries, such as Japan, USA, Russian, Argentina, Thailand, China and Chile, minced fish is a main product for human consumption (Sheviclo 1997). Japanese companies try to increase the mince fish production in the world, from small and low-value fish since the raw material is cheap. The value-added effect will therefore be great but the product is still offered to consumers at a relatively low price (SEAFDEC 1997).

2.2 Various species for the mince

Many different species are used for mince. The characteristics that make these species especially suitable are their colour and fat content. These species are (Sheviclo 1997):

- Menhaden
- Herring
- Capelin
- Sardine
- Anchovy
- Alaska Pollock
- Cod
- Atlantic Pollock
- Merluza
- Southern Blue Whiting
- Pacific Whiting
- Salmon
- Whiplail
- Atka Mackerel
- Alfonsino
- Golden thread fin Bream
- Cutlass Fish
- Sharp Totted Eel
- Shark

These species give three different varieties of mince meat colour:
- White
- Dark
- Medium (in between)

A. White coloured minced fish: This category is produced from flesh with low fat content and highly gelatinous. Fish that give this type of raw material included Alaska pollock. This explains the reason why Alaska Pollock is widely used for the production of minced fish. About 80% of Alaska Pollock goes into minced fish production.

B. Dark coloured minced fish: This category is a product of high fat flesh. The dark coloration has made this product less popular to consumers. Species of fish commonly used to produce this category include Sardines, Atka Mackerel, and among others. These species are readily available in Japan and therefore are used for the production of mince. However, it must be mixed with flesh of other species to lessen the dark colouration.
C. Medium (red) coloured mince fish: This category product is produced from fish high myoglobin and haemoglobin content of flesh. These fish species include: Tuna,

Catfish, and Atlantic Pollock (Sheviclo 1997). Red coloured minced fish is commonly used for the production of sausages.

2.3 Production process of minced fish

It is recommended to keep fish to be minced below 5°C. This is followed by beheading and gutting. In the case of small fish and low investment companies, beheading and gutting are done manually while in large processing companies machines are used. On average, about 30 - 50% becomes waste, depending on the species being processed. Various factors affect the amount of waste in the process including the season of catch and freshness of the raw material (FAO 2001).

Washing of minced fish is an important step in the production process. Washing removes blood and other excess material. Unproperly washed minced fish spoils rapidly (Sheviclo 1997).

The subsequent steps in the process are filleting, deboning and mixing, which, like beheading and gutting, can be dome manually or mechanically. This process also includes washing, which removes the remaining filth and some enzymes (such as proteolytic enzymes), and any possible parasite. (FAO 2001, Lloyd and Ernstand1995)

Washed fillets are then directed to the deboning machine, which has a drum of 4 – 8mm mesh sizes. Deboning is a very important process because here all dark coloured tissue is removed as well as very small bones. This improves the quality of the product and therefore enhances consumer satisfaction (FAO 2001).

2.4 Packaging and storage of minced fish

Immediately after the production of minced fish the next step is packaging. There are two methods of packaging, commonly called **primary** and **secondary** packaging. Primary package is usually in 10 kg polyethylene bags and secondary package is the packing of the primary in cartons. Usually the secondary package contains 2 - 10 kg polyethylene packages. The product is storage at – 20°C. At this temperature the shelf life of the product is estimated at 90 days (FAO. 2001). By lowering the temperature to –30°C the shelf-life increases to 180 days.

2.5 Quality water

High quality water is required for the production of minced fish, thus potable water is recommended. The water should also have low level of metals such as Ca, Mg, Cu, and Fe since these metals can affect the colour of the minced fish and may also cause rapid colour changes in minced fish during storage (Lloyd and Ernstand 1971).

2.6 Utilisation of minced fish

Minced fish is a base material many products including:

A. ROUND TYPE CATEGORY PRODUCT:

- Fish ball

- Fish cake
- Fish patty
- Fish burger

B. ELONGATED TYPE CATEGORY PRODUCT:
- Fish sausage
- Fish loaf
- Fish finger
- Fish nugget
- Fish chikuwa

C. IMITATION TYPE CATEGORY PRODUCT:
- Fish imitation products

D. NOODLE TYPE CATEGORY PRODUCT:
- Fish noodle products

E. PASTE TYPE CATEGORY PRODUCT:
- Fish spread

Of the round category production, fish burgers are most common. This product includes 41.6% minced fish after mixing with (58.4%) other material such as fillers, improvers, bonders, extender materials and other cryoprotectants, shown in Table 1.

Table 1: Fish burger formula of minced fish (Sheviclo 1997)

Raw material composition	**Material %**
Minced fish	41.6
Bread meal	24
Onion	20
Yolk	3
Salt	2
Corn meal	4
liquid oil	4
Papper	0.5
Galley meal	0.4
Monosodiumglutamate (MSG)	0.5

Fish sausages, which belong to the elongated category, are 50% minced fish, 20% plant proteins, 2% fat and 10% starch. After mixing the mince goes to the filler and is packed and cooked (Table2).

Table 2: Fish sausage formula of minced fish (Sheviclo 1997)

Raw material composition	Material %
Minced fish mixed	50
Corn meal	8
Fish gelatine	0.64
Margarine	3
Onion meal	0.2
Salt	2
Liquid smoke	0.08
Liquid oil	7
Sponsors	1.58
Cold water	27.65

Fish mince is used to produce imitation foods. The mince serves as base in the product and extracts are added to bring out the specified flavour and colour. An imitation carp product is 55% minced fish mixing with 25% water, 2.5% carp extract, and other sponsor and filler materials. Then the product is shaped and packaged (Table 3).

Table 3: Formula for imitated carp (Sheviclo 1997)

Raw material composition	Material %
Minced fish	55
Water	25
Carp extract	2.5
Yolk	8
Starch	5
Sponsor	2.3
Salt	1.5
Oil	0.7

2.7 Quality control for minced fish

A. Interobacteriaceae

In this family there are many pathogens, such as *Escherichia coli, Salmonella Sp. and Shigella sp.* (Bonnell 1994).

Escherichia coli and other faecal coliforms are found in the fecies of humans and other warm blooded animals. *E.coli* is a typical mesophile growing at 7 – 50°C with an optimum temperature of 37°C. *E. coli* grows below pH4.4 and with water activity at 0.92 (Adams and Moss 1995).

Shigella sp. is the cause of shigellosis and more common in warmer climates and in travellers returning from warmer climates. Optimum temperature for Shigella growth is 37°C and pH minimum is 5.5 (Adams and Moss 1995)

Salmonella sp. are regarded as human pathogens, although they differ in the characteristics and the severity of the illness they cause. The most severe Salmonella infection is Typhoid fever. Optimum temperature for Salmonella growth is 37°C and pH minimum is 4.0 and water activity is 0.92 (Adams and Moss 1995). Characteristics of interobactericeae are shown in table 4.

Table 4: Growing condition of some Interobacteriaceae (Adams and Moss 1995)

Bacteria	Tem.(°C) optimum	pH minimum	Water activity minimum
E. coli	37	4.4	0.95
Shigella sp.	37	5.5	0.92
Salmonella	37	4	0.92

B. Psychrotrophic bacteria

Psychrotrophic bacteria grow at much lower temperatures than Interobacteriaceae. The pathogenic psychrotrophic bacteria can grow below 5°C and common ones are *Aeromonas hydrophila, Clostidium botulinum type E and nonproteolytic type B and F, Listeria monocytogenes, Vibriocholera, Yersinia enterocolitica*. The major psychrotrophic bacteria are found in milk, meats and poultry, and fish and other seafood (Vanderzant and Splittstoesser 1992).

3. MATERIALS AND METHODS

The procedures of the project are in three parts:

1. Samples for chemical and microbiological analysis were collected from a minced fish processing line:
   - After deskinning of the fish
   - From fish cuts processing to mincing
   - From the new mince
   - After filler and pump
   - After packaging

2. Samples for chemical and microbiological analysis were collected from a mince sample that had been frozen for 5 days (-30°C) and then thawed for 24 h. The sampling intervals were 1, 2, 3, 4 and 7 days.

3. Frozen minced fish was analysed for microbiological and chemical parameters

   - 1 day after freezing (control sample)
   - 2 weeks after freezing
   - 4 week after freezing
   - 6 week after freezing

3.1 Microbiological measurement of mince fish

3.1.1 Count of total psychrotrophic bacteria and H_2S – producing bacteria by the pour – plate method

Iron agar (LA) containing sodium thiosulfate and L-cysteine was used for determination of total psychrotrophic bacteria and H2S – producing organisms. Bacteria able to produce H2S form black colonies when S^{-2} reacts with Fe^{+2} in the medium (FeS).
The samples were minced fish, 25 g of mince sample first mixed with 225g of dilution buffer in a stomacher for 1 minute resulting in 1/10 dilution and diluted futher using three tubes with 9ml buffer from 1/100, 1/1000 and 1/10000 dilution. One plate was used for each dilution. Of each dilution used 1 ml was transferred with pipettes to the plates. Melted 45°C iron agar was poured on the plate and the content was mixed. After solidification the plates were covered with overlay of iron agar and incubated at 22°C for 72 hours (Vanderzant and Splittstoesser 1992).

3.1.2 Total coliforms and Faecal Coliforms counts by the most probably number method (MPN).

Faecal coliforms ferment lactose and produce acid and gas. Lauryl sulphate tryptose (LST) broth was used as a pre-enrichment media. Brilliant green lactose bile (BGLB) broth was used for total coliforms and EC broth for faecal coliforms. All media contained lactose. Three tubes of LST media used for each dilution, and each dilution was transferred with pipettes to tubes, for first three tubes using 10 ml from 1/10 dilution and then from other six tubes using 1ml from 1/100 and 1/1000 dilution and were incubated at 35°C for 48 hours. After primary incubation one loopful of positive tubes (gas formation tubes) were transferred to BGLB media for total coliforms (incubation at 35°C for 48 hours) and EC broth for faecal coliforms (incubation at 44.5°C for 24 hours).

Table 5: Media dilution for TPC psychrotrophic bacteria and H_2S producing bacteria culture of the raw material minced fish from Atlantic pollock.

Sample	**Total Psychrotrophic bacteria (Level dilution / gram)**	**H_2S – producing bacteria (Level dilution /gram)**
Fish	10^1 - 10^5	10^1 - 10^5
After filleting	" "	" "
After deskining	" "	" "
Fish cuts	" "	" "
New mince	" "	" "
After packaging	" "	" "

Table 6: Media dilution for TPC psychrotrophic bacteria and H_2S producing bacteria culture of the minced fish after thawing from Atlantic pollock

Sample	**Total Psychrotrophic bacteria (Level dilution / gram)**	**H_2S – producing bacteria (Level dilution /gram)**
1 day after thawing	10^1 - 10^5	10^1 - 10^5
2 day after thawing	10^1 - 10^6	10^1 - 10^6
3 day after thawing	10^1 - 10^7	10^1 - 10^7
4 day after thawing	10^1 - 10^7	10^1 - 10^7
5 day after thawing	10^1 - 10^7	10^1 - 10^7

Table 7: Media dilution for TPC psychrotrophic bacteria and H_2S producing bacteria culture of the minced fish after frozen from Atlantic pollock

Sample	**Total Psychrotrophic bacteria (Level dilution / gram)**	**H_2S – producing bacteria (Level dilution /gram)**
1 day after frozen	10^1 - 10^6	10^1 - 10^6
15 day after frozen (2 weeks)	10^1 - 10^5	10^1 - 10^5
30 day after frozen (4 weeks)	10^1 - 10^5	10^1 - 10^5
60 day after frozen (6 weeks)	10^1 - 10^3	10^1 - 10^3

3.2 Chemical measurements of mince fish

3.2.1 TVB-N Method

100 g of the minced fish was weighed and 200 ml of 7.5 % aq Trichloroacetic Acid was added. Mixed for 1 min. in Waring blender, let stand for 10 min., and then filtrated to make an extract. 25 ml of the extract was transferred into distillation flask and 6ml of 10% NaOH added. Distilled for 4 min, and distillate gathered into 10ml of 4% Boric Acid with indicator. Distillate was titrated with 0.05 N H_2SO_4.

3.2.2 TMA Method

100 g of the minced fish was weighed and 200 ml of 7.5 % aq Trichloroacetic Acid was added. Mixed for 1 min. in Waring blender, let stand for 10 min., and then filtrated to make an extract. 25 ml of the extract was transferred into distillation flask and 6ml of 10% NaOH and 20ml 35% formaldehyde added. Distilled for 4 min, and distillate gathered in to 10ml of 4% Boric Acid with indicator. Distillate was titrated with 0.05 Na H_2SO_4.

3.2.3 Protein measurement (Kjeltec Method)

2 g of the minced fish was weighed and transferred in to Kjeldahl digestion flask withcatalyst (2 tablets) and 17.5 ml $H_2SO_{4.}$ Heated for 3 hours at 420°C, cooled and measured in Kjeltec auto distillation unit (ISO 1979).

3.2.4 Fat measurement (Soxtec Method)

Crucible with dry sample and sand (drying method), is transferred into extraction thimble and fat extracted in soxtec system with petroleum ether (boiling point 40–60°C) for 82 min (AOAC 1979).

3.2.5 pH measurement

5 g of the minced fish was weighed and 5 ml distillate water was added, mixed with a magnetic stirrer and measured with pH meter.

3.2.6 Moisture measurement

5 g of the minced fish weighed into a crucible with sand and blended with a glass stick and put into an oven at 103 ± 2°C for 4 hours, cooled in desiccator and weighed (ISO 1999).

3.3 Scheme for mince fish processing from pollock in Iceland

The process of fish mince from Pollock in Iceland is shown in Figure 1 and further pictures from the production process are in Figures 2-8.

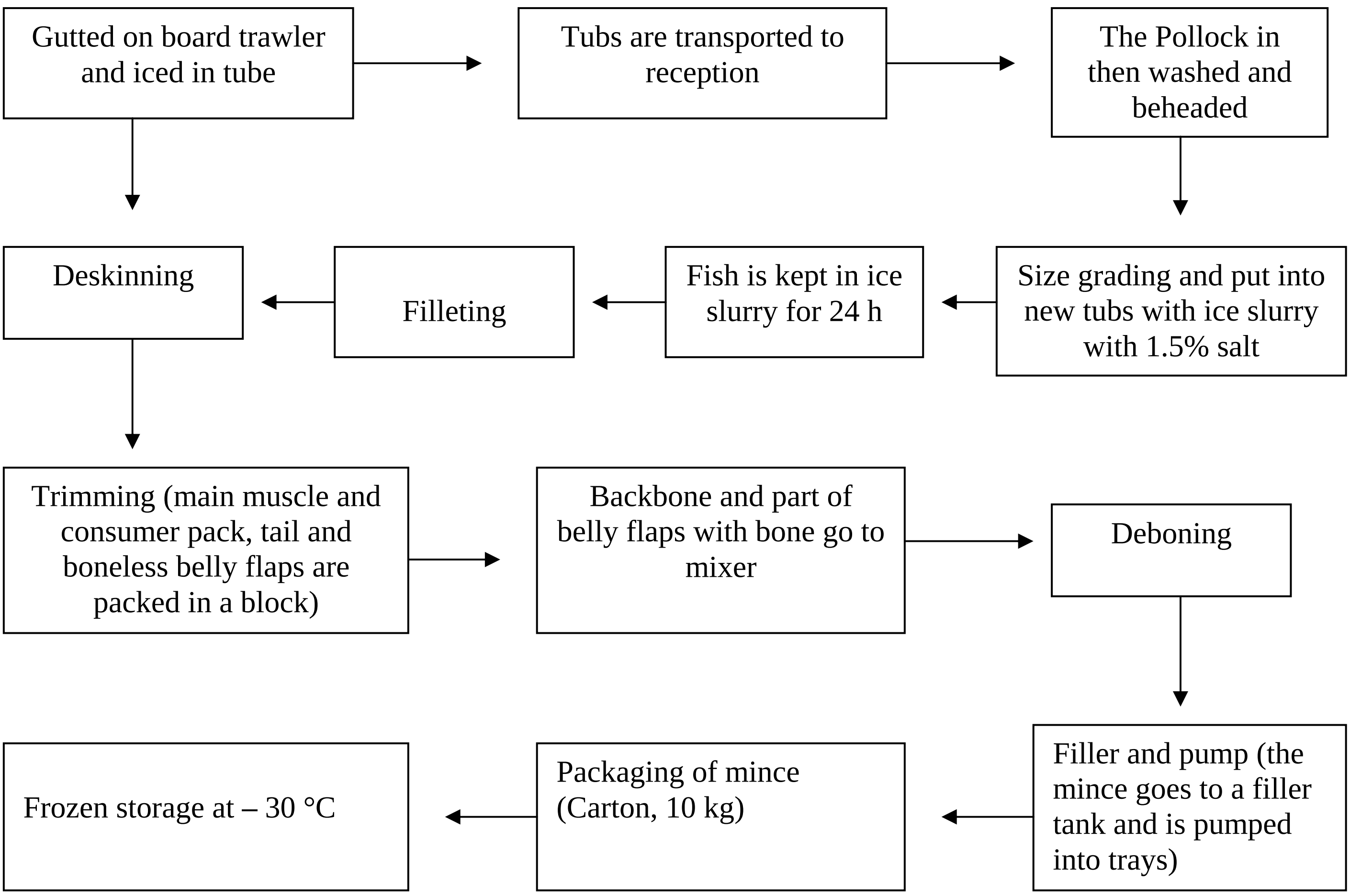

Figure 1: The production process of fish mince from Pollock in Iceland.

Figure 2: Fish after beheading and gutting

Figure 3: Filleting

Figure 4: Fish after filleting

Figure 5: Mixing

Figure 6: Mixing and Deboning

Figure 7: Minced fish in a packaging

Figure 8: Minced fish after packaging

4. RESULTS

4.1 Raw material

4.1.1 Temperature and pH

Temperature and pH of raw material, at the beginning of processing (whole fish) and end line (minced fish after packaging) was measured and is shown in Figures 9 and 10 (table 12 and 13 in Appendix). The results showed that pH and temperature increased but only after filler temperature decreased.

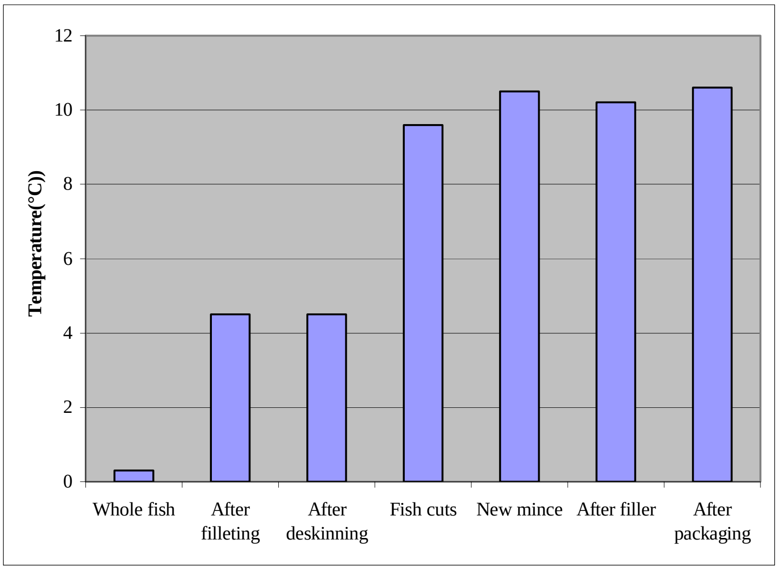

Figure 9: Temperature (°C) for mince fish processing line for pollock

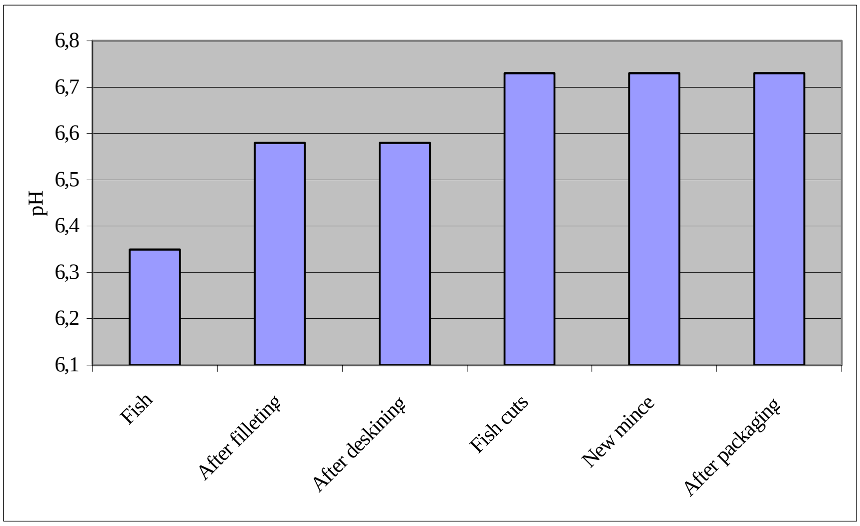

Figure 10: pH measurement in processing

4.1.2 Bacterial counts of raw material

Results of bacterial counts for raw material showed that the total number of psychrotrophic bacteria increased (Figure 11 and Table 13 in Appendix). In whole fish (first stage), the log no. of bacteria was 1/g and after packaging (last stage), the log no. of bacteria was 5.24/g. Number of H_2S–producing bacteria increased throughout the processing with no bacteria detected at the first stage of processing (whole fish) and about 3.5 (log no./g) at news mince stage (Figure 12 and Table 14 in Appendix). Results for total coliforms and faecal coliform showed that number of these bacteria increased during processing of the pollock mince, indicating that cleaning and disinfecting operation were insufficient (Figure 13 and Table15 in Appendix).

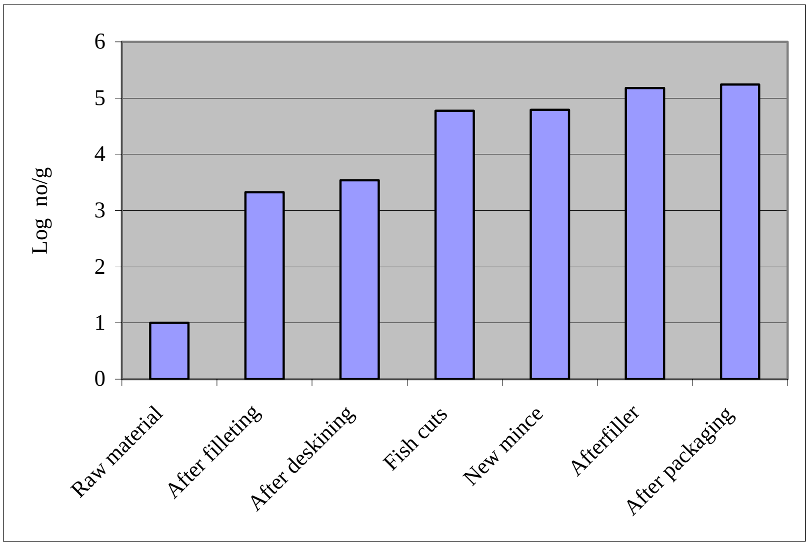

Figure 11: Log number of psychrotrophic bacteria (total / gram) during processing

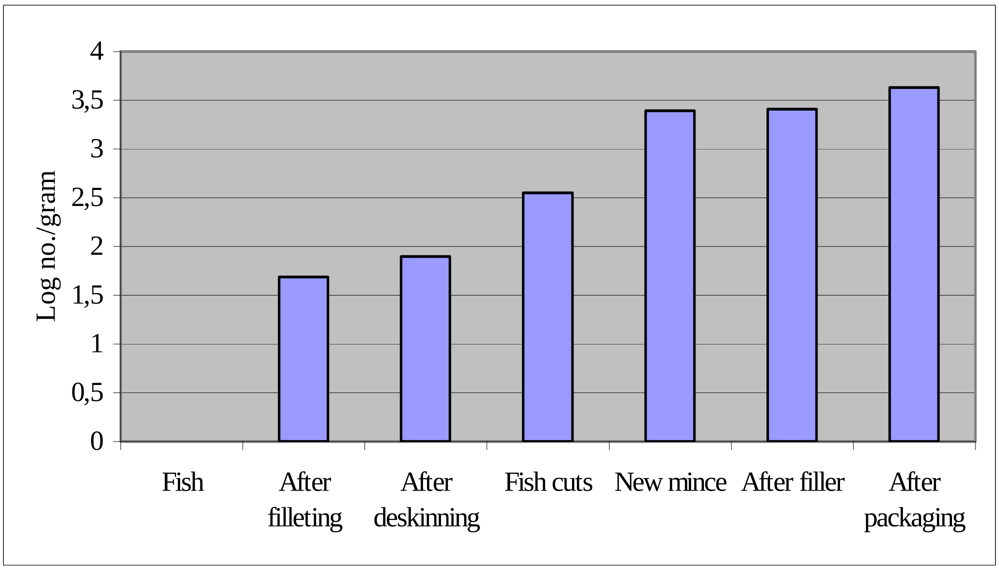

Figure 12: Log number of psychrotrophic bacteria H_2S producing (total /during processing of minced pollock

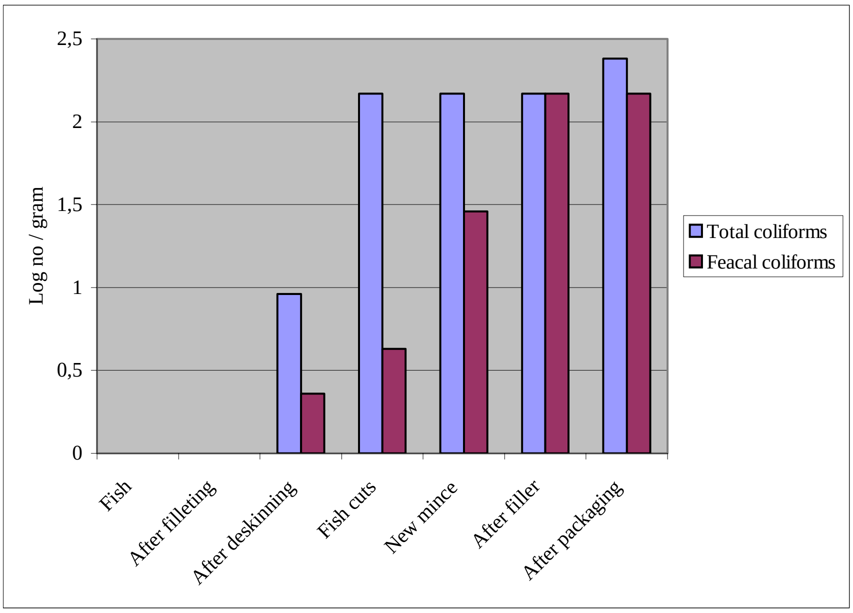

Figure 13: Log number total coliforms and faecal coliforms per gram for mince processing.

4.1.3 Chemical measurements of raw material

Nutritional value measurement show that Atlantic pollock is a lean fish with high protein content (Table 8).

Table 8: Protein, fat and moisture content in the whole fish and mince fish

Sample	Protein %	Fat %	Moisture %
Whole fish	17.5	0.3	79.5
Minced fish	17.1	0.4	81.57

Results of TMA and TVB-N measurements for whole fish and raw material are shown in Figure 14. Variation for fish fillets and minced after packaging was insignificant and this range is normal and acceptable.

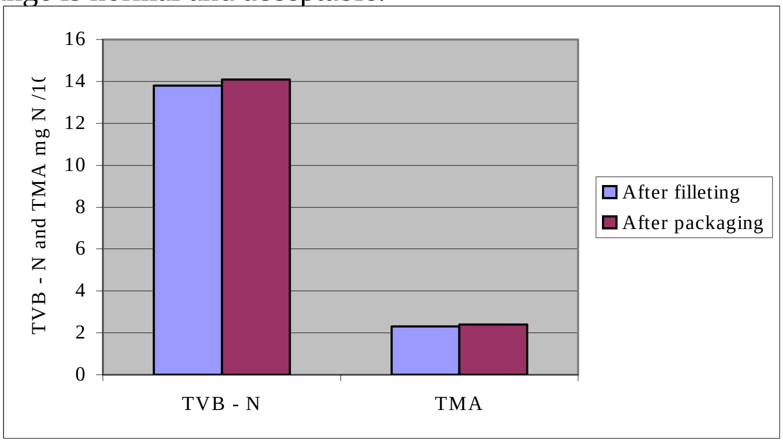

Figure 14: TVB – N and TMA (mg/100g) variation line processing minced fish from pollock

4.2 Thawed minced fish

4.2.1 Bacterial counts of mince after thawing

Results for thawed minced fish kept at 1–2°C for up to 7 days are shown in Figures 15– 17. Total psychrotrophic and H_2S-producing bacteria increased by about 23% during 7 days of storage. The increased was pronounced between day 4 and 7. Number of total and faecal coliforms, however, decreased during the stage period.

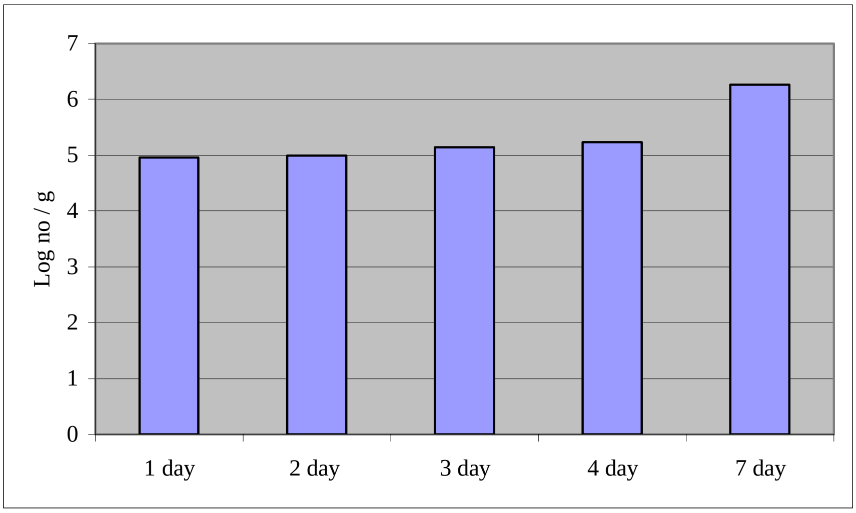

Figure 15: Log number psychrotrophic bacteria (total/g) in minced fish after thawing.

The number of H_2S producers increased more gradually throughout the period. Yet, a sudden increased was observed between day 3 and 4.

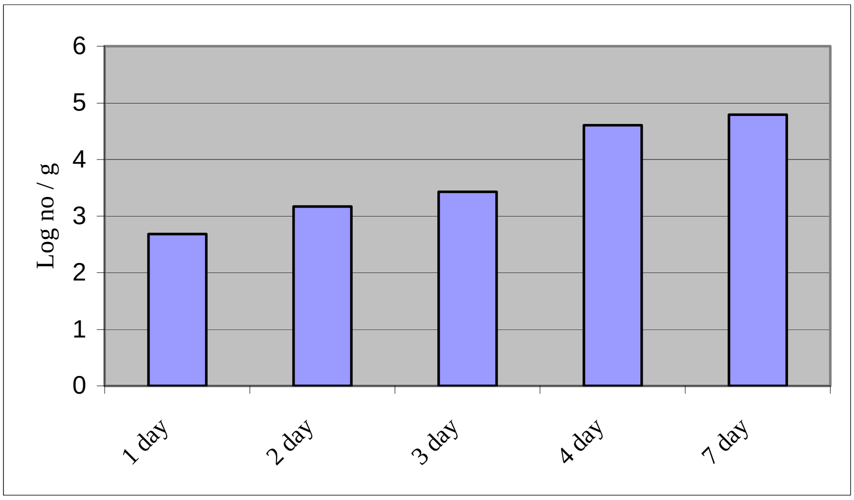

Figure 16: Log number of psychrotrophic bacteria (H_2S producing) (total/g) in minced fish after thawing.

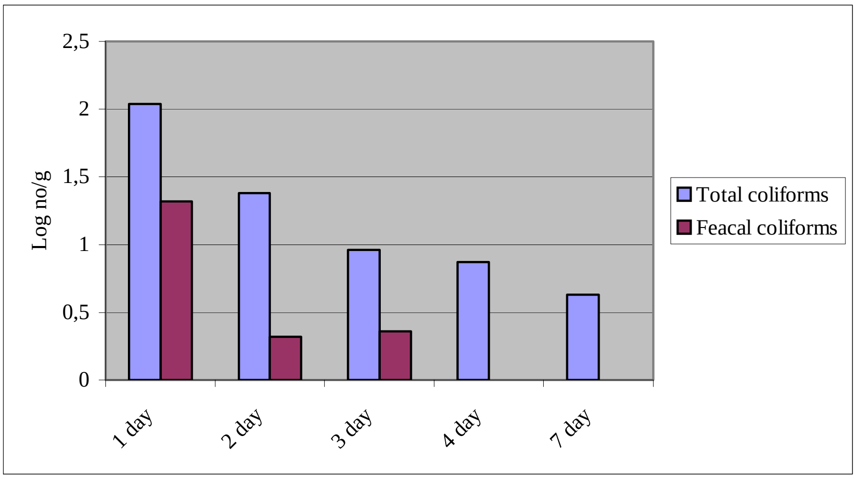

Figure 17: Log number of total coliforms and faecal coliforms per gram minced fish after thawing.

The number of total coliforms decreased from day one to day seven and no faecal coliforms were found after day 3.

4.2.2 Chemical measurements of mince after thawing

Results of chemical spoilage measurements for minced fish after thawing are shown in Figure18.

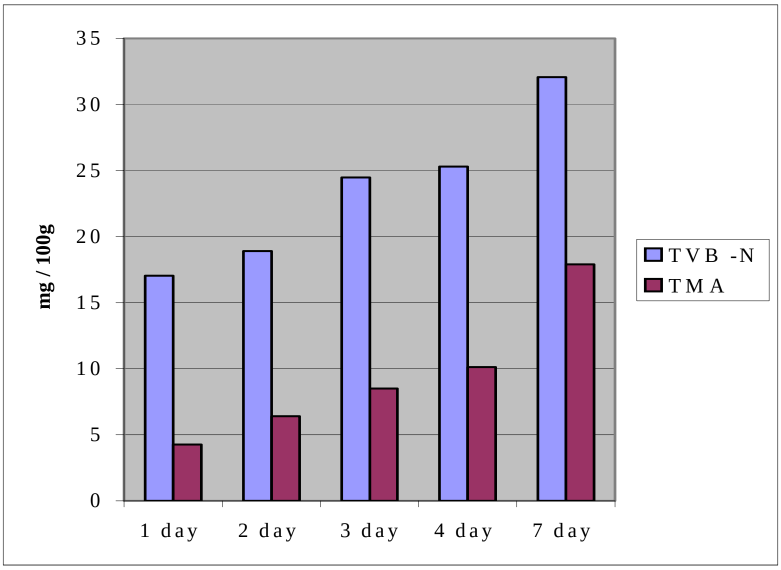

Figure 18: TVB – N and TMA (mg / 100) over 7 days period in minced fish after thawing from pollock (in temperature 0 – 2 °C)

TMA and TVB – N increased during the 7days period. Results indicate that levels of TVB - N and TMA were acceptable the first 4 days (<10 mg / 100 g. N) and < 30 mg for TMA) but after seven days the levels were too high for consumption of the minced fish.

4.3 Frozen minced fish

4.3.1 Bacterial counts of frozen mince

Results from bacterial counts for frozen minced fish (Table 22 in Appendix and Figure 19) showed that total psychrotrophic bacteria decreased during freezer storage. Log no. of bacteria was 4.86/g in the beginning and decreased to 4.02/g after 50 days. The results for H_2S–producing bacteria showed (Figure 20), that on day 1 in freezer the log no. of bacteria/g was 3.2 and after 50 days it was 2.11/g.

Results for total and faecal coliforms showed (Table 24 in Appendix and Figure 21) that the number of these bacteria decreased considerably during freezer storage.

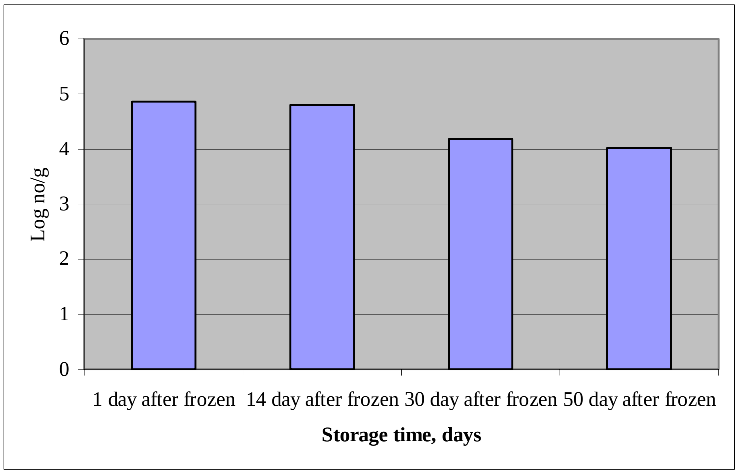

Figure 19: Log number psychrotrophic bacteria (total/g) in frozen minced fish.

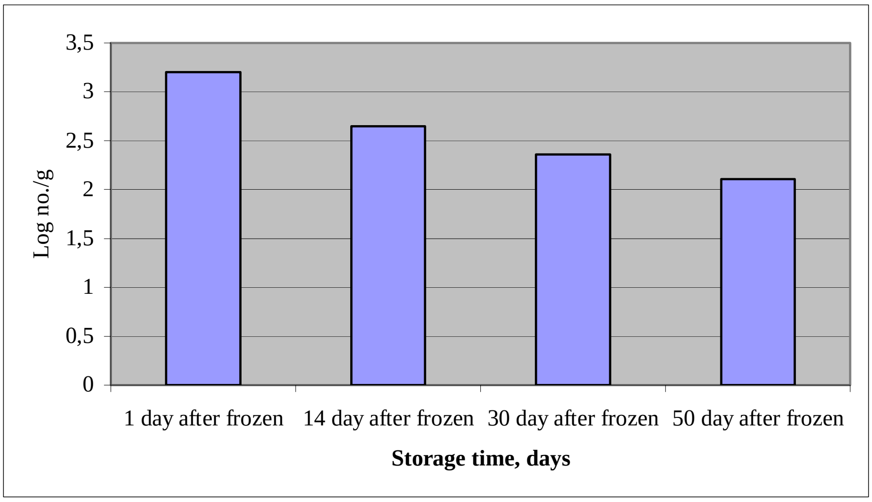

Figure 20: Log number of psychrotrophic bateria H_2S producing (total/g) in frozen minced fish.

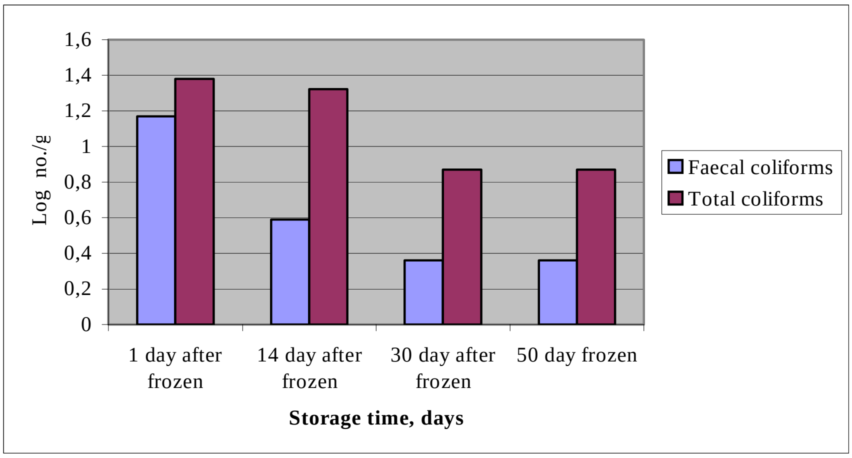

Figure 21: Log number total and faecal coliforms per gram in frozen minced fish.

4.3.2 Chemical measurements of frozen mince

The chemical results of frozen minced fish showed that after 50 days pH was 6.73, protein 16.9%, fat 0.4 – 0.5% and moisture was 81.9 %. Results for chemical spoilage measurement showed that TVB–N for frozen mince was recorded 17.2 (mg / 100g) and for TMA 4.5 (mg / 100g) and all characteristics were acceptable (Table 9).

Table 9: Frozen minced fish characteristics after 50 days storage at – 30 °C

Sample	**Protein (%)**	**Fat (%)**	**Moisture (%)**	**TVB – N (mg /100)**	**TMA (mg /100)**
Frozen minced fish	16.9	0.4 – 0.5	81.9	17.2	4.5

4.4 Statistical analysis

Statistical analysis of data by using t–test with 95% level of confidence of the microbiological measurement for total psychrotrophic count showed for raw material and fish minced after thawing was insignificant ($p > 0.05$), H_2S producing bacteria count between raw material and fish minced after thawing was significant ($p < 0.05$).

For total coliform count between raw material and fish minced after thawing showed significant, also faecal coliforms count between raw material and fish minced after thawing was significant.

Statistical analysis of the total and faecal coliforms count between raw material and fish minced after thawing was insignificant.

Data analysis for TVB – N and TMA from raw material and fish minced after thawing was insignificant. Also t – test analysis for pH data between raw material and fish minced after thawing was insignificant.

Chemical statistical analysis shown, that t – tests for, protein and fat between raw material and minced fish after frozen was insignificant. But data analysis showed moisture, TVB – N and TMA between raw material and minced fish after frozen significant. Statistical analysis for microbiological measurement between raw material and minced after frozen was insignificant.

First and **last** chart refers to the whole fish and minced product in **part 1,** and in **part 2** measurement were taken on first day and last, and **part 3** of the mince was frozen for approximately **50 days** shown in the table 10 and 11.

Table 10: Comparison of microbiological characteristics of the samples

Sample	**pH** first and last stage	**Temp(°C)** first and last stage	**Total psychrotrophic bacteria log no./g** first and last stage	**H2S – producing bacteria log no./gr** first and last stage	**Total coliforms bacteria log no./g** first and last stage	**Faecal coliforms bacteria log no./g** first and last stage
part I Raw material for minced fish	6.35 6.73	0.3 10.6	1 5.24	0 3.63	0 2.38	0 2.17
part 2 Minced fish after thawing	6.65 6.86	1 2	4.96 6.26	2.68 4.79	2.04 0,63	1.32 0
part 3 Frozen minced fish	6.59 6.73	No data - 30	4.86 4.02	3,2 2.11	1,38 0.87	1.38 0.36

Table 11: Comparison of chemical characteristics of the samples

Sample	**Protein (%)** first and last stage	**Fat (%)** first and last stage	**Moisture (%)** first and last stage	**TVB – N (mg /100)** first and last stage	**TMA (mg/100)** first and last stage
part 1 Raw material for minced fish	17.5 18.1	0.3 0.4	79.5 79.9	13.8 14.1	2.3 2.4
part 1 Minced fish after thawing	17.1 17.8	0.4 0.5	81.75 82.05	17.04 32.06	4.26 17.91
part 3 Frozen minced fish	16.9 17.6	0.4 0.5	81.9 82.3	17.2 17.9	4.5 4.8

5. DISCUSSION

Spoilage of fish and fishery products can be attributed to the following:
1 – Micro-organisms and their enzymes.
2 – Chemical spoilage such as lipid oxidation (rancidity).
3 – Autolysis(enzymes from fish).

The main spoilage of thawed mince is due to bacteria. Keeping mince at 0°C or over results in rapid growth of psychrotrophic bacteria and same of these bacteria are active spoil agents. They produce various enzymes which result in the production of various chemicals which give bad smell and taste. Best known of these reactions is the bacterial breakdown of TMAO to TMA. The main constituents of TVB-N are TMA and ammonia (NH_3). In this project, Atlantic Pollock was found to have fat content in the range of 0.3 – 0.4%. That means that this species of fish is very lean which makes spoilage due to lipid oxidation (rancidity) very unlikely.

Spoilage due to autolytic enzyme in this product should be of minor importance compared to bacterial spoilage since such enzymes are mainly found in the intestine of the fish. This could be a big problem in fish, where the intestines are not removed prior to mincing. There are several important processing factors which are involved in the manufacture of minced fish. They include processing steps such as beheading, gutting or filleting for medium size fish (Pollock), fish temperature and moisture content in the minced fish and the processing line was partly responsible for increase in microbiological measurements.Since quality assessment can not be done under deep frozen conditions, minced fish must be thawed.

There are four main reasons for the increase in the total, H_2S–producing psychrotrophic bacteria and total coliforms and faecal coliforms for raw material and minced fish after thawing:

1- Temperature increase in the processing line that affects psychrotrophic bacterial growth and growth of the coliforms.

2- Improper cleaning system can be lead to high bacterial counts. It is of great importance to clean all machinery thoroughly before and after processing. Floors, walls, etc. should also be cleaned.

3- Water can be contaminated.

4- Low hygiene of staff for processing the mince can be a reason for increasing total coliforms and faecal bacteria. Clean gloves and clothes are essential in fish processing. People suffering from bad colds or stomach illnesses should not work in food production.

The observed low total and faecal coliform count for thawed minced fish might have been due to the sharp drop of temperature from –30°C to room temperature. This sharp drop could have caused stress to the bacteria resulting in low metabolic rate of the bacteria (Silliker and Bryan 1995).This decrease was probably also due to competition to the growth of psychrotrophic bacteria. Temperature was kept at 1 - 2°C and coliforms do not grow at low temperature.

Although there can be a high bacterial count in the initial mince fish products, this is not problem for they can be inactivated in heat processing. The coliforms bacteria are all killed during heat processing of mince product like fish burger, sausage, etc.

Keeping food frozen prolongs the shelf-life of the food and also inhibits the growth of micro-organisms. It is worth noting that for minced products –30°C is the best temperature for prolonging shelf–life.

The reasons for decreasing bacterial count of the frozen minced fish are different tolerance of bacteria to low temperature. Micro-organisms differ in their responses to freezing. Some survive unharmed while others are sensitive to freezing and freezer storage. Most micro-organisms are killed in the temperature range from –2°C to -10°C. It is however apparent from this study that some bacteria were killed during the 50 day storage of the mince at –30°C. Coliforms were very sensitive. TMA and TVB–N did not change significantly in this project during freezer storage.

6. CONCLUSIONS

1. This study shows that Atlantic pollock is a lean fish and good raw material for the preparation of minced fish products, which include fish sausage, fish burger, fish ball, etc., and it also gives an attractive colour. The final product is cheap and can be used for consumption.

2. Many categories of fish are suitable for the production of minced fish but lean fish is considered the best. Fatty fish muscle can also be used for minced products, but thorough washing is necessary for removing the excess fat globules.

3. The results indicate that the –30°C is the optimum temperature for the storage of minced fish to have longer shelf–life.

4. Minced fish compared to whole one has much larger surface area, which makes it more vulnerable to environmental factors. Thus, the mincing process must have a corresponding higher standard to quality control. The initial relatively high bacterial count isn’t very important in the mince and raw material, because further processing will take place such as cooling which reduces microbial contamination considerably.

5. Bacterial count of minced fish is usually higher than other fish products. To avoid high bacterial content it is advisable that processing plant constantly check temperature, cleaning system and staff hygiene during processing.

ACKNOWLEDGMENTS

I would like to thank Dr. Tumi Tomasson, The Director of the Fisheries Training Programme at the United Nation University for providing the opportunity to attend this programme.

I would like to thank the Icelandic Fisheries Laboratories for their co-operation.

I would like to thank Mr. Hannes Magnusson for helping with this project and his valuable advice to me.

I would also like to thank Mr. Thor Asgeirsson, Deputy Director of the UNU-Fisheries Training Programme for his advice on doing this project.

I would like to thank the Programme Officer, Mrs Sigríður Kr. Ingvarsdóttir UNU-Fisheries Training Program for her advice and co-operation in taking samples in the Icelandic Company.

I would like to thank Mrs Ingibjörg Jónsdottir and Ingibjörg Rósa Þorvaldsdóttir and Jarmíla Hemannsdóttir for chemical and microbiological measurements of the minced fish samples.

I would like to thank Grandi Company manager for takes samples of line processing minced fish and staff of that company. I am grateful to all the fellows of the UNU/FTP for their help during my training in Iceland.

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APPENDIX

Table 12: Temperature (°C) for line mince fish processing from pollock

Sample	Temperature
Fish	0.3
After filleting	4.5
After deskinning	4.5
Fish cuts	9.6
New mince	10.5
After filler	10.2
After packaging	10.6

Table 13: pH measurement for line processing minced fish from pollock

Sample	pH
Fish	6.35
After filleting	6.58
After deskinning	6.58
Fish cuts	6.73
New mince	6.7
After packaging	6.73

Table 14: Number psychrotrophic bacteria (total / gram) duration
Processing of minced pollock

Sample	Log no.bacteria
Raw material	1
After filleting	3.32
After deskinning	3.54
Fish cuts	4.78
New mince	4.79
After filler	5.18
After packaging	5.24

Table 15: Log number of psychrotrophic bacteria H_2S producing

(Total / gram) during processing of minced pollock

Sample	Log no./ bacteria
Fish	0
After filleting	1.69
After deskinning	1.9
Fish cuts	2.55
New mince	3.39
After filler	3.41
After packaging	3.63

Table 16: Log number total and faecal coliforms per gram for line processing
Minced fish from pollock

Line processing	Total coliforms	Faecal coliforms
Fish	0	0
After filleting	0	0
After deskinning	0.96	0.63
Fish cuts	2.17	0.36
New mince	2.17	1.46
After filler	2.17	2.17
After packaging	2.38	2.17

Table 17: Number psychrotrophic bacteria (total / gram) minced fish thawing From pollock

Sample	Log no. / gram
1 day	4.96
2 day	4.99
3 day	5.14
4 day	5.23
7 day	6.26

Table 18: Log number of psychrotrophic bacteria H_2S producing (total / gram) minced fish thawing pollock

Sample	Log no. / gram
1 day	2.68
2 day	3.17
3 day	3.43
4 day	4.6
7 day	4.79

Table 19: Log number total and faecal coliforms per gram minced fish thawing from pollock

Day	Total coliforms	Faecal coliforms
1 day	2.04	1.32
2 day	1.38	0.32
3 day	0.96	0.36
4 day	0.87	0
7 day	0.63	0

Table 20: TVB – N and TMA (mg / 100gram) variation line processing minced fish from pollock

Sample	TVB - N	TMA
After filleting	13.8	2.3
After packaging	14.1	2.4

Table 21: TVB – N and TMA (mg / 100) variation minced fish after thawing from pollock

Sample	TVB -N	TMA
1 day after thawing	17.04	4.26
2 day after thawing	18.9	6.39
3 day after thawing	24.49	8.52
4 day after thawing	25.29	10.11
7 day after thawing	32.06	17.91

Table 22: Log number psychrotrophic bacteria (total / gram) frozen minced fish
From pollock

Sample	Log no / gram
1 day after frozen	4.86
14 day after frozen	4.8
30 day after frozen	4.18
50 day after frozen	4.02

Table 23: Log number of psychrotrophic bacteria H_2S producing (total / gram)
For frozen minced fish pollock

Sample	Log no / gram
1 day after frozen	3.2
14 day after frozen	2.65
30 day after frozen	2.36
50 day frozen	2.11

Table 24: Log number total and faecal coliforms per gram for frozen minced
fish from pollock

Sample	Faecal coliforms	Total coliforms
1 day after frozen	1.17	1.38
14 day after frozen	0.59	1.32
30 day after frozen	0.36	0.87
50 day frozen	0.36	0.87