Improving the utilization of Silver carp (Hypopthalmichthys Molitrix) and or other under-utilized fish species, especially Fresh water Bream (Abramis brama)

Possibilities for value adding supply chains and international trade of Silver carp (*Hypopthalmichthys Molitrix*) in the Islamic Republic of Iran.

Final report of the research between 2005 and 2007

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Abstract

Iran is a country with extended rural areas of which most are very dry and warm. Therefore the catch, production and consumption of fish does mainly occur at the scarcely available places nearby water in the north and south. Due to the limited places available in Iran where aquatic food could be cultured or caught and the ancient problem in arid areas to transport fish over large distances, fish is not a major part of the diet for most inhibitants causing the domestic market for fish to be very small, even today. On the contrary cheese has been and still is an important food for the Iranian people. Recently the production of Silver Carp has been growing fast, but currently the growth does collapse due a limiting demand. The Iranian government does see fish as one of their main internal protein sources and is eager to see that the proteins from fish as for example Silver Carp are better utilized and more available throughout the whole country.

Bilateral research priorities has been established during a fact finding mission during 2004. The research has been carried out within the framework of the DLO-research program international co-operation: Iranian-Dutch collaborating R&D projects related to food safety and food chains. The relevancy is cleared by the M.O.U. and the agreements between LNV and WUR (ref Adj/LJ/03/0021920). As the availability of Silver Carp has not been sufficient during the project all experiments within the project were executed using fresh-water bream as a model species for Silver carp.

Production of a proper fillet from Silver Carp without the unpleasant fatty tissue bones and skin using specific parts of the fish has been researched by investigating the Silver Carp's anatomy by the use of X-rays. Due to lack of Silver carp in the Netherlands, this task is mainly executed by the Iranian project partners. X-rays did show specific parts could be used to produce pieces of a high quality boneless fillet. The size of these high quality fillet pieces is expected to be dependent on the size of the fish used for processing. The challenge is to find a method to remove these boneless parts from the whole fish. The by-products of this process can be used for the production of the other products developed during this project. Sous vide packaging of fish is well possible as a preservation method but did not give a solution for disintegrating the small pin bones in Freshwater Bream and it is unlike the process will work with Silver Carp.

The development of new products by restructuring fish proteins, processing and preservation methods using minced meat of Silver carp has been researched by producing several fish protein structures, such as soft cheese alike, Surimi alike and ice cream alike protein structures using different process strategies. To improve the utilization of fresh water fish with many bones, new procedures for processing have been developed giving added value by creating products apart from a better quality and yield. Both mince as proteins recovered from the wash water have been used to produce products. The processes developed do relate to intermediate products, such as mince, freeze dried proteins and freeze texturized proteins, as to final products for the consumer.

Procedures are given for both the production of mince as a frozen half fabricate and using the half fabricate as raw material for the production of value added products in different physical forms (sausage, sliceable products, etc), such as Kamaboko, Surimi, oil enriched products and extreme fatty oil in fish gels. Sliceable cheese alike structures were produced successfully using a blend of fresh water Bream and Salmon. Addition of 40% water and 10% of oil makes this product interesting from an economical point of view. Mince also has been cooked and formed by means of extrusion technology together with corn starch to produce direct expanded products. These products show that snacks can be produced with a better-balanced nutritional value together with a long shelf live.

Procedures are given to use the recovered soluble proteins to produce emulsions, pate, cheese alike and imitation meat alike textures. Butter, pate and cheese alike textures with high oil content have been created using both heat gelation and cold setting, but results are far from edible. More research is needed to optimize and further explore the use of the precipitated water-soluble fish proteins as high attractive products. Fish ice has been produced from freeze dried water soluble proteins, but the results obtained were not optimal. More research is needed to develop fish ice acceptable for the consumer. Imitation meat or restructured fillet pieces were produced from proteins recovered from the wash water. When fried the pieces can be used as snacks, when cooked the pieces can be used for stir fry dishes, soups and or a kind of "fish kebab". To our surprise the meat analogue showed a structure and colour almost equal to that of turkey meat.

Contents

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1. Overview of the project

1.1 Introduction

Iran is a country with extended rural areas of which most are very dry and warm. Therefore the catch, production and consumption of fish mainly occur at the scarcely available places nearby water in the north and south of the country. Due to the limited places available in the country where aquatic food could be cultured or caught and the ancient problem in arid areas to transport fish over large distances, fish is not a major part of the diet for most Iranian people, causing the domestic market for fish to be very small even today. On the contrary, cheese has been and still is an important part of the food for Iranian people, because the proteins from milk were widely available and the making of cheese as a preservation method worked well. Recently the production of Silver Carp has been growing fast, but currently the growth does collapse due a limiting demand. The Iranian government does see fish as one of their main internal protein sources and is eager to see that the proteins from fish as for example Silver Carp are better utilized and more available throughout the whole country.

1.2 Objectives

The research priorities and objectives as agreed by the researchers of both countries during the fact finding and goal establishing mission of the project (13) are depicted in the scheme below. The yellow boxes mark the approved research objectives, which are covered in the current project.

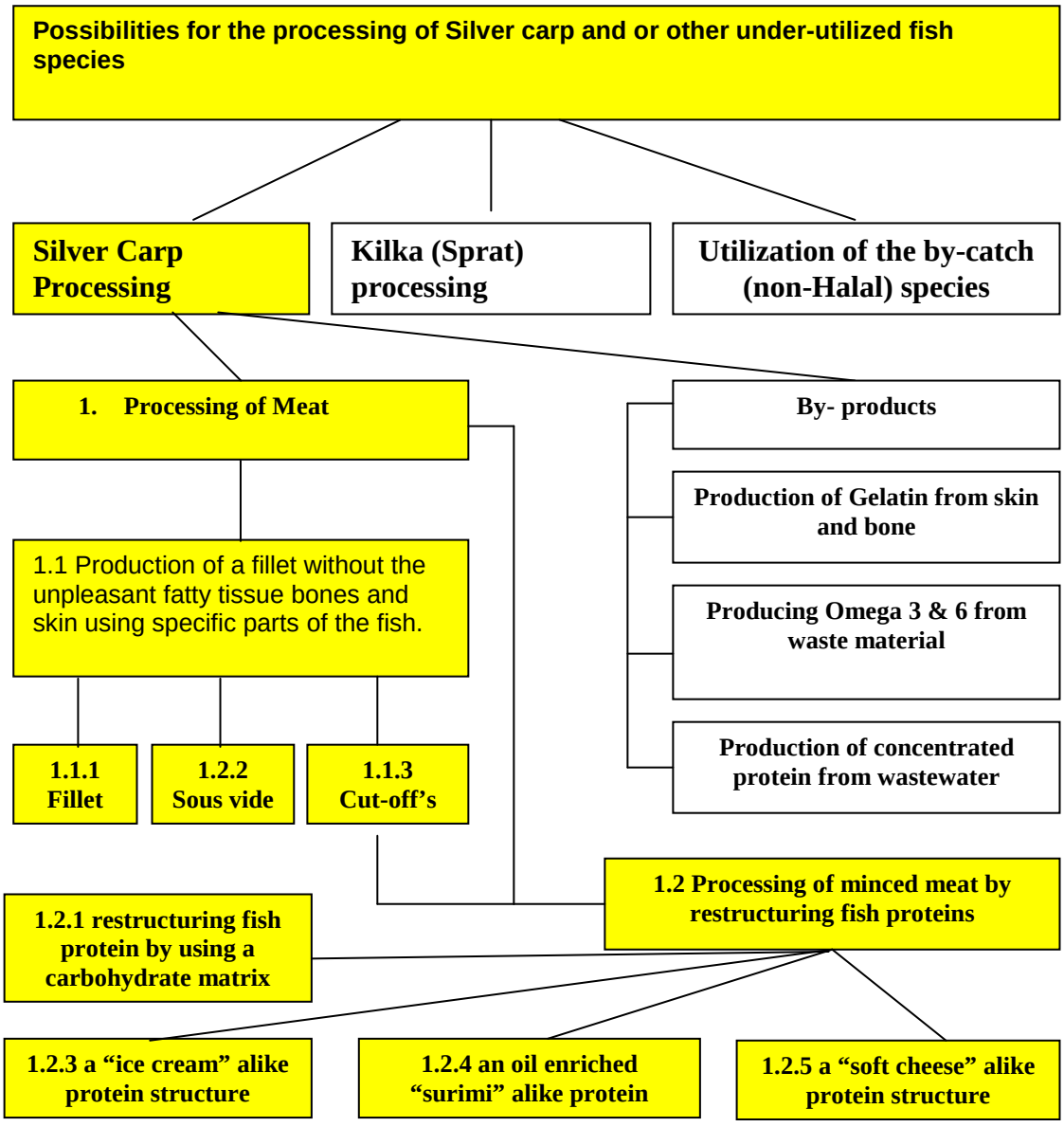


Figure a: The research priorities and objectives as agreed. The yellow boxes mark the prior research objections

In summary the two main objectives

1. The production of a fillet from Silver Carp without the unpleasant fatty tissue, bones and skin using specific parts of the fish.

2. The development of new products by restructuring fish proteins, processing and preservation methods using minced meat of Silver carp to enlarge the supply area and thereby the demand of the internal market and stimulate export. The new structured products will enhance the added value by decreasing the amount of valueless fish material and by increasing the convenience for the user.

1.3 Work plan

1.1 To achieve the first objective: “The production of a proper fillet from Silver Carp without the unpleasant fatty tissue bones and skin using specific parts of the fish”, the Silver Carp’s anatomy will be investigated by the use of imaging techniques, such as X-rays to evaluate if some parts of the fish contain less bones and can be used to produce a high quality boneless fillet.

1.2 For the second objective: “The development of new products by restructuring fish proteins, processing and preservation methods using minced meat of Silver carp”, several fish protein structures, such as the soft cheese alike, surimi alike and ice cream alike protein structures and the processing techniques mentioned below will be investigated for their feasibility:

1.2.1) Restructuring fish proteins together with a carbohydrate matrix, using extrusion technology

1.2.2) The use of sous vide preservation technology to produce vacuum packed cooked fillets from Silver Carp decreasing the problem of the sharp pine bones by disintegrating their structure and making them edible.

1.2.3) The ice cream like protein structure will be produced by the (partly) replacement of milk proteins by the proteins originating from Silver carp.

1.2.4) The production of an oil enriched surimi alike protein structure by using emulsion technology and additives.

1.2.5) The soft cheese alike protein structure will be created by the use of acid coagulation of proteins from Silver Carp

1.4 Reasons for performing the project

1.4.1 Relevancy to international policy development LNV

The reseach has been carried out within the scope and objectives of the framework of the DLO-research program international co-operation: Iranian-Dutch collaborating research project related to food safety and food chains. Resultes of this collaboration will stimulate the addition of value to the fish meat, the sustainability, enlargement of the internal market, and export of (farmed) aquatic products from Iran. Specific keywords are: Regional value adding supply chains, enlargement of internal market and international trade, food quality and safety, under utilised fish species and Silver carp. Tools are upgrading, technology, restructuring of fish proteins, preservation, higher added value, traditional products, sensory evolution and partner shipping upgrading, technology, higher added value, traditional products, and partner shipping.
The relevancy is cleared by the M.O.U. and the agreements between LNV and WUR (ref Adj/LJ/03/0021920).

1.4.2 Urgency of the issue, contribution to community and social objectives: economical, industry, safety, social and environmental impact

This project benefits to the Iran consumers and the fish supply chain as a whole (fishermen, fish farmers, processors, the food ingredient, functional food industry, and retailer). The better utilisation of, high valued, raw materials and or under utilised fish species does result in a better use of the natural resources, which has a positive effect on the health state of the people, environment and image of the fish industry. Introduction of the developed processes in the fish industry or in companies affiliated with industry will lead to increased employment and technological know-how. Environmental problems related to fish offal's are reduced as transport over long distances of putrefying offal's can be avoided and by-products that are momentarily used as low valued animal feed can be upgraded to food grade ingredients.

1.4.3 Spin-off: Economic impact and exploitation potential

The fish processors will use the new products to have better possibilities for distribution internally and for export and will be able to increase their margins and market share. The consumers will have more opportunities to include fish in their diet, which could increase the health of the whole population and may cause savings on community medical expenses. The development of a soft cheese alike protein structure from fish could give a great contribution to the goal to increase the amount of fish protein in the diet of the people who historically seen would not consider to eat fish at all. The expectation is that a soft cheese alike protein structure made from fish protein as an "anonymous" protein source would be more readily accepted, because cheese is a major part of their diet, and will significantly increase the domestic demand for fish together with its health improving effects and new impulse for further growth of the Silver Carp industry. The ice cream like protein structure can also contribute to an increasing demand for fish proteins. The current available fish ice is a luxury product with high added value. In some parts of Asia ice cream with a fish flavour is very popular. It would be a challenge to replace the fish flavour by real fish protein. The oil enriched surimi alike protein structure and the restructuring of fish proteins together with a carbohydrate matrix are important for two reasons. The combination of carbohydrates and fish proteins and oil and proteins are excellent combinations from a nutritional value point of view. The production of a fillet without the unpleasant fatty, bones and skin using specific parts of the fish or the production of sous vide products from Silver Carp could cause a major boost in the export of Silver Carp as premium product. Silver Carp is a very tasty fish, with unfortunately many bones, which is one of the major reasons for the consumer not to buy this type of fish. To summarise the project can contribute to a better utilisation and also division of the fish protein available in Iran. Because it is expected that the proteins from fish will be more available for both the domestic as export market it will increase demand, which can be the start of a growing and sustainable Silver Carp industry

1.5 Executed research between 2005 and 2007

As the availability and supply of Silver Carp has not been sufficient during the project all experiments were executed by using fresh-water bream as a model species for Silver carp. Preliminary result of the project has been reported in the progress report of 2006 (14)

1.1) To obtain results for the first objective: “The production of a proper fillet from Silver Carp without the unpleasant fatty tissue bones and skin using specific parts of the fish”, the Silver Carp’s anatomy will be investigated by the use of imaging techniques, such as X-rays to evaluate if some parts of the fish contain less bones and can be used to produce a high quality boneless fillet. Due to lack of Silver carp specimen in the Netherlands, this task is mainly executed by the Iranian project partners. A short description is given in paragraph 2.2.

1.2) To achieve the second objective: “The development of new products by restructuring fish proteins, processing and preservation methods using minced meat of Silver carp”, several fish protein structures, such as the soft cheese alike, surimi alike and ice cream alike protein structures and the processing techniques mentioned below will be investigated for their feasibility:

1.2.1) Restructuring fish proteins together with a carbohydrate matrix, using extrusion technology.

This task is successfully executed with Freshwater Bream, as a model species for Silver Carp. Results are shown in paragraph 2.3

1.2.2) The use of sous vide preservation technology to produce vacuum packed cooked fillets from Silver Carp decreasing the problem of the sharp pine bones by disintegrating their structure and making them edible.

This task is executed with Freshwater Bream and it can be concluded that “Sous vide” as preservation technology cannot be used to soften the bones and thereby decrease the problem of the sharp pine bones in Freshwater Bream. The method is too mild to disintegrate the bones. It is unlikely the technology will work with Silver Carp.

1.2.3) The ice cream like protein structure will be produced by the (partly) replacement of milk proteins by the proteins originating from Fresh water Bream as a model species for Silver Carp. This task is executed with only limited success. Results are shown in paragraph 2.3.3.

1.2.4) The production of an oil enriched surimi alike protein structure by using emulsion technology and additives.

This task is successfully executed with Freshwater Bream, as a model species for Silver Carp. Detailed results and procedures are shown in paragraph 2.3.4

1.2.5) The soft cheese alike protein structure will be created by the use of acid coagulation of proteins from Silver Carp

Experiments were executed using proteins originating from Fresh water Bream. Several experiments were executed using proteins recovered from the wash water, some with surprising results, e.g. “Imitation Meat” as described in paragraph 2.5, others with limited success. A different approach to create a sliceable cheese alike structure is shown in paragraph 2.4.5.3.2. Detailed results and procedures of all experiments executed are described in paragraph 2.4.5 and 2.5

2. Results of executed research

2.1 Introduction

Due to problems regarding the supply of Silver carp the raw material used in the experiments executed during the project was produced from Fresh water Bream, *Abramis brama.* This decision was made after comparing tissue composition of several related cyprinids (8). When putting the different evaluated species in order of most comparable tissue composition it results in the following list.

1) Silver Carp
2) Bighead Carp
3) Grass Carp
4) Fresh water Bream
5) Roach
6) Common Carp

Bighead appears to be the best option for replacing Silver Carp, but this fish is just as hard to get as Silver Carp, which is also the case for Grass Carp. This does leave Fresh water Bream as the best and most practical alternative, because the fish is easy to obtain. Therefore Fresh water Bream is used as a model species for Silver Carp.

2.2 Task 1.1 The production of high quality fillet pieces from Silver Carp without the unpleasant fatty tissue bones and skin using specific parts of the fish

Intramuscular bones in fish often cause problems for the fish processors to produce boneless fillets. This is also the case with carp species, especially Silver Carp. According to our Iranian colleagues Silver carp does posses 43 intramuscular bones on each side of the fish; 26 above the lateral line of which 20 of them have a typical Y-shape (figure b) and 17 below the lateral line. The later can be found in the flesh approximately 1/3 of the depth below the body surface. An overview is given in figure c. As can be seen in figure d en e some parts of the fish are free of bones. These specific parts could be used to produce pieces of a high quality boneless fillet. One could think of product names like “saddle of Silver carp” or “lion fillet” referring to the best meat parts available from Silver Carp serving the top end of the market and creating higher added value. The size of these high quality fillet pieces is expected to be largely dependent on the size of the fish used for processing. The challenge is to find a method to mechanically remove these boneless parts from the whole fish. The by-products of this process could be used for the production of products developed during this project, which are described in this report. The X-rays in the figures shown are made by our Iranian partners and taken from the presentation of Jalili, which is a part of the Iranian progress report of 2006.

Figure b: Silver carp does posses 43 intramuscular bones on each side of the fish; 26 above the lateral line of which 20 of them have a typical Y-shape and 17 below the lateral line, of which only one side is shown.

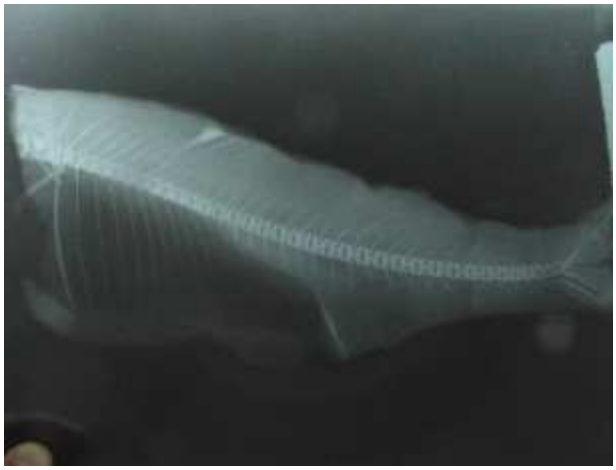
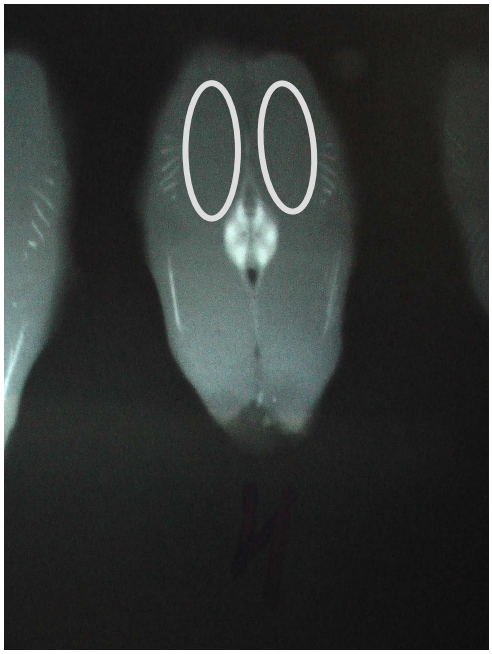


Figure c: An overview of the bones in Silver Carp.



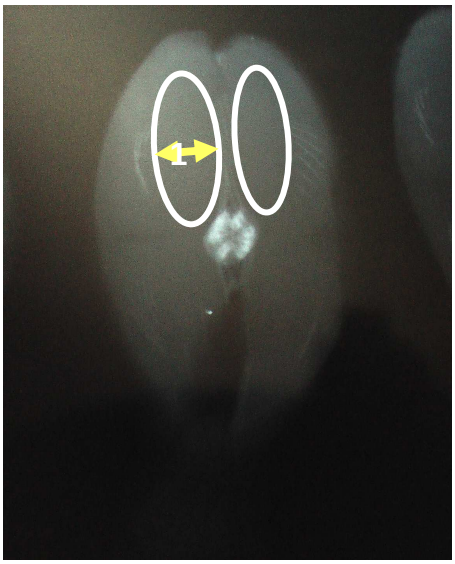


Figure d en e: These two x-ray figures do show the parts of Silver Carp, which are free of bones and could be used for the production of some specific high quality products.

2.3 Task 1.2 The development of new products by restructuring fish proteins, processing and preservation methods using minced meat of Silver carp

Several fish protein structures, such as cheese alike, surimi alike and ice cream alike structures and processing techniques were investigated for their feasibility. The results are given in the next sections.

2.3.1 Task 1.2.1 Use of extrusion technology for the production of a direct expanded fish product

2.3.1.1 Introduction

As experienced in earlier projects it is possible to produce direct expanded fish products from raw materials as cornflower, minced fish and salt (2,7). This kind of product can be used as a snack or used as half fabricate to produce soup or (pap) products. The restructuring of fish proteins together with a carbohydrate matrix is important because the combination of carbohydrates and fish proteins is an excellent combination from a nutritional value point of view. The process takes place in a so-called high shear extruder. The raw material does expand immediately after leaving the mouth of the extruder and only needs to be dried or grinded. The expansion is caused by the extensive heating (140-180℃) of the raw material due to the friction in the extruder under extreme pressures, which vary between 40-120 bars. Under these kind of conditions the water is perfectly mixed in the starch smelt and stays a fluid as long as the mixture is in the extruders barrel. Directly after leaving the mouth of the extruder entering atmospheric conditions the overheated water immediately evaporates and causes a quick and three-dimensional expansion of the starch matrix creating a light and crispy product as shown in figure 3 and 4.

The following process conditions are important when producing a direct expanded product:

- The water content of the raw material mixture, especially because fish material is used.
- Barrel temperature profile of extruder (T1-T5)
- Screw configuration
- Die geometry
- Turning speed of the screw in rpm's.
- Throughput and feeding rate from feeder
- Amount of added water; rate of the water pump

During the experiments the co-turning Clextral BC 45, with a screw length of 1.25 m has been used (figure 1).

The screw configuration used is based on 4 different functions in one process, namely

1) Transport with light compression,
2) Mixing,
3) RSE (reverse screw elements),
4) And compression towards the die.

Table 1) Screw configuration as used in these experiments (see also figure 2 and7):

TZ	TZ	TZ	RSE	TZ	TZ	TZ	TZ	MIX	RSE	RSE	RSE	TZ	TZ
200	100	50	50	100	100	100	100	100	50	50	50	100	100
50	35	35	-25	35	25	25	15	∞	-15	-15	-15	25	15
			12						8R	6R	6R		

-The turning speed (rpm) are varied between 100-200 with an expected optimum of 200 rpm.
-Feeder level varies between 1-28, with an optimum of 9.
-Dies: 4x2mm or 1x2mm with a divergent configuration, which has the preference.
-Best end temperatures are 120-130 degrees Celsius.
-Power requirement is an indication of the shear forces and has to be around 60-80 A.
-A completely filled barrel is necessary to achieve the necessary shear forces.

The process need extra water to start up (Clextral pump), which will later go to zero to when the process is running. Premix is made with the use of the Nautamixer (figure 2). Calibration of both the feeder as the water pump is necessary. Due to high throughputs it is necessary to make batches of raw material of minimal 100 kg.

The general recipe to be tested contains 20% of minced fish (Freshwater Bream), 78.6% of corn flour and 1.4% salt. The corn flour itself contains approximately 11% water and the water content of the fish will be most likely higher than 80%, which results in corn:water:salt of approximately 72%:25%:3%. During the experiments the total water content appeared to be to high and it had to be brought down by either lower the amount of fish, drying the fish or drying the corn flour. The following recipes were executed:

2.3.1.2 Recipes, process conditions and pictures of produced products:

Recipe A): 20% minced fish (Freshwater Bream), 78.6% of corn flour and 1.4% salt, which does result in a dry matter content of approximately 75%:

1. Set temperature barrel: T1: 40 T2: 140 T3: 140 T4: 140 T5: 140,
   Measured temperature barrel: T1: 20 T2: 140 T3: 112 T4: 141 T5: 147, N=126, Die: 4*2mm, Feed=4, I=33 A, Extra water=0, Throughput 525 g/min. Pressure 27 bar. Result (product I) is shown in figure 5.
2. Set temperature barrel: T1: 40 T2: 140 T3: 140 T4: 140 T5: 140,
   Measured temperature barrel: T1: 26 T2: 140 T3: 119 T4: 140 T5: 145, N=200, Die: 4*2mm, Feed=4, I=31 A, Extra water=0, Throughput 525 g/min. Pressure 21 bar. Result (product II) is shown in figure 6.
3. Set temperature barrel: T1: 40 T2: 140 T3: 140 T4: 140 T5: 160,
   Measured temperature barrel: T1: 23 T2: 138 T3: 116 T4: 139 T5: 160-170, N=200, Die: 4*2mm, Feed=8, I=41 A, Extra water=0, Throughput 1.1 kg/min. Pressure 33 bar. Result (product III) is shown in figure 7.Important is that the temperature at the Die does not exceed 160 ºC.
4. Set temperature barrel: T1: 40 T2: 140 T3: 140 T4: 140 T5: 160,
   Measured temperature barrel: T1: 22 T2: 139 T3: 97 T4: 136 T5: 167, N=200, Die: 2*2mm, Feed=8, I=37 A, Extra water=0, Throughput 1.1 kg/min Pressure 47 bar. Result (product IV) is shown in figure 8.
5. Set temperature barrel: T1: 40 T2: 140 T3: 140 T4: 140 T5: 160,
   Measured temperature barrel: T1: 29 T2: 139 T3: 98 T4: 142 T5: 170, N=200, Die: 2*2mm, Feed=8, I=43 A, Extra water=0, Throughput 1.1 kg/min Pressure 58 bar. Result (product V) is shown in figure 8.

Recipe B): 20% minced fish (Freshwater Bream), 78.6% of <u>dried</u> corn flour and 1.4% salt, which does result in a dry matter content of approximately 78%:

6. Set temperature barrel: T1: 40 T2: 140 T3: 140 T4: 140 T5: 160,
   Measured temperature barrel: T1: 24 T2: 139 T3: 98 T4: 142 T5: 170, N=201, Die: 2*2mm, Feed=8, I=43 A, Extra water=0, Throughput 1.1 kg/min. Pressure 58 bar. Result (product VI) is shown in figure 9.

   Recipe C): 12% minced fish (Freshwater Bream), 86.6% of <u>dried</u> corn flour and 1.4% salt, which does result in a dry matter content of approximately 81%:

7. Set temperature barrel: T1: 40 T2: 140 T3: 140 T4: 140 T5: 160,
   Measured temperature barrel: T1: 19 T2: 139 T3: 128 T4: 139 T5: 155, N=200, Die: 2*2mm, Feed=8, I=70 A, Extra water=0, Throughput 1.1 kg/min. Pressure 71 bar. Result (product VII) is shown in figure 10.
8. Set temperature barrel: T1: 40 T2: 140 T3: 140 T4: 140 T5: 160,
   Measured temperature barrel: T1: 20 T2: 139 T3: 133 T4: 142 T5: 154, N=200, Die: 2*2mm, Feed=9, I=75-80 A, Extra water=0, Throughput Pressure 82-90 bar. Result (product VIII) is shown in figure 10.
9. Set temperature barrel: T1: 40 T2: 140 T3: 140 T4: 140 T5: 160,
   Measured temperature barrel: T1: 23 T2: 133 T3: 136 T4: 140 T5: 151, N=200, Die: 2*2mm, Feed=8, I=71 A, Extra water=0, Throughput 1.1 kg/min. Pressure 71 bar. Result (product IX) is shown in figure 11 and 12.

All extrudates are shown in figure 4 to 14 in enclosure VIII. The expansion ratio's are written in table 2.

Table 2: Expansion ratio's of the different direct expanded products produced.

Product	Average diameter	Average expansion ratio
I	5.37	2.69
II	5.30	2.65
III	5.47	2.74
IV	5.98	2.99
V	5.83	2.91
VI	7.35	3.67
VII	8.73	4.37
VIII	8.43	4.21
IX	8.90	4.45

2.3.2 Task 1.2.2 The use of sous vide preservation technology to produce vacuum packed cooked fillets from Silver Carp decreasing the problem of the sharp pine bones by disintegrating their structure and making them edible.

In this exploratory experiment "Sous vide" preservation technology is tested for the production of vacuum packed cooked fillets from Freshwater Bream. The idea is that a heat treatment will disintegrate the structure of the bones, because collagen, which is a part of the bone structure, is heat sensitive and will turn into gelatin, which will most likely decrease the problem of the sharp pine bones by making them edible. The effect of the treatment was evaluated by visually inspecting the spinal bones, pine bones, belly and tailbones. The experimental set-up and results are shown in table 3.

Table 3: The experimental set-up and results

Treatment	Temperature	Time(min)	Comments	Picture
A	70ºC	45	All Bone's still hard, sharp no disintegration observed.	15
B	70ºC	90	All Bone's still hard, sharp no disintegration observed.	15
C	95ºC	30	All Bone's still hard, sharp no disintegration observed.	15
D	95ºC	60	All Bone's still hard, sharp no disintegration observed.	15

The whole treatment process and evaluation is shown in figure 15 to 22. Non of the temperature time combinations used had any effect on softening the bones. Sous vide as preservation technology cannot be used to soften the bones and thereby decrease the problem of the sharp pine bones in Freshwater Bream. The method is too mild to disintegrate the bones. It is unlikely it will work with Silver Carp.

2.3.3 Task 1.2.3 The ice cream alike protein structure will be produced by the (partly) replacement of milk proteins by proteins originating Fresh water Bream, as a model species for Silver carp

2.3.3.1 Ice cream

Ice cream (originally iced cream) is a frozen dessert mainly made from dairy products such as cream combined with flavourings and sweeteners such as sugar. This mixture is cooled while stirring to prevent large ice crystals from forming. Although the term "ice cream" is sometimes used to mean frozen desserts and snacks in general, it is usually reserved for frozen desserts and snacks made with a high percentage of milk fat.

2.3.3.2 Structure of ice-cream

The structure of ice-cream is both fascinating and confusing. The way we perceive the texture of ice cream when we consume it (smooth, coarse, etc.) is based on its structure, and thus structure is probably one of its most important attributes (12).

Ice cream is both an *emulsion* and a *foam.* An emulsion are liquid droplets dispersed in another immiscible liquid. The dispersed phase droplet size ranges from 0.1 - 10 µ m. Important oil-in-water food emulsions, ones in which oil or fat is the dispersed phase and water is the continuous phase, include milk, cream, ice cream, salad dressings, cake batters, flavour emulsions, meat emulsions, and cream liquors. Examples of food water-in-oil emulsions are butter or margarine. Emulsions are inherently unstable because free energy is associated with the interface between the two phases. As the interfacial area increases, either through a decrease in particle size or the addition of more dispersed phase material, i.e. higher fat, more energy is needed to keep the emulsion from coalescing. Some molecules act as surface active agents (called surfactants or emulsifiers) and can reduce this energy needed to keep these phases apart).A foam is a gas dispersed in a liquid where the gas bubbles are the discrete phase. There are many food foams including whipped creams, ice cream, carbonated soft drinks and mousses. A foam is likewise unstable and needs a stabilizing agent to form the gas bubble membrane. The milk fat exists in tiny globules that have been formed by the homogenizer. There are many proteins which act as emulsifiers and give the fat emulsion its needed stability. The emulsifiers (The emulsifiers are a group of compounds in ice cream which aid in developing the appropriate fat structure and air distribution necessary for the smooth eating and good meltdown characteristics desired in ice cream). Since each molecule of an emulsifier contains a hydrophilic portion and a lypophilic portion, they reside at the interface between fat and water. As a result they act to reduce the interfacial tension or the force which exists between the two phases of the *emulsion***.** The emulsifiers actually promote a destabilization of the fat emulsion which leads to a smooth, dry product with good meltdown properties (12).

The original ice cream emulsifier was egg yolk**,** which was used in most of the original recipes. Today, two emulsifiers predominate most ice cream formulations (12):

- mono- and di-glycerides derived from the partial hydrolysis of fats or oils of animal or vegetable origin.
- polysorbate 80, a sorbitan ester consisting of a glucose alcohol (Sorbitol) molecule bound to a fatty acid, oleic acid, with oxyethylene groups added for further water solubility

Other possible sources of emulsifiers include buttermilk, and glycerol esters. All of these compounds are either fats or carbohydrates, important components in most of the foods we eat and need. Together, the stabilizers and emulsifiers make up less than one half percent by weight of our ice cream. They are all compounds which have been exhaustively tested for safety and have received the "generally recognized as safe" or GRAS status) are added to ice cream to actually reduce the stability of this fat emulsion by replacing proteins on the fat surface. When the mix is subjected to the whipping action of the barrel freezer, the fat emulsion begins to partially break down and the fat globules begin to flocculate or destabilize. The air bubbles which are being beaten into the mix are stabilized by this partially coalesced fat. If emulsifiers were not added, the fat globules would have so much ability to resist this coalescing, due to the proteins being adsorbed to the fat globule, that the air bubbles would not be properly stabilized and the ice cream would not have the same smooth texture (due to this fat structure) that it has.

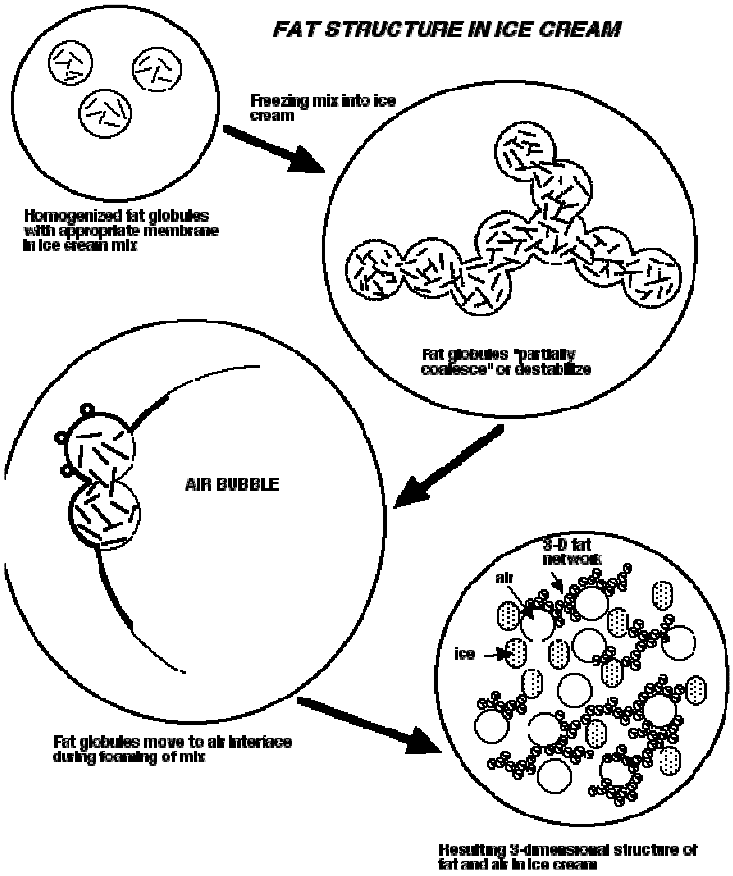


Figure f, the fat structure in ice-cream (12)

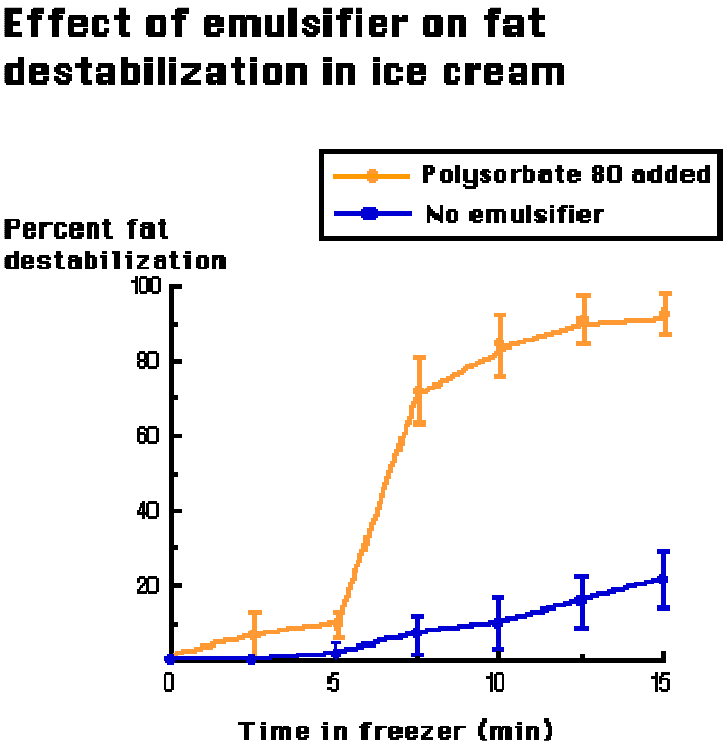


Figure g: Effect of emulsifier on fat destabilization in ice cream (12).

2.3.3.3 Ice Cream Meltdown

One of the important manifestations of ice cream structure is its melt-down. When ice cream is put in an ambient environment to melt (as in a scoop on a plate), two events occur; the melting of the ice and the collapse of the fat-stabilized foam structure. The melting of the ice is controlled by the outside temperature and the rate of heat transfer. However, even after the ice crystals melt, the ice cream does not "melt" (collapse) until the fat-stabilized foam structure collapses, and that is a function of the extent of fat destabilization/partial coalescence, which is controlled mostly by the emulsifier concentration. This process is shown in figure h, which shows ice cream sitting on a mesh screen at ambient temperature:

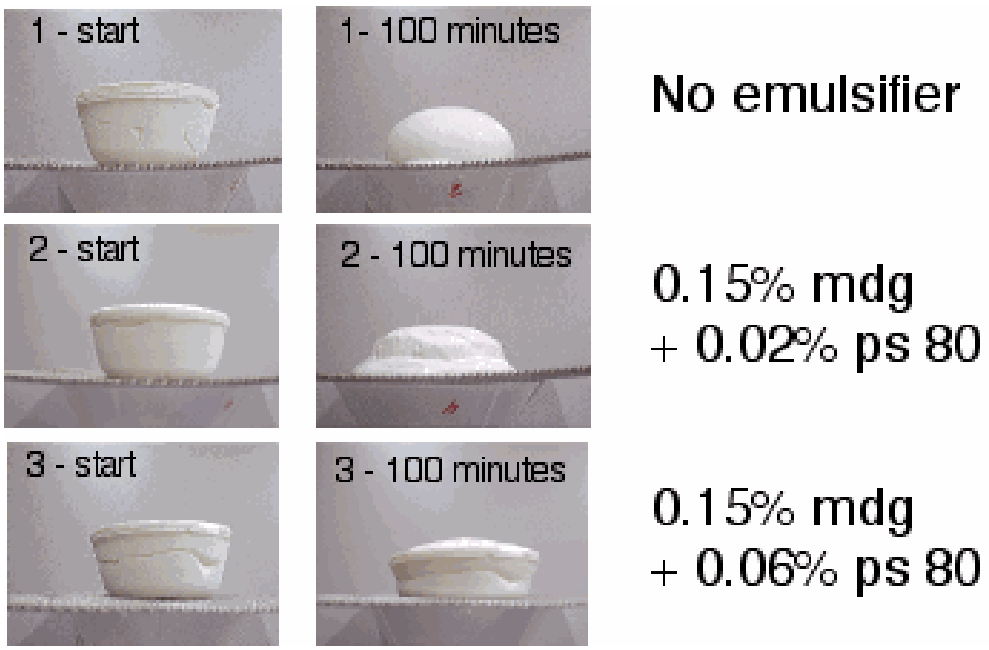


Figure h: Ice cream containing both mono, di- glycerides and polysorbate 80. The increased amount of shape retention and slowness of melt is due to the added emulsifiers, particularly polysorbate 80 (12).

2.3.3.4 Structure from the Ice crystals

Also adding structure to the ice cream is the formation of the ice crystals. Water freezes out of a solution in its pure form as ice. In a sugar solution, such as ice cream, the initial freezing point of the solution is lower than 0℃ due to these dissolved sugars (*freezing point depression*), which is mostly a function of the sugar content of the mix. As ice crystallization begins and water freezes out in its pure form, the concentration of the remaining solution of sugar is increased due to water removal and hence the freezing point is further lowered. This process is shown schematically in figure i.

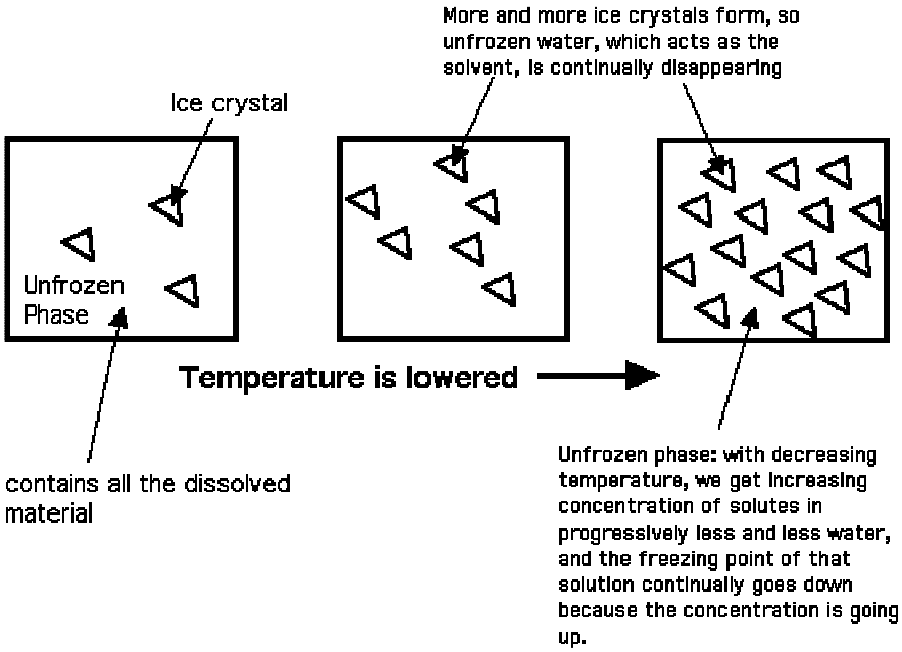


Figure i: The process of freezing point depression (12).

This process of freeze concentration continues to very low temperatures. Even at the typical ice cream serving temperature of -16°C, only about 72% of th e water is frozen. The rest remains as a very concentrated sugar solution. Thus when temperature is plotted against % water frozen, you get the phase in figure j. This helps to give ice cream its ability to be scooped and chewed at freezer temperatures. The air content also contributes to this ability.

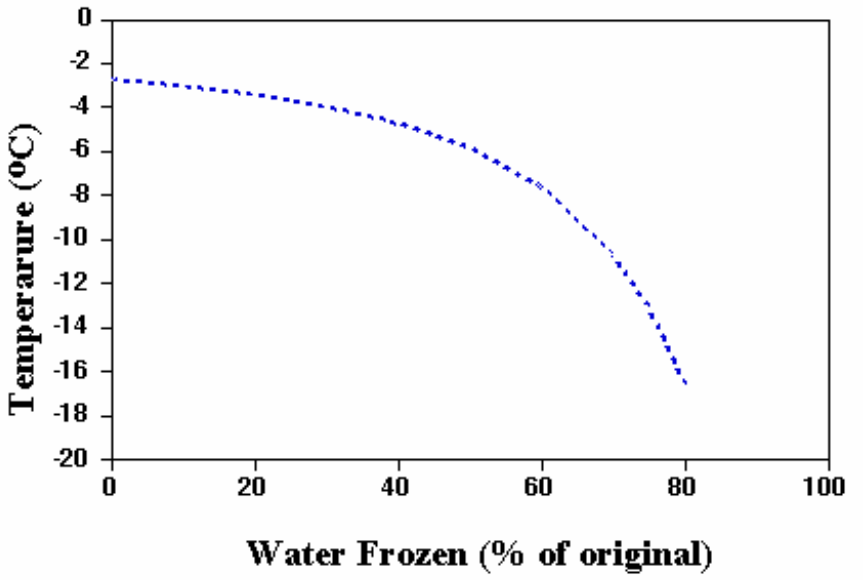


Figure j: Ice temperature is plotted against % water frozen (12).

The effect of sweeteners on freezing characteristics of ice cream mixes is demonstrated by the figure k showing the ice cream freezing curve. Also critical to ice cream structure is ice crystal size, and the effect of recrystallization (heat shock, temperature fluctuations) on ice crystal size and texture. Ice crystals are relatively unstable, and during frozen storage, they undergo changes in number, size, and shape, known collectively as recrystallization. This is probably the most important reaction leading to quality losses in all frozen foods. Some recrystallization occurs naturally at constant temperatures, but by far the majority of problems are created as a result of temperature fluctuations. If the temperature during the frozen storage of

ice cream increases, some of the ice crystals, particularly the smaller ones, melt and consequently the amount of unfrozen water in the serum phase increases. Conversely, as temperatures decrease, water will refreeze but does not renucleate. Rather, it is deposited on the surface of larger crystals, so the net result is that the total number of crystals diminish and the mean crystal size increases. Temperature fluctuations are common in frozen storage as a result of the cyclic nature of refrigeration systems and the need for automatic defrost. However, mishandling of product is probably the biggest cause. The sight of ice cream sitting unrefrigerated on a loading dock, in the supermarket aisle, in a shopping cart, or in someone's grocery bag is too common. If one were to track the temperature history of ice cream during distribution, retailing, and finally consumption, one would find a great number of temperature fluctuations. Each time the temperature changes, the ice to serum content changes, and the smaller ice crystals disappear while the larger ones grow even larger. Recrystallization is minimized by maintaining low and constant storage temperatures. Figure k provides data to show the increase in size of ice crystals that occurs with temperature cycles.

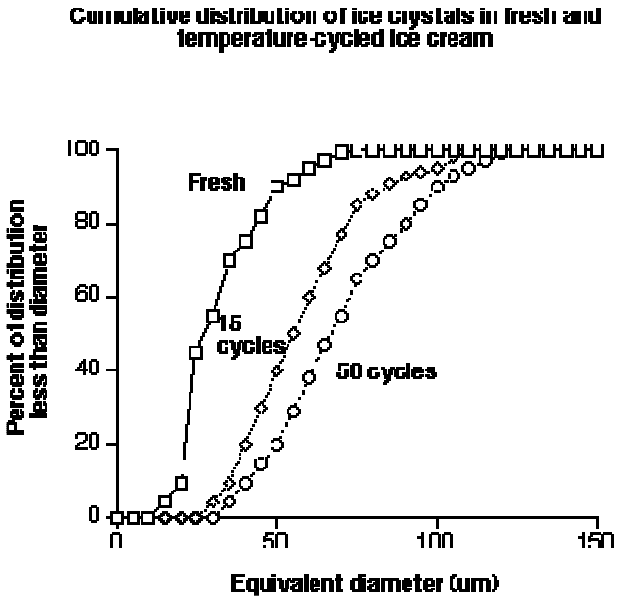


Figure k: The increase in size of ice crystals between fresh ice, and ice which went through several temperature cycles (12).

Thus the structure of ice cream can be described as a partly frozen foam with ice crystals and air bubbles occupying a majority of the space. The tiny fat globules, some of them flocculated and surrounding the air bubbles also form a dispersed phase. Proteins and emulsifiers are in turn surrounding the fat globules. The continuous phase consists of a very concentrated, unfrozen solution of sugars. One gram of ice cream of typical composition contains 1.5 x 10exp12 fat globules of average diameter 1µ m that have a surface area of greater than 1 square meter (in a gram!), 8 x 10exp6 air bubbles of average diameter 70 µ m with a surface area of 0.1 sq. m., and 8 x 10exp6 ice crystals of average diameter 50 µ m with a surface area of another 0.1 sq. m. The importance of surface chemistry becomes obvious (12)

2.3.3.5 Production and composition

Ice cream is sold in a variety of different forms. Before the development of modern refrigeration, ice cream was a luxury item reserved for special occasions. Making ice cream was quite laborious. Ice was cut commercially from lakes and ponds during the winter and stored in large heaps in holes in the ground or in wood-frame ice houses, insulated by straw. Ice cream was made by hand in a large bowl surrounded by packed ice and salt. The temperature of the ingredients was reduced by the mixture of crushed ice and salt. The salty water is cooled by the ice, and is liquid below the freezing point of pure water. The immersed container can make better contact with the salty water and ice mixture than it could with ice alone.

In the 18th century cream, milk, and egg yolks began to feature in the recipes of previously dairy-free flavoured ices, resulting in ice *cream* in the modern sense of the word. After the 1830s when ice-making machines became available, ice cream gradually became more widely available. Ice cream became popular throughout the world in the second half of the 20th century after cheap refrigeration became common, which allowed commercial mass production of ice cream and the birth of the modern ice cream industry. There was an explosion of ice cream stores and of flavours and types.

Modern industrially-produced ice cream is made from a mixture of ingredients, which are listed below:

- minimum of 10% milk fat
- 9-12% milk solids: this component, also known as the serum solids, contains the proteins (caseins and whey proteins) and carbohydrates (lactose) found in milk
- 12-16% sweeteners: usually a combination of sucrose and/or glucose-based corn syrup sweeteners
- 0.2-0.5% stabilizers and emulsifiers
- 55%-64% water which comes from milk solids or other ingredients

These ingredients, along with air incorporated during the stirring process, make up ice cream. Generally, less expensive ice creams contain lower-quality ingredients, and more air is incorporated, sometimes as much as 50% of the final volume. Artisan-produced ice creams, such as Berthillon's, often contain very little air, although some is necessary to produce the characteristic creamy texture of the product. In general, the finest ice creams have between 3-15% air. Because ice cream is sold by volume, it is economically advantageous for producers to reduce the density of the product in order to cut costs. In the United Kingdom today, much of the lower-priced ice cream sold, has little milk or milk solids content, being made with vegetable oil, usually hydrogenated palm kernel oil. The use of stabilizers rather than cream and the incorporation of air also decrease the fat and energy content of less expensive ice creams, making them more appealing to those on diets. Ice creams come in a wide variety of flavors, often with additives such as chocolate flakes or chips, nuts, fruit, and small candies/sweets.

Persia

Bastani, Persian rosewater ice cream, is typically served between wafers as an ice cream sandwich. The Persians mastered the technique of storing ice inside giant naturally-cooled refrigerators known as yakhchals. These structures kept ice brought in from the winter, or from nearby mountains, well into the summer. They worked by using tall wind catchers that kept the sub-level storage space at frigid temperatures. In 400 BCE, Persians invented a special chilled pudding-like dish, made of rosewater and vermicelli which was served to royalty during summers. The ice was mixed with saffron, fruits, and various other flavours. The treat, widely made today in Iran, is called *"faludeh"*, and is made from starch (usually wheat), spun in a sieve-like machine which produces threads or drops of the batter, which are boiled in water. The mix is then frozen, and mixed with rosewater and lemons, before serving.(10, 11 and 12).

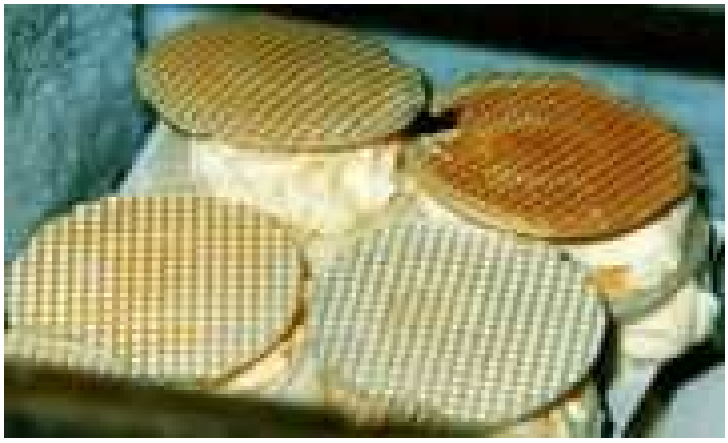


Figure I: Bastani, Persian rosewater ice cream, is typically served between wafers as an ice cream sandwich.

A modern production method: "using liquid nitrogen"

Using liquid nitrogen to freeze ice cream is an old idea that is only recently starting to see commercialization. The preparation results in a column of white condensed water vapour cloud. The ice cream, dangerous to eat while still "steaming," is allowed to rest until the liquid nitrogen is completely vaporized. Some ice cream is often frozen to the sides of the container, and must be allowed to thaw. Making ice cream with liquid nitrogen has advantages over conventional freezing. Due to the rapid freezing, the crystal grains are smaller, giving the ice cream a creamier texture, and allowing one to get the same texture by using less milk fat. Some ice creams are made without milk; for example with soy milk, coconut milk or rice milk instead. A minority of non-dairy ice creams are based on nut butter.

2.3.3.6 The production of "Fish ice"

As one of the many ideas to use fish proteins for the production of new "out the box" products, four different recipes were developed to produce fish ice. The recipes are developed based on the theory and thoughts as written above. Recipes and process conditions are summarized in table 4 and 5. An impression of the ingredients used, especially the freeze dried water soluble fish proteins are shown in figure 96, 97 and 98. Sensory evaluation of different recipes at different temperatures are described in table 6, 7 and 8.

Table 4: The four basic recipes to produce ice using fish proteins.

Basic recipes			
Recipe	**Composition**	**Approximate nutritive value**	**Comments**
A	• 60 gram fish protein • 250 ml cream • 75 gram sugar • 165 gram water • 1 gram emulsifier (E471) • 2 egg yolks • Flavours and colorants	• Protein 13% • Carbohydrates 16.5% • Fat 17.5% • Water 53 %	Result see picture 102, 103 and 104.

Table 4: The four basic recipes to produce ice using fish proteins; continuation

B	• 60 gram of fish protein • 250 ml cream • 75 gram of sugar • 215 ml water • 1 gram emulsifier (E471) • 2 egg yolks • Flavours and colorants	• Protein 11% • Carbohydrates 13.5% • Fat 15% • Water 60.5%	Result see picture 102, 103 and 104.
C	• 60 gram fish protein • 415 gram of water • 75 gram of sugar • 90 gram of butter • 1 gram of emulsifier (E471) • 2 egg yolks • Flavours and colorants	• Protein 9% • Carbohydrates 12% • Fat 14% • Water 65%	Result see picture 102, 103 and 104.
D	• 60 gram fish protein • 415 g of water (325 g of water, 90 g of flake ice). • 75 gram of sugar • 90 gram of olive oil • 2 gram of emulsifier • 2 egg yolks • Flavours and colorants	• Protein 9% • Carbohydrates 12% • Fat 14% • Water 65%	Result see picture 102, 103 and 104.

Table 5: The process variations to produce ice using fish proteins.

Process	***Process***
Recipe 1: Composition see recipe A	Step 1) Dried sacroplasmatic fish proteins are grinded into a powder Step 2) 165 gram of water and 100 ml of cream is added towards 60 grams of grinded fish proteins Step 3) Mixture is heated towards 80 ℃ and cooled down for 10 minutes. Step 4) Mixture is added towards egg yolk, emulsifier and sugar and stirred using the Robocoupe (horizontal cutter). Flavours and or colorants can be added according to personal wishes. Step 5) Mixture needs to be pre-cooled towards 3℃. Step 6) 150 ml of cream with a temperature of approximately 5℃ is stirred thoroughly until it becomes solid. Step 7) The pre-cooled mixture is added towards the solid cream while stirring thoroughly. The homogenized mixture is put into the ice machine were it will become ice (-4℃) within appro ximately 10-20 minutes.

Table 5: The process variations to produce ice using fish proteins; continuation

Recipe 2: Composition see recipe B	Step 1) Dried sacroplasmatic fish proteins are grinded into a powder Step 2) 215 gram of water and 100 ml of cream is added towards 60 grams of grinded fish proteins Step 3) pH of Mixture is adjusted from approximately 4.2 towards 6.5 using NaOH. Step 4) Mixture is heated towards 70℃ and cooled d own for 10 minutes Step 5) Mixture is added towards egg yolk, emulsifier and sugar and stirred using the Robocoupe (horizontal cutter). Flavours and or colorants can be added according to personal wishes. Step 6) Mixture need to be pre-cooled towards 3℃. Step 7) 150 ml of cream with a temperature of approximately 5℃ is stirred thoroughly until it becomes solid. Step 8) The pre-cooled mixture is added towards the solid cream while stirring thoroughly. The homogenized mixture is put into the ice machine were it will become ice (-4℃) within appro ximately 10-20 minutes.
Recipe 3: Composition see recipe C	Step 1) Dried sacroplasmatic fish proteins are grinded into a powder Step 2) 415 gram of water is added towards 60 grams of grinded fish proteins Step 3) pH of Mixture is adjusted from approximately 4.2 towards 6.5 using NaOH. Step 4) Mixture is heated towards 70℃ and cooled d own for 10 minutes Step 5) Mixture is added towards egg yolk, emulsifier, sugar and 90 grams of melted butter and stirred using the Robocoupe (horizontal cutter). Flavours and or colorants can be added according to personal wishes. Step 6) Mixture needs to be pre-cooled towards 3℃. Step 7) The homogenized mixture is put into the ice machine were it will become ice (-4℃) within approximately 10-20 m inutes.

Table 5: The process variations to produce ice using fish proteins; continuation

Recipe 4: **Composition see recipe D**	Step 1) Dried sacroplasmatic fish proteins are grinded into a powder Step 2) 325 gram of water is added towards 60 grams of grinded fish proteins Step 3) pH of mixture is adjusted from approximately 4.2 towards 6.7 using NaOH. Step 4) Mixture is heated towards 30℃ cooled down for 10 minutes Step 5) Sugar is added and mixture stirred using the Robocoupe (horizontal cutter). Flavours and or colorants can be added during this step according to personal wishes. Step 6) Mixture needs to be pre-cooled towards 3℃. Step 7) The mixture of water and flake ice (90 grams at 0℃) is added towards the egg yolk (figure 99) together with the emulsifier and homogenized using the Robocoupe (stand 2). During homogenization, the pre-cooled olive oil (3℃) is s lowly added and the mixture needs to be homogenized until an artificial cream (vegetable olive oil cream) is formed (Robocoupe stand 2 for 20 seconds). Step 8) The pre-cooled protein mixture is added towards the artificial olive oil cream while stirring thoroughly using the Robocoupe (2 times 5 seconds). The homogenized mixture is put into the ice machine were it will become ice (-4℃) within appro ximately 10-20 minutes.
Recipe 5: **Composition see recipe A**	Step 1) Dried sacroplasmatic fish proteins are grinded into a powder Step 2) 165 gram of water and 100 ml of cream is added towards 60 grams of grinded fish proteins and pH is adjusted from approximately 4.2 towards 6.7 using NaOH. Step 3) Mixture is heated towards 30℃ and cooled d own for 10 minutes. Step 4) Mixture is added towards egg yolk, emulsifier and sugar and stirred using the Robocoupe (horizontal cutter). Flavours and or colorants can be added according to personal wishes. Step 5) Mixture needs to be pre-cooled towards 3℃. Step 6) 150 ml of cream with a temperature of approximately 5℃ is stirred thoroughly until it becomes solid. Step 7) The pre-cooled mixture is added towards the solid cream while stirring thoroughly. The homogenized mixture is put into the ice machine were it will become ice (-4℃) within appro ximately 10-20 minutes.

Results

Results are summarized in table 6, 7 and 8.

Table 6: results of the sensory evaluation of the different recipes at -20℃.

Recipe	Sensory evaluation of ice at -20℃.
R1	Sliceable, soft and creamy, but contains granular protein
R2	Sliceable, breakable, fatty and contains granular proteins although less than R1.
R3	Hardly sliceable, very watery and granular structure.
R4	Hardly sliceable, very watery, but drier than R3.
R5	Less sliceable, breakable, granular watery and fatty feeling when melting the sample between fingers.
	A visual impression is shown in figure 102

Table 7: results of the sensory evaluation of the different recipes at -7℃.

Recipe	Sensory evaluation of ice at -7℃.					
	Plasticity	**Yield/ stress**	**Homo-geneity**	**Hard-ness***	**Creaminess**	**Sensory/ characteristics**
R1	++	-/+	3	5	+	Granular, but soft and creamy
R2	+++	+	2	3	++	Granular and creamy, but thicker and drier than R1. Is also breakable
R3	-	+++	1	1	-	Very granular, dry, hard and not creamy.
R4	-/+	++	5	2	-	Not granular, dry and not creamy, but softer than 3.
R5	+++	+	4	4	++	More granular than 1, 2 an 4, but more creamier and drier than 1. Also soft and breakable.

*Hardness: 5 is soft and 1 is hard.

Table 8: Sensory evaluation of the different recipes at room temperature (melted ice).

Recipe	Sensory evaluation of melted ice cream	
	Separated layer visible*	**Sensory/ characteristics**
R1	-	Watery solution with granular pieces of proteins
R2	++	Watery solution with granular pieces of proteins
R3	-	Granular pieces of proteins and separation of water
R4	+++	Homogenous solution with very few granular pieces of proteins
R5	++++	Mouse structure
	A visual impression is shown in figure 104.	

*- separated layers are visible, ++++ no separation visible.

It appeared to be very difficult to dissolve the freeze dried water soluble fish proteins in water, the first and very important step in the described processes to produce fish ice. The first challenge encountered during this part of the project was the fact that the freeze dried fish proteins appeared to be able to absorb much larger quantities of water than that could be added considering the original recipe (recipe A). Considering recipe A, the starting point to develop a recipe, only 165 grams of water could be added as it is important to add the cream (an oil in water emulsion), which mainly contains water, just before the final step of the processing procedure.

The main reason to add the cream in the final step is the fact that cream largely losses its functional characteristics during the in the process proposed heat treatment to pasteurize the fish proteins. It is also a necessity to add the cream towards the mixture under cold conditions (temperature < 7℃) to optimize t he use of the emulsifying and foam forming characteristics to become whipped cream (a food foam) necessary to create a mixture firm enough for a successful transition into “creamy fish ice” (frozen food foam), during the ice making process using an ice machine. It was clear that more water needed to be added in the recipe. At first, because it was decided to keep the total water content below 55%, it was tried to add only a part of the cream together with the water in step 1, but this idea appeared not to be able to beat the challenge. Addition of extra water was necessary, but even after adding sufficient amounts of water the proteins didn’t dissolve. After measuring pH values of the protein suspension it became clear that the limited solubility of the proteins was mainly due to low pH values of the suspension, which were laying around 4. These low pH values could only be explained by acetic acid. Acetic acid, the key ingredient of vinegar, which was used to precipitate the water soluble fish proteins in the first place (paragraph 2.4.5). Apparently sufficient acetic acid did stay behind in the protein concentrate even after centrifuging the precipitated proteins to increase the dry matter content of the precipitate. Off course the same amount of acetic acid will remain in the final freeze dried half fabricate. Protein functionality (e.g. solubility) of the freeze dried fish proteins in suspension improved significantly after raising the pH towards values between 6.5 and 7 by adding NaOH (1 M). Solubility of the protein did improve and thereby its potential to used the freeze dried proteins to produce fish ice, but decreased drastically again as soon as the mixture was pasteurized. Therefore it was decided not to pasteurize the protein, but to focus on the process of making ice cream. With the thought that the fish proteins might be able to completely replace the milk solids (casein and whey proteins) and butter cold replace the necessary milk fat as both are originally added toward the recipe by using cream, only butter and extra water was added (recipe C or R3) instead. The problem encountered with this recipe was the difference in density and viscosity between the protein solution and butter, even though the mixture was heated and the butter was melted before homogenization. Therefore it was decided to replace butter by an “artificial cream”; the vegetable olive oil cream (recipe 4). To produce this artificial cream, an oil in water emulsion the mixture of water and flake ice (90 grams at 0℃) is added towards the egg yolk (figure 99) together with the emulsifier and homogenized using the Robocoupe (stand 2). During homogenization, the pre-cooled olive oil (3℃) is s lowly added and the mixture needs to be homogenized until an artificial cream (vegetable olive oil cream) is formed (Robocoupe stand 2 for 20 seconds) (recipe 4 step 7) and although the final result did produce an ice cream with a the highest homogeneity of all recipes (table 7), its plasticity was very poor, hardness was high and did show no creamy characteristics at all. Most likely the amount of stabilized air bubbles in the final product were insufficient and inadequate to produce a smooth and creamy texture. This also might be the cause of the observed colour difference of the products made by recipe 3 and 4 compared to the other recipes.

Looking at the sensory evaluation and the pictures, the product made by using recipe 5 is, although far from optimal is the best “fish ice” produced within this product, but further research is necessary to develop an “fish ice” which is acceptable for the consumer.

2.3.4 Task 1.2.4 The production of an oil enriched surimi alike protein structure by using emulsion technology and additives.

2.3.4.1 Introduction

Water washing of fish meat during production of fish gels or frozen minced meats like surimi is necessary to remove blood, fishy odours and the sacroplasmatic proteins, although not inferior nutritiously to myofibrillar proteins. The heat coagulative sacroplasmatic proteins will bind to the myofibrillar proteins in the protein matrix when fish meat is heated. This impedes the formation of a gel and is one of the reasons why it is difficult to make a strong elastic gel form un-washed fish meat, especially from pelagic fish species (5).

Myofibrillar protein is the protein, which contains myosin, actin, tropomyosin, troponin and actinin. Myofibrillar proteins cover 66-77% of the total protein in fish meat and play an important role in coagulation and gel forming when the (washed) fish meat is being processed (5).

Frozen surimi used as a starting material for the production of Kamaboko. Washing, grinding with salt and heating are the three fundamental steps in processing of Kamaboko. The characteristic of Kamaboko is its resilient texture or “Ashi” (5). The essential element to create this kind of texture is the forming of actomyosin. Therefore eliminating the components, like all the water-soluble proteins, that obstruct the gel forming capacity by washing is a necessity. Besides rinsing of the blood washing also decreases the amount of substances causing bad odor and color.

Grinding of the de-boned and washed meat with sodium chloride is essential for gel formation. Salt is necessary to increase the ionic strength of the fish meat causing the actomyosin to solubulise and form a sol. Heating this actomyosin sol has two goals, it will result in a network structure creating the specific texture and make the product microbiological safe to consume.

2.3.4.2 Production of raw material and optimization of the washing and de-watering process

2.3.4.2.1 Pre-processing; De-heading, gutting and removal of slime

The head constitutes 10-20% of the total fish weight and it is cut off as an inedible part. Although many mechanized de-heading machines had been developed for processing marine fish, freshwater fish are usually de-headed manually as shown in figure 23 and 24. The main reason is the lack of inexpensive equipment offering minimal tissue loss during this procedure. De-heading is necessary when producing de-boned meat from Freshwater Bream as it is with most species (1).

The purpose of gutting is to remove those fish body parts most likely to reduce product quality, as well as to remove gonads and sometimes the swim bladder. Evisceration of freshwater fish is labour-intensive and usually performed by hand (figure 24, 25 and 26). Gutting consists of cutting down the belly (fish may be de-headed or not), removal of internal organs, and, optionally, cleaning the body cavity of the peritoneum, kidney tissue and blood. Fish is cut longitudinally up to the anal opening, and special care is taken to avoid cutting the gall bladder. This procedure is performed on a table made of special material, which is hard, easy to wash and does not absorb fluids. The table surface should be frequently rinsed and periodically disinfected.

Specialized gutting work stations available on the market nowadays, allows to safely cut fish down the belly (used mainly during processing of trout), remove the guts by vacuum suction and quickly wash and rinse the body cavity with a rotational brush and a water spray, including kidney tissue removal (figure 25 and 26), which is of prime importance for the production of a good quality de-boned mince. It eases the washing process and improves the edibility of the mince produced. Simple systems consisting of rotating brushes and water sprays are also widely used. They facilitate the work and increase the product quality. Protective gloves, periodically disinfected and replaced, should be worn during gutting, especially when mechanized devices are used. It is likely that the vacuum suction tools (kidney and blood removal) used to clean the body cavity in processing salmonids, can find application for other freshwater fish species, as for example Silver Carp. Gutting machines for processing trout, eel and a couple of other species, have been constructed in several countries, but high price renders them unsuitable for smaller plants. The cutting of the body cavity, removal of guts and kidney tissue with brushes and vacuum suction can be performed in these multi-application machines. Some freshwater fish species, in particular bream, perch, roach, carp of length 20-40 cm, can be de-headed and gutted in a machine which employs a so-called American cut. Although the technological efficiency of this cut is not high, the processing speed reaches up to 40 fishes/minute (1).

Slime accumulating on the skin surface of dying fish is a protection mechanism against harmful conditions. In some freshwater species slime constitutes 2-3% of body weight. Slime excretion stops before *rigor mortis*. Slime creates a perfect environment for micro-organism growth and should be removed by thorough washing. Eel, trout and carp require special care with regard to slime removal. Slime can be removed from fish by manual washing them with added salt (figure 26). Fish, especially eel can also be washed in machines, which originally served as scalers (1). The machine needs to be loaded with 30 kg of fish and several kilograms of salt, and after about 2-3 minutes the slime is completely removed from the fish skin. This procedure is more efficient than manual washing (1). Soaking fish in a 2% solution of baking soda and then washing in a cylindrical rotating washer can also remove slime from eel, trout and other freshwater species (1).

Many freshwater species are routinely scaled; this is extremely labour-intensive when done manually. Some sources estimate that manual scaling of larger animals requires almost 50% of the total time necessary to produce headed and gutted fish without fins. It is not necessary to scale fish to be minced in a mincing/deboning separator (1).

The average yield of the pre-processing process of Freshwater Bream, which consisted of de-heading, gutting, flushing kidneys and removal of slime was 72% as is calculated in enclosure 1. The final result of pre-processing is shown in figure 27.

2.3.4.2.2 Deboning

Minced meat can be produced from less valuable fish species after de-heading, their body cavities carefully cleaned and kidney tissue removed as mentioned earlier. Meat is separated from the bones, skin and scales, in automated devices called separators. In the separator shown in figure 28, meat is squeezed through holes into the cylinder under pressure applied by a conveyor belt partially encircling the cylinder (about 25% of the cylinder's perimeter). The cylinder rotates slightly faster than the conveyor. The openings in the cylinder are usually 3-7 mm in diameter. For processing of freshwater fish, the holes are 4 and 5 mm in diameter in general the rule applies that the smaller the holes, the stronger the grinding action. Pressure applied by the conveyor to the cylinder can be regulated depending on the type and size of the raw product and on the hole diameter. The use of separators for processing such freshwater species as perch, bream and tench, offers a new perspective on production of novelty products, which could gain customer approval and be successfully marketed. Minced meat can be either frozen in cardboard or foil containers, turned into surimi by washing or used immediately to produce fish burgers, fish sticks, canned fish, vegetable mixes and fish dumplings etc. The technological efficiency attained during the production of ground meat from bream not larger than 1 kg, was 40% of total body weight (1).

The average yield of de-boning Freshwater Bream by using the Baader 694 is 71% as calculated in enclosure VII. The raw material used for de-boning is shown in figure 27. The process is shown in figure 28 and the un-washed de-boned meat from Freshwater Bream is shown in figure 30. The technological efficiency achieved during the production of ground meat from bream in this experiment was 51% of total body weight (0.71*0.72), which appears to be very good compared to the average efficiency of bream mentioned by the FAO (1).

2.3.4.2.3 Washing and de-watering

Freshly caught fresh water Bream was stored in flake ice until further processing. Mince from Fresh water Bream, the raw material, was produced in bulk by five different procedures:

1) **Production of a washed and frozen half fabricates; surimi products**

   All fish were stripped and de-headed. Special attention was made to remove kidney tissue by washing the abdominal cavity with a water jet. All fish were de-slimed by washing with salt and de-boned by the use of a Baader 694 bone separator (drum pores 3mm). Immediately antioxidants 2.5 g/kg were added to the de-boned meat.

   As mentioned earlier the water washing of de-boned fish meat intended for the production of frozen mince (surimi) is necessary to remove the sacroplasmatic proteins, blood and fishy odours. To optimise the wash- and dewater process for bream the following experiments were executed.

   A) The de-boned meat was washed three times for 10-15 minutes using tap water with 0.04M NaCl in a ratio meat: water of 1:1 (meat/water) and anti-oxidants 2.5 g/l. Water temperature was kept

below 10ºC. After each wash cycle, the Baader 523 screw press was used to dewater the mince. The last step needed three dewatering cycles to produce a nice “heavy” mince.

B) The de-boned meat was washed three times for 10-15 minutes. One time using tap water with 0.01M NaCl and two times using tape water with 0.04M NaCl. In all wash cycles the ratio meat: water was kept on 1:1 (meat/water) and anti-oxidants 2.5 g/l were added to prevent oxidation. Water temperature was kept below 10ºC. After each wash cycle, the Baader 523 screw press was used to dewater the mince. The last step needed three dewatering cycles to produce a nice “heavy” mince.

C) The de-boned meat was washed three times for 10-15 minutes. One time using demi-water, one time using tap water with 0.01M NaCl and one time using tap water with 0.04M NaCl. In all wash cycles the ratio meat: water was kept on 1:1 (meat/water) and anti-oxidants 2.5 g/l were added to prevent oxidation. Water temperature was kept below 10ºC. After each wash cycle, the Baader 523 screw press was used to dewater the mince. The last step needed three dewatering cycles to produce a nice “heavy” mince.

The three different washed minces together with cryoprotectants (0.2% polyphosphate and 4% Sorbitol) and 0.25% antioxidants (see mixture below) was homogenization, vacuum packed and frozen at –20ºC until further processing(3, 4, 6).

To prevent oxidation of polyunsaturated fatty acids (PUFA’s) in the raw material it is necessary to use a mixture of anti-oxidants. The mixture does contain the ingredients listed below (3, 4):

-1,47 g of TBHQuinone
-214g of EDTA
-976g of Na ascorbate

The washing and de-watering process is shown in figure 31-34. Figure 31 does show a mixture of un-washed de-boned meat mixed with the same weight of water. Washing and de-watering of the de-boned mince is done in three steps; wash cycle one, two and three, which are shown in chronological order by respectively figure 32, 33 and 34. The results of the different washing procedures A, B and C to produce surimi are shown in respectively enclosure III, IV and V.

All three procedures clearly washed and de-watered the mince, which could be judged by their appearance especially colour. In all procedures the colour of brown to red before washing (figure 30) is changed to gray-white colour comparable to that of mince made from Pollock (*Pollachius virens*). The consistency of all minces produced was firm and their functional properties as for example their cold setting gelling capacity were all good and above expectation. The dry matter (DM) content of the washed and de-watered minces produced were for procedure A, B and C respectively 24.3, 23.4 and 24.7 of which in all cased 4.55% consists of cryo-protectants (4% Sorbitol and 0.2% phosphates) and antioxidants (0.25%). The yield of the different procedures are summarized in Table 9 and the calculations are shown in enclosure VII.

Table 9: Yields of the different washing processes.

Process	Yield (%) by weight	Yield (%) by DM of mince	Yield (%) of water soluble proteins
A	48	69	28
B	38	55	40
C	29	41	47

Process A gives the highest yield and could be chosen as the optimal process, however less water soluble proteins are removed, which, although not observed on the raw material, could have an effect on the quality of the Kamaboko products produced by heat gelation. The yields of the different procedures are summarized in enclosure XII.

2) **Production of an un-washed half fabricate**

All fish were de-scaled, stripped and de-headed. Special attention was made to remove kidney tissue by washing the abdominal cavity with a water jet. The cleaned fish were de-boned by the use of a Baader 694 bone separator (drum pores 3mm). The de-boned meat together with cryoprotectants (0.2% polyphosphate E450b and 4% Sorbitol E420) and 0.25% antioxidants (see mixture below) was homogenized, vacuum packed and frozen at –20ºC until further processing.

From two raw materials separately (1A and 2) the following protocols were used to produce three kinds of end products. All percentages mentioned are based on the total weight of mince.

2.3.4.2.4 Production of value added products using washed mince as a raw material

A) Production of a surimi product

After the mince was thawed 1% salt (w/w) (NaCl), 0.4% (w/w) ActivaWM, 0.5% Caseinate and 0.2% of polyphosphate was added and the cutter was used to homogenize the mixture. After homogenization the farce was vacuum packed to remove the air and put into its final shape (3, 4).

B) Production of an oil enriched surimi product (±10%)

After the mince was thawed 7% cooled tap water, 1% salt (w/w) (NaCl), 0.4% (w/w) ActivaWM, 0.5% Caseinate, 0.2% polyphosphate, 2.5% frying fat and 7.5% vegetable oil was added while cuttering to homogenize the mixture. After homogenization the farce was vacuum packed to remove the air and put into it's final shape (3, 4).

C) Production of extreme fatty oil in fish gel

5% of caseinate and 0.4% of ActivaWM were dissolved in 40% cooled tap water T<5ºC. While cuttering this solution, 30% of cooled vegetable oil (T<5ºC) was added slowly until a milk alike solution did appear. Then the mince together with 1% salt en 0.2% polyphosphate was added to the milk alike solution and cuttered severely for approximately 30-45 seconds until a homogenized paste was created. After homogenization the farce was vacuum packed to remove the air and put into it's final shape (3, 4).

Two sizes of collagen casings, calibre 23 and 93 and a meatball were used to give the product its final shape. To achieve a significant pre-setting the mixture has been rested for at least 12 hours at 3-4ºC. Pre-setting was followed by pasteurization, which in this case is also the inactivation of the enzyme and final heat gelation of the proteins.

Pasteurization protocols differ for the different shapes:

- 20 minutes at T>85ºC for the 23-caliber casing to create a temperature of 85ºC in the middle of the product for at least 1 minute.
- 90 minutes at T>85ºC for the 93-caliber casing to create a temperature of 85ºC in the middle of the product for at least 5 minutes.
- 75 minutes at T>85ºC for the meatball to create a temperature of 85ºC in the middle of the product for at least 15 minutes.

After pasteurization all products are cooled down in flake ice, deep-frozen using a blast freezer (-30 ºC, ventilation point 5 (on a scale of 10), vacuum packed and stored by **–20**ºC. Results: 21 different products (In product 1-18 olive oil was used, in product 19-21 sun flower oil was used:

1) 1 AA, casing caliber-23 (see figure 41)
2) 1 AA, casing caliber-93 (see figure 42)
3) 1 AA, meatball of approximately 60 mm (see figure 35).
4) 1 AB, casing caliber-23 (see figure 41)
5) 1 AB, casing caliber-93 (see figure 42)
6) 1 AB, meatball of approximately 60 mm (see figure 37).
7) 1 AC, casing caliber-23 (see figure 41)
8) 1 AC, casing caliber-93 (see figure 42)
9) 1 AC, meatball of approximately 60 mm (see figure 39).
10) 2A, casing caliber-23 (see figure 41)
11) 2A, casing caliber-93 (see figure 42)
12) 2A, meatball of approximately 60 mm (see figure 36).
13) 2B, casing caliber-23 (see figure 41)
14) 2B, casing caliber-93 (see figure 42)
15) 2B, meatball of approximately 60 mm (see figure 38).
16) 2C, casing caliber-23 (see figure 41)
17) 2C, casing caliber-93 (see figure 42)
18) 2C, meatball of approximately 60 mm (see figure 40).
19) Extra, casing caliber-23 (see figure 41)
20) Extra, casing caliber-93 (see figure 42)
21) Extra, meatball of approximately 60 mm.

Colour of products, as can be seen on the figure 35-43:
-1AA: lightly brown, 1AB: greyish and 1AC white.
-2A: Dark gray, 2B, gray,

Texture, oil and water leakage after cold setting:
Cold all mixture were already firm after cold setting. No oil or water leaking could be observed.

Texture, oil and water leakage after heat gelation and thawing
This task will be executed in 2006. At this point no data is available for the pasteurized products.

2.3.5 Tasks 1.2.5 the soft cheese alike protein structure will be created by the use of acid coagulation of proteins from Fresh water Bream, as a model species for Silver Carp

Exploratory experiments with water-soluble proteins collected from the wash water

2.3.5.1 Introduction

Sacroplasmatic or water-soluble proteins contain many kinds of water-soluble proteins, as for example myo-albumine, globulin and all kind of enzymes. In fish water soluble proteins made up 18-25% of the total protein content. The water-soluble proteins can be obtained by using pressure or extracting fish meat with a low ionic strength salt solution. The specific content of sacroplasmatic protein in fish meat varies with the fish species, but is generally higher in pelagic fish species and lower in demarsal species. Generally the water-soluble proteins in the water-extracted fraction of pelagic species can be obtained by a heat treatment at 90ºC for 10 minutes causing most of it to coagulate (5).

Goal: How to concentrate and utilize the water-soluble proteins dissolved in the wash water.

To evaluate four possible precipitation methods 1000 ml of wash water (0.04M NaCl) from the first and third wash step in the cycle of three (see washing and de-watering) was put in a one Liter Erlenmeyer and treated as written in table 10:

Table 10: Treatments used, pH and results to precipitate the water soluble proteins dissolved in wash water.

Code	Treatment	pH	Visual effect after 2 hours	Photo
Wash water step one in cycle of three				
1)	Addition of ActivaWB (1%)	5.5	Colour wash water is brown; precipitation is marginal consisting out of a few big pieces.	**44**
2)	0.1 M NaCl	5.3	Colour wash water is brown; precipitation is marginal consisting out of small pieces	**45**
3)	Vinegar 5%	4.2	Colour wash water is red; high level of precipitation (400 ml) consisting of intermediate white pieces.	**46**
4)	Vinegar 10%	4.0	Colour wash water is brown; precipitation is reasonable consisting out of a rather large white pieces.	**47**
5)	Blanco	5.4	Colour wash water is brown; precipitation is marginal consisting out of small pieces	**48**
Wash water step three in cycle of three				
6)	Vinegar 5%	3.5	Colour wash water is white; intermediate level of precipitation (300 ml) consisting of intermediate white coloured pieces.	**49**
-	Vinegar 100%	2.4	-	**--**

After evaluation it was decided that addition of 5% vinegar was the best method to precipitate the water-soluble proteins out to the wash water. This method procedure was used to precipitate the water-soluble proteins from all the wash waters collected from the wash and de-water steps in collected in procedure 1A, 1B and 1C. All data is written in table 11 and details can be found in enclosure VI. The yields are respectively 28, 40 and 47% for procedure A, B and C. The calculation can be found in enclosure VI.

Table 6: The data of the collected precipitate of all collected wash waters is written below.

Day	Experiment nr.	Wash step nr. And Molarity	Total water	Weight barrel	Net weight	% Dry matter	Amount of Dry matter
Precipitate after sedimentation							
1	1	1 (0.04 M)	2850	341	2509	8.0	201
1	1	2 (0.04 M)	4052	339	3713	7.1	264
1	1	3 (0.04 M)	5509	336	5173	6.0	310
						Total	**775**
2	1	1 (0.01 M)	4828	339	4490	9.08	408
2	1	2 (0.04 M)	5738	343	5395	6.70	361
2	1	3 (0.04 M)	9183	735	8448	5.97	504
						Total	**1273**
2	2	1 (Demi)	5280	337	4943	9.00	445
2	2	2 (0.01 M)	10187	692	9495	4.86	746
2	2	3 (0.04 M)	16085	730	15355	4.26	404
						Total	**1595**
							3643
Precipitate centrifuging, used centrifuge is shown in figure 50 and 51.							
1	1	1 (0.04 M)	1251	368	883	21.6	190
1	1	2 (0.04 M)	1748	357	1391	Av. 19	264
1	1	3 (0.04 M)	2463	352	2111	Av. 19	401
						Total	**855**
2	1	1 (0.01 M)	2123	360	1763	Av. 19	335
2	1	2 (0.04 M)	2208	349	1859	Av. 19	353
2	1	3 (0.04 M)	3537	116	3421	Av. 19	650
						Total	**1338**
2	2	1 (Demi)	2332	362	1970	Av. 19	374
2	2	2 (0.01 M)	3323	118	3205	17.9	574
2	2	3 (0.04 M)	2407	117	2290	Av. 19	435
						Total	**1383**
							3576
Supernatant of all samples is centrifuged again delivering							
			37605	-	-	2.5	**940**
Conclusions: 4.6 kg of water soluble protein could be collected 54.6 kg of mince ≈ 8.4% Or from 9.3 kg DM in washed mince ≈ 49.4%. The recovered water soluble proteins are shown in picture 52							

2.3.5.2 Production of a cream cheese and or feta alike product using water-soluble fish protein

Based on the composition of milk type of cheeses, two types of cheeses has been created.

Composition raw cream cheese
4.5% as (3% lactose and 1% salt, etc), 7.5% protein, 35% vet, 54% water (9).

Composition feta cheese
5% as (4% lactose and 1% salt, etc), 14% protein, 21% vet, 55% water (9).

Step 1) Do the proteins coagulate and still show functional properties, as gelation and emulsification?

The functional properties and the possibility of using the collected water soluble fish proteins for the production of a cheese a like product has been tested by three :

1) Effect of heat treatment 90ºC/30 minutes on gelation and oil in protein matrix.
2) The effect of pH on the test executed in test 1.
3) Effect of addition of casein on the tests above.

The experiments are worked out in the next tabels:

Table 12. Behaviour of precipitate after sedimentation (Dry matter sediment used is approx. 9%)

Experiment	pH	Pasteurization T/time	Visual observation
Behaviour of precipitate after sedimentation without added oil			
1)	4.2	-	Consistency of milk
2)	4.2	90ºC/30 minutes	Little pieces of coagulated protein, which precipitate quickly.
3) a	Adapted from 4.2 to 7.5 by using NaOH (5.0 M).	90ºC/30 minutes	Big pieces of coagulated protein, which precipitate quickly
4) b	Adapted from 4.2 to 11 back to 7.3	-	Consistency of fruit pulp. At pH 11 the water soluble protein solution does form a gel.
Behaviour of precipitate after sedimentation with added oil (3.5 times the weight of available protein), emulsified by using the ultra-torax.			
1)	4.2	90ºC/30 minutes	Most of the added oil is and stays emulsified, even after the heat treatment, creating a firm solution, but not a gel. The colour is not white.
2)	4.2	90ºC/30 minutes	Most of the added oil is not emulsified and running out of the coagulated protein. Remarkable is the observation that after addition of 5 M NaOH the coagulated protein does dissolve again creating a gel.
3) a	7.5	90ºC/30 minutes	Oil stays totally emulsified in a raw cheese a like appearance of the coagulated proteins. Colour is not white, but crème.
4) b	7.3	90ºC/30 minutes	Not a gel, oil does not stay emulsified. Colour is dark crème.
Behaviour of precipitate after sedimentation with added oil (3.5 times the weight of available protein), emulsified by using the Hobar cutter 15 seconds at slow and 30 seconds at high speed.			
5) a	Adapted from 4.2 to 11 back to 7.3 without casein and polyphosphate	Cold setting	Solution does show minor gelation, oil stays emulsified and colour is white
5) b		90ºC/30 minutes	Protein does coagulate into big pieces (curdled milk), which are floating. The stays into the protein matrix. The floated protein was collected, pressed and salt was added trying to imitate feta cheese process. The protein didn't form a firm gel. Colour was crème.
6) a	Adapted from 4.2 to 11 back to 7.3, with 5% casein and 0.4% phosphate	Cold setting	Solution does not show any gelation, oil stays emulsified and colour is white
6) b		90ºC/30 minutes	Solution looks like biest.

Table 13: Behaviour of precipitate (figure 52) after centrifugation (Dry matter precipitate used 19%).

Code	Recipe	pH	Gelation (hot 90ºC /cold 1ºC)	Visual observation	Picture
1a	300 g water soluble protein, 120 g oil	4.2	Cold	After 4 hrs: colour; beige, a paste no gel, consistency like pudding, which sticks to finger. After 48 hrs: colour; beige, a paste no gel, consistency like pudding or cream cheese. Is well spreadable like cream cheese. Little bite granular.	61
1b		4.2	Hot	After heat treatment see picture	53
2a	300 g water soluble protein, 120 g water and120 g oil	4.2	Cold	After 4 hrs: colour; beige, a paste no gel, consistency like pap, which sticks to finger. After 48 hrs: colour; beige, a paste no gel, consistency like a soft pudding or cream cheese. Sticks to finger. Is well spreadable like a soft cream cheese. Less granular that 1A.	61+62
2b		4.2	Hot	After heat treatment see picture	54
3a	300 g water soluble protein, 120 g water and120 g oil	7.5	Cold	After 4 hrs: colour; crème white, a paste no gel, consistency like pudding, which sticks to finger. After 48 hrs: colour; crème white, a paste no gel. Consistency like pudding, doesn't stick to finger, well spreadable.	61+62
3b		7.5	Hot	After heat treatment see picture	55
4a	300 g water soluble protein, 120 g oil	7.3	Cold	After 4 hrs: colour; crème white, gel is occurring, consistency like cheesecake. After 48 hrs: colour; crème white, a firm gel, consistency like cheesecake, doesn't stick to finger. Is less spreadable than 5 and 3A	6+62, 63
4b		7.3	Hot	After heat treatment see picture	56
5	300 g water soluble protein, 120 g water and250 g oil	4.2-7.5	Cold (addition of 8.25 g 5M NaOH).	Oil didn't emulgate at pH 4.2 but did at pH 7.5. After 4 hrs: colour; crème white, gel is occurring, consistency like cheesecake, but it sticks to the finger. After 48 hrs: colour; crème white, a firm gel, consistency like cheesecake, doesn't stick to finger, but sticks to spoon. Is very well spreadable.	61+62+ 63

Table 14: Results after using cheese press to compress the heat-treated water-soluble proteins

Sample	Weight before pressing (g)	Weight after pressing (g)	Weight fluids (g)	Comments	Picture
1B) 300 g water-soluble protein, 120 g oil	343	159	131	Oil doesn't stay in the matrix, but a beige cheese like product does emerge, which is dry and granular, not pliable. (Parmesan cheese?)	58+60
2B) 300 g water soluble protein, 120 g water and120 g oil	348	181	147	Oil doesn't stay in the matrix, but a beige cheese like product does emerge, which is pliable.	58+59
3B) 300 g water soluble protein, 120 g water and120 g oil	540	170	75.2	Oil stays in the protein matrix, but the emulsion is pressed through the cheese cloth, no hard structure does emerge (cream cheese?)	58
4B) 300 g water soluble protein, 120 g oil	256	-	-	A nice emulsion, but the emulsion is pressed through the cheese cloth, no hard structure does emerge (cream cheese?)	59
Looking at the pictures it looks like the big difference in behavior and texture is mainly caused by the difference in pH. (4.2 – 7.5)					

2.3.5.3 Production of cheese alike structures using a different approach

2.3.5.3.1 Use of proteins recovered from the wash water

Cheese alike structures were produced by using "water soluble" proteins. The proteins were recovered from the wash water using vinegar (acid aided precipitation) followed by centrifugation. Centrifuged proteins were finally stored vacuum packed at -24 ℃. The differe nt recipes used are summarized in table 15.

Table 15: recipes used for the production of the cheese alike structures using water soluble proteins.

Recipe	all recipes are based on using 100 grams of water soluble proteins
R1	40 grams of olive oil, 40 grams of water
R2	40 grams of olive oil
R3	80 grams of olive oil
R4	40 grams of olive oil, 40 grams of water, 6 gram of casein, 0.4 gram of ActivaWM and 0.2 gram of phosphates
R5	40 grams of olive oil, 6 gram of casein, 0.4 gram of ActivaWM and 0.2 gram of phosphates
R6	80 grams of olive oil, 40 grams of water, 6 gram of casein, 0.4 gram of ActivaWM and 0.2 gram of phosphates

*Dry matter content of half fabricate was approximately 13%

Results

All recipes (R1 to R6) were evaluated after cold setting (figure 105-107) as well as heat setting (figure 108-110). Results are summarized in table 16 and table 17 for respectively the cold as heat settled products.

Table 16: The results of the sensory evaluation of the cheese alike structures using cold setting.

Recipe	Sensory evaluation of cheese alike structures (cold setting or gelation)		
	Texture (1-6)*	**Colour**	**Sensory/ characteristics**
R1	1	Beige	Porridge , separation of water visible: figure 105 and 106
R2	3	Beige or sand a like	Like a spreadable mouse, no separation of water or oil, see figure 105 and 106
R3	3	Yellow	Like R2, see figure 105 and 106
R4	2	Beige	Like R2 and R3 see figure 105 and 107
R5	6	Beige, but lighter 1-4	Sliceable, like a bavarois, bounce able, no separation of water or oil, see figure 105 and 107
R6	6	Creamy	Sliceable, like a bavarois, bounce able, no separation of water or oil, see figure 105 and 107

* 1 is soft no gel, 6 is a very firm gel

As can be seen from the sensory evaluation as well as from the different figures addition of ActivaWM together with casein has an improving effect on the consistency of the cold settled products. Also of prime importance is the ratio of water to oil, which should be between 1.5 and 2. Taking into account the results of the sensory evaluation, recipe R6 is preferred.

Table 17: The results of the sensory evaluation of the cheese alike structures using heat setting.

Recipe	Sensory evaluation of cheese alike structures (heated or heat gelation; 2.5 hours to reach a temperature of 70℃)		
	Texture	**Colour**	**Sensory/ characteristics**
R1	1	Beige	Sliceable, separation of water visible figure 108 and 109
R2	3	Beige or sand a like	Sliceable, separation of water and a little oil visible figure 108 and 109
R3	2	Yellow	Sliceable, separation of water and a little oil visible figure 108 and 109
R4	Porridge	Beige	Not sliceable, separation of water visible no separation of oil figure 108 and 110
R5	5	Beige, but lighter than 1-4	Good sliceable and a dry gel, no separation of water or oil figure 108 and 110
R6	6	Creamy	Very good sliceable and a dry gel, no separation of water or oil. Figure 108 and 110

As can be seen from the sensory evaluation (table 17) as well from the different pictures presented in figure 08, 109 and 110, recipe 5 and 6 are preferred. This mainly because these two recipes do produce a sliceable dry gel, without drip loss and oil leakage.

2.3.5.3.2 The use of a blend of fresh water Bream and Salmon mince to create sliceable cheese alike products with a nice texture, colour, flavour and taste

The goal of this experiment was to evaluate if a blend made of lean mince from freshwater Bream and fatty mince from salmon could be used to produce products with a nice texture, colour, flavour and taste. To make a proper evaluation the following questions are of importance:

- Is the ratio of 1:1 between fresh water Bream and Salmon sufficient to emulsify all the oil originating from the Salmon meat, to produce products with a firm and sliceable texture, colour, flavour and taste?
- What is the preferable amount of water, which could be added to this blend of Bream and Salmon without negatively affecting the final texture?
- Is the use of 0.4% ActivaWM, a source of transglutaminase, sufficient to create a firm and sliceable product?

The products were produced using recipes containing at least the following ingredients: 0.4% ActivaWM, 5% caseinate, cryo-protectants (4% Sorbitol, 0.2% poly-phosphates), salt (0.8% NaCl en 0.2% poly-phosphates) and 20 or 40% of extra water. The processing conditions are developed to create a firm and sliceable product using de-boned mince as a raw material. The mince is produced as described in paragraph 2.3.4. An important note is that the caseinate must be completely solved in the water before it is added towards the mince. The experimental set-up and process conditions are shown in table 18.

Table 18: The process conditions to produce products using blends of fresh water Bream and Salmon.

Ingredient	**Amount (g)**	**Processing**
Recipe 1 A		Step 1: Caseinate and ActivaWM are added towards the water (T<5°C) homogenized and stored in the fridge. Step 2: Both minces, together with the Sorbitol, poly-phosphates and necessary salts are put together and homogenized by using a cutter. Step 3: The milky substance of water, caseinate and ActivaWM as produced in step 1 is added towards the homogenized mince from step 2 and homogenized again until a firm farce is formed. Step 4: The air in the farce is removed by using a vacuum apparatus. Step 5: At this point the half-fabricate needs to be formed in its final shape as quickly as possible as the next step is the pre-setting of the gel, which starts as soon as the ActivaWM is added. Step 6: Pre-setting: cooling (12 hrs, 2°C<T<4°C), Step 7: Heating (T > 85°C, 20 minutes) Step 8: Freezing (-20°C).
Water	200	
Mince freshwater Bream*	500	
Mince Salmon	500	
Caseinate	50	
Sorbitol	20	
Poly-phosphates	3	
NaCl	8	
ActivaWM		
Recipe 1 B; 2A with addition of 10% oil		
Recipe 2 A		
Water	400	
Mince freshwater Bream*	500	
Mince Salmon	500	
Caseinate	50	
Sorbitol	20	
Poly-phosphates	3	
NaCl	8	
ActivaWM	4	
Recipe 2 B; 2A with addition of 10% oil		

* Raw material of fresh water bream does already contain cryo-protectants

Results

Half-fabricates were formed in two different final shapes; sausages and cheese balls. Both products can be used as ready to eat products, but the cheese balls are preferable used to produce slices of “cheese”, which can be used as sandwich fillings. Results of all products are shown in figure 80 to 95. The sensory evaluation is described in table 19.

Table 19; The sensory evaluation of the cheese alike products produced out of a blend from Bream and Salmon.

Recipe	Texture	Drip loss	Oil leakage	Colour	Smell	Taste
1A	Firm, but granular	None	No	Pink	+	Fishy salmon
1B	Firm, but granular	None	“”	“”	+	Neutral
2A	Firm less granular	Little sweaty	“”	“”	+	Neutral, juicy, fresh
2B	Firm	Little sweaty	“”	Lightly pink	+	Neutral, juicy, boiled egg alike, less granular

The pictures depicted in figure 85 to 95 show that all the recipes developed for the production of “cheese balls” are very well sliceable. No oil leakage was observed during the process of slicing the products, even for the oil enriched recipes 1B and 2B, proving all the available oil is absorbed into the protein matrix. Drip loss of the products after slicing was negligible, the recipes with 40% of extra water (2A and 2B) included, although at room temperature these “high water content” products did show became a little sweaty.

Conclusion

The ration of 1:1 between fresh water Bream and Salmon is sufficient to emulsify all the oil originating from the Salmon meat to produce products with a firm cheese alike and sliceable texture. The use of 0.4% ActivaWM is also sufficient to create a firm and sliceable product. Taking into account the results of the sensory evaluation and the expected extra revenue by adding 40% extra water and 10% of oil recipe 2B is preferred.

2.4 Production of “imitation meat” products from proteins recovered from wash water

During the execution of the experiments to process the “water soluble proteins” it appeared that the proteins apparent in the wash water from the wash process as described in paragraph 2.3.4.2 can be used to produce “imitation” meat products with a fibre alike texture. This result was rather unexpected as the general idea is that the majority of the proteins in the wash water are sacroplasmatic proteins or globular and not myofibrillar proteins. The hypothesis that the wash water does also contain myofibrillar proteins, which during the process of freezing do form fibre alike structures and thereby capture the available sacroplasmatic proteins within their network does seem a plausible one, but can’t be proven at this time.

The formation of these fibre alike protein structures took place during freezing of the wash water (pH 6.0) at -24˚C. A process comparable to the well known Genco process (freezing out of water) is most likely responsible for the results found as during thawing it appeared that in the storage bucket the proteins were precipitated and the floating ice appeared to be consisting of almost pure water (figure 67). During the process of freezing out the wash water, only the water in this mixture of water and proteins starts to freeze causing an increase in the concentration of the dissolved proteins. At a certain concentration the proteins start to precipitate and form a thick layer on to the bottom of the barrel. As the process of freezing continues, veins of ice will grow through the precipitated protein layer forcing the proteins to form a network around the veins of ice. This process, also known as freeze-textured fibre formation did most likely, created the unexpected fibre a like texture.

As described in paragraph 2.3.4.2 , the wash procedure of the de-boned meat did consist out of three different steps, 1, 2 and 3 respectively each creating an unique extract (wash water) considering the type of proteins (the blend) and protein concentration. Thereby an unique freeze textured protein half fabricate for each type of wash water is shown in figure 64, 65, 66, 68. A sensory description c.q. evaluation of the thawed half fabricate is shown in table 20.

Table 20: Sensory description of the wash water and thawed half fabricates

Type of wash water		**Sensory description wash water**	**Sensory description half fabricate**	**Weight in grams**
Step 1		Flaky suspension of proteins in water	Moderately fibrous, figure 68	683
Step 2		Clear wash water, with proteins concentrated at the bottom	Very fibrous, multilayer structure, figure 64 and 70	778
Step 3	**3a**	“ “	Very fibrous laminated layer kind of texture, see figure 65 and 74	905
	3b	“ “	Very fibrous laminated layer kind of texture, see figure 66, 76 and 79	1165
	3c	discarded	-	-
	3d	Flaky suspension of proteins in water	More like a mince kind of texture	109

* Dry matter content of half fabricate was approximately 13%

To evaluate the potential for the use of the half fabricates produced all freeze textured protein extracts were cooked in water as well as fried in vegetable oil. The results are described in table 21:

Table 21: The results of the sensory evaluation of the different half fabricates produced raw, cooked as well as fried.

Protein source (code)*	Elasticity of raw proteins (1-5)**	Raw	Cooked	Fried
1	1	Juicy, short fibers, as fresh mince, not granular. A visual is shown in figure 68 and 69.	Shredded, like kebab, taste is slightly fishy. A visual is shown in figure 69.	Crispy and slightly fishy. A visual is shown in figure 69.
2	4	Small pieces, like turkey kebab with meat alike texture and high elasticity. Figure 70 and 71.	Looks like turkey kebab, pieces have a nice elasticity. Figure 71.	Juicy chunks, crispy and slightly fishy. A visual is shown in figure 71.
3	2	Texture like mince, not granular, more fibers like sample 1. Figure 72 and 73.	Though, not as nice as sample 1. Figure 73.	As sample number 1, but to much fibers. Figure 73.
3a	3	As sample from protein source number 2. Kebab, better color as 2, but sample was less elastic. Visual impression is given in figure 74 and 75.	As sample from protein source number 2. Figure 75.	Nice fried chunks, very juicy. Figure 75.
3b	3	Sample forms membranous alike structures; thin layers of proteins, almost film forming. Moderate elasticity. Visual impression; figure 76 and 77.	As sample from protein source number 2. Figure 77.	As sample from protein source number 2. Figure 77.
3b* **	2	Like a turkey fillet (schnitzel), nice and juicy. Texture build up out of clearly long fibers. (Figure 78)	During cooking fibers did shrink and texture became though, layered and dry. Sample was cooked to long. (Figure 78)	Looks like a fried chicken or turkey schnitzel, juicy but though. Sample was fried to long. (Figure 78)

*) Protein concentrate precipitate from wash water; 1) wash water step 1, wash water step 2, wash water step 3 (See paragraph 2.3.4.2). **) 1 is no elasticity 5 is high elasticity. *** Protein is restructerized into an imitation meat fillet (schnitzel).

Conclusion

The fried samples can be used as snacks, the cooked samples can be used as “imitation meat” for stir fry dishes, soups and or for the production of a kind of “fish kebab”. As can be seen, looking at the different pictures of the products (figure 68-79), the colour of the raw material equals towards that of turkey meat.

3. Conclusions

To improve the utilization of fresh water fish with many bones, new procedures for processing has been developed giving added value by creating products apart from a better quality and yield.
The developed processing relates to intermediate products, such as mince and proteins, and to final products for the consumer.

- **Production of fillet**
  For the production of a proper fillet from Silver Carp without the unpleasant fatty tissue bones and skin using specific parts of the fish, the Silver Carp's anatomy was investigated by the use of X-rays to evaluate if some parts of the fish contain less bones and can be used to produce a high quality boneless fillet. Due to lack of Silver carp specimen in the Netherlands, this task is mainly executed by the Iranian project partners. X rays did show specific parts could be used to produce pieces of a high quality boneless fillet. The size of these high quality fillet pieces is expected to be largely dependent on the size of the fish used for processing. The challenge is to find a method to mechanically remove these boneless parts from the whole fish. The by-products of this process can be used for mince and proteins.

Mince and proteins from the wash water have been used to produce several products, which showed that both the myofibrillar as sacroplasmatic proteins recovered from Freshwater Bream, which serves as a model species for bony fish, could be utilized. More specific conclusions are written below.

- **Pre-processing and deboning**
  -The average yield of the pre-processing process of Freshwater Bream, which consisted of de-heading, gutting, flushing kidneys and removal of slime, is 72%.
  -The average yield of de-boning Freshwater Bream by using the Baader 694 is 71%
  -The technological efficiency achieved during the production of ground meat from bream in this experiment was 51% of total body weight (0.71*0.72), which is good compared to the average efficiency of bream mentioned by the FAO (1).

- **Washing and de-watering of de-boned meat**
  On dry matter base 55% of the fish proteins will be found in the mince. The yield of the washing and de-watering process is high with the tested procedures. Yield wise process A is the most optimal with a yield of 48% on weight and 69% on dry matter content. Dry matter content of the washed and dewatered mince is 24%, being in the same range as fish meat, deducting the 4.4 % of added cryoprotectants and anti-oxidants. The color of the washed and dewatered proteins is gray to white and the functionality is high. It is very well possible to create larger structures from this mince.

- **Production of surimi**
  Procedures are given for both the production of mince as a frozen half fabricate (surimi) and using the half fabricate as raw material for the production of value added products in different physical forms (sausage, burger, meatball etc) such as:
  -Kamaboko,
  -Surimi,
  -Oil enriched surimi products
  -Extreme fatty oil in fish gels.

- **Utilization of the water-soluble proteins**
  As a preliminary result the use of 5% vinegar was the best method to precipitate the water-soluble proteins from the wash water. The yields are respectively 28, 40 and 47% for procedure A, B and C. On dry matter base 40% of the fish proteins (water soluble) have been recovered from the wash water. Using the recovered soluble proteins different procedures were tested to produce emulsions, pate and cheese alike textures. Butter, pate and cheese alike textures with high oil content have been created using both heat gelation and cold setting, but the results are far from edible. More research is needed to optimize and further explore the use of the water-soluble fish proteins as high attractive products.

- **Extrusion**
  Mince has been cooked and formed by means of extrusion together with starches (based on corn in this case). These products show that snacks can be produced with a better-balanced nutritional value. The products have a long shelf live. The extrusion process has been described for a twin-screw extruder. Essential is the end temperature in the extruder of 160°C, necessary for the expansion of the product. The moisture content of the feed has to be as low as possible. The maximum allowable amount of moisture in the recipe for producing an acceptable direct expanded product with Freshwater Bream appeared to be 20%. The whole process gives a dry product with a crispy texture after further drying. There are strong indications that lowering the moisture content of the recipe can produce products with higher expansion rates. Either lower the amount of fish, drying the fish or drying the cornstarch, can lower the moisture content.

- **Sous vide**
  Sous vide packaging of fish is well possible as a preservation method but doesn't give any solutions for disintegrating or softening the small pin bones in Freshwater Bream. Therefore it is unlike it will work with Silver Carp.

- **Fish ice**
  Fish ice has been produced, but the results obtained were not optimal. More research is needed to develop fish ice acceptable for the consumer

- **Sliceable cheese alike structure**
  Sliceable cheese alike structures were produced successfully. The ration of 1:1 between fresh water Bream and Salmon is sufficient to emulsify all the oil originating from the Salmon meat to produce products with a firm cheese alike and sliceable texture. The use of 0.4% ActivaWM is also sufficient to create a firm and sliceable product. Taking into account the results of the sensory evaluation and the expected extra revenue by adding 40% extra water and 10% of oil recipe 2B is preferred.

- **Imitation meat**
  "Imitation meat" or restructured fillet pieces were produced from proteins recovered from the wash water. When fried the pieces can be used as snacks, when cooked the pieces can be used for stir fry dishes, soups and or a kind of "fish kebab".

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Figures

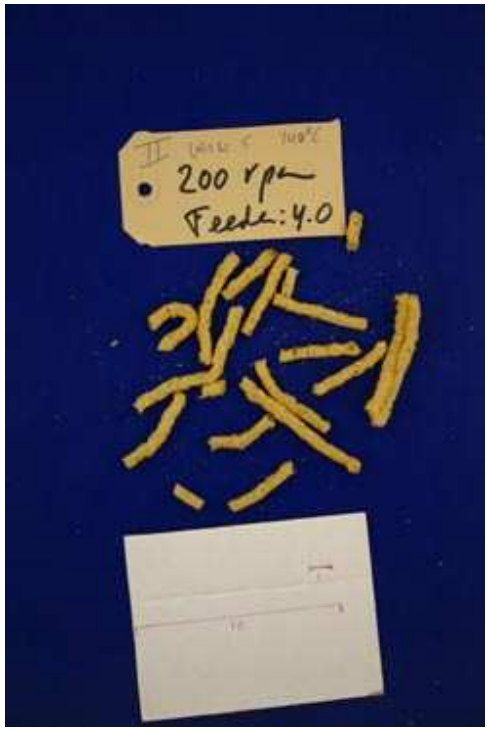

Figure 1


Figure 2

Figure 3


Figure 4

Figure 5



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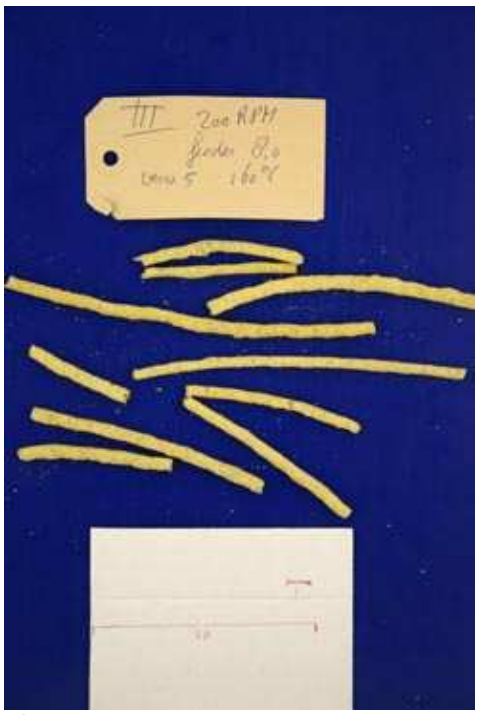
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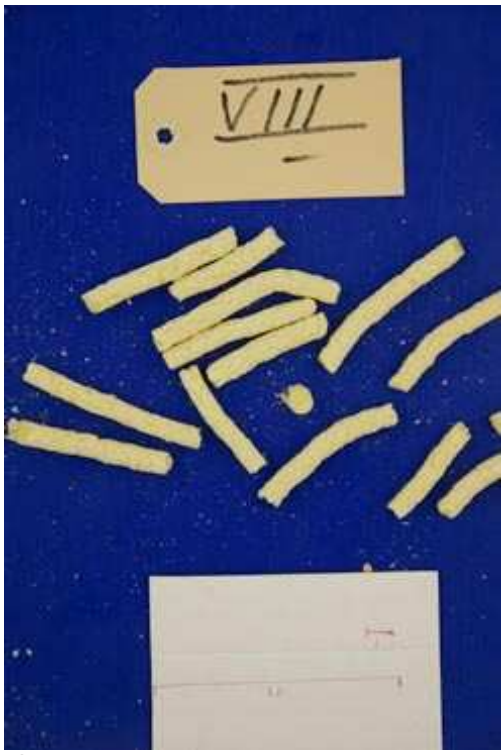

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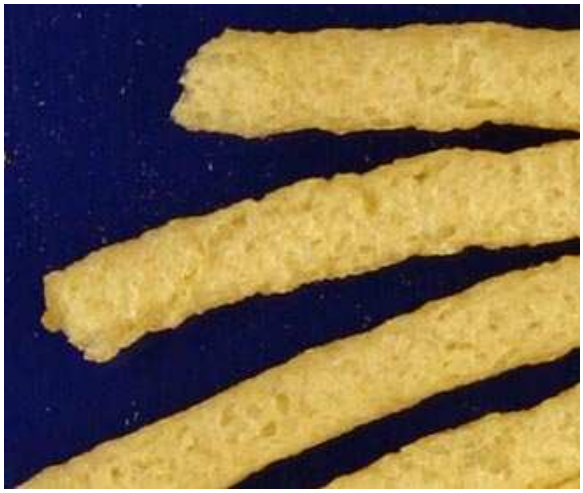

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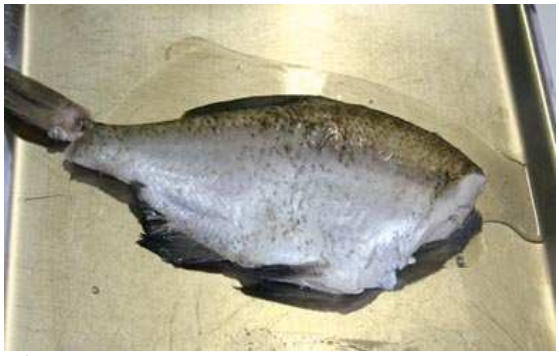

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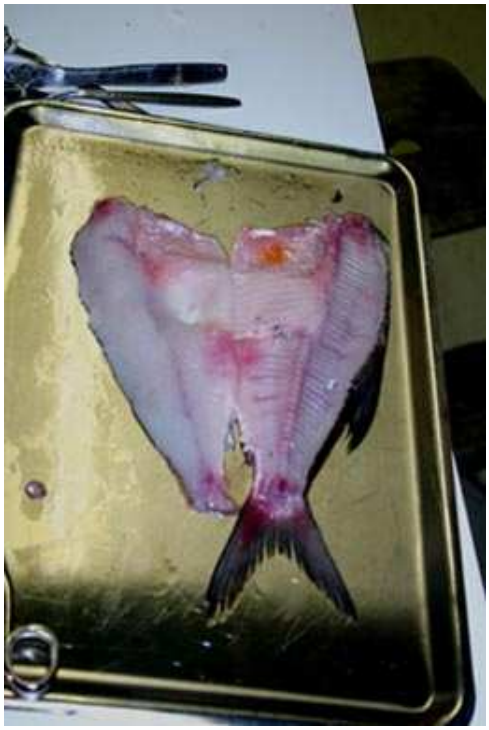

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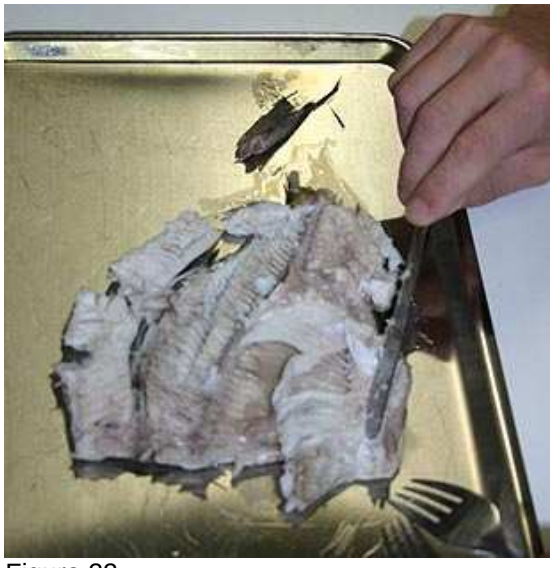

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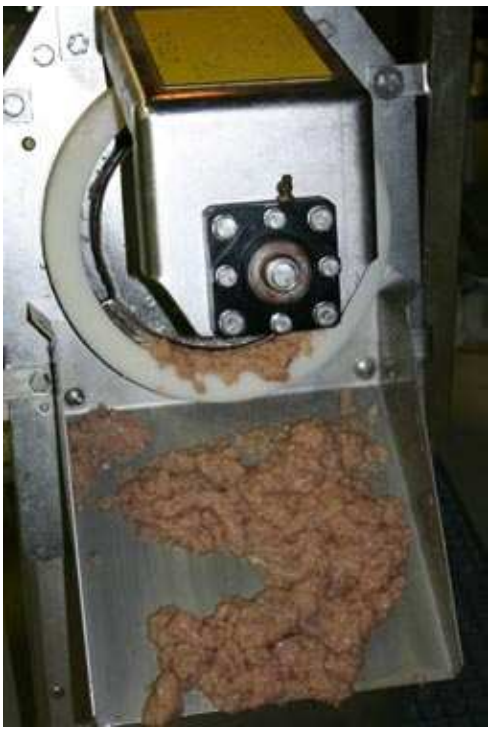

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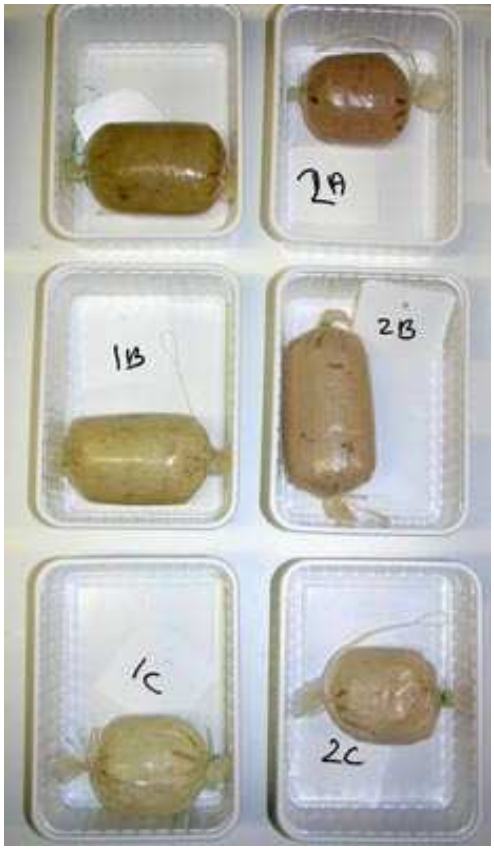

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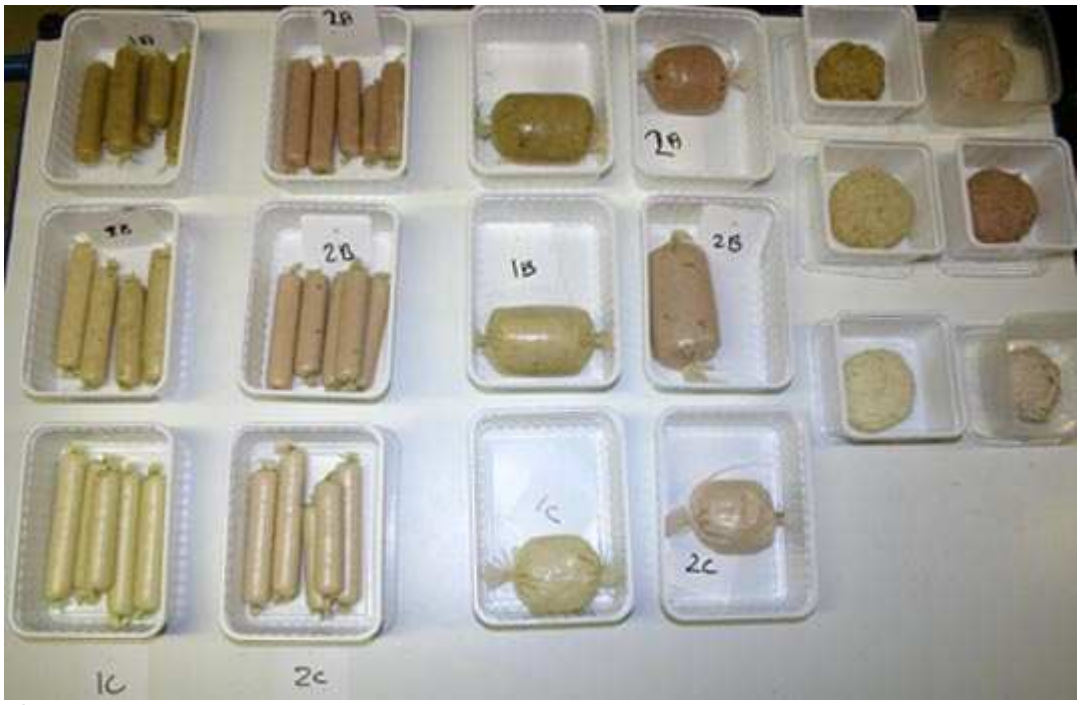

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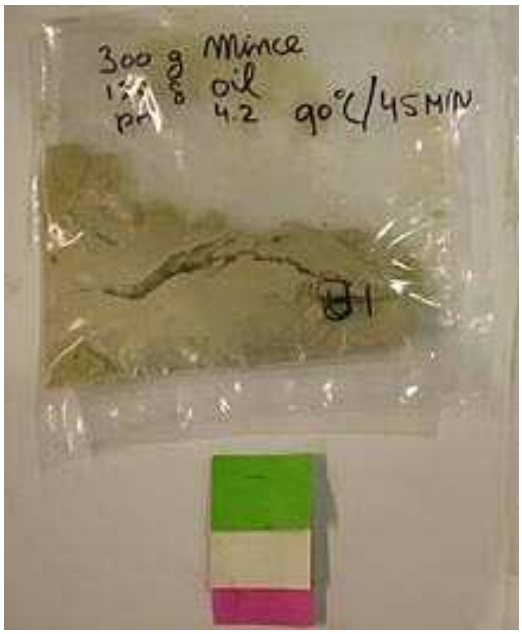

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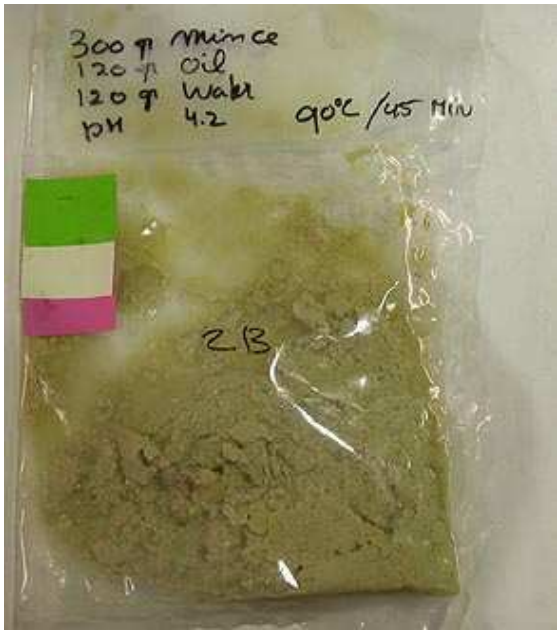

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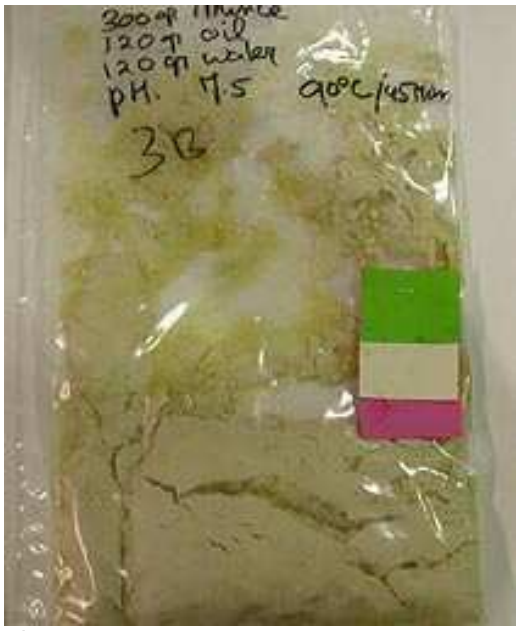

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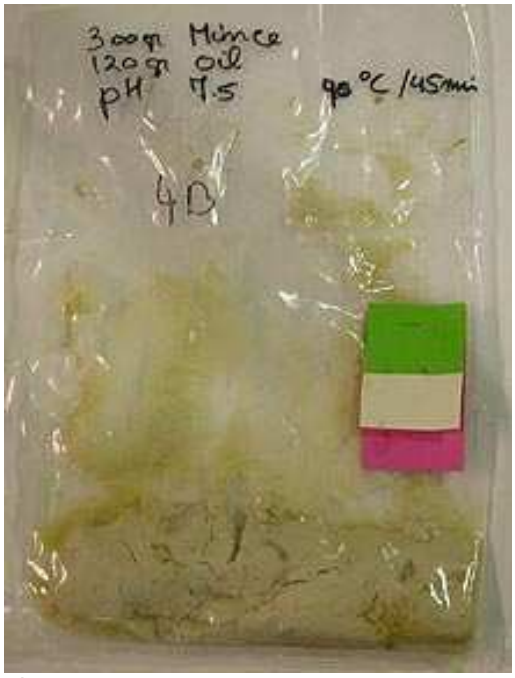

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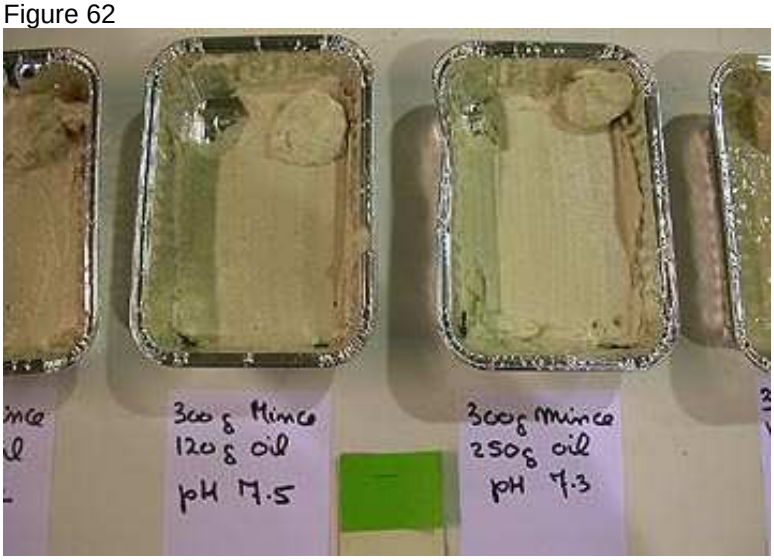

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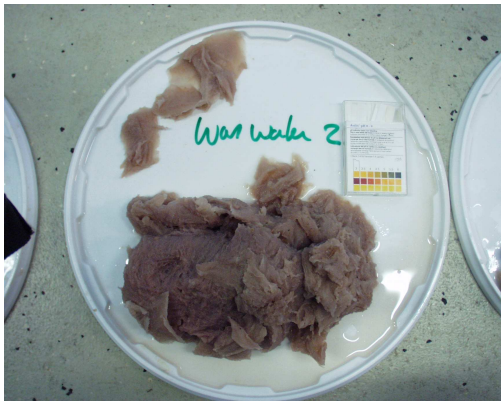

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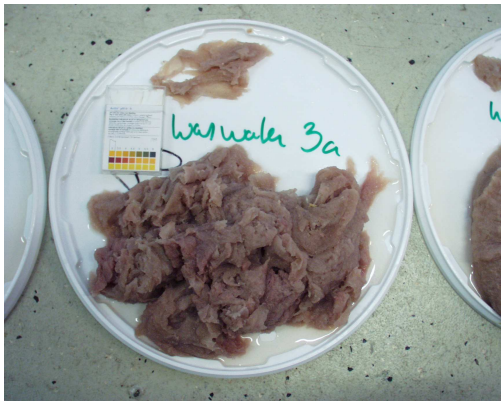

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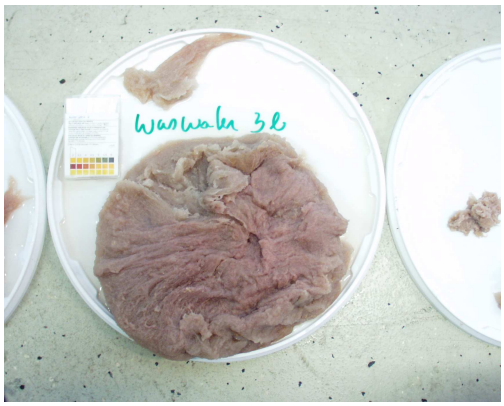

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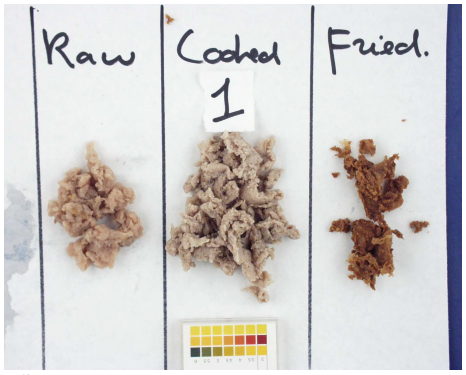

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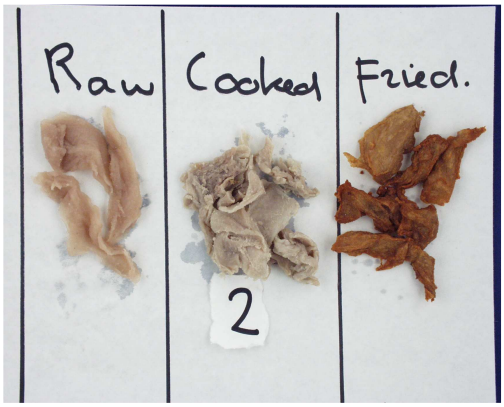

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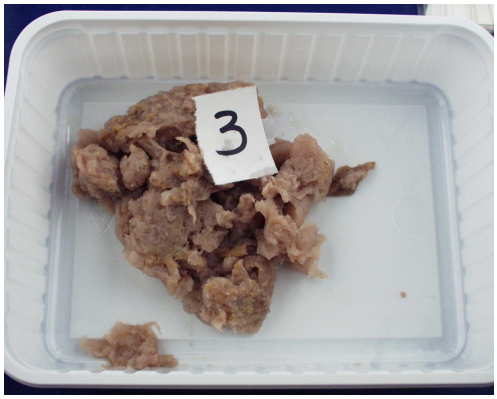

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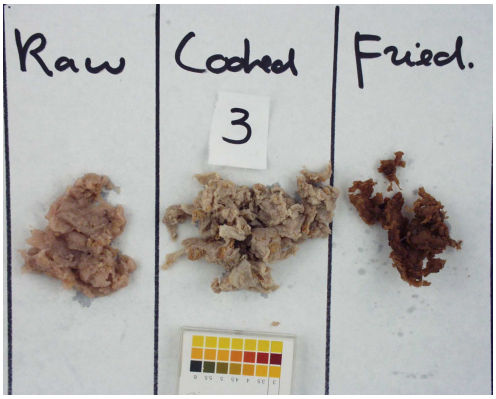

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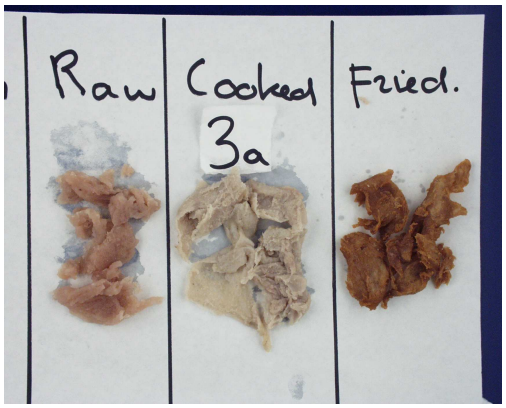

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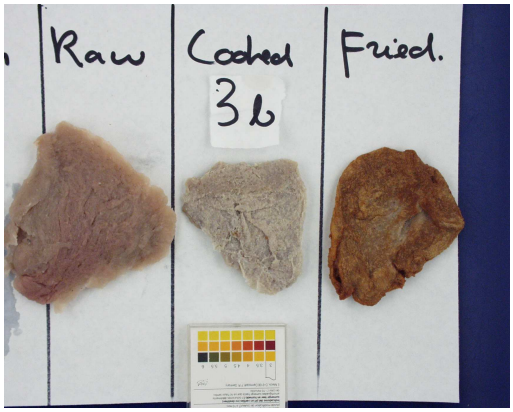

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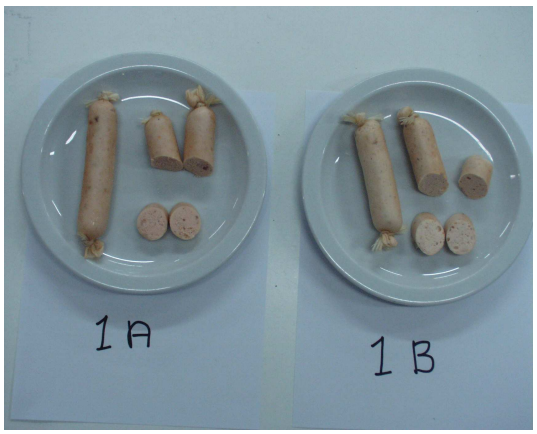

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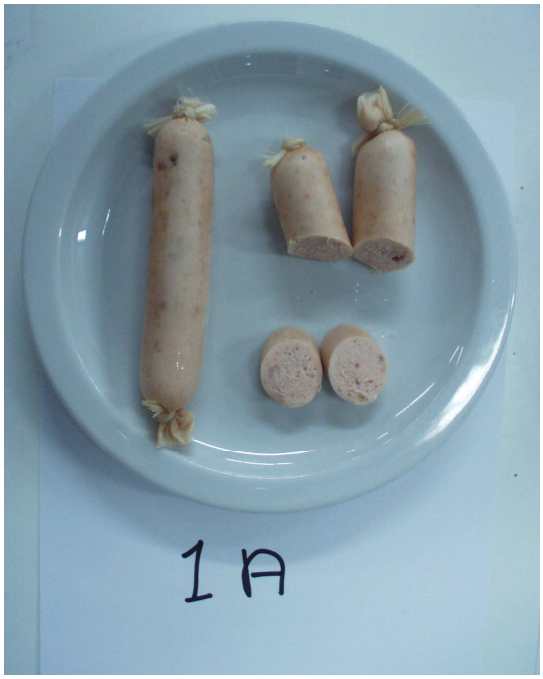

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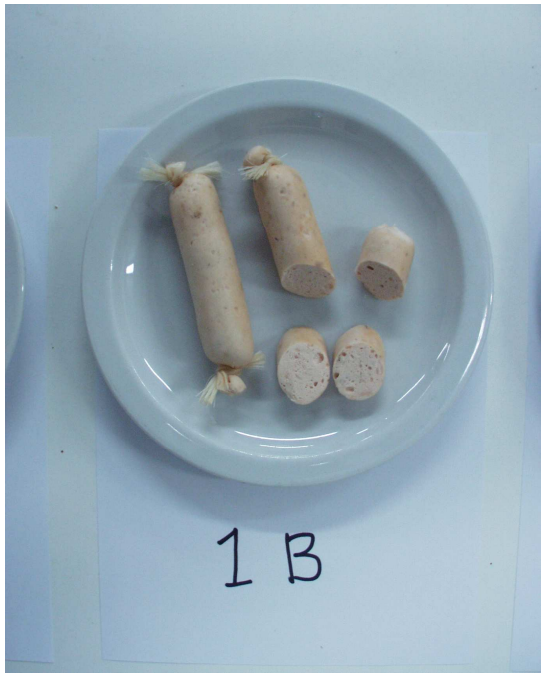

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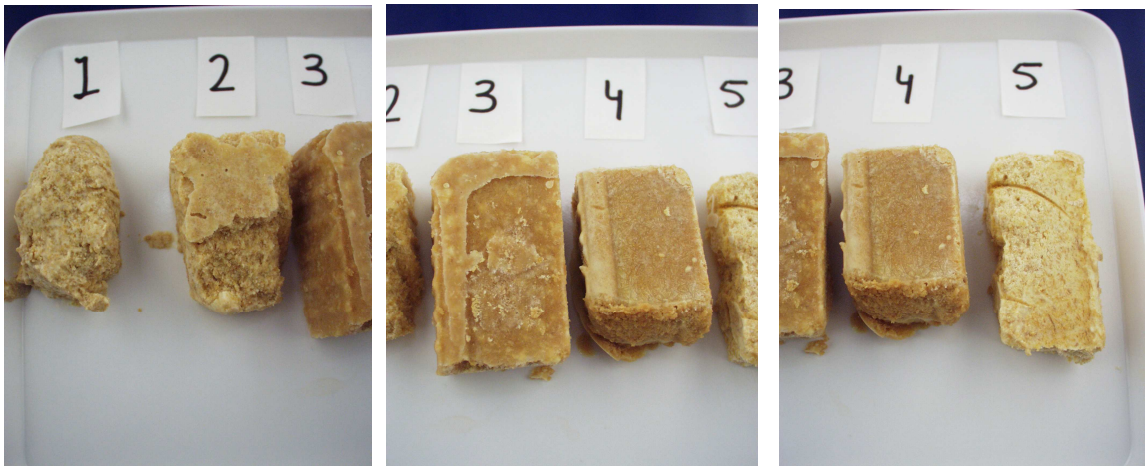

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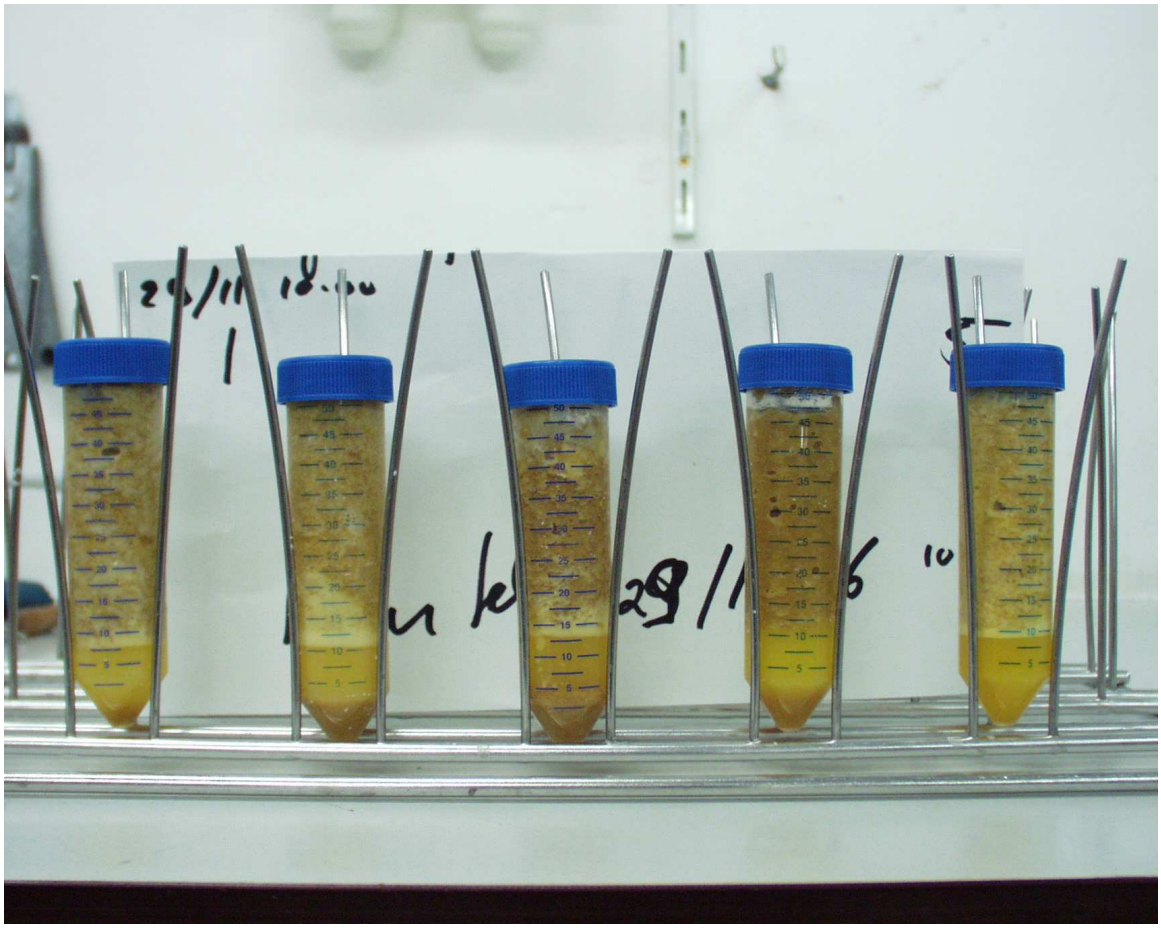
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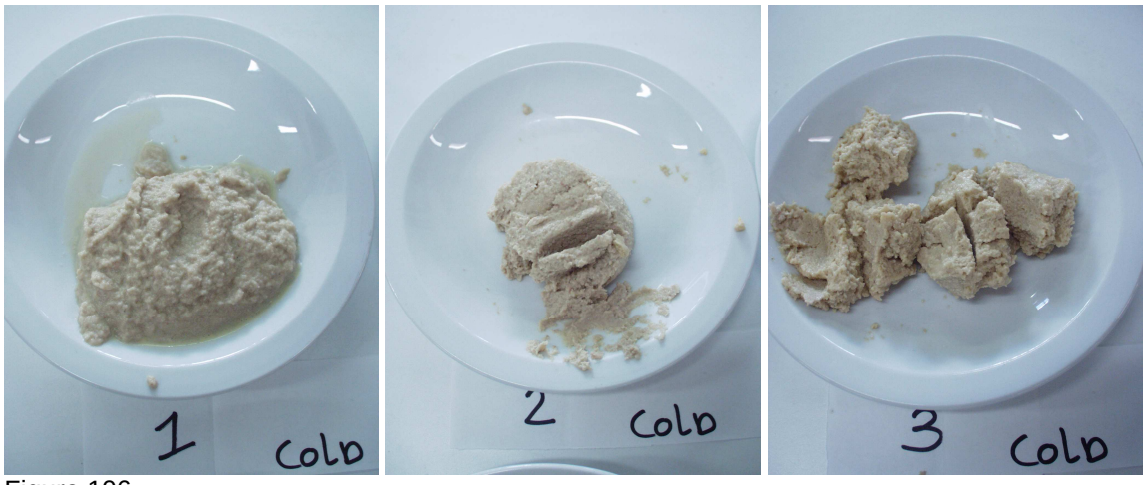

Figure 106

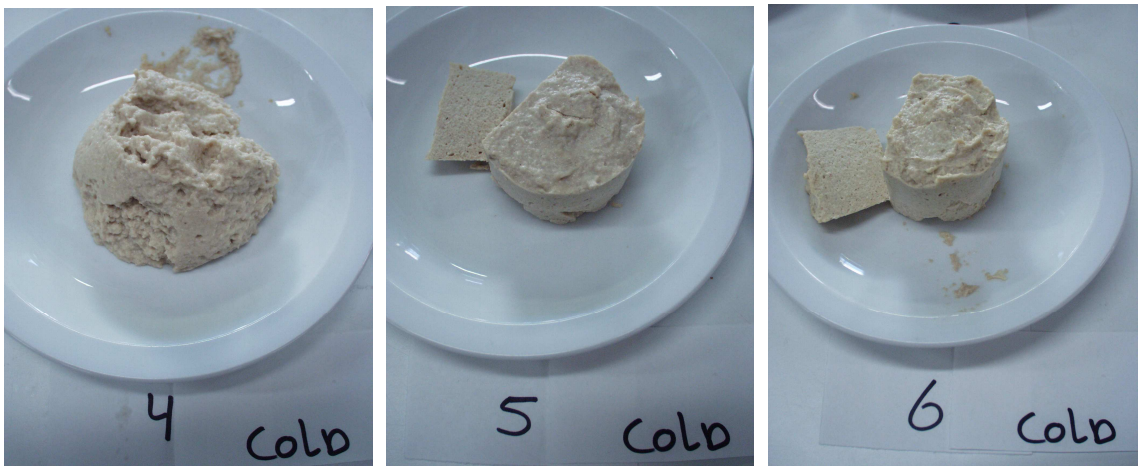

Figure107

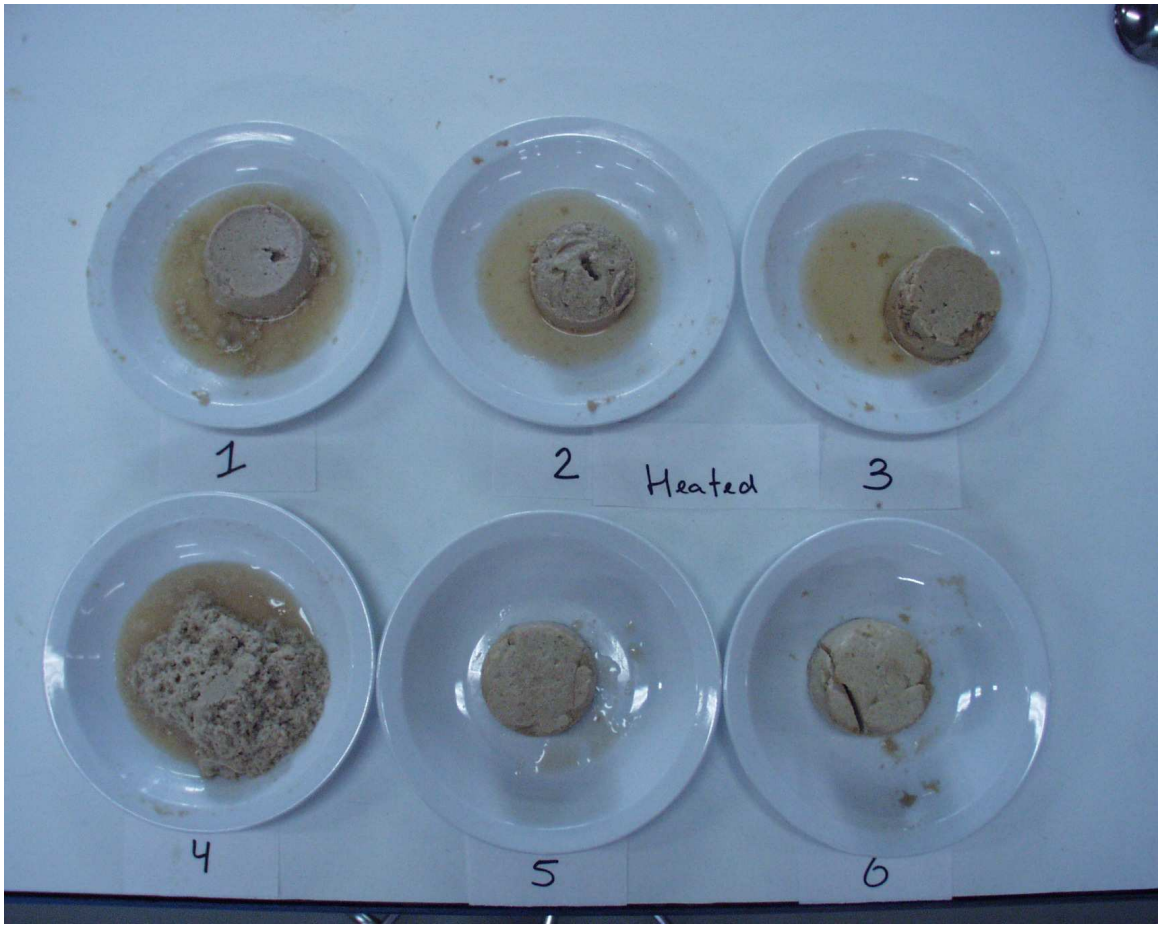

Figure 108

Figure 109

Figure 110