

**P.O. Box 1390, Skulagata 4**

**120 Reykjavik, Iceland**

**Final Project 2007**

## **THE EFFECTS OF STORING AND DRYING ON THE QUALITY OF CURED, SALTED COD**

Minh Nguyen Van  
Nha Trang University  
02 Nguyen Dinh Chieu, Nha Trang  
Vietnam  
[minh280477@yahoo.com](mailto:minh280477@yahoo.com)

Supervisors  
Ásbjörn Jónsson, [asbjorn.jonsson@matis.is](mailto:asbjorn.jonsson@matis.is)  
and Sigurjón Arason, [sigurjon@matis.is](mailto:sigurjon@matis.is)  
Icelandic Food Research

### **ABSTRACT**

Changes in physical and chemical properties of different cured, salted cod fillets at different storage times (1, 3 and 6 weeks) and temperatures (+2, -4, -12, -18 and -24 °C) were investigated. In the curing process, part of the fillets was injected with protein and phosphates. A part of the fillets was dried in a heat pump dryer. During the storage period, the lightness of the salted cod increased, and samples in all groups stored at -18 °C and -24 °C had higher lightness values compared to other samples. However, the phosphates and protein injection did not significantly affect the lightness of the salted cod during storage. The formation of total volatile basic nitrogen and trimethylamine increased with time of storage, but the values were low and acceptable. The samples stored at -4 °C had a higher drip loss than other samples. The cooking yield and water holding capacity increased slightly after 6 weeks of storage, but varied much between the samples during the storage time. Higher water holding capacity and higher cooking yield were observed in fillets treated with phosphates. The results were in correlation with the water holding capacity and cooking yield of desalted cod after 3 weeks and 6 weeks of storage. The water content decreased slightly after storage for 6 weeks. Nevertheless, phosphates did not contribute significantly to changes in colour and water loss during heat pump drying.

*Keywords:* Salted cod, storing, heat pump drying, water content, water holding capacity, cooking yield, NMR.

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## 1 INTRODUCTION

Currently, the demand for traceability of food quality and food safety for consumers is increasing (Ólafsdóttir 2005). The quality of fish and fish products is an important concern of the industry and consumers. Deterioration of fish products mainly occurs as a result of bacteriological activity and chemical changes during processing and storage. Storage conditions are the most important factors that affect the quality of fishery products.

The effects of different storage methods on the quality of different products have been studied and promising results have been found. Cold storage, chill storage, vacuum packaging and modified atmosphere packaging (MAP) are often considered. Chill storage is based on lowering the temperature close to the freezing point of the products using different cooling agents such as refrigerated air, refrigerated sea water (RSW) and chilled sea water (CSW) (Smith *et al.* 1980, Kraus 1992); flake ice or slurry ice (Yamada *et al.* 2002, Zeng *et al.* 2005); or liquid ice and brine solution (Huidobro *et al.* 2002). Cold storage is based on lowering the temperature below the freezing point of the products using refrigerated air agent. At chilled and freezing temperatures, the spoilage of bacterial growth is reduced resulting in extended shelf life and good quality (Chapman 1990). However, each method has its advantages and disadvantages depending on the characteristics of the products and storage conditions. Therefore, studies are needed to find out appropriate storage conditions for each fish product.

Vietnam is a seafood export country. The export turnover increased from US\$ 971 million in 1999 to US\$ 3.4 billion in 2006 (Fistenet 2006). Shelf life extension of fish products and quality are therefore of great interest to Vietnamese fish producers. Although chill storage and cold storage have been researched and applied in the world for a long time, these methods have not been researched and applied properly for different seafood products in Vietnam. Most producers apply storage methods based on their experience or customers' requirements. Better understanding of the storage conditions would offer prospects for quality improvement of seafood products in Vietnam.

The overall objective of this project was to research the effect of different storage temperatures and storage times on the quality of salted cod. It is a novel product in Vietnam. Thus, a better understanding of this product could be applied to fish species in Vietnam.

In addition, a drying experiment of salted cod was carried out using a heat pump dryer. At the same time, physical and chemical changes in dried salted cod were studied to evaluate the quality changes in the product.

### Objectives:

The objectives of the project were to observe the effect of different storage temperatures and times on the quality of salted cod and to evaluate the quality changes of salted cod during heat pump drying. The following aims were established for the project work:

- To study the physical characteristics of salted cod during storage and during drying.
- To determine the chemical changes of salted cod during storage and during drying.

## **2 STATE OF THE ART**

### **2.1 Salted fish**

Salted fish products are popular in many countries around the world. Salted fish products have been shown to be safe for millenniums, even in developed countries (Turan *et al.* 2007). Atlantic cod (*Gadus morhua* L.) is by far the most important species for salted fish processing (Erikson *et al.* 2004). Atlantic cod has been the most important commercial species in the North Atlantic for centuries and is now regarded as a very promising species in cold water fish farming (Duun and Rustad 2007). Salted cod is one of the most important processed products for the economies of Iceland and Norway (Rodrigues *et al.* 2005) Heavily salted cod is a traditional product from the North-Atlantic fisheries and is highly regarded as ripened fish product in many countries (Lauritzsen and Martinsen 1999). Traditional production of salted cod is a simple process in which cods are piled up with alternate layers of dry salt crystals. Regular salted cod reaches a salt concentration of 0.20 (w/w) and a decrease of 65–80% (w/w) of water. The final product shows a high microbiological stability and specific sensorial features (Del Valle and Gonzales-Inigo 1968). Nowadays, there are two types of commercial salted cod: “dry salted cod” with 47% water content, and “wet salted cod” with more than 50% water content (Andrés *et al.* 2005a). Salted cod is one of the principal salted products consumed in Mediterranean countries and is used in the preparation of many traditional dishes (Barat *et al.* 2002)

Salting is one of the oldest treatments in food preservation. It decreases the water activity and consists of transporting salt into food structures and is governed by various physical and chemical factors such as diffusion, osmosis and a series of complicated chemical and biochemical processes (Turan *et al.* 2007). Salt causes plasmolysis and alters protein and enzyme states in such a way that proteins become impervious to enzyme action and lose their efficacy. It also has bacteriostatic and bactericidal effects (Ismail and Wootton 1982). Salting is mainly used to preserve products and also to promote important sensorial changes that make the final product appreciated by consumers (Andrés *et al.* 2005b).

Salting is usually performed by dry, brine, or injection salting or a combination of these methods. The most commonly used methods by the industry are dry salting (pile) and brine salting (Boeri *et al.* 1982, Birkeland and Bjerkeng 2005). The main features of salting are the removal of some of the water from the fish flesh and its

partial replacement by salt (Turan *et al.* 2007). When salt brine or dry salt are used as salting agents, two main simultaneous flows are usually generated; water loss and salt uptake.

The salt uptake and water loss depend on the contact area and initial weight (Fuentes *et al.* 2007). The properties of fish muscle vary due to changes in water and salt content: the muscle gains salt, whereas water is lost or gained depending on the salting procedure (Thorarinsdottir *et al.* 2002, Thorarinsdottir *et al.* 2004 and Sannaveerappa *et al.* 2004). Diffusion is said to be the most important mass transfer mechanism responsible for sodium and chloride transport. The brine concentration showed an important effect on the rate of diffusion in to the muscle and weight changes during the salting processing. Solute diffuses from the salting agent into fish muscle and water diffuses out of the fish, due to the differences in osmotic pressures between inter-cells and salting agent (Raoult-Wack 1994, Yao and Le Maguer 1996).

Salt uptake depends on many factors including species, muscle type, fish size, fillet thickness, weight, composition (lipid content and distribution), physiological state, salting method, brine concentration, duration of salting step, fish-to-salt ratio, ambient temperature, and freezing and thawing (Wang *et al.* 1998), Jittinandana *et al.* 2002). The rates of the salt and water diffusion are positively correlated with increasing the brine concentration (Poernomo *et al.* 1992, Bellagha *et al.* 2007). The rate of salt uptake is very important with regard to weight change, water holding capacity (WHC) and quality of the final product. The fast rate of salt uptake may lead to inactivation of enzymes and bacteria. The rate of salt uptake depends on the type of process and product.

The rigor mortis of fish affects the salt uptake and water loss during brine salting, pre-rigor salting leads to a higher water loss and lower salt uptake than in fish salted post-rigor and in-rigor (Wang *et al.* 1998, Lauritzen *et al.* 2004). The rigor mortis also affects the equilibrium salt concentration in fish muscle during brine salting. The equilibrium salt concentration in fish muscle is lower for pre-rigor mortis fish than either in-rigor or post-rigor mortis fish, due to the degradation of cellular structures by enzymes (Røså *et al.* 2004, Birkeland *et al.* 2007).

The protein content could also influence the rate of salt uptake as salt penetration decreases with increasing total protein (Zugarranmurdi and Lupin 1980). During the salting process, the changes in protein structure such as protein denaturation of cod occurred when brine concentration was raised from 20 to 25% due to the protein salting-out and the yield got lower than that obtained when using 20% brine (Barat *et al.* 2002). The brine concentration also affects the water holding capacity. A lower brine concentration is known to increase the water holding capacity and higher yields are obtained when compared to salting with saturated brine (Slabjy *et al.* 1987). The yield or the weight gain of salted products depends not only on the brine concentration, but also on the brining time and temperature. The weight gain of salted herring at low brining temperature was higher than that at high brining temperature and increased weight gain seemed possible by further extension of the brining time (Birkeland *et al.* 2005).

Generally, the quality of the salted fish depends on the quality and chemical composition of raw material. Freshness is an important parameter that affects the

quality of salted cod during the salting process, as the freshest raw material gives the lowest overall yield and the lowest salt uptake (Barat *et al.* 2006). The quality of salted fish also depends on origin, feeding, and maturity (Solberg *et al.* 2000). In addition, the salt composition affects the quality of salted fish. Usually, sea salt (NaCl) is used, and sodium (Na), as the main component, is considered essential to produce the desired texture and flavour and guarantee the safety of the fish (Ismail and Wootton 1982).

Salted cod is usually dried before storage and it is desalted and cooked before consuming (Andrés *et al.* 2005b). Salted cod is usually stored at 0±2 °C and during storage time, salt penetrates into the muscle. The changes in salt penetration rates closely follow changes in extractable actomyosin in muscle, indicating a dependence of the change on the degree of denaturation of fish muscle proteins. The brine concentration and frozen storage time have an impact on water transfer of salted fish. Water migrates from the brine into the flesh if fresh mullet fillets are dipped in brine at a concentration up to 15%. When the concentration of the brine is 20% or higher, water migrates from the fish muscle to the brine. However, after two months of frozen storage, fish muscle gains water if dipped in brine of 20% or below and loses water when the brine was 25% or higher (Deng 1977).

In recent years, the salted cod industry has suffered heavy losses by the deterioration of the product caused by colour; microbiological and chemical changes because of the storage temperature, for example lipid oxidation, protein denaturation and weight changes. A major quality problem in such skin-on products is yellow/brownish discolouration of the flesh surface. The yellow/brown colour may cover the whole muscle surface or only sections of it (Lauritzsen and Martinsen 1999). The salting of cod causes protein denaturation, leading to a small loss of protein during the re-hydration process, due to protein aggregation and precipitation and the short soaking time (Ito *et al.* 1990, Tambo *et al.* 1992, Thorarinsdottir *et al.* 2002). The high salt concentration initially causes protein loss by the osmotic effect of the salt (Yao and Le Maguer 1996, Ooizumi *et al.* 2003).

## **2.2 Methods for storage of fish and fish products**

Fish and fish products are highly perishable because of high water activity, the lipid oxidation, autolytic enzymes and metabolic activities of microorganisms. The degradation process is carried out at first by muscle enzymes and later by microbial enzymes (Ólafsdóttir *et al.* 1997). The rate of deterioration during storage depends on temperature and it can be slowed down by the low storage temperature such as chilling (chilled storage) and freezing (cold storage). Low temperature is important to retain the quality of fish and fish products (Jain and Pathare 2007). Low temperature storage is one of the primary methods to maintain fish freshness, based on the reduction in the rates of microbiological, chemical and biochemical changes (Chapman 1990).

Chilled storage is based on lowering temperature close to the freezing point of the products. Chilled storage is a useful technique that has been applied to extend the shelf life of fish and fish products. The low temperature will reduce the activities of spoilage bacteria, enzyme activity and lipid oxidation reaction, so the fish and fish products remain edible longer. However, bacterial biomass and types of bacteria



affect the success of storage (Chow 1982). Multiple interactions between biological, physical and technical factors affect the efficiency of chilled storage (Babiak *et al.* 2006). During chilled storage of fish and fish products, deterioration of sensory quality and loss of nutritional value have been detected as a result of changes in chemical constituents (Ashie *et al.* 1996, Olafsdóttir *et al.* 1997).

Cold storage is based on temperature below the freezing point of the products. Cold storage of fish and fish products has been largely used to retain their sensory and nutritional properties before consumption (Erickson 1997). Some deterioration of the frozen product takes place during cold storage, but the changes are small under the right storage conditions (FAO 2001).

### 2.3 Heat pump drying

Drying is one of the thermal treatments applied in many food industries, and the use of dried foods is expanding rapidly (Senadeera *et al.* 2005). The goals of the drying process research in the food industry are three-fold: economic considerations, environmental concerns and product quality aspects (Okos *et al.* 1992). In many agricultural countries, large quantities of food products are dried to improve shelf-life, reduce packaging costs, lower shipping weights, enhance appearance, encapsulate original flavour and maintain nutritional value (Sokhansanj and Jayas 1987, Chou and Chua 2001). Commonly used methods are sun drying and hot air drying. The disadvantage of these methods is that deterioration in quality often occurs during the drying process (Cho *et al.* 1989, Skonberg *et al.* 1998). The drive towards improved drying technologies is promoted by the needs to produce better quality products. Improvement in quality of most food products translates into a significant increase in their market value. On the other hand, in most industrialised countries, the energy used in drying accounts for 7-15% of the total industrial energy used often with a relatively low thermal efficiency ranging from 25% to 50% (Dincer 1998, Chua *et al.* 2001). Some highly industrialised countries use over one third of their primal energy for drying operations (Dirk and Markus 2004). Consequently, in order to reduce the energy consumption, it is necessary to select an efficient heating system. The heat pump presents an efficient and environmentally friendly technology due to its low energy consumption (Strømme *et al.* 2000) and the high coefficient of performance of the heat pump and the high thermal efficiency of a properly designed dryer (Braun *et al.* 2002, Dirk and Markus 2004). In addition, heat pump drying can operate independently of outside ambient weather conditions (Perera and Rahman 1997).

Heat pump drying has the potential to operate more efficiently, and at lower temperatures than conventional drying (Filho and Strømme 1996). In heat pump drying, both sensible and latent heat can be recovered from the dryer exhaust humid air, improving the overall thermal performance. Heat pump drying can be operated over a wide range of temperature, providing good conditions for the drying of heat sensitive materials (Hawlder *et al.* 2006).

Heat pump drying can improve the quality of fish products due to its low drying temperature and independence from outdoor air. In recent years, interest in applying heat pump drying to foods and biomaterials has been growing to enhance the quality of the final products (Chou and Chua 2001). Usually, dried products have a low aroma volatile content, suffer loss of heat-labile vitamins and have a high incidence of

colour degradation (Perera and Rahman 1997). Although, heat pump drying has been used extensively in the industry for many years, application of heat pump drying to dry fish and fish products is still limited and there is a lack of heat pump dryer characteristics data (Shi *et al.* 2007).

Principles of a heat pump dryer: The heat pump dryer system is a combination of two sub-systems: a heat pump and a dryer. The flow chart of the heat pump dryer system is shown in Figure 1 (Phani *et al.* 2002). In a heat pump system, the working fluid (refrigerant) at low pressure is vaporised in the evaporator by receiving the heat from the dryer exhaust humid air. The compressor raises the enthalpy of the working fluid of the heat pump and discharges it as superheated vapour at high-pressure. At the condenser, heat is removed from the working fluid and released to the dried air. The working fluid is then throttled to the low-pressure line (using an expansion valve) and enters the evaporator to complete the cycle. In the dryer system, hot and dried air exiting the condenser is passed through the drying chamber where it releases heat to the damp material and also extracts the moisture from the damp material by convective transfer to the air. The humid air exit of the drying chamber is passed through the evaporator where the moisture in the air will be condensed into water as the air goes below dew point temperature. After passing through the evaporator, the dried air is passed through the condenser to reheat. The reheated air goes to the drying chamber again.

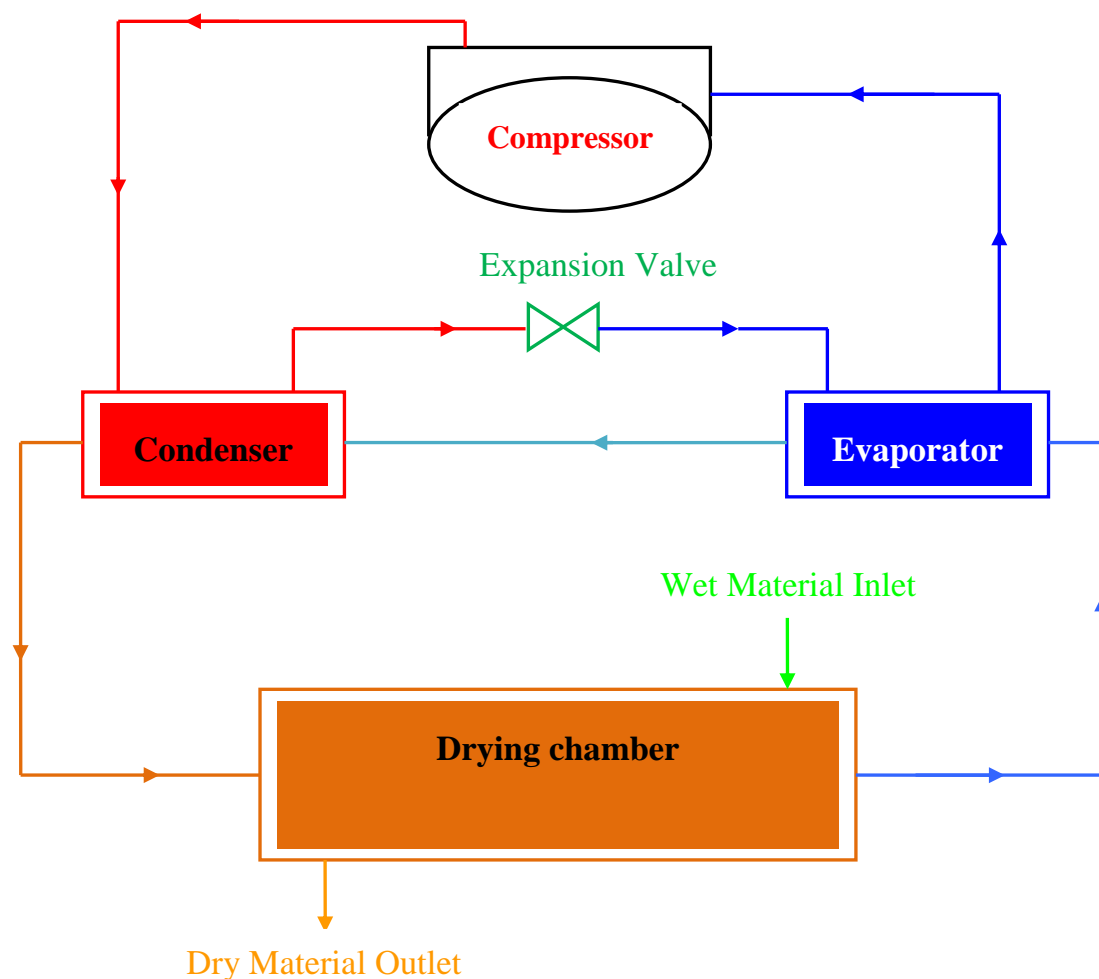


Figure 1: The heat pump dryer system

### 3 MATERIALS AND METHODS

#### 3.1 Experimental design

A total of 400 fillets of salted cod was used in this study. The samples were received from Visir hf., Djúpvogur in Iceland and contained four groups of differently cured fish (A, B, C and D). Four different curing methods were used:

- A: Injecting and brining.
- B: Injecting, phosphates (\*CARNAL 2110) and brining.
- C: Injecting, protein and brining.
- D: Injecting, protein, phosphates (\*CARNAL 2110) and brining.

Each group of fish was stored at different temperatures of 2 °C, -4 °C, -12 °C, -18 °C and -24 °C. The samples were stored for one week, 3 weeks and 6 weeks until they were analysed for physical and chemical properties (Table 1 and Figure 2).

\*Potassium, sodium and di-, triphosphates.

Table 1: The experimental groups of four different curing types of salted cod (A, B, C and D) stored at five different temperatures (2 °C, -4 °C, -12 °C, -18 °C and -24 °C).

Group	Type of fish	Storage temp. (°C)	Code
1	A	2	2A
2	A	-4	4A
3	A	-12	12A
4	A	-18	18A
5	A	-24	24A
6	B	2	2B
7	B	-4	4B
8	B	-12	12B
9	B	-18	18B
10	B	-24	24B
11	C	2	2C
12	C	-4	4C
13	C	-12	12C
14	C	-18	18C
15	C	-24	24C
16	D	2	2D
17	D	-4	4D
18	D	-12	12D
19	D	-18	18D
20	D	-24	24D

Part of the cured, salted cod was dried using a heat pump dryer. Before drying, the excess salt on the surface of the fish was rinsed and the fish was dried for 5 days (Figure 3). The conditions of the drying chamber are shown in Table 2.

Table 2: The temperature, velocity and relative humidity of the chamber in five days of drying

Drying parameter	Day 1	Day 2	Day 3	Day 4	Day 5
Temperature (°C)	19	19	19	19	19
Velocity (m/s)	2	2	2	2	2
Relative humidity (%)	60	60	55	55	55

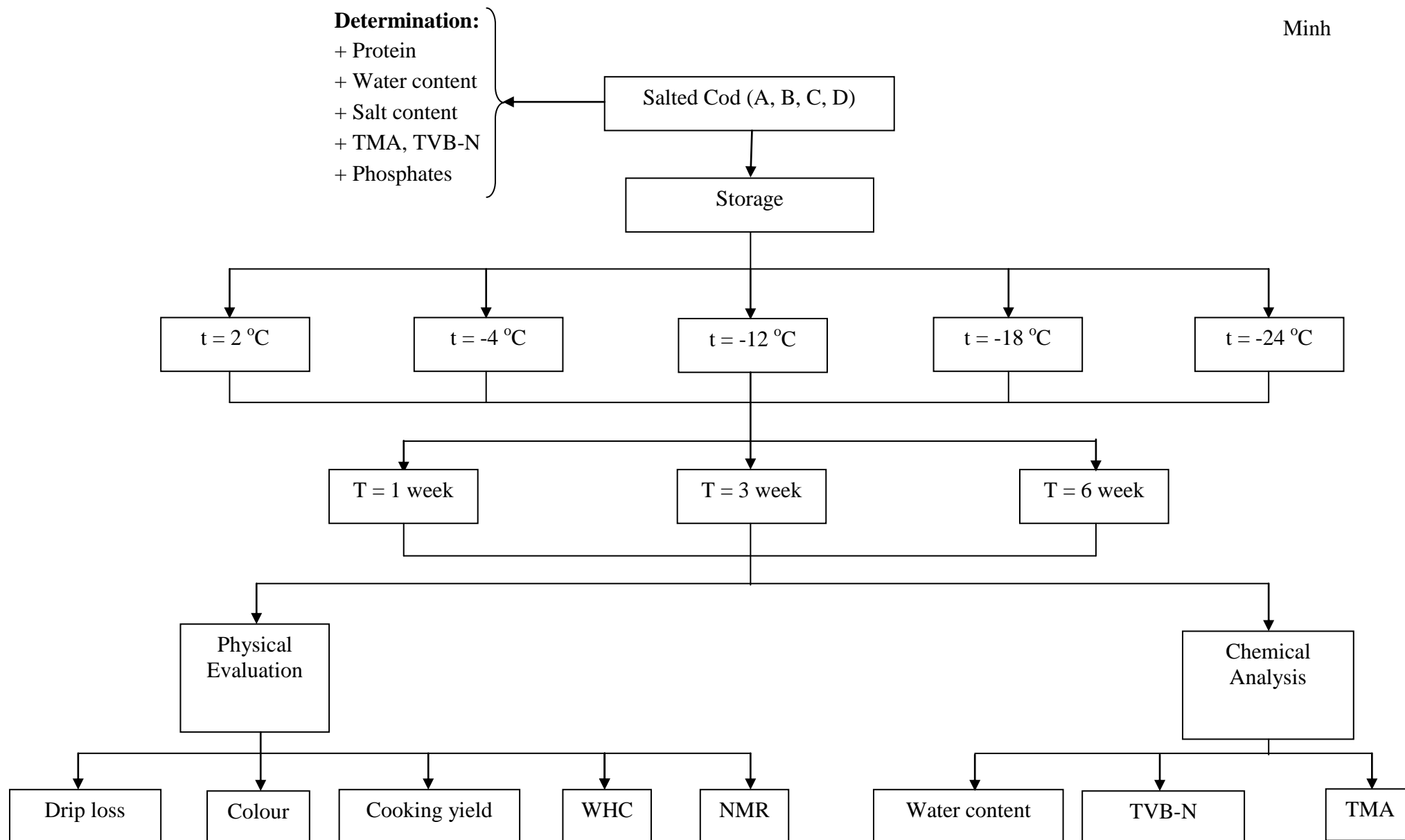


Figure 2: The flowchart of the experiments for storage of salted cod

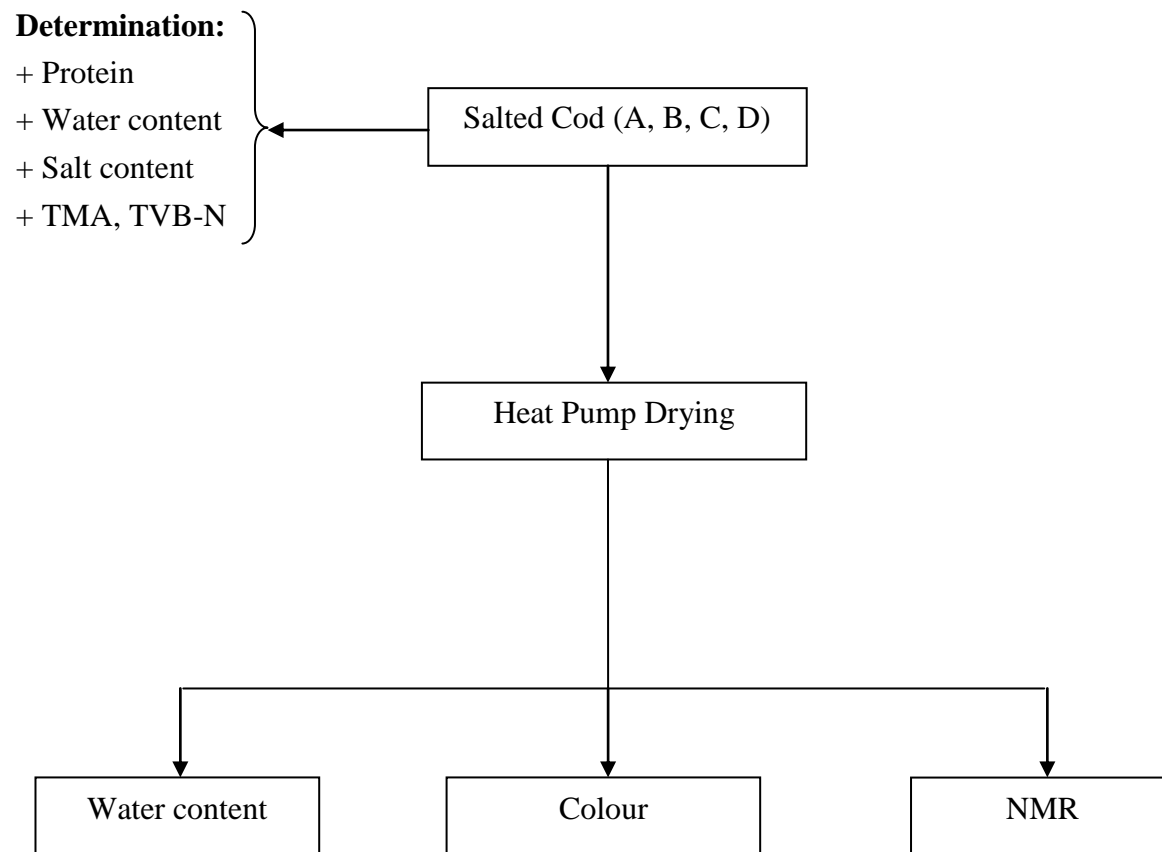


Figure 3: The flowchart of the experiments drying of salted cod using a heat pump dryer

## 3.2 Sampling

Samples were taken on the arrival of the salted cod (week 0) and after 1, 3 and 6 weeks of storage for evaluation of colour, water holding capacity (WHC), water content, trimethylamine (TMA), total volatile basic nitrogen (TVB-N) and cooking yield. Additionally, the amount of protein, salt and phosphates were measured on the arrival of the salted cod. The drip loss was determined at the end of the storage.

The cod was desalted, after 3 weeks and 6 weeks of storage respectively, with water at a ratio of 1:5 (fish: water) for 24 hours and then at a ratio of 1:6 (fish: water) for 72 hours. The total weight changes ( $\Delta W$ ), amount of salt, phosphates and content were measured. In the desalted cod, water holding capacity (WHC) and cooking yield were also determined.

The colour and nuclear magnetic resonance (NMR) were determined in the dried cod after five days of drying.

## 3.3 Methods of analyses

### 3.3.1 Physical analyses

#### Drip loss

Drip loss is defined as the amount of liquid loss during storage and is expressed as a percentage of weight loss. It was determined by the weight of the fillets before and after storage.

#### Colour

The intensity of the flesh colour was measured with a Minolta CR-300 chromameter (Minolta camera Co., Ltd; Osaka, Japan) in Lab\* system (CIE, 1976) with CIE Illuminant C. The instrument records the  $L^*$  value, lightness on the scale of 0 to 100 from black to white;  $a^*$  value, (+) red or (-) green  $b^*$  value, (+) yellow or (-) blue.

#### Cooking yield

Cooking yield is defined as the amount of liquid loss during cooking. About 35 g of sample was weighed and heated in a steaming oven at 90 °C for 10 minutes. Samples were drained for 5 minutes and weighed (Rattanasatheirn *et al.* 2008). The cooking yield (CY) was calculated by the formula:

$$CY = \frac{W_{cooked}}{W_{raw}} \times 100\%$$

Where:

$W_{cooked}$  is the weight of cooked sample.

$W_{raw}$  is the weight of sample before cooking.

### Water holding capacity (WHC)

Water holding capacity was determined according to a method by Børresen (Eide *et al.* 1982). The samples were prepared by chopping them in a Braun Mixer (Type 4262, Germany) for 10-15 seconds (until homogenous) and 2 g of the sample were weighted in the glass. Samples were centrifuged at 1500 rpm for 5 minutes in special sample glasses made from plexi-glass. Water removed during centrifugation was drained through a nylon membrane in the sample glasses.

The WHC of the sample was then calculated using the following formula:

$$\text{WHC} = \frac{W - \Delta r}{W} \times 100\%$$

Where:

W is the water content of the sample before centrifugation (%).

$\Delta r$  is the weight lost by centrifugation (%) and was calculated by the formula:

$$\Delta r = \frac{W_1 - W_2}{W_1} \times 100\%$$

Where:

$W_1$  is the weight of the original sample (g)

$W_2$  is the weight of the sample after centrifugation (g)

### Nuclear magnetic resonance (NMR)

The behaviour and distribution of water throughout the salted cod samples was determined with low field nuclear magnetic resonance (LF-NMR). A low-field Bruker Minispec mq 20, bench top NMR-analyzer (Bruker Optics GmbH, Am Silberstreifen D-76287 Rheinstetten, Germany), with test tubes of 10 mm width, were used for the measurements. All measurements were performed at STP using a Receiver Gain of 70 dB and a Recycle Delay of 10 s. The transverse relaxation time, T2, was measured with a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence with an interpulse spacing of 250  $\mu$ s, 16 scans and 8100 fitted points. The longitudinal relaxation time, T1, was measured with an Inversion Recovery (IR) pulse sequence with 30 measuring points, 4 scans and a duration factor of 1.322.

### Total weight changes after desalting

Total weight changes after desalting are defined as the amount of water intake after desalting and expressed as a percentage of weight gains. The total weight changes of the salted cod after desalting were determined as shown in formula (Barat *et al.* 2002)

$$W = (W_2 - W_1) / W_1 \times 100\%$$

Where:

+  $W_1$ : weight of fish before desalting.

+  $W_2$ : weight of fish after desalting.

### 3.3.2 Chemical analyses

#### Water content

Water content was determined according to ISO 6496:1999. About 5 g of sample were weighed accurately ( $\pm 1$  mg) and placed in an aluminium foil dish which was prepared with a thin layer of sea-sand and a glass rod. The samples were mixed thoroughly with the sand. The glass rod was kept on the dish and then left to dry for  $4 \pm 0.1$  h in the oven at  $103^\circ\text{C}$ . The dish was removed from the oven and allowed to cool to ambient temperature in a desiccator for about 15 minutes. The water content was calculated by the formula as follows:

$$W = \frac{m_1 - (m_3 - m_2)}{m_1} \times 100\%$$

Where:

$m_1$  is the mass of the test portion (g).

$m_2$  is the mass of the dish, test portion, sand and glass rod (g).

$m_3$  is the mass of the dish, dried test portion, sand and glass rod (g).

#### Protein content

Protein content of all samples was determined by the Kjeldahl method (ISO 20483:2006). A sample of 5 g was digested in sulphuric acid in the presence of copper as a catalyst. Thereafter, the sample was placed in a distillation unit, 2400 Kjeltac Auto Sample System. The acid solution was made alkaline by a sodium hydroxide solution. The ammonia was distilled into boric acid and the acid was simultaneously titrated with diluted  $\text{H}_2\text{SO}_4$ . The nitrogen content was multiplied by the factor 6.25 to get the ratio of crude protein.

#### Total volatile basic nitrogen (TVB-N) and trimethylamine (TMA)

The TVB-N was determined by dissolving 100 g of the salted cod fish sample extract in 200 ml 7.5% aqueous trichloroacetic acid, filtering the mixture, and then mixing 25 ml of the extract in a distillation flask with 6 ml 10% NaOH. Steam distillation was then carried out using the Kjeldahl-type distillator (Struer TVN) and the TVB-N condensed is mixed with 10 ml 4% boric acid and 0.04 ml of methyl red and bromocresol green indicator which turns green when alkalized by the TVB-N (Malle and Poumeyrol 1989). The solution was then titrated with 0.0372 N sulphuric acid until there was a complete neutralisation of the base which was indicated by a colour change to pink on the addition of a further drop of sulphuric acid. The TVB-N content was calculated by the formula:

$$\frac{14 \frac{\text{mg}}{\text{mol}} \times a \times b \times 300}{25 \text{ ml}} \left( \frac{\text{mgN}}{100\text{g}} \right)$$

Where:

a: volume of sulphuric acid (ml)

b: normality of sulphuric acid (%)



To measure TMA the same method as for TVB-N was used but 20 ml of formaldehyde was added to the distillation flask to block the primary and secondary amines.

#### Salt content

Salt content of all the samples was determined by titration according to AOAC (1995). Soluble chloride was extracted from the samples with water containing nitric acid. The chloride content of the solution was titrated with silver nitrate and the end point was determined potentiometrically.

#### Phosphates content

The method is based on reaction of ortophosphates in acidic solution with ammonium molybdate and ammonium vanadate with the formation of vanadomolybdophosphoric acid complex. Absorbance of the complex is measured at 420 nm.

The sample is weighted out in a crucible, CaO added to form Ca-phosphates, dried at 100 °C, ignited and ashed over night at 500 °C. The ash is then hydrolysed with HCl and HNO<sub>3</sub> for 10 min. The liquid is made up to certain volume with water, filtered and finally reacted with vanadate/molybdate solution. Solutions of potassiumhydrogenphosphates (KH<sub>2</sub>PO<sub>4</sub>) as standard are reacted following the same procedure as samples to make a standard curve. Results are given as mg/g P<sub>2</sub>O<sub>5</sub>.

### **3.4 Statistical analysis**

Microsoft Excel 2007 was used to calculate the means and standard deviations for all multiple measurements and to generate graphs. The obtained data sets were compared by multiple comparisons ANOVA by using all pair wise comparison by Sigmastat 3.5 (Jandel Scientific Software, Ontario, Canada). Significance of difference was defined at  $p < 0.05$ . Principal component analysis (PCA) was performed by the Unscrambler version 9.1, from Camo, Norway. PCA transforms original variables into new axes or principal components (PCs), which are orthogonal, so that the data presented in those axes are uncorrelated with each other, therefore, PCA expresses as much as possible of total variation in the data in a few principal components and each successively derived PC expresses decreasing amounts of the variation (Smith 1991).

## **4 RESULTS**

### **4.1 Effects of temperature and storage time on the quality of salted cod**

#### **4.1.1 Physical changes**

##### Drip loss

Drop loss was measured as the weight loss in salted cod during the storage time. Generally, the drip loss was low for all the samples (0-1.5%). Samples stored at -4 °C were an exception (Table 3). In those samples the drip loss increased tenfold compared to the other samples.

Table 3: The drip loss (%) of the salted cod (group A: injecting and brining; group B: injecting, phosphates and brining; group C: injecting, protein and brining; and group D: injecting, protein, phosphates and brining) stored for 6 weeks at different temperatures.

Storage temperature	Drip loss (%)			
	A	B	C	D
2°C	0.81	1.33	0.66	1.16
-4°C	12.44	12.46	11.20	13.88
-12°C	0.03	1.54	1.30	2.36
-18°C	0.02	0.83	0.36	0.68
-24°C	0.05	0.00	1.02	1.46

### Colour analysis

The lightness was measured on a scale from 0 to 100 (from black to white). Generally, the lightness of the salted cod increased with storage time for all treatments independent of different temperatures. Comparison between the treatments showed that treatments B and D had higher values of lightness than treatments A and C. Generally, the lightness for all treatments stored at 2 °C and -4 °C was lower than that of all treatments at other temperatures (Table 4).

In treatment A (injecting and brining), there was a decrease in  $L^*$  value after 1 week of storage at all temperatures, although it was only statistical significant ( $p < 0.05$ ) at -4 °C. The  $L^*$  value increased in all cases from week 1 to week 6. The  $L^*$  value of the samples stored at -12 °C and -24 °C increased significantly ( $p < 0.05$ ) from 1 week to 6 weeks storage. No significant differences ( $p > 0.05$ ) were found between different storage times in the samples stored at 2 °C.

In treatment B (injecting, phosphates and brining), the  $L^*$  value increased for all samples at different temperatures, except the sample stored at -4°C. The sample stored at -24 °C increased significantly ( $p < 0.05$ ) after 1 week of storage compared to the sample stored at -18 °C. From week 1 to week 6 of storage, the  $L^*$  value of samples stored at -12 °C, -18 °C and -24 °C increased significantly ( $p < 0.05$ ).

In treatment C (injecting, protein and brining), the  $L^*$  value of the samples stored at -12 °C and -24 °C increased significantly ( $p < 0.05$ ) after 6 weeks of storage. No significant differences ( $p > 0.05$ ) in  $L^*$  value between different storage times were found in the samples stored at 2 °C and -4 °C.

In treatment D (injecting, protein, phosphates and brining), the lightness of the samples stored at -12 °C and -24 °C increased significantly ( $p < 0.05$ ) after storage for 6 weeks. Meanwhile, the lightness of samples stored at -4 °C decreased significantly ( $p < 0.05$ ) after 6 weeks of storage.

Table 4: The lightness ( $L^*$  value) of the salted cod (group A: injecting and brining; group B: injecting, phosphates and brining; group C: injecting, protein and brining; and group D: injecting, protein, phosphates and brining) stored for 6 weeks at different temperatures.

Storage time (week)	Group A				
	2 °C	-4 °C	-12 °C	-18 °C	-24 °C
0	52.48 ± 2.28	52.48 ± 2.28	52.48 ± 2.28	52.48 ± 2.28	52.48 ± 2.28
1	50.29 ± 2.67	46.23 ± 1.71	48.44 ± 1.92	51.17 ± 2.85	49.47 ± 3.39
3	51.44 ± 1.75	48.25 ± 2.90	53.69 ± 6.30	49.67 ± 3.95	53.12 ± 2.67
6	51.00 ± 2.80	51.51 ± 3.08	56.25 ± 3.99	52.15 ± 3.95	55.57 ± 4.49
Storage time (week)	Group B				
	2 °C	-4 °C	-12 °C	-18 °C	-24 °C
0	50.44 ± 2.52	50.44 ± 2.52	50.44 ± 2.52	50.44 ± 2.52	50.44 ± 2.52
1	52.99 ± 2.57	51.00 ± 2.63	51.52 ± 3.11	48.37 ± 2.31	54.68 ± 4.19
3	51.90 ± 2.96	50.64 ± 3.11	54.45 ± 3.36	53.94 ± 4.74	53.93 ± 3.23
6	52.57 ± 4.05	48.72 ± 3.17	58.78 ± 4.47	54.63 ± 7.21	56.26 ± 4.07
Storage time (week)	Group C				
	2 °C	-4 °C	-12 °C	-18 °C	-24 °C
0	51.07 ± 2.69	51.07 ± 2.69	51.07 ± 2.69	51.07 ± 2.69	51.07 ± 2.69
1	49.00 ± 1.35	48.62 ± 3.20	50.34 ± 2.84	48.21 ± 4.03	48.29 ± 2.91
3	52.19 ± 3.37	46.35 ± 1.52	50.10 ± 3.67	55.15 ± 4.96	50.37 ± 3.96
6	51.83 ± 1.74	50.94 ± 2.14	58.32 ± 4.10	51.24 ± 3.31	64.16 ± 4.17
Storage time (week)	Group D				
	2 °C	-4 °C	-12 °C	-18 °C	-24 °C
0	54.06 ± 2.56	54.06 ± 2.56	54.06 ± 2.56	54.06 ± 2.56	54.06 ± 2.56
1	50.43 ± 2.28	51.31 ± 2.36	52.69 ± 2.09	52.63 ± 3.64	52.35 ± 2.97
3	54.24 ± 4.19	49.07 ± 2.53	54.29 ± 3.54	56.78 ± 5.00	56.32 ± 4.21
6	53.01 ± 3.76	50.04 ± 3.86	56.60 ± 2.73	56.47 ± 2.11	65.88 ± 3.81

The  $a^*$  value describes the intensity in green colour (negative) and in red colour (positive) of the salted cod. Generally, the  $a^*$  value of samples for all treatments after 6 weeks of storage decreased when samples were stored at 2 °C and -4 °C, but increased in samples stored at other temperatures. No significant differences ( $p>0.05$ ) in  $a^*$  value were found for any of the samples (Table 5).

Table 5: The  $a^*$  value of the salted cod (treatment A: injecting and brining; treatment B: injecting, phosphates and brining; treatment C: injecting, protein and brining; and treatment D: injecting, protein, phosphates and brining) stored for 6 weeks at different temperatures.

Storage time (week)	Group A				
	2 °C	-4 °C	-12 °C	-18 °C	-24 °C
0	-2.65 ± 0.28	-2.65 ± 0.28	-2.65 ± 0.28	-2.65 ± 0.28	-2.65 ± 0.28
1	-2.92 ± 0.31	-2.46 ± 0.16	-2.46 ± 0.36	-2.84 ± 0.51	-2.58 ± 0.61
3	-2.97 ± 0.32	-2.82 ± 0.32	-2.69 ± 0.53	-2.63 ± 0.47	-2.68 ± 0.31
6	-2.86 ± 0.36	-2.76 ± 0.58	-2.27 ± 0.57	-2.34 ± 0.47	-2.20 ± 0.32
Storage time (week)	Group B				
	2 °C	-4 °C	-12 °C	-18 °C	-24 °C
0	-2.61 ± 0.15	-2.61 ± 0.15	-2.61 ± 0.15	-2.61 ± 0.15	-2.61 ± 0.15
1	-2.89 ± 0.53	-2.85 ± 0.25	-3.11 ± 0.43	-2.76 ± 0.19	-3.09 ± 0.47
3	-2.93 ± 0.31	-2.90 ± 0.31	-2.09 ± 0.47	-2.28 ± 0.26	-2.98 ± 0.40
6	-3.00 ± 0.48	-2.77 ± 0.27	-2.16 ± 0.46	-2.41 ± 0.29	-2.08 ± 0.39
Storage time (week)	Group C				
	2 °C	-4 °C	-12 °C	-18 °C	-24 °C
0	-2.50 ± 0.40	-2.50 ± 0.40	-2.50 ± 0.40	-2.50 ± 0.40	-2.50 ± 0.40
1	-2.58 ± 0.31	-2.64 ± 0.24	-2.91 ± 0.20	-2.48 ± 0.23	-2.36 ± 0.30
3	-3.01 ± 0.41	-2.56 ± 0.20	-2.36 ± 0.53	-1.96 ± 0.39	-2.10 ± 0.27
6	-2.97 ± 0.38	-2.88 ± 0.29	-2.17 ± 0.25	-2.13 ± 0.38	-1.45 ± 0.28
Storage time (week)	Group D				
	2 °C	-4 °C	-12 °C	-18 °C	-24 °C
0	-2.99 ± 0.31	-2.99 ± 0.31	-2.99 ± 0.31	-2.99 ± 0.31	-2.99 ± 0.31
1	-2.61 ± 0.17	-2.89 ± 0.30	-2.95 ± 0.54	-2.77 ± 0.39	-2.51 ± 0.44
3	-3.17 ± 0.43	-2.78 ± 0.20	-3.04 ± 0.38	-2.27 ± 0.37	-2.78 ± 0.51
6	-3.21 ± 0.35	-2.83 ± 0.39	-2.49 ± 0.19	-2.38 ± 0.41	-1.56 ± 0.17

The  $b^*$  value describes intensity in blue (negative) and in yellow (positive) of the salted cod. In all cases, the  $b^*$  value increased with time for all treatments stored at different temperatures. The  $b^*$  value of the samples stored at 2 °C was lower than that for all treatments at other temperatures. There was a statistically significant increase ( $p < 0.05$ ) in yellow colour for treatments C and D stored at -24 °C (Table 6).

Table 6: The  $b^*$  value of the salted cod (treatment A: injecting and brining; treatment B: injecting, phosphates and brining; treatment C: injecting, protein and brining; and treatment D: injecting, protein, phosphates and brining) stored for 6 weeks at different temperatures.

Storage time (week)	Group A				
	2 °C	-4 °C	-12 °C	-18 °C	-24 °C
0	-3.64 ± 1.64	-3.64 ± 1.64	-3.64 ± 1.64	-3.64 ± 1.64	-3.64 ± 1.64
1	-4.70 ± 1.84	-5.39 ± 1.16	-4.79 ± 1.48	-3.24 ± 1.39	-2.55 ± 1.12
3	-5.32 ± 0.95	-5.11 ± 1.44	-2.83 ± 1.65	-3.79 ± 1.17	-3.58 ± 0.94
6	-4.87 ± 1.34	-1.01 ± 1.81	-2.88 ± 0.90	-3.10 ± 1.26	-1.93 ± 1.42
Storage time (week)	Group B				
	2 °C	-4 °C	-12 °C	-18 °C	-24 °C
0	-6.53 ± 0.94	-6.53 ± 0.94	-6.53 ± 0.94	-6.53 ± 0.94	-6.53 ± 0.94
1	-7.25 ± 1.02	-5.81 ± 1.13	-6.13 ± 0.80	-6.52 ± 1.05	-5.27 ± 1.40
3	-7.24 ± 1.21	-6.19 ± 1.27	-4.82 ± 0.93	-4.21 ± 1.00	-4.87 ± 1.38
6	-5.69 ± 1.23	-5.08 ± 1.47	-3.19 ± 0.96	-3.42 ± 1.44	-1.31 ± 1.00
Storage time (week)	Group C				
	2 °C	-4 °C	-12 °C	-18 °C	-24 °C
0	-4.89 ± 0.79	-4.89 ± 0.79	-4.89 ± 0.79	-4.89 ± 0.79	-4.89 ± 0.79
1	-5.48 ± 1.39	-4.35 ± 1.37	-5.23 ± 0.65	-5.42 ± 1.57	-3.92 ± 1.20
3	-4.08 ± 1.50	-5.29 ± 0.83	-5.05 ± 0.60	-2.81 ± 0.71	-1.53 ± 0.63
6	-3.44 ± 1.19	-2.30 ± 0.93	-1.67 ± 1.42	-3.03 ± 1.21	0.57 ± 1.37
Storage time (week)	Group D				
	2 °C	-4 °C	-12 °C	-18 °C	-24 °C
0	-5.40 ± 1.18	-5.40 ± 1.18	-5.40 ± 1.18	-5.40 ± 1.18	-5.40 ± 1.18
1	-7.05 ± 0.83	-6.17 ± 0.98	-5.92 ± 0.58	-5.49 ± 1.09	-5.82 ± 0.96
3	-5.49 ± 1.70	-6.55 ± 1.11	-5.06 ± 0.81	-3.38 ± 1.70	-3.01 ± 1.81
6	-4.69 ± 1.58	-3.19 ± 1.46	-3.29 ± 1.45	-3.05 ± 0.76	0.32 ± 1.26

### Cooking yield

Cooking yield was measured as the weight loss during cooking. In the beginning of the storage (week 0), the cooking yield was higher for salted cod in treatment C (~58.5%) and D (~61.0%) where the protein was injected, compared to treatments A (~58.0%) and B (~58.0%). Generally, the cooking yield (CY) for all treatments stored at different temperatures increased after 1 week of storage, then decreased after 3 weeks of storage and increased slightly after 6 weeks of storage. The CY in treatment D was significantly higher ( $p < 0.05$ ), compared to other treatments. The CY of the samples stored at 2 °C for all treatments were lower than samples stored at other temperatures (Figure 4).

The CY of the samples in treatment A (Figure 4a) stored at different temperatures, except -18 °C decreased significantly ( $p < 0.05$ ) from week 1 to week 3 of storage and then increased at the end of the storage period. From week 3 to week 6, the CY of the

sample stored at  $-4\text{ }^{\circ}\text{C}$  increased significantly ( $p<0.05$ ), meanwhile, the CY of the sample stored at  $-18\text{ }^{\circ}\text{C}$  was stable during the storage time.

In treatment B, the CY after 1 week of storage increased for all samples stored at different temperatures and then decreased between week 1 and week 3. From week 3 to week 6 of storage, the CY of the sample stored at  $-4\text{ }^{\circ}\text{C}$  increased significantly compared to other samples at different temperatures ( $p<0.05$ ) (Figure 4b).

The CY of all samples in treatment C (Figure 4c) stored at different temperatures varied after storage for 1 week, decreased after storage for 3 weeks and then increased slightly after 6 weeks of storage. However, the CY of the sample stored at  $-24\text{ }^{\circ}\text{C}$  increased significantly ( $p<0.05$ ) during the storage time, compared to other samples at different temperatures.

The CY increased slightly after 1 week of storage for all samples in treatment D, decreased after storage for 3 weeks and then varied after 6 weeks of storage (Figure 4d). The CY of the sample stored at  $-24\text{ }^{\circ}\text{C}$  increased significantly ( $p<0.05$ ) after storage for 6 weeks compared to other samples at other temperatures.

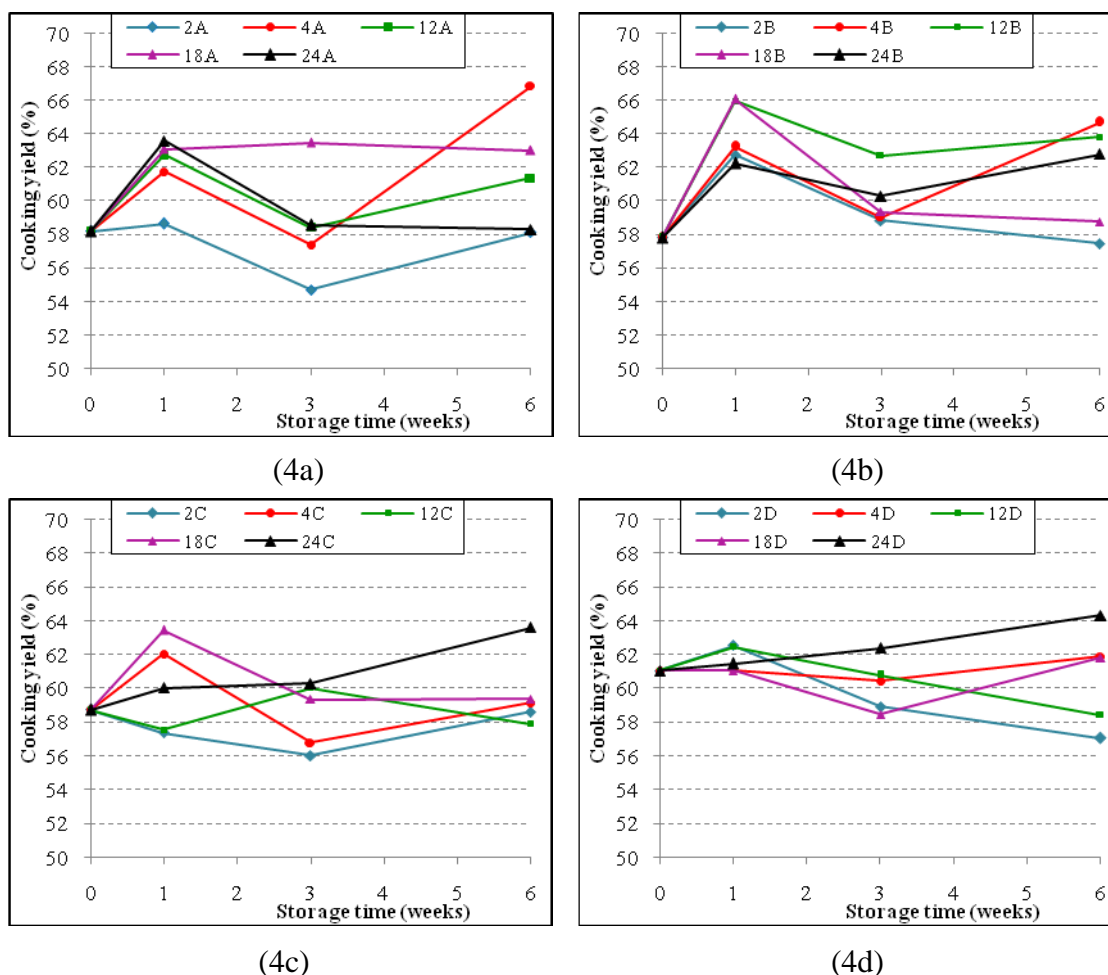


Figure 4: The cooking yield of the salted cod (group A: injecting and brining (4a); group B: injecting, phosphates and brining (4b); group C: injecting, protein and brining (4c); and group D: injecting, protein, phosphates and brining (4d)) stored at different temperatures ( $2\text{ }^{\circ}\text{C}$ ,  $-4\text{ }^{\circ}\text{C}$ ,  $-12\text{ }^{\circ}\text{C}$ ,  $-18\text{ }^{\circ}\text{C}$  and  $-24\text{ }^{\circ}\text{C}$ ).

### Water holding capacity

Water holding capacity (WHC) is the ability of a muscle to retain fluid under specific conditions. Generally, the water holding capacity (WHC) at the beginning (week 0) was significantly higher ( $p < 0.05$ ) in salted cod treated with phosphates (B and D), compared to the A and C treatments. During the storage time the WHC in all the samples varied between different temperatures. After 3 weeks of storage the samples stored at  $-18^{\circ}\text{C}$  had the lowest WHC for all treatments. The samples stored at  $-4^{\circ}\text{C}$  distinguished from the other samples stored at different temperatures, by increasing in WHC during the storage time for all treatments (Figure 5).

The WHC of all samples in treatment A (Figure 5a) increased after 1 week of storage. The WHC of the sample stored at  $2^{\circ}\text{C}$  increased significantly ( $p < 0.05$ ) after storage for 1 week, and then decreased significantly ( $p < 0.05$ ) after storage for 3 weeks. The WHC of the samples stored at  $-18^{\circ}\text{C}$  and  $-24^{\circ}\text{C}$  increased significantly ( $p < 0.05$ ) from week 3 to week 6. Meanwhile, the WHC of the sample stored at  $-4^{\circ}\text{C}$  increased significantly ( $p < 0.05$ ) during the storage period.

In treatment B (Figure 5b), the WHC of samples stored at different temperatures  $2^{\circ}\text{C}$ ,  $-12^{\circ}\text{C}$  and  $-24^{\circ}\text{C}$  increased after 1 week of storage, and then decreased from week 1 to week 3. The WHC of the sample stored at  $-24^{\circ}\text{C}$  increased significantly ( $p < 0.05$ ) after 6 weeks of storage. The WHC of the sample stored at  $-4^{\circ}\text{C}$  increased significantly ( $p < 0.05$ ) during the storage period. The WHC of the sample stored at  $-18^{\circ}\text{C}$  decreased after storage for 3 weeks and increased significantly ( $p < 0.05$ ) after 6 weeks of storage.

The WHC of all samples in treatment C increased after storage for 1 week at all temperatures and then decreased after 3 weeks and finally increased at the end of the storage period (Figure 5c). The WHC of the sample stored at  $-18^{\circ}\text{C}$  decreased significantly from week 1 to week 3, and then increased significantly ( $p < 0.05$ ) at the end of the storage time. The WHC of the samples stored at  $-4^{\circ}\text{C}$  increased significantly ( $p < 0.05$ ) during the storage period.

There was a small variation in the WHC of samples in group D (Figure 5d) for different temperatures through the storage period, except for the WHC of the samples stored at  $2^{\circ}\text{C}$  and  $-12^{\circ}\text{C}$  increased significantly ( $p < 0.05$ ) after 1 week of storage.

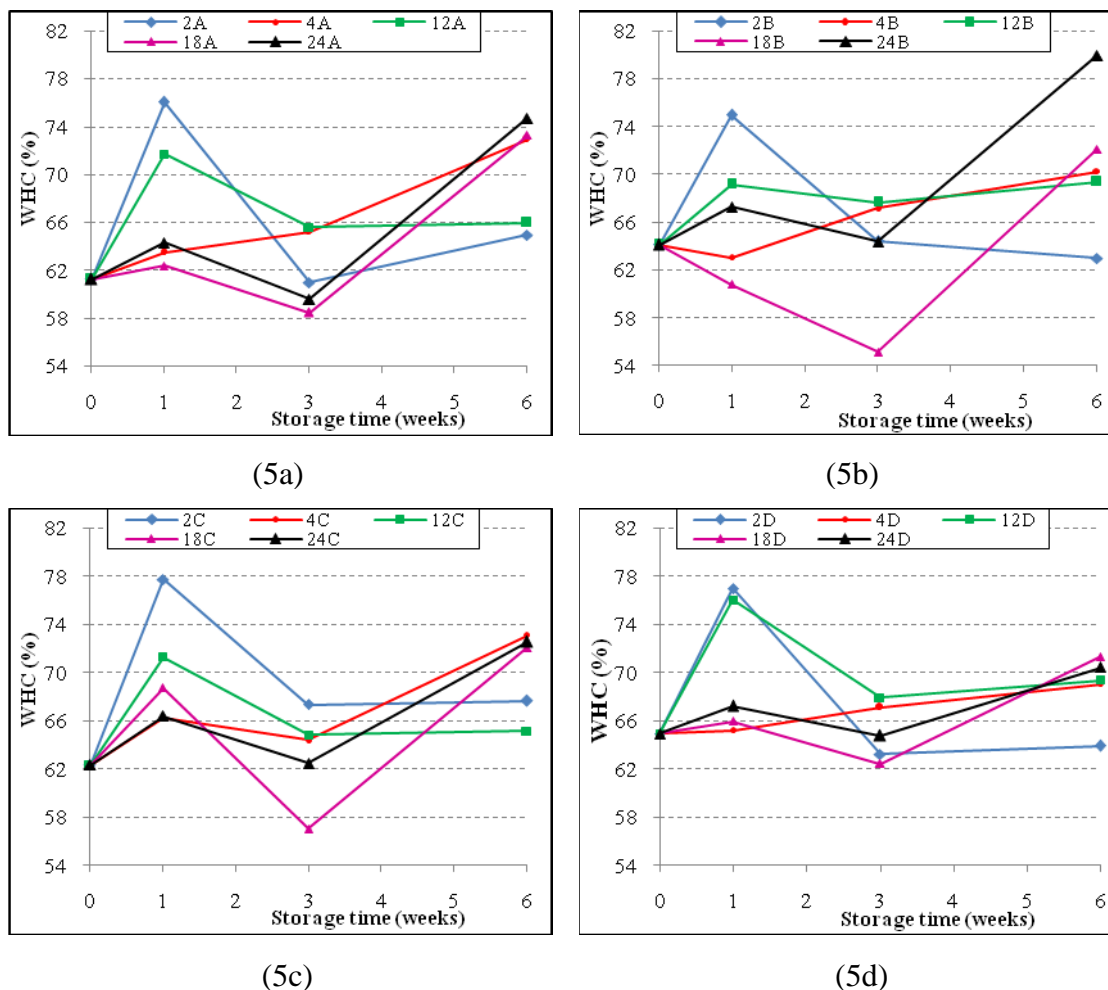


Figure 5: Water holding capacity of the salted cod (group A: injecting and brining (5a); group B: injecting, phosphates and brining (5b); group C: injecting, protein and brining (5c); and group D: injecting, protein, phosphates and brining (5d)) stored at different temperatures (2 °C, -4 °C, -12 °C, -18 °C and -24 °C).

### Nuclear magnetic resonance

Low field nuclear magnetic resonance (LF-NMR) measurements were performed on samples from all four groups after processing (week 0), after 3 and 6 weeks of storage at -24 °C respectively. The results from the NMR analysis can be viewed in the following passage. The NMR measurements were performed at ambient temperatures.

The results from the longitudinal relaxation measurements in the storage experiment of the dry salted cod groups can be viewed in Figure 6 for the storage temperature of -24 °C.



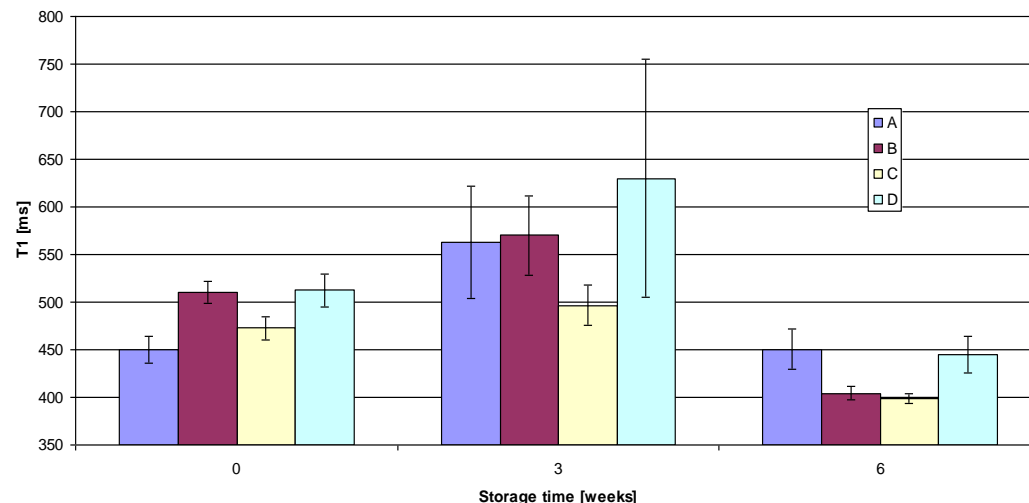


Figure 6: Longitudinal relaxation times  $T_1$  measured after processing (groups A, B, C and D) at week 0 and after 3 and 6 weeks of storage at  $-24\text{ }^{\circ}\text{C}$ .

The figure shows a significant difference in the longitudinal relaxation time between the groups after processing. The polyphosphates treated groups (B and D) showed longer relaxation times than the polyphosphates lacking groups (A and C), indicating that the polyphosphates results in a decrease in water mobility in the samples. This is in correlation with the water holding capacity results, which state a significantly higher water holding capacity in the polyphosphates groups. The protein addition did, on the other hand, not contribute significantly to a change in water mobility in the samples.

The figure also shows that the longitudinal relaxation time  $T_1$ , increased after 3 weeks of storage in all groups, to later decrease in all groups after 6 weeks of storage. This change was though not significant in group D due to a large standard deviation in the samples stored for 3 weeks. Reasons for this behaviour are unknown.

The results from the transversal relaxation times,  $T_2$  can be viewed in Figures 7 to 8. The relaxation data was fitted with a bi-exponential model giving two apparent water populations,  $A_1$  and  $A_2$  with relaxation times,  $T_{21}$  and  $T_{22}$  respectively. The shorter relaxation time,  $T_{21}$  and the connected water population  $A_1$  described the behaviour and relative amount of water that was tightly bound in the muscle structure, for example bound within cells or to proteins. The longer relaxation time,  $T_{22}$  and the connected water population  $A_2$  described on the other hand the behaviour and relative amount of more freely moving water, for example water in the intracellular space. Higher transversal relaxation times indicated and increased in the water mobility of the samples.

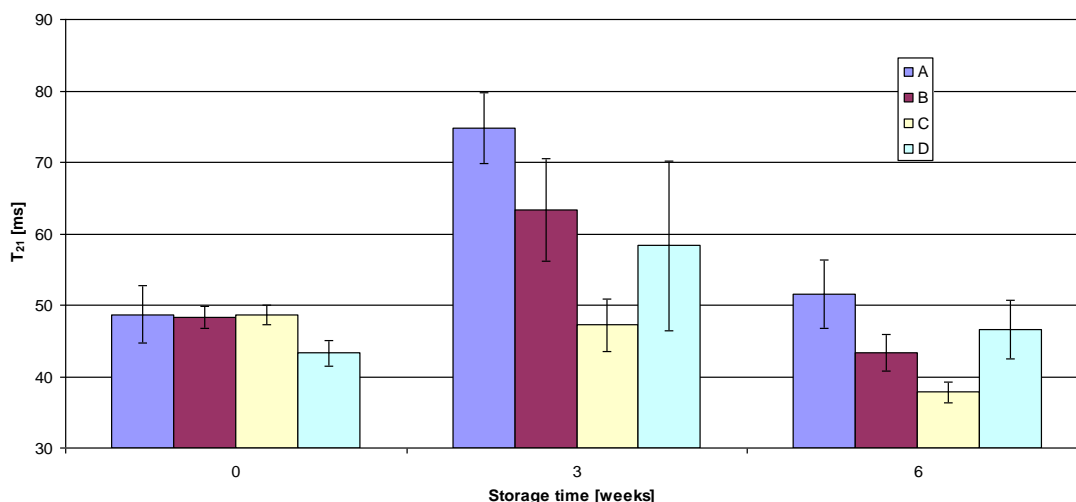


Figure 7: Shorter relaxation time,  $T_{21}$  measured after processing (groups A, B, C and D) at week 0 as well as after 3 and 6 weeks of storage at  $-24\text{ }^{\circ}\text{C}$ .

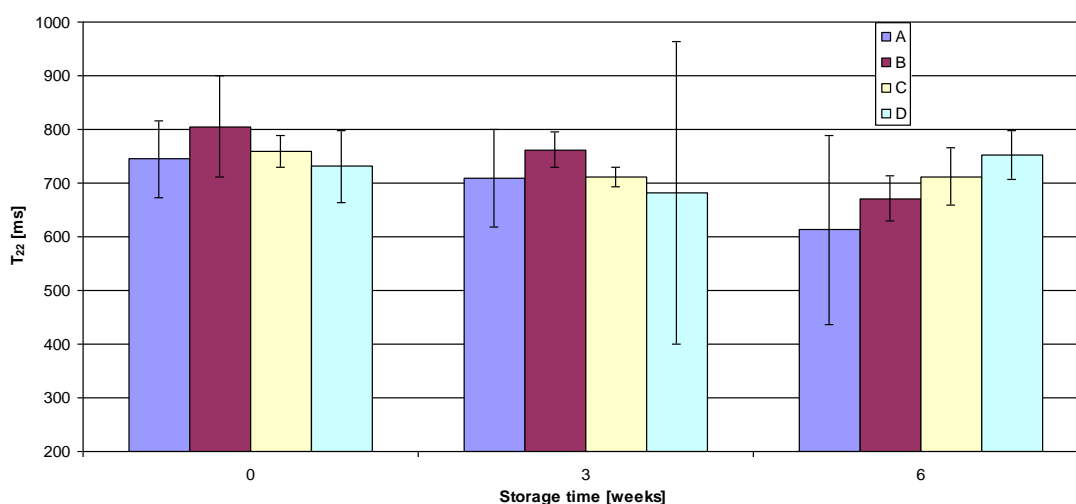


Figure 8: Longer relaxation time,  $T_{22}$  measured after processing (groups A, B, C and D) at week 0 as well as after 3 and 6 weeks of storage at  $-24\text{ }^{\circ}\text{C}$ .

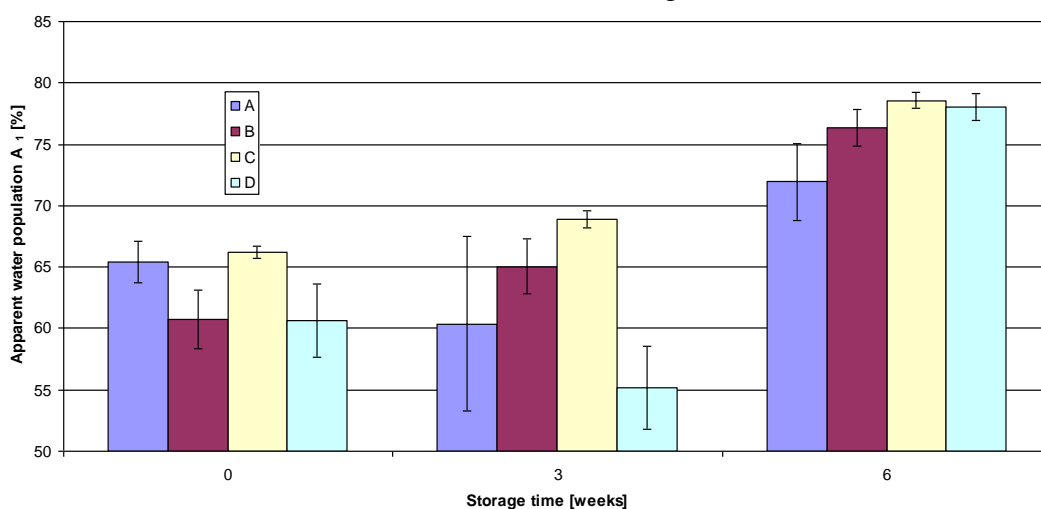


Figure 9: Apparent relative water population of tightly bound water  $A_1$  measured after processing (groups A, B, C and D) at week 0 as well as after 3 and 6 weeks of storage at  $-24\text{ }^{\circ}\text{C}$ .

Figure 7 shows the shorter transversal relaxation times measured in the experiment. No significant difference was found between the groups after processing (week 0) except in group D (injecting, protein phosphates and brining), which had a shorter relaxation time than the other groups indicating slightly lower water mobility in the tightly bound water in group D compared to the other groups. A similar behaviour was observed in the  $T_{21}$  as in the longitudinal relaxation time  $T_1$  with storage time, where samples stored for 3 weeks showed longer relaxation times than after processing (week 0), to decreased again after 6 weeks of storage in all groups. As for the  $T_1$  measurements the reason for this behaviour is unknown.

No significant differences could be found in the longer transverse relaxation time,  $T_{22}$  neither between the groups nor with storing time (Figure 8). This indicated that the mobility of the freely moving water was independent of the processing methods as well as the storage time.

The apparent relative amount of tightly bound water,  $A_1$  can be viewed in Figure 9. It could be seen that the polyphosphates groups (B and D) had less tightly bound water than the polyphosphates free groups (A and C). This indicated that water was drawn out of the cells due to the addition of phosphates and into the intracellular space. The protein addition did on the other hand not affect the apparent water population of tightly bound water in the samples.

The apparent relative amount of tightly bound water increased in all groups after 6 weeks of storage at  $-24\text{ }^{\circ}\text{C}$ , while changes were not significant after 3 weeks of storage. This was in correlation with a slight increase in water holding capacity and cooking yield with storage time as well as a slight decrease in water content with storage time. This can be explained by the fact that the freely moving water was lost first due to drip loss during storage, while the more tightly bound water of the samples remained. Since this water was more tightly bound to the muscular structure of the sample this water was harder to remove, resulting in higher water holding capacity and higher cooking yields.

#### **4.1.2 Chemical changes**

##### Water content

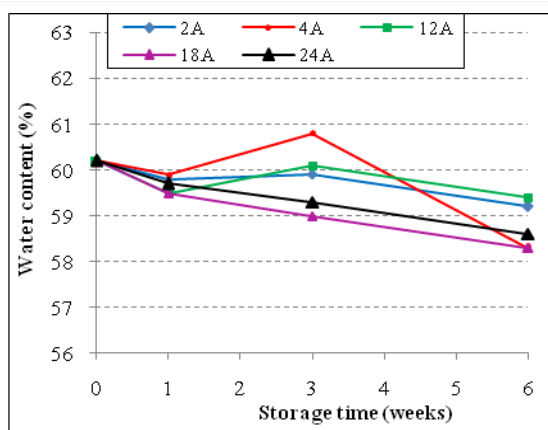
Generally, the water content of the salted cod for all treatments decreased during the storage period for most of the temperatures. The samples in the four groups stored at  $-4\text{ }^{\circ}\text{C}$  were excluded, where the water content increased after the third week. In the beginning of the storage time the water content was similar between different treatments ( $\sim 60\%$ ) (Figure 10).

The water content of the samples in group A (Figure 10a) decreased slightly during the storage time; except for the sample stored at  $-4\text{ }^{\circ}\text{C}$ , where the water content increased after storage for 3 weeks.

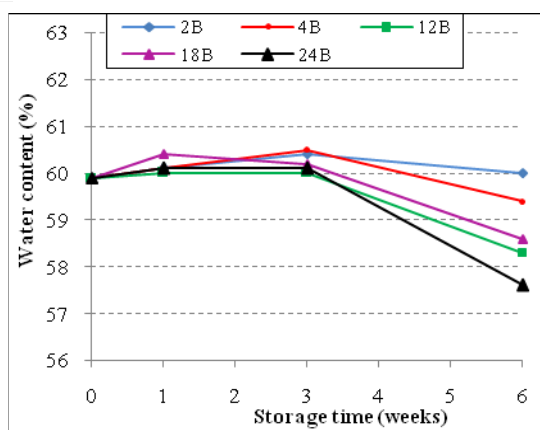
In group B, the water content of all samples for all storage temperatures increased slightly from week 1 to week 3 and then decreased after 6 weeks of storage (Figure 10b).

Generally, the water content of the salted cod group C (Figure 10c) stored at different temperatures decreased during the storage period. The sample stored at -4 °C and -12 °C increased slightly from week 1 to week 3 of storage.

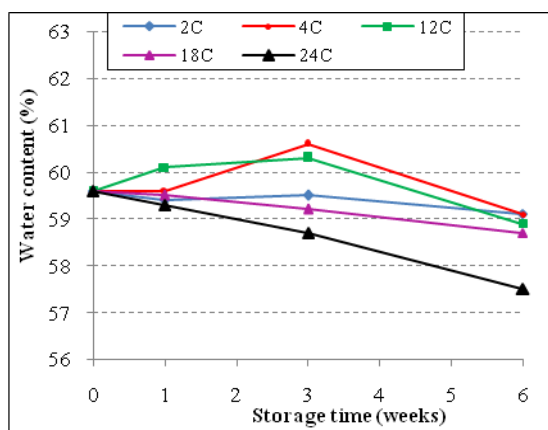
The water content of all samples in group D (Figure 10d) stored at different temperatures increased slightly from week 1 to week 3 of storage. After 3 weeks of storage, the water content of all samples decreased. However, the water content of the sample stored at 2 °C was stable throughout the storage period.



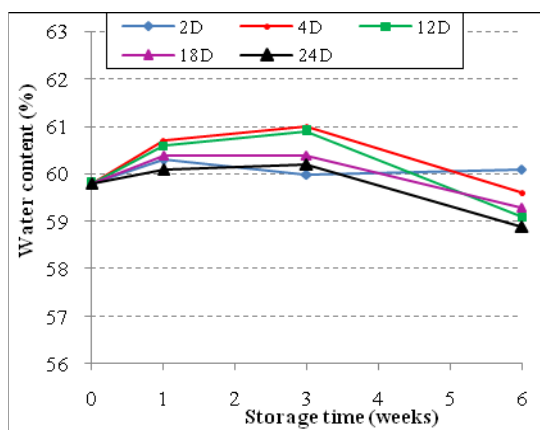
(10a)



(10b)



(10c)



(10d)

Figure 10: Water content of the salted cod (group A: injecting and brining (10a); group B: injecting, phosphates and brining (10b); group C: injecting, protein and brining (10c); and group D: injecting, protein, phosphates and brining (10d)) stored at different temperatures (2 °C, -4 °C, -12 °C, -18 °C and -24 °C).

Protein content

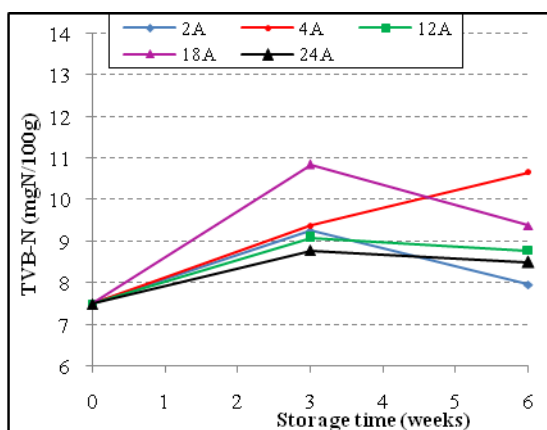
The protein content varied from 18.8% to 21.0% in all samples (Table 7). The injected protein in the fillets seemed not to have any affect on the protein content.

Table 7: The protein content (%) of all groups at the beginning (week 0)

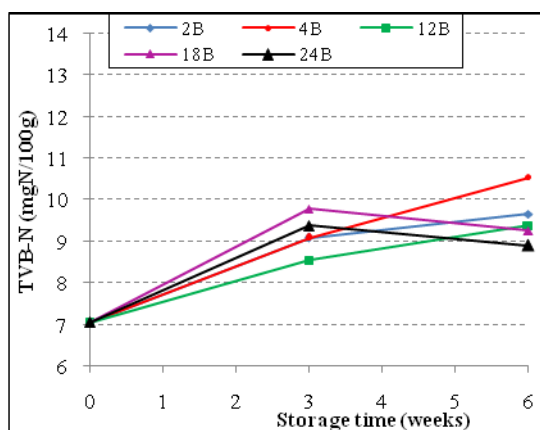
	Treatment			
	A	B	C	D
Protein content (%)	21.0	19.1	20.3	18.8

### Total volatile base nitrogen

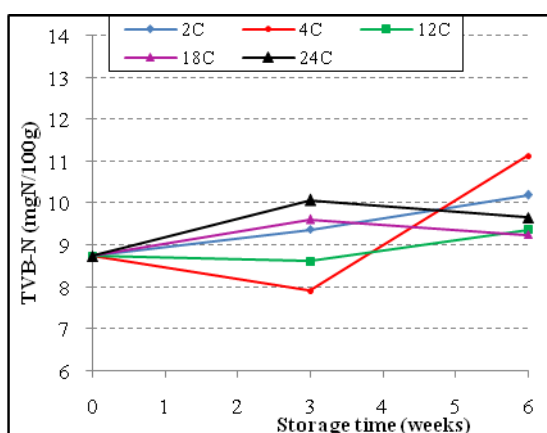
Total volatile base nitrogen (TVB-N) consists of trimethylamine (TMA), dimethylamine (DMA), formaldehyde (FA) and other volatile bases. Generally the TVB-N of the salted cod increased during the storage time for all the samples (Figure 11). In the beginning of the storage (week 0) the samples from group C had the highest content of TVB-N (~8.8 mg N/100 g), compared to other groups (Figure 11c). The salted cod in group B had the most rapid increase in TVB-N (Figure 11b) compared to other groups. A small change in TVB-N content was observed in samples in group D (Figure 11d).



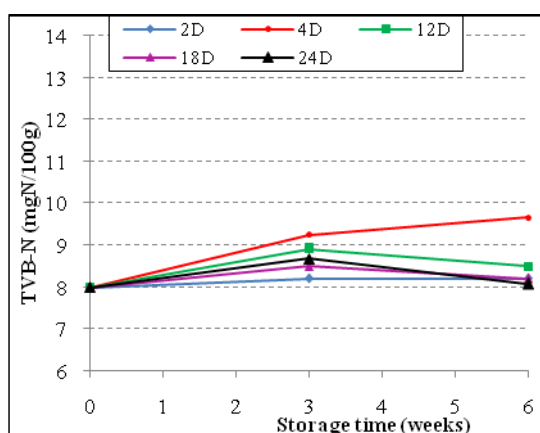
(11a)



(11b)



(11c)



(11d)

Figure 11: Changes in TVB-N content of the salted cod (group A: injecting and brining (11a); group B: injecting, phosphates and brining (11b); group C: injecting, protein and brining (11c); and group D: injecting, protein, phosphates and brining (11d)) stored at different temperatures (2 °C, -4 °C, -12 °C, -18 °C and -24 °C).

### Trimethylamine

Trimethylamine (TMA) in seafood is degraded from trimethylamineoxide (TMAO) by microorganism. The results showed that the TMA content of the salted cod was stable through the storage time for all samples (Figure 12). TMA content in the beginning of the storage time showed various values between different treated groups. The TMA content was ~1.70 mg N/100 g in group A, ~0.95 mg N/100 g in group B,

~1.40 mg N/100 g in group C and ~1.40 mg N/100 g in group D. The TMA values showed much variation between samples during the storage period. The TMA content of the samples stored at -4 °C was higher in A, B and C groups compared to the D group.

The TMA content of all samples in group A (Figure 12a) decreased after storage for 3 weeks. After that, the TMA content of the samples stored at 2 °C and -4 °C increased again. Meanwhile, the TMA content of other samples continued decreasing at the end of the storage period.

In group B, the TMA content of the samples stored at -4 °C increased during the storage time. Meanwhile, the TMA content of the sample stored at -24 °C increased after 3 weeks of storage and then decreased at the end of the storage period (Figure 12b).

The TMA content of the salted cod in group C (Figure 12c) decreased after storage for 3 weeks. At the end of the storage period, the TMA content of the samples stored at 2 °C and -4 °C increased much higher compared to other samples at other storage temperatures.

The TMA content of all samples in group D (Figure 12d) decreased after storage for 3 weeks. From week 3 to week 6, the TMA content varied much between the samples. The TMA content of the samples stored at 2 °C and -4 °C increased much higher, meanwhile, the TMA content of the samples stored at -18 °C and -24 °C was stable from week 3 to week 6 of the storage time.

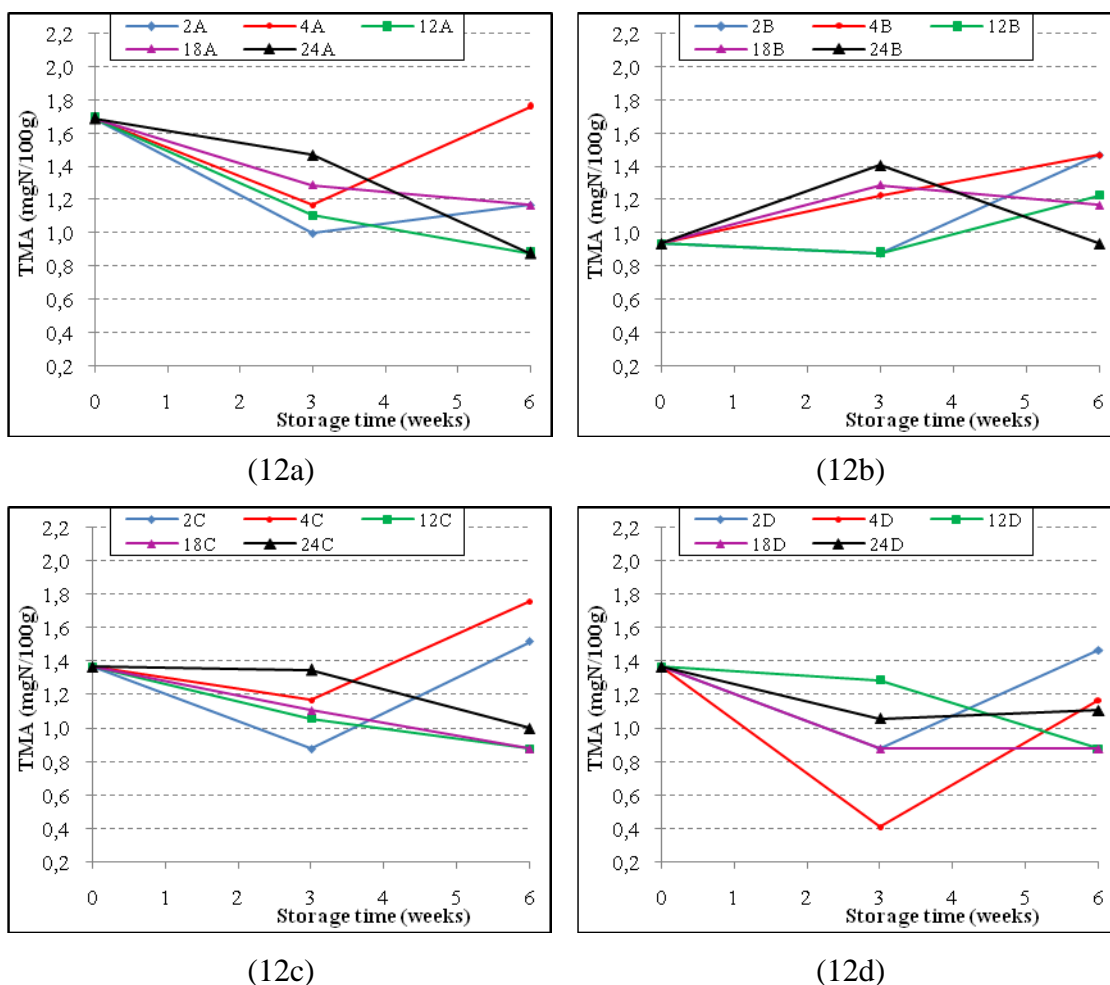


Figure 12: Changes in TMA content of the salted cod (group A: injecting and brining (12a); group B: injecting, phosphates and brining (12b); group C: injecting, protein and brining (12c); and group D: injecting, protein, phosphates and brining (12d) stored at different temperatures (2 °C, -4 °C, -12 °C, -18 °C and -24 °C).

#### 4.1.3 Multivariate data analysis of salted cod

In order to describe the main characteristics of the samples and to highlight their main differences, a multivariate statistical procedure, principal component analysis (PCA) was performed on the data obtained.

Scores plot for the PCA (PC1 and PC2) of all the data analysis for all samples (Figure 13) showed that the PC1 explained 55% of the total variance between the samples and the PC2 explained 25% of the total variance between the samples. Together PC1 and PC2 explained 80% of the variance in the data set. Four groups were observed corresponding with different storage periods. Those differences could be explained due to the different initial quality of the fish.



Figure 13: Score plot for the different groups of all samples of salted cod. Blue=A group, red=B group, green=C group and light blue=D group.

The physical parameters cooking yield and water holding capacity explained most of the variations in the data set, and were closely aligned along the first principal component (PC1). A second PC was explained mostly by spoilage mechanism (TMA and TVB-N) and colour related differences (Figure 14). Cooking yield and water holding capacity were positively correlated and when the WHC increased the water content decreased. Colour L and colour b were also closely related together. Colour L (lightness) was governed by another phenomenon, resulting in a higher PC2 (correlation) loading. The redness ( $a^*$  value) could not be interpreted in this plot.

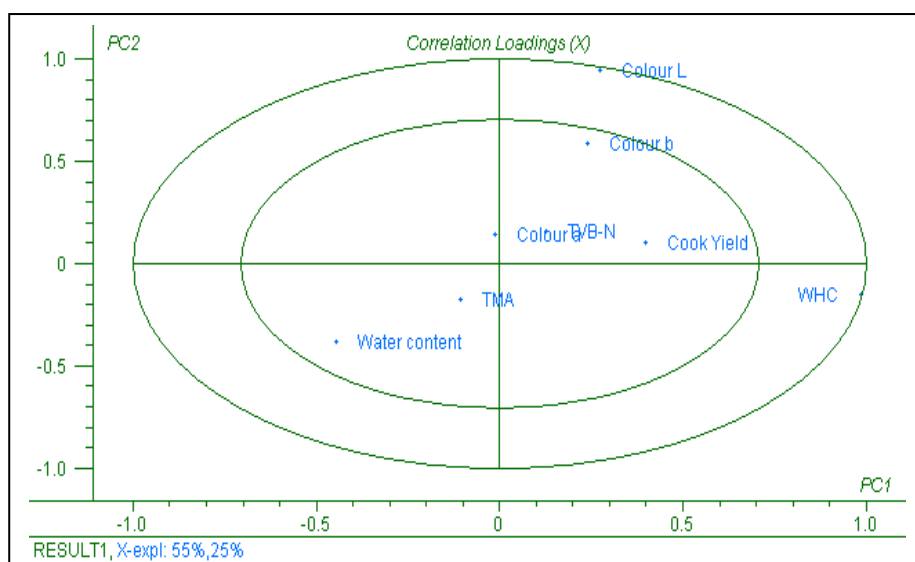


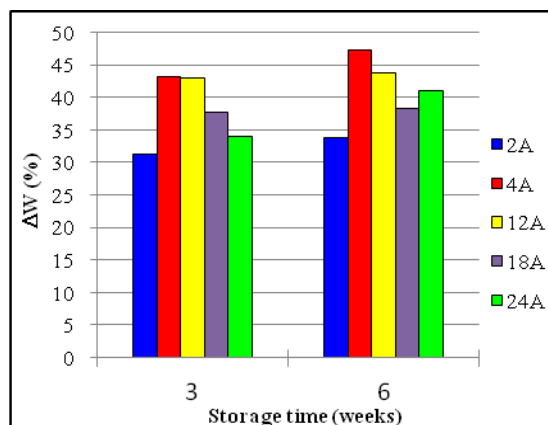
Figure 14: The correlation loading plot for all the data analysis for all samples of salted cod.



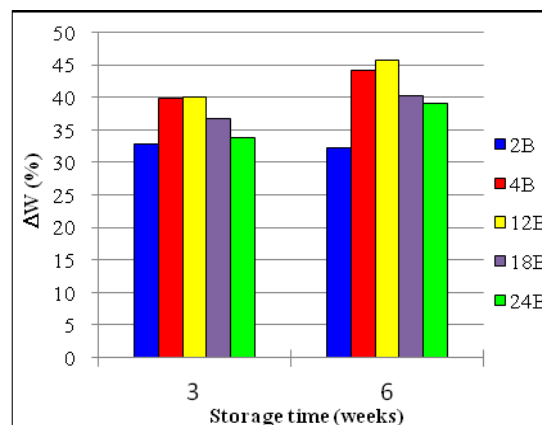
## 4.2 Effects of temperature and storage time on the quality of desalted cod

### 4.2.1 The total weight changes of the salted cod after desalting

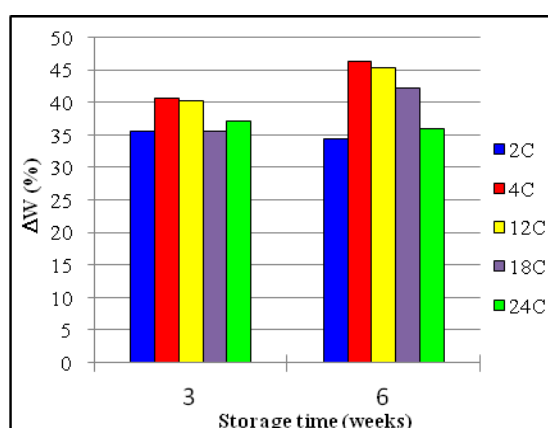
Generally the total weight changes of samples in all groups stored at  $-4^{\circ}\text{C}$  and  $-12^{\circ}\text{C}$  were higher compared to samples at different temperatures. The total weight changes increased slightly from week 3 to week 6 of the storage time for most samples (Figure 15).



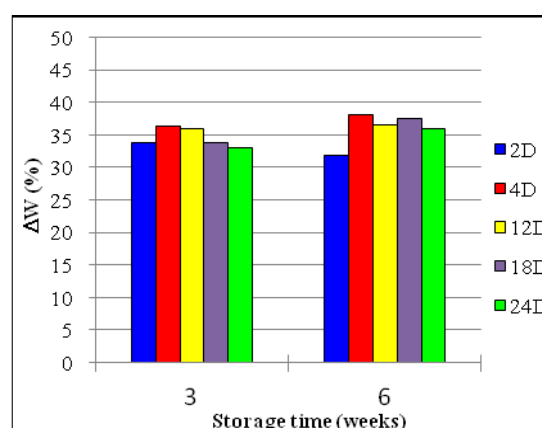
(15a)



(15b)



(15c)



(15d)

Figure 15: The total weight changes of the salted cod after desalting (group A: injecting and brining (15a); group B: injecting, phosphates and brining (15b); group C: injecting, protein and brining (15c); and group D: injecting, protein, phosphates and brining (15d) stored at different temperatures ( $2^{\circ}\text{C}$ ,  $-4^{\circ}\text{C}$ ,  $-12^{\circ}\text{C}$ ,  $-18^{\circ}\text{C}$  and  $-24^{\circ}\text{C}$ ).

### 4.2.2 Cooking yield

Generally, the cooking yield (CY) varied much between the samples in all groups after 3 weeks and 6 weeks of storage. The CY of all samples in groups B and D was higher, compared to samples in groups A and C (Figure 16).

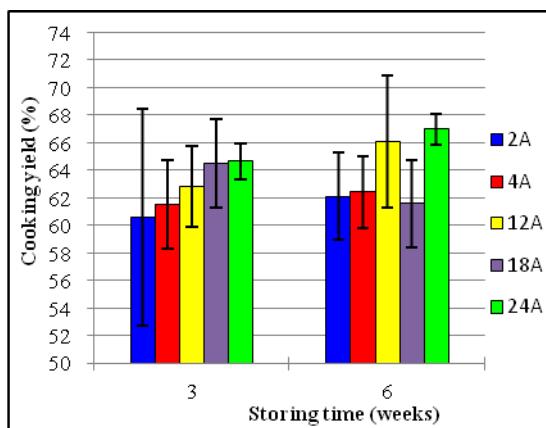
The CY of the samples in group A after 3 weeks of storage increased when storage temperature decreased (Figure 16a), but no significant differences ( $p > 0.05$ ) in CY

between these samples was found. After 6 weeks of the storage period, the CY of the samples stored at  $-12^{\circ}\text{C}$  and  $-24^{\circ}\text{C}$  increased, compared to other samples. From week 3 to week 6, the CY of the sample stored at  $-24^{\circ}\text{C}$  increased significantly ( $p<0.05$ ). Meanwhile, no significant differences ( $p>0.05$ ) in CY between other samples were found from week 3 to week 6.

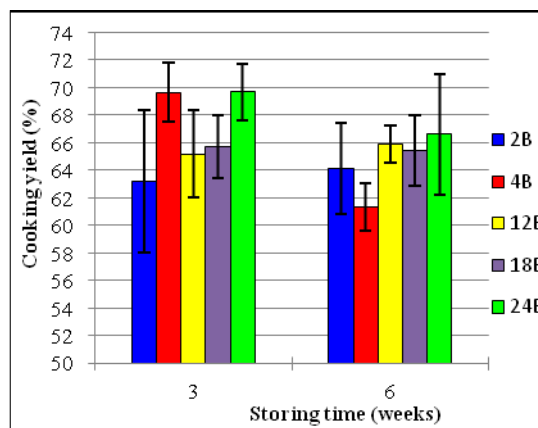
In group B, significant differences ( $p<0.05$ ) were found in CY for samples stored at  $-4^{\circ}\text{C}$  as the CY decreased from week 3 to week 6 (Figure 16b).

The CY of the desalted cod in group C (Figure 16c) varied between the samples during the storage time. The CY of all samples, except the sample stored at  $2^{\circ}\text{C}$  increased from 3 weeks to 6 weeks. After storage for 6 weeks, a significant difference ( $p<0.05$ ) in CY was found between the samples stored at  $2^{\circ}\text{C}$  and  $-18^{\circ}\text{C}$ . From week 3 to week 6 of the storage period, the CY of the samples stored at  $-4^{\circ}\text{C}$  and  $-18^{\circ}\text{C}$  increased significantly ( $p<0.05$ ).

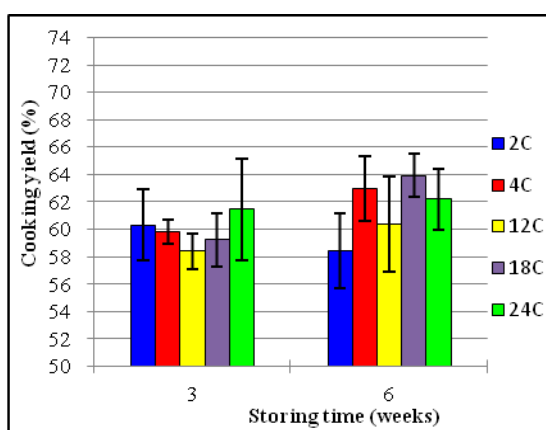
The CY of the desalted cod type D (Figure 16d) showed that the CY did not change much during the storage period and no significant differences ( $p>0.05$ ) in CY between the samples was found.



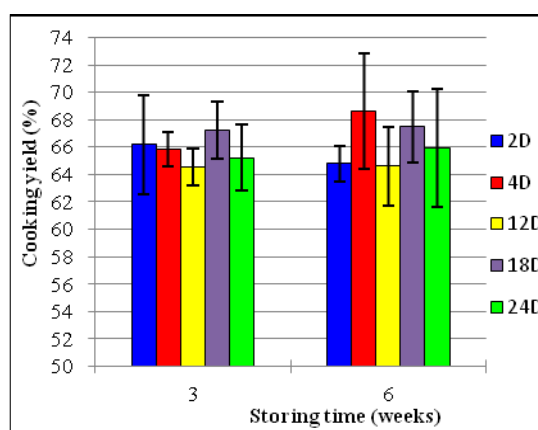
(16a)



(16b)



(16c)



(16d)

Figure 16: The cooking yield of desalted cod (group A: injecting and brining (16a); group B: injecting, phosphates and brining (16b); group C: injecting, protein and brining (16c); and group D: injecting, protein, phosphates and brining (16d)) stored at different temperatures (2 °C, -4 °C, -12 °C, -18 °C and -24 °C).

#### 4.2.3 Water holding capacity

The water holding capacity (WHC) varied much between the samples in groups A and C after 3 weeks and 6 weeks of storage, while there were no differences in samples in groups B and D (Figure 17). The results were in correlation with the cooking yield results.

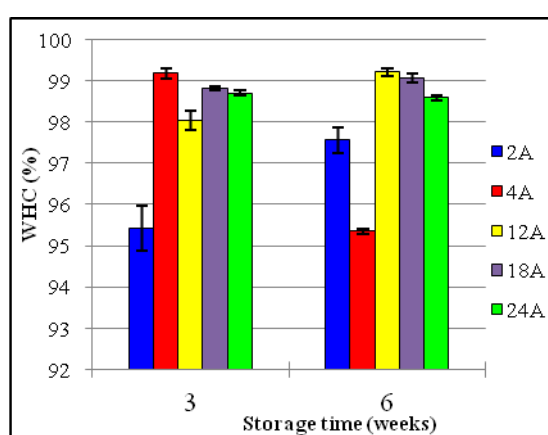
In group A, the WHC of the sample stored at 2 °C was lower after 3 weeks of storage, compared to other samples and was significantly lower ( $p < 0.05$ ) than the sample stored at -4 °C. However, the WHC of the sample stored at -4 °C decreased after storage for 6 weeks and was significantly lower in WHC, compared to the sample stored at -12 °C. Meanwhile, the WHC of the samples stored at -18 °C and -24 °C was stable through the storage time (Figure 17a).

The WHC of all samples in group B (Figure 17b) seemed to be stable from week 3 to week 6 of the storage period. After 3 weeks of storage, a significant difference ( $p < 0.05$ ) in WHC was found between the samples stored at -18 °C and -24 °C. A

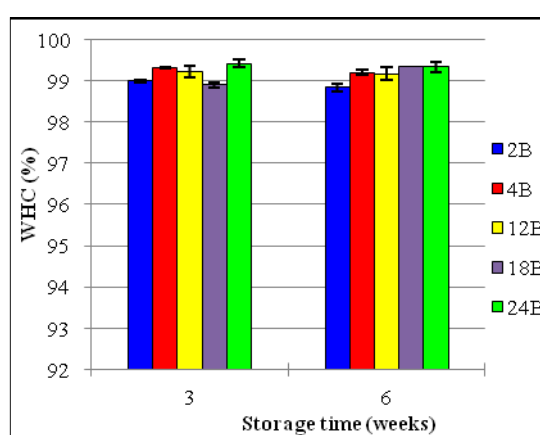
significant differences ( $p < 0.05$ ) in WHC were also found between the samples stored at 2 °C and -18 °C at the end of the storage period.

The WHC of the desalted cod in group C (Figure 17c) showed that after storage for 3 weeks, the WHC varied much between the samples and there was a significant differences ( $p < 0.05$ ) in WHC between the samples stored at -18 °C and -24 °C. After 6 weeks of storage, the WHC of the samples stored at 2 °C and -24 °C decreased and there was a significant differences ( $p < 0.05$ ) in WHC between the samples stored at -18 °C and -24 °C.

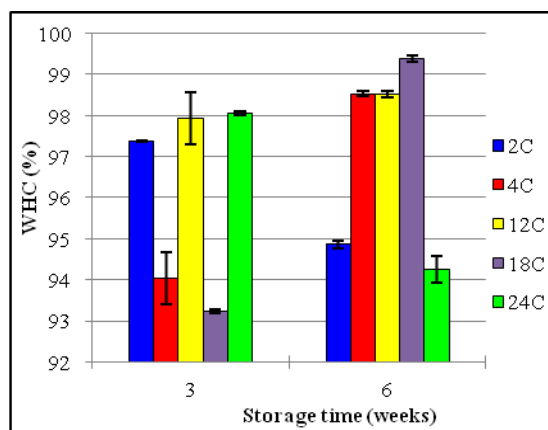
Generally, the WHC of samples in group D (Figure 17d) was stable from week 3 to week 6 of the storage period. After storage for 3 weeks, a significant difference ( $p < 0.05$ ) in WHC was found between the samples stored at 2 °C and -24 °C.



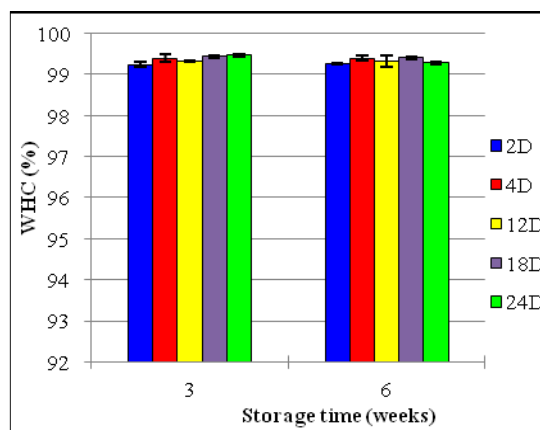
(17a)



(17b)



(17c)



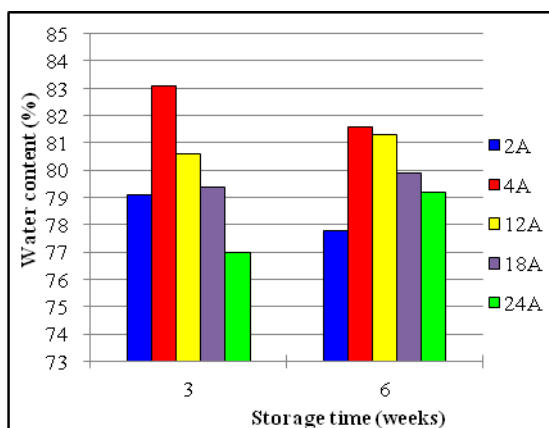
(17d)

Figure 17: The water holding capacity of the desalted cod (group A: injecting and brining (17a); group B: injecting, phosphates and brining (17b); group C: injecting, protein and brining (17c); and group D: injecting, protein, phosphates and brining (17d) stored at different temperatures (2 °C, -4 °C, -12 °C, -18 °C and -24 °C).

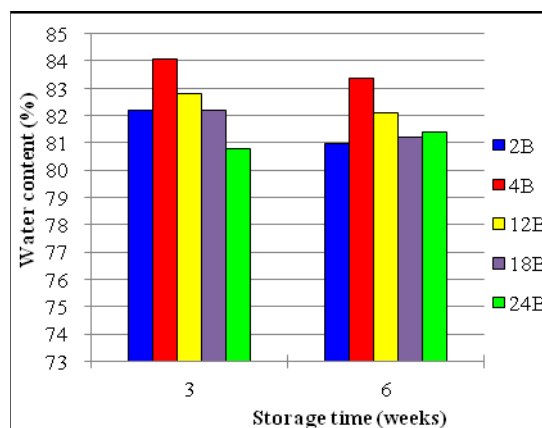
#### 4.2.4 Water content

The water content of desalted cod in all groups stored at -4 °C and -12 °C was higher, compared to other samples at different storage temperatures. The water content of all the samples increased slightly from week 3 to week 6 of the storage (Figure 18).

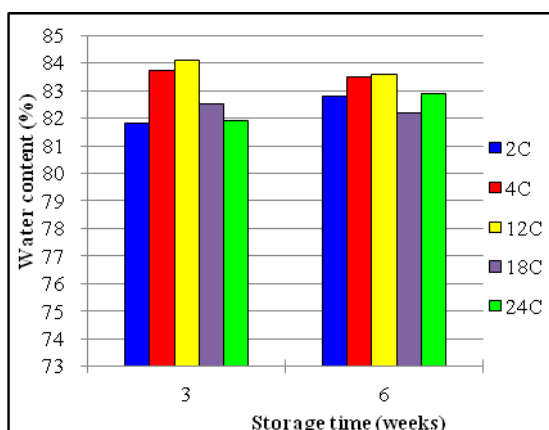
The water content of the desalted cod in group D (Figure 18d) was higher compared to other samples in other groups and was more stable between the storage times.



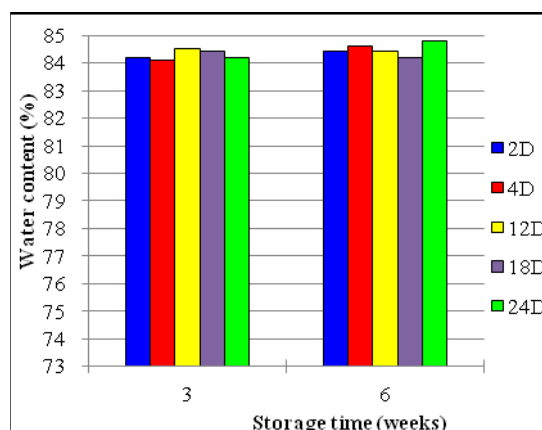
(18a)



(18b)



(18c)



(18d)

Figure 18: The water content of the desalted cod (group A: injecting and brining (18a); group B: injecting, phosphates and brining (18b); group C: injecting, protein and brining (18c); and group D: injecting, protein, phosphates and brining (18d) stored at different temperatures (2 °C, -4 °C, -12 °C, -18 °C and -24 °C).

#### 4.2.5 Salt content

The salt content of salted and desalted cod was not different between the groups (Figure 19).

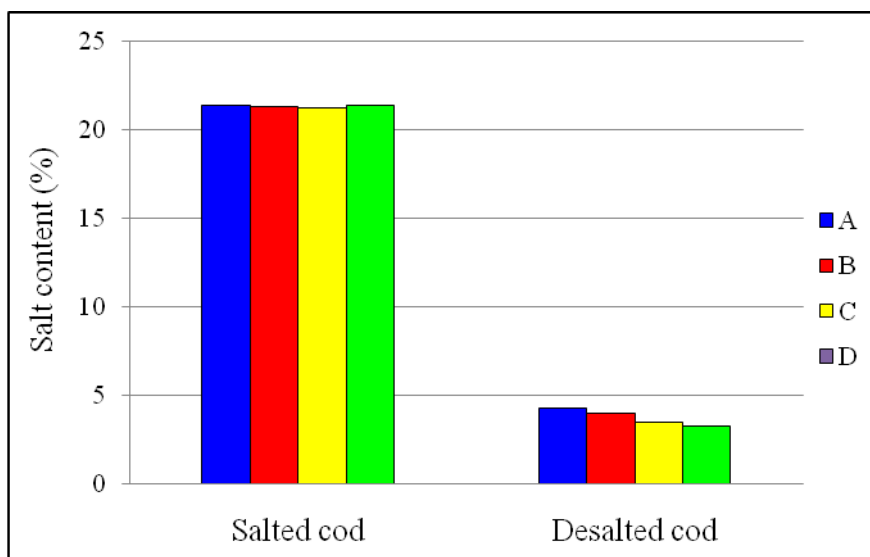


Figure 19: The salt content of salted cod and desalted cod (group A: injecting and brining; group B: injecting, phosphates and brining; group C: injecting, protein and brining; and group D: injecting, protein, phosphates and brining).

#### 4.2.6 Phosphates content

The phosphates content of salted and desalted cod was higher in groups B and D, compared with A and C as expected, because of the injection of phosphates in the fillets of the former groups (Figure 20).

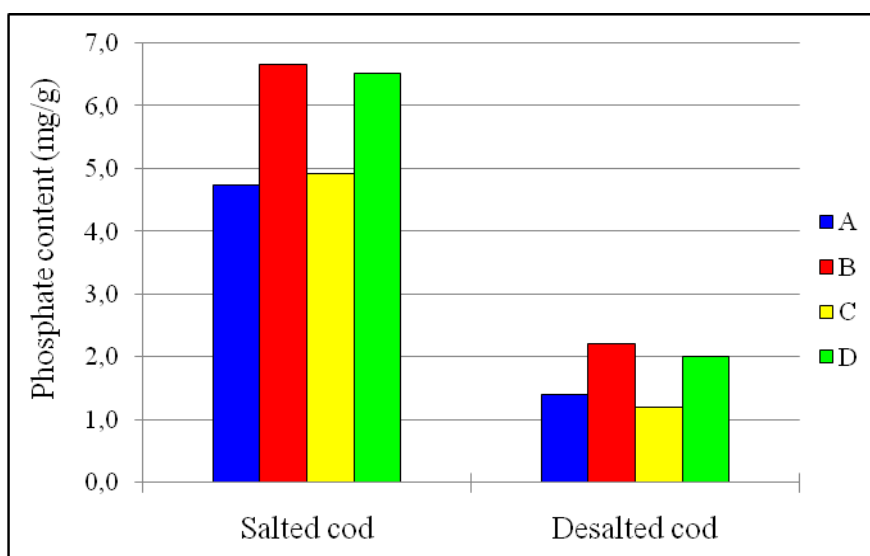


Figure 20: The phosphates ( $P_2O_5$ ) content of salted cod and desalted cod (group A: injecting and brining; group B: injecting, phosphates and brining; group C: injecting, protein and brining; and group D: injecting, protein, phosphates and brining).

#### 4.2.7 Multivariate data analysis of desalted cod

In order to describe the main characteristics of the samples and to highlight their main differences, a multivariate statistical procedure, principal component analysis (PCA) was performed on the data obtained.

Scores plotted for the PCA (PC1 and PC2) of all the data analysis for all samples (Figure 21) showed that the PC1 explained 56% of the total variance between the samples and PC2 explained 29% of the total variance between the samples. Together PC1 and PC2 explained 85% of the variance in the data set. Four groups were observed corresponding with different storage periods. Those differences could be explained due to the different initial quality of the fish.

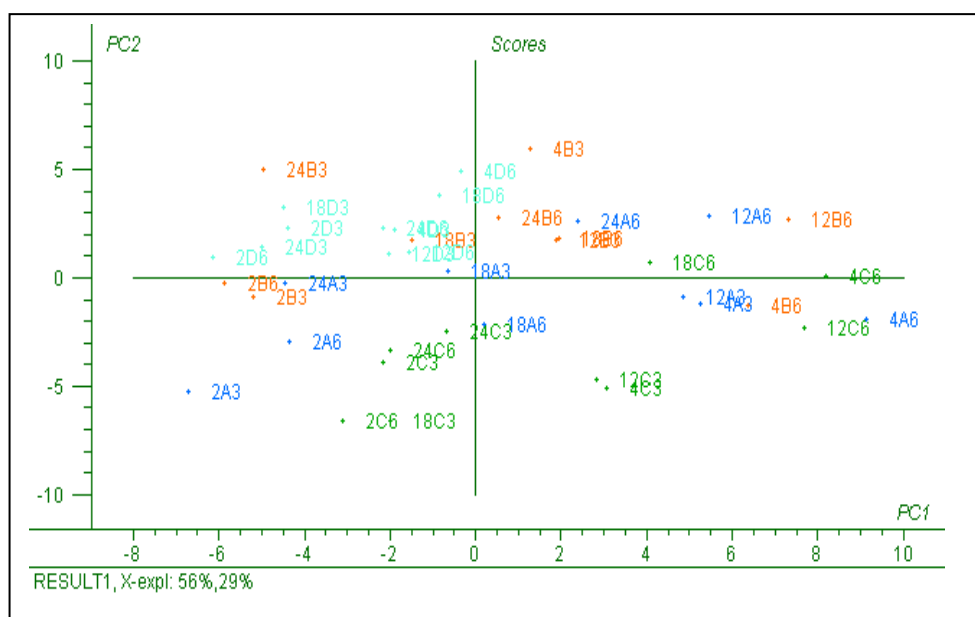


Figure 21: Score plot for the different groups of all samples of desalted cod. Blue=A group, red=B group, green=C group and light blue=D group.

The water content and total weight changes after desalting ( $\Delta W$ ) explained most of the variation in the data set, and were closely aligned along the first principal component (PC1). A second PC was explained mostly by the parameters cooking yield and water holding capacity (Figure 22). Cooking yield and water holding capacity are positively correlated.

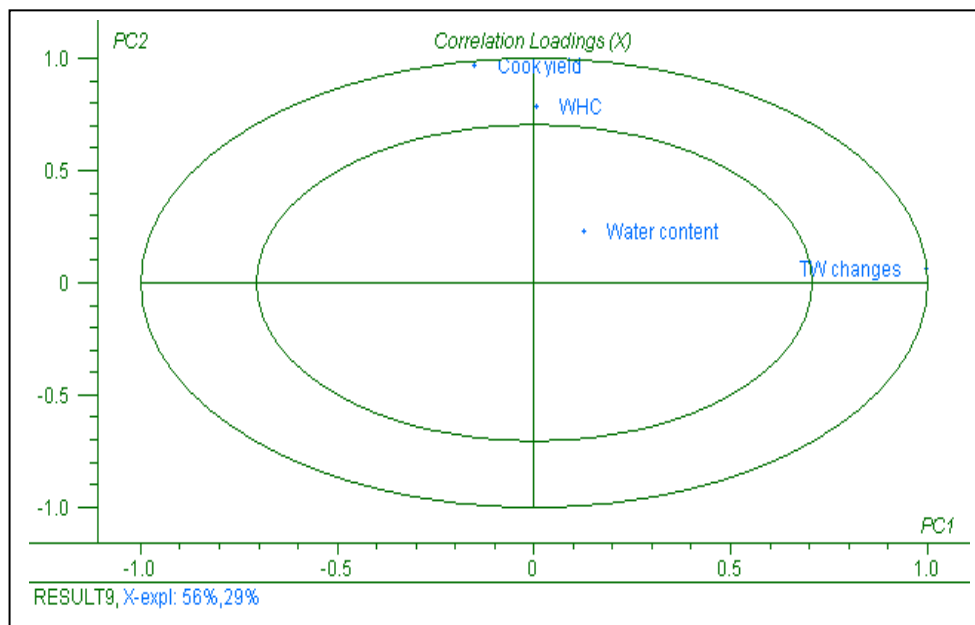


Figure 22: The correlation loading plot for all the data analysis for all samples of desalted cod. (TW changes = Total weight changes).

### 4.3 The effect of heat pump drying on the quality of salted cod

#### 4.3.1 Change in ratio of weight loss during heat pump drying

The ratio of the weight loss of the salted cod fillets (groups A, B, C and D) during heat pump drying (Figure 23) showed that as the drying time increased, the ratio of weight loss decreased. During drying time, no significant differences ( $p>0.05$ ) in ratio of weight loss between the groups of salted fish were found.

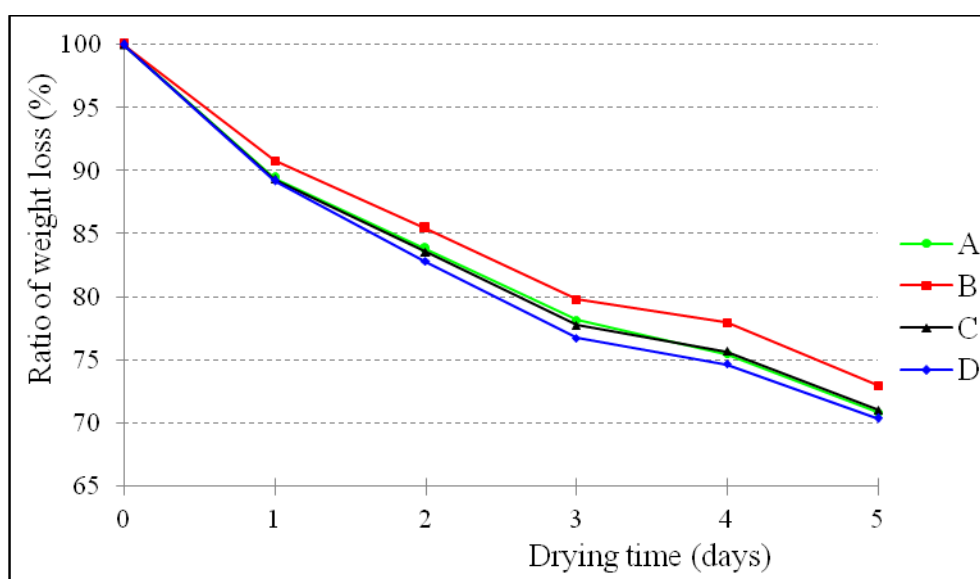


Figure 23: The ratio of weight loss of salted cod fillets (group A: injecting and brining; group B: injecting, phosphates and brining; group C: injecting, protein and



brining; and group D: injecting, protein, phosphates and brining) during heat pump drying.

#### 4.3.2 Change in water content during heat pump drying

The variations in the water content with the drying time are illustrated in Figure 24. Generally, as the drying time increased, the water content decreased. No significant differences ( $p>0.05$ ) in water content between the groups of the salted fish were found during drying time.

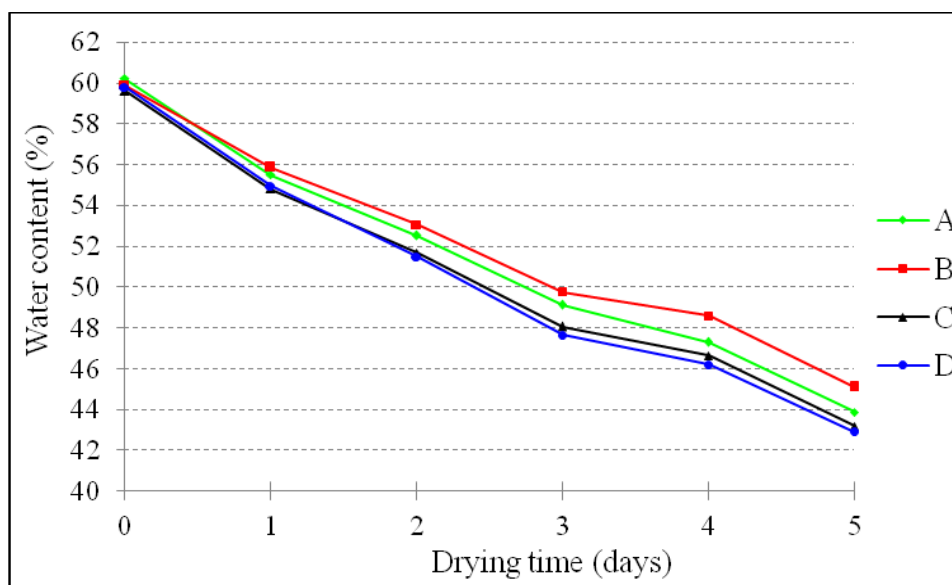


Figure 24: The water content of salted cod fillets (group A: injecting and brining; group B: injecting, phosphates and brining; group C: injecting, protein and brining; and group D: injecting, protein, phosphates and brining) during heat pump drying.

#### 4.3.3 Colour analysis

The  $L^*$  value or lightness of the salted cod in all samples increased significantly ( $p<0.05$ ) during the drying time (Figure 25).

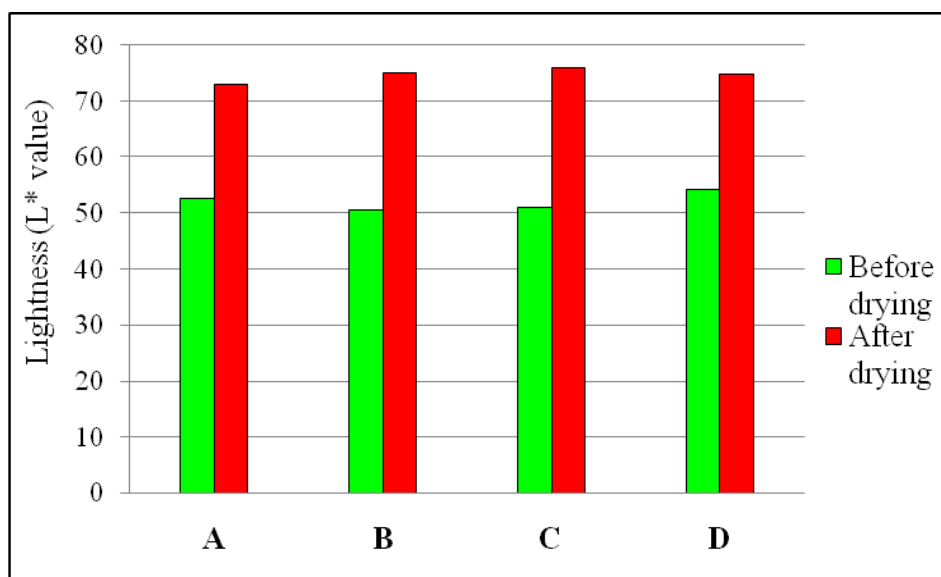


Figure 25: The lightness of salted cod (group A: injecting and brining; group B: injecting, phosphates and brining; group C: injecting, protein and brining; and group D: injecting, protein, phosphates and brining) before and after heat pump drying.

The  $a^*$  value describes the intensity in green colour (negative) and in red colour (positive) of the salted cod (groups A, B, C and D). The red colour intensity of the salted cod increased significantly ( $p < 0.05$ ) during the drying time (Figure 26).

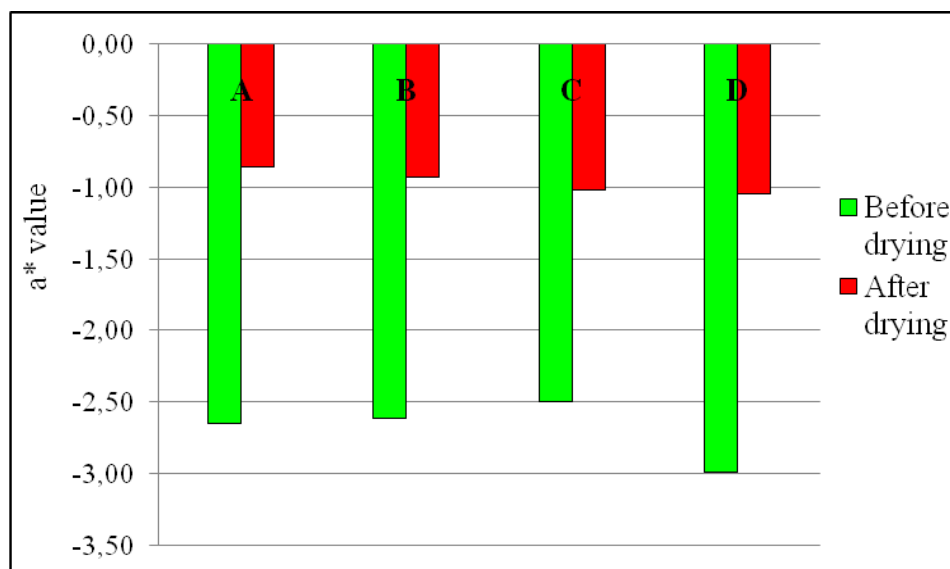


Figure 26: The  $a^*$  value of salted cod (group A: injecting and brining; group B: injecting, phosphates and brining; group C: injecting, protein and brining; and group D: injecting, protein, phosphates and brining) before and after heat pump drying.

The  $b^*$  value describes the intensity in blue colour (negative) and in yellow colour (positive) of the salted cod (groups A, B, C and D). The red colour intensity increased significantly ( $p < 0.05$ ) (Figure 27).

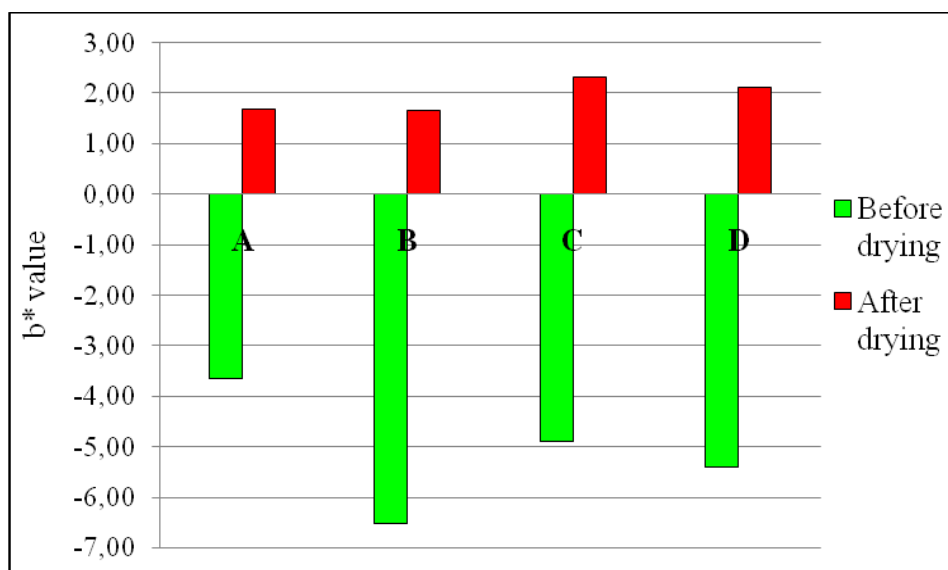


Figure 27: The  $b^*$  value of salted cod (group A: injecting and brining; group B: injecting, phosphates and brining; group C: injecting, protein and brining; and group D: injecting, protein, phosphates and brining) before and after heat pump drying.

#### 4.3.4 Nuclear magnetic resonance analysis

The relaxation times were also measured after heat pump drying for 5 days. The measured values before and after the drying can be viewed in Figures 28 to 31.

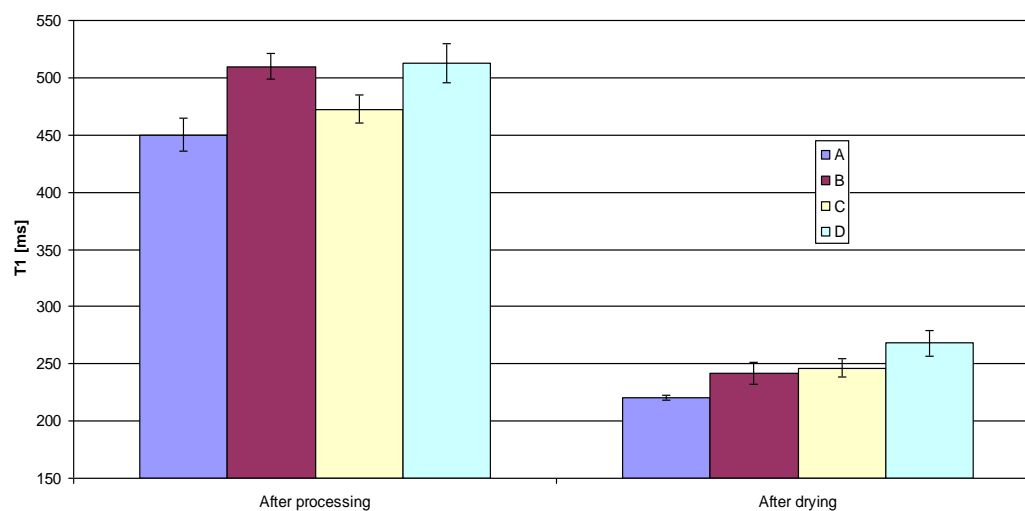


Figure 28: Longitudinal relaxation times  $T_1$  measured after processing and after drying.

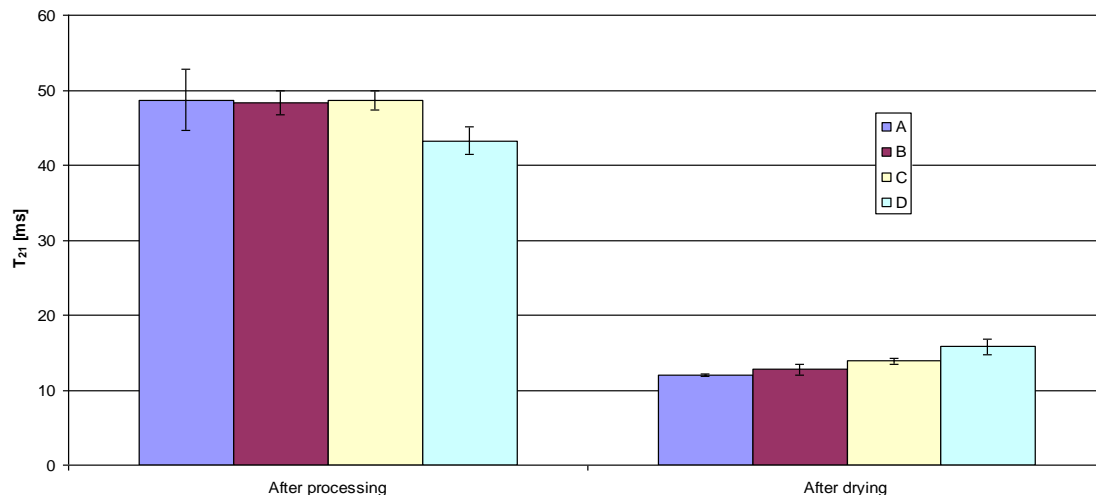


Figure 29: Shorter relaxation time,  $T_{21}$  measured after processing and after drying.

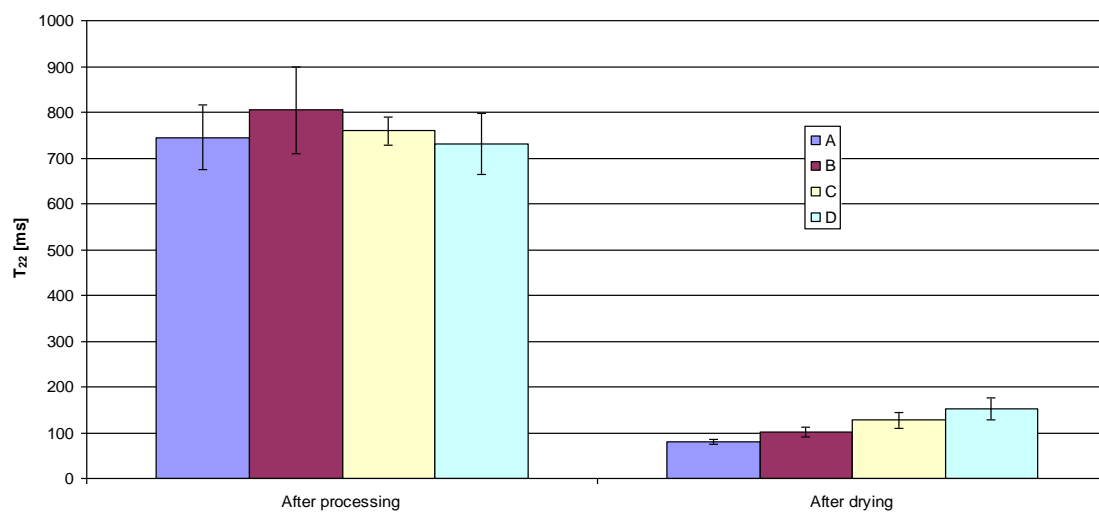


Figure 30: Longer relaxation time,  $T_{22}$  measured after processing and after drying.

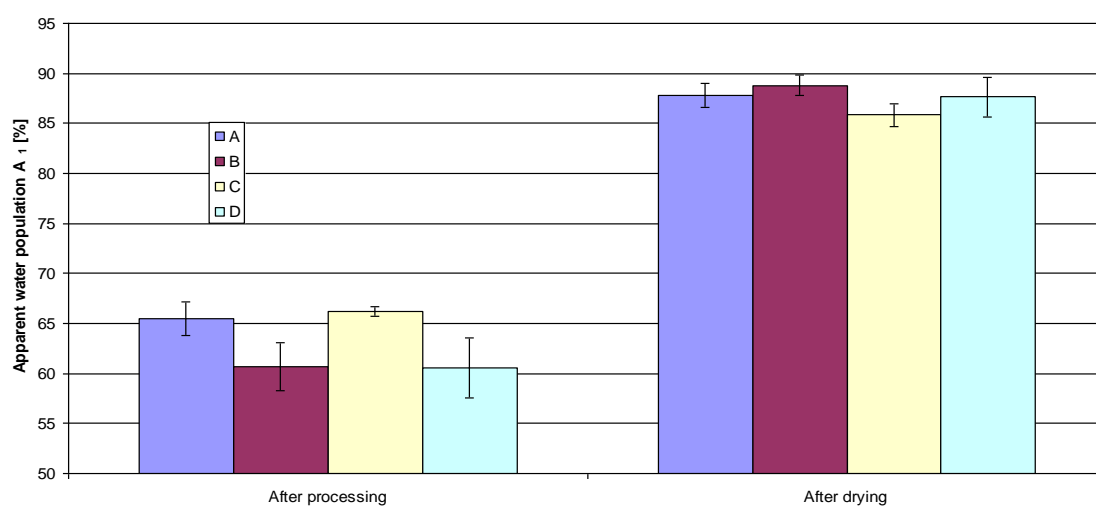


Figure 31: Apparent relative water population of tightly bound water  $A_1$  measured after processing and after drying.

Figures 28 to 30 show a large decrease in the measured relaxation times during the drying. This was simply correlated to the water loss during the drying process, resulting in a lower relaxation time.

Figure 31, on the other hand, indicates a large change in the apparent relative water population of tightly bound water during the drying. Before the drying 60-66% of the water in the sample was tightly bound to the muscular structure, but after the drying this ratio went up to 85-89% indicating that the freely moving water was removed more easily in the drying process, while more energy was needed to remove the tightly bound water.

## 5 DISCUSSION

### 5.1 The effect of temperature and storage time on the quality of salted cod

After the storage of the salted cod for 6 weeks, a much higher drip loss was observed for different treated groups (A, B, C and D) stored at  $-4^{\circ}\text{C}$ , compared to other temperatures (Table 3). This was caused by the enzyme activity in the muscle which is controlled by the temperature (Huss 1995). Generally, as the temperature decreases, the enzyme activity decreases because of the protein denaturation. However, the rate of several enzymatic reactions in the muscle of foods is higher in the critical temperature zone  $-1^{\circ}\text{C}$  to  $-6^{\circ}\text{C}$  than above the freezing point (Sikorski and Kolakowski 2000). Therefore, the salted cod for all groups stored at  $-4^{\circ}\text{C}$  had a higher drip loss compared to cod stored at other temperatures.

Change in colour of salted cod is a problem in the fishing industry concerning the storage of the fish. This project showed that after 6 weeks of storage, the lightness of salted cod in the four groups stored at  $-4^{\circ}\text{C}$  decreased, meanwhile, the lightness of fishes stored at other temperatures increased (Table 4). This is mainly due to the protein denaturation rate in the muscle of the cod stored at  $-4^{\circ}\text{C}$ , which is higher than for other temperatures used in this project, along with high enzyme activity in the muscle (Sikorski and Kolakowski 2000). Generally, the lightness increased during the storage and the highest value was observed in the samples for all groups stored at  $-24^{\circ}\text{C}$ . The decrease in  $a^*$  value in samples for all groups stored at  $2^{\circ}\text{C}$  and  $-4^{\circ}\text{C}$  was associated with the decrease of red colour and increase of green colour on the surface. In samples for all groups stored at other temperatures, the  $a^*$  value increased, it means that the red colour on the surface increased (Table 5). The  $b^*$  value of all samples for four groups increased after 6 weeks of storage, it means that the yellow colour increased on the surface (Table 6). The increase in yellow colour was presumed to be due to the oxidation of pigment in the fish muscle by oxygen and enzyme oxidation (Khayat and Schwall 1983, Hamre *et al.* 2003).

Cooking yield of food products and seafood products is an important concern to consumers. At the beginning of the storage time, the cooking yield of the salted cod fillets injected with protein (groups C and D) was higher than for the other groups. It means that protein and phosphates injected together in the muscle had more effect than injection of phosphates alone. During the storage time, the cooking yield of salted cod in all groups stored at  $2^{\circ}\text{C}$  was lower compared to other fillets stored at different temperatures (Figure 4). That can mainly be caused by degradation of

organic substances in the muscle by enzymes, rather than by microorganisms. Magnusson (2006) has studied the microbiological changes in salted cod during storage. His results showed that salted cod containing 20% salt and kept at 5 °C was a stable product microbiologically. According to the literature (Horner 1997, Kolodziejaska *et al.* 2002) most microorganisms normally associated with fish spoilage are halophobic and will not grow in salt concentration exceeding 5%. The cooking yield of the salted cod in groups B, C and D stored at -24 °C increased during the storage time, meanwhile, a difference was observed in salted cod group A. However, the results of the CY measurements showed great variance at the end of the storage time.

The water holding capacity (WHC) is one of the quality factors which are appreciated by the consumers. The WHC of the salted cod in all groups stored at five different temperatures increased at the end of the storage period (Figure 5). This may be explained by the degradation of cytoskeletal protein such as vinculin, desmin and talin. The cytoskeletal protein consists of large numbers of connections between myofibrils and between myofibrils and the sarcolemma. When the cytoskeletal protein degrades, the cell shrinkage can be halted and extracellular water is able to flow into the muscle cell with a following increase in water holding capacity. Moreover, the water holding capacity is affected by storage time and storage temperature and there is a relationship between WHC and other factors (Kristensen and Purslow 2001). During the storage time, the WHC varied between the samples. This can be caused by the changes in proteolytic activity in the muscle due to the changes in myofibrillar proteins. The changes in myofibrillar proteins which affect the quality of fish muscle have been related to proteolytic activity in the muscle of fish (Benjakul *et al.* 1997). At the beginning, the WHC of samples in groups B and D were 64.1% and 64.9% respectively, were significantly higher ( $p < 0.05$ ) than that of the samples in groups A and C (61.2% and 62.3% respectively). This can be explained by the fact that the phosphates content in salted fish groups B and D (6.7% and 6.5% of  $P_2O_5$  respectively) was higher than that of the salted cod groups A and C (4.7% and 4.9% of  $P_2O_5$  respectively). The higher phosphates content in the muscle of fish, the higher binding water on proteins was found. The polyphosphates results in a decrease in water mobility in the fish muscle (Keith and Fred 1998). The WHC of the samples in all groups stored at -4 °C increased during the storage period. The results can be affected by the decrease of the water content during the storage time. The WHC of the samples stored at 2 °C was lower than other samples at other temperatures. Thorarinsdottir *et al.* (2002) explained that the WHC is affected by the muscle protein denaturation. Mackie (1993) and Sigurgisladottir *et al.* (2000) reported that protein denaturation and water loss of fish flesh are associated with frozen storage. It has also been found that the lower WHC at the higher temperature may be due to increased thermal denaturation of proteins at the higher compared with the lower temperature (Sankar and Ramachandran 2005).

The water content of the salted cod in groups B and D is more stable in the first 3 weeks, compared to groups A and C (Figure 10). This can be explained by the phosphates content in the muscle of the salted cod in groups B and D. According to Aitken (2001) the main value of phosphates is to improve the retention of water by the protein in fish. Keith and Fred (1998) studied the effect of the phosphates on shrimp. They stated that shrimp treated with a solution of sodium tripolyphosphates produced the greatest weight gains due to water in shrimp. Phosphates treatment also helped

reduce water migration during frozen storage. In this study, higher drip loss was observed in salted fish stored at  $-4\text{ }^{\circ}\text{C}$  related to the water content of the salted cod during the storage period. The water content of the samples in four groups stored at  $-4\text{ }^{\circ}\text{C}$  decreased rapidly at the end of the storage time.

The total volatile basic nitrogen (TVB-N) is one of the most widely used measurements of seafood quality. TVB-N value is an important parameter for determining the freshness of fish products. TVB-N value is affected by species, catching region and season, age and sex of fish (Gökoğlu *et al.* 1998). It is a general term which includes the trimethylamine, dimethylamine, ammonia and other volatile basic nitrogenous compounds associated with seafood spoilage (Huss 1995). At the beginning, the TVB-N values of the salted cod in groups C and D were 8.8 mgN/100 g and 8.2 mgN/100 g flesh fish, respectively, were higher than samples in groups A and B (7.5 mgN/100 g and 7.0 mgN/100 g, respectively). This was probably due to the protein injection during salting process in groups C and D. The TVB-N of the samples in all groups stored at  $-4\text{ }^{\circ}\text{C}$  increased significantly ( $p < 0.05$ ) during the storage period and was higher than in other samples (Figure 11). This may be caused by the higher enzyme activities at  $-4\text{ }^{\circ}\text{C}$  compared to other storage temperatures. The TVB-N of all the samples increased during the storage time but the values were not high and were acceptable according to Connell (1990) (the limiting level for rejection of TVB-N is 20 mgN/100 g). The highest TVB-N value was observed in the sample in group C stored at  $-4\text{ }^{\circ}\text{C}$  (11.1 mgN/100 g flesh fish). The results could be explained that the salt content in the fish muscle was high (about 21%) so it could inhibit the growth of microbiological organisms. As reported by Magnusson (2006), salted cod fillets containing 20% salt are stable microbiologically. Leroi *et al.* (2000) also reported that salt could prevent the growth of both spoilage and pathogenic bacteria. Generally, the TVB-N content of the salted cod in groups B and D with phosphates injection was slightly lower than that of the salted cod in groups A and C without phosphates injection. As the explanation of Masniyom *et al.* (2005) the phosphates in the fish muscle can control the changes in nonprotein fraction of the muscle as evidenced by lower TVB formation.

Trimethylamine (TMA) content is often used as a biochemical index to assess keeping quality and shelf-life of fish (Connell 1990). It is a pungent volatile amine often associated with the typical fishy odour of spoiling seafood. The level of TMA found in fresh fish rejected by sensory panels varies between fish species, but is typically around 10-15 mgN/100 g in aerobically stored fish (Huss 1995). The variation in the acceptability level can be attributed to the fact that TMA values vary with species, season, storage conditions, bacteria and intrinsic enzyme activity (Debevere and Boskou 1996). In this study the TMA values of all samples in all groups varied substantially. The same with the TVB-N values, the TMA value of the samples in all groups stored at  $-4\text{ }^{\circ}\text{C}$  increased through the storage time and had a higher value compared to the other samples (Figure 12). At the end of the storage time, the TMA values of all the samples were significantly lower than the general TMA limit for fish reported by Connell (1990) (10-15 mg N/100 g). The low values of TMA obtained in this study could be explained by high salt content in the cod (21%), and low temperature storage, resulting in low microorganisms activity. In addition, the TMA is formed from trimethylamine oxide (TMAO) as the result of endogenous enzymes, but at chilled and frozen storage TMA is produced by the bacterial enzyme activity (Ashie *et al.* 1996, Rodriguez *et al.* 1999 and Sivertsvik *et al.* 2002). Nevertheless, the

storage temperatures are low, so that the bacterial enzyme activity is reduced. Furthermore, it could be attributed to the preservative effect of salt (Leroi *et al.* 2000, Kolodziejaska *et al.* 2002).

## 5.2 The effect of temperature and storage time on the quality of desalted cod

The high total changes ( $\Delta W$ ) of salted cod after desalting of the samples in all groups was observed when samples were stored at  $-4\text{ }^{\circ}\text{C}$  after 3 weeks and 6 weeks of storage (Figure 15). This can be explained by the fact that the desalting operation can be considered as a solid-liquid extraction, in which solutes are the  $\text{Na}^+$  and  $\text{Cl}^-$  ions, the solvent is water and the inert matrix is constituted by the fish proteins. The mass transport during desalting was due to activity gradients between the cod liquid and the surrounding desalting, and pressure gradients (Barat *et al.* 2004). The samples stored at  $-4\text{ }^{\circ}\text{C}$  had a high drip loss so that the activity gradients and pressure gradients were higher than in other samples. Generally, the samples in four groups stored at  $2\text{ }^{\circ}\text{C}$  after 3 weeks and 6 weeks of storage got a low total weight change. The total weight changes of the samples in groups A and C were slightly higher than those in groups B and D. This was maybe due to the effects of polyphosphates in the fish muscle. The phosphates would increase the yield after the salting process by increasing the water intake (Thorarinsdottir *et al.* 2001) so that it reduces the mass transfer during desalting. The results were in correlation with the water content of salted cod after 3 weeks and 6 weeks of storage, which indicated that the water content of samples in the phosphates groups (B and D) were slightly higher than in groups A and C.

Generally, the cooking yield (CY) of all samples in groups B and D after 3 weeks and 6 weeks of storage was higher than in groups A and C (Figure 16). This result correlated with the NMR results of salted cod, which stated that the polyphosphates groups (B and D) showed longer relaxation times than the polyphosphates lacking groups (A and C). It can be explained by reduced water mobility in the samples by phosphates (Keith and Fred 1998). The cooking yield of the samples in all groups was always high when the samples were stored at  $-24\text{ }^{\circ}\text{C}$ . It was due to lower temperature resulting in a lower microbiological activity, enzyme activity and protein denaturation.

The water holding capacity (WHC) in groups B and D was more stable than in groups A and C during the storage time (Figure 17). The results were probably due to the effects of phosphates in the fish muscle. The phosphates in the fish muscle have induced water retention by change pH. The WHC is minimal around the isoelectric point, but increases with either increasing or decreasing pH, which results were caused by the stronger electrostatic repulsion forces and increased space for water to be held in the muscle (Offer and Knight 1988, Molins 1991). In addition, phosphates can affect the bacterial growth during storage and desalting. The effects of phosphates in the fish muscle on growth of microorganisms have been discussed by many scientists. Marshall and Jindal (1997) reported that trisodium phosphates reduced aerobic and total coliform counts in the meat from catfish frames. Masniyom *et al.* (2005) researched the effect of phosphates and modified atmosphere packaging (MAP) on the quality of seabass slices. The results showed that phosphates showed the synergistic effect on the retardation of bacterial growth in the samples kept under MAP. The differences in water content between groups were due to the different salting processes (Andrés *et al.* 2005).



Generally, the water content of samples in groups B, C and D was higher than samples in group A. The water content after 3 weeks and 6 weeks of storage was always high when samples were stored at -4 °C and -12 °C (Figure 18). This was in correlation with the water holding capacity results and total weight changes of desalted cod after 3 and 6 weeks of storage.

### 5.3 The effect of heat pump drying on the quality of salted cod

The ratio of weight loss and water content of all samples in groups A, B, C and D were similar and no significant differences in weight loss were found between the groups (Figure 23 and 24). This means that phosphates and protein injection during the salting process did not affect the weight loss and water content during drying. Nevertheless, more weight loss (from 100% to 77%) and a decrease in water content (from 60% to 47%) in all samples were observed during the first 3 days of drying compared to the last 2 days of drying. The results could be explained by the fact that during the first 3 days of drying the surface of the fish was initially wet and a continuous film of water existed on the drying surface. Evaporated water during the first 3 days was free water (unbound water). During the last 2 days the evaporated water was bound water and the surface of the fish was dry.

The colour values of samples in the four groups changed after drying. The lightness of all samples in all groups increased significantly ( $p < 0.05$ ) after drying (Figure 25). This can be caused by the water removal during drying. After drying, the salt crystals in the fish muscle were formed.

The  $a^*$  value of all samples in all groups increased significantly ( $p < 0.05$ ) after drying, it meant that the red colour of fish increased (Figure 26). The significant increase ( $p < 0.05$ ) in  $b^*$  value after drying meant that the yellow colour increased on the surface. This was presumed to be due to the oxidation of pigment in the fish muscle by oxygen and enzyme oxidation (Khayat and Schwall 1983, Hamre *et al.* 2003).

## 6 CONCLUSIONS

The results indicated that protein and phosphates injection during the salting process increased the water holding capacity and cooking yield of the salted cod and desalted cod during storage.

The salted cod, stored at -24 °C had a lower TVB-N, TMA values and higher WHC and CY, compared to other storage temperatures. The salted cod stored at -4 °C had lower CY and WHC and higher drip loss, TVB-N and TMA, compared to other storage temperatures. This can mainly be caused by the enzymes activity in the fish muscle. Consequently, the storage temperature of -4 °C was not suitable for storing salted cod.

The results during drying of salted cod demonstrated that the phosphates and protein injection during salting process did not affect the colour and water loss during drying.

## ACKNOWLEDGEMENTS

I would like to give special thanks to my supervisors Mr. Ásbjörn Jónsson and Mr. Sigurjón Arason for their elaborate guidance, enthusiastic assistance and valuable advice.

I am particularly grateful to Dr. Tumi Tómasson, Mr. Þór Ásgeirsson, and Ms. Sigríður Kr. Ingvarsdóttir for their endless support during the programme.

I am grateful to Ms. María Guðjónsdóttir for her great input about NMR in this report, Mr. Irek Adam Klonowski, and the staff of Icelandic Fisheries Laboratories for their guidance and their help during this project.

I am also grateful to the library staff, computer service staff, UNU fellows and my friends for their help and encouragement. Special thanks go to the UNU-Fisheries Training Programme for funding this course.

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