Universidade do Minho
Escola de Engenharia

Tiago António da Silva Pereira Fernandes

Study of the variables affecting the thickness of an edible coating applied on frozen fish

Agosto de 2014

Study of the variables affecting the thickness of an edible coating applied on frozen fish
Tiago António da Silva Pereira Fernandes
UMinho | 2014

Universidade do Minho
Escola de Engenharia

Tiago António da Silva Pereira Fernandes

Study of the variables affecting the thickness of an edible coating applied on frozen fish

Dissertação de Mestrado
Mestrado Integrado em Engenharia Biológica
Ramo Tecnologia Química e Alimentar

Trabalho Efetuado sob a orientação do
Professor Doutor Engenheiro António Augusto Martins de Oliveira Soares Vicente
e do
Engenheiro Nuno Miguel Ferreira Soares

Agosto de 2014

Universidade do Minho
Escola de Engenharia

Declaração RepositóriUM: Dissertação de Mestrado

Nome: Tiago António da Silva Pereira Fernandes

N.º do Cartão de Cidadão/BI: 13619821 Telefone/Telemóvel: 912047512

Correio eletrónico: Tiago.spf9@gmail.com

Curso: Mestrado Integrado em Engenharia Biológica Ano de conclusão da dissertação: 2014

Área de Especialização: Tecnologia Química e Alimentar

Escola de Engenharia, Departamento/Centro: Engenharia Biológica

TÍTULO DA DISSERTAÇÃO/TRABALHO DE PROJETO

Título em PT: Estudo das variáveis que afetam a espessura de um revestimento edível aplicado em pescado congelado.

Título em EN: Study of the variables affecting the thickness of an edible coating applied on frozen fish.

Orientador: António Augusto Martins de Oliveira Soares Vicente

Coorientador: Nuno Miguel Ferreira Soares

Número de Unidades ECTS da Dissertação: 30 Classificação em valores (0 a 20): 19

Classificação ECTS com base no percentil (A a F): A

Declaro sob compromisso de honra que a dissertação agora entregue corresponde à que foi aprovada pelo júri constituído pela Universidade do Minho, UM.

Declaro que concedo à Universidade do Minho e aos seus agentes uma licença não-exclusiva para arquivar e tornar acessível, nomeadamente através do seu repositório institucional, nas condições abaixo indicadas, a minha dissertação, em suporte digital.

Concordo que a minha dissertação seja colocada no repositório da Universidade do Minho com o seguinte estatuto (assinale um):

1. ☐ Disponibilização imediata do trabalho para acesso universal;
2. ☐ Disponibilização do trabalho para acesso exclusivo na UM, durante o período de
   ☐ 1 ano, ☐ 2 anos ou ☐ 3 anos, sendo que após o tempo assinalado autorizo o acesso universal.
3. ☐ Disponibilização do trabalho de acordo com o **Despacho RT-98/2010 c**) (embargo anos)

Braga, 6 de Agosto de 2014

Assinatura:

_____

Agradecimentos

À família e amigos, pelo apoio e paciência, com um agradecimento especial ao João Pedro e a Tânia.

Ao Professor António Vicente por toda a ajuda e disponibilidade e também pela alegria, optimismo e boa disposição.

Ao Engenheiro Nuno Soares pelos ensinamentos, companheirismo e presença constante, mas também pelo apoio e preocupação permanente.

À Vanibru pela recepção e simpatia dos seus trabalhadores e pela cedência das suas instalações e equipamentos.

Ao Departamento de Engenharia Biológica, em especial ao Laboratório de Instrumentação e Processo, em particular ao Ricardo e a Ana Cristina pela cooperação e paciência, mas também à Plataforma de Microscopia e à Doutora Ana Nicolau pela ajuda e disponibilidade.

Ao Departamento de Engenharia de polímeros, em especial ao Engenheiro Maurício Malheiro pela ajuda e disponibilização do equipamento DSC.

À Marina e à Cristina pelo apoio e simpatia.

A todos os que de qualquer forma tenham colaborado comigo e que permitiram a realização deste trabalho, muito obrigado.

Resumo

O consumo de peixe tem aumentado progressivamente nos últimos anos, devido sobretudo às suas características nutricionais únicas e aos seus beneficios para a saúde dos consumidores. A vidragem é uma técnica usado como suplemento à congelação e a sua finalidade é retardar a deterioração do pescado. Este trabalho foca-se no estudo das variáveis (temperatura do peixe, temperatura do revestimento, tempo de mergulho) que afectam a espessura de um revestimento edivel (água e quitosano 1.5%) aplicado em peixe congelado. Amostras de salmão do Atlântico congelado (*Salmo salar*) a -15, -20, -25 °C foram vidradas com água a 0.5, 1.5, 2.5 °C e revestidas com quitosano a 2.5, 5, 8 °C durante 10 a 60 segundos. Tanto na água como no quitosano a descida da temperatura do salmão e do revestimento resulta num aumento da espessura. A espessura obtida nos revestimentos usados foi sempre superior no quitosano, atingindo uma espessura máxima de (1.41±0.05) mm para o quitosano e (0.84±0.03) mm na água. A análise DSC permitiu constatar que tanto a temperatura de congelação como o calor de cristalização são inferiores na solução de 1.5% quitosano em comparação com a água, o que favorece a mudança de fase. Os perfis de temperatura do salmão permitiram confirmar, para diferentes condições de mergulho, se a temperatura do salmão se encontra dentro dos limites de segurança alimentar, de modo a evitar o crescimento de microorganismos patogénicos. O tempo máximo que o salmão pode estar mergulhado no revestimento (tempo de mergulho seguro) para as várias condições testadas nunca ultrapassa os 40 segundos. A temperatura média do revestimento que aderiu ao salmão foi determinada experimentalmente, sendo importante para tentativas futuras de previsão da espessura de revestimento.

Palavras-chave: salmão, vidragem, água, quitosano, espessura.

Abstract

Fish consumption has gradually increased in recent years, mainly due to its unique nutritional characteristics and their benefits to the health of consumers. Glazing is a technique used in addition to freezing and is intended to retard the deterioration of fish. This work focuses in the study of the variables (fish temperature, coating temperature, dipping time) that affect the thickness of an edible coating (water glaze and 1.5% chitosan) applied on frozen fish. Samples of frozen Atlantic salmon (*Salmo salar*) at -15, -20, -25 °C were glazed with water at 0.5, 1.5, 2.5 °C and coated with chitosan at 2.5, 5, 8 °C during 10 to 60 seconds. For both water and chitosan lowering the salmon and coating temperature results in an increase of the thickness. The thickness of the coatings used was always higher for chitosan than for water, reaching a maximum thickness of (1.41±0.05) mm for chitosan and (0.84±0.03) mm in water. By DSC analysis it was found that both freezing temperature and cystallization heat are lower in 1.5% chitosan solution than in water, which favors the phase change. Salmon temperature profiles allowed confirming, for different dipping conditions, if the salmon temperature is within the limits of food safety for the growth of microorganisms pathogens. The maximum time salmon may be dipped in the coating (safe dipping time) for the tested conditions never exceeds 40 seconds. The average temperature of the coating that adhered to the salmon was determined experimentally, being important for future attempts to predict the coating thickness.

Keywords: salmon, glazing, water, chitosan, thickness.

List of contents

List of Nomenclature

Abbreviations

a* - Red and green direction

b* - Yellow and blue direction

Da - dalton

DL - Decree-Law

DSC - Differential Scanning Calorimetry

EFSA - European Food Safety Authority

FAO - Food and Agriculture Organization

FDA - Food and Drug Administration

GlcN - 2-amino-2-deoxy-β-D-glucose

GlcNAc - N-acetyl glucosamine

GRAS - Generally Recognized As Safe

K-value - ATP breakdown products

L* - Lightness

-NHCOCH3 - acetamide group

-OH - hydroxilic group

Op+ - Olympus pro plus

p - Significance

pK_a - Acid dissociation constant

PCA - Principal component analysis

TBA - Thiobarbituric acid

TVB-N - Total volatile basic nitrogen

TVC - Total viable counts

$ - US dollar

Symbols

a_w - Water activity

C_p - Specific heat

h - Convective coefficient

k - Thermal conductivity

L - Wall semi-thickness

m - mass

Q - Heat

t - time

T - Wall temperature

T_o - Initial wall temperature

T_{av} - Average temperature

T_i - Initial temperature

T_w - Surface temperature

v/v - volume/volume

w/v - mass/volume

W_f - final mass

W_i - initial mass

x - Depth

α – Thermal diffusivity

ΔH - Crystallization heat

ΔT - Temperature change

ρ - Density

Σ - Sum

List of Figures

Figures in Appendixes

List of Tables

Introduction

Fish is a very perishable product and is essential to improve its preservation processes. Freezing is nowadays the main method of preserving fish, however it does not stop completely the deterioration. In order to decrease this undesirable changes, it is common to use a thin layer of ice on the surface of the product (glazing) to reduce the risk of deterioration. The amount of glazing necessary to protect the fish is not defined but the glaze content is normally in the range from 8-12% of the gross weight (Jacobsen & Fossan, 2001). However larger amounts are used, leading to excessive application in the frozen fish industry looking at obtaining commercial advantages. Although water is used as coating, other coatings have properties that can optimize this technique. To master the process it is necessary to know how the variables that affect the thickness of the glazing (coating temperature, product temperature, superficial area and the dipping time). In this context arises this work that intends to apply chitosan solutions and water on frozen Atlantic salmon (*Salmo salar*) to study how those variables affect the resulting thickness.

This dissertation was organized in two parts: Part I - State of the art and Part II - Experimental work. Part I is divided in three chapters and Part II is also composed by three chapters.

Chapter 1 provides an overview about the fish industry, in particular the reasons for the high consumption of fish but also the evolution of capture and fish production over the years. This chapter also refers freezing and water glazing of fish, which are the traditional methods of preservation of frozen fish. Chapter 2 introduces the concept of edible packaging, differentiating edible films and coatings. Several compounds capable of being used in edible packaging are presented, such as the main features of each one. From these the focus goes to chitosan, explaining its isolation method and also its physicochemical and biomedical characteristics that make it a solution to various industries, including the processing of frozen fish. The application of coatings to frozen fish results in the exchange of heat between fish and coating. Chapter 3 explains how this energy transfer occurs and under which conditions.

Chapter 4 describes the methods, such as salmon and coating solution preparation, salmon dipping in chitosan and water, samples preparation and photographs taken and differential scanning calorimetry of chitosan solution. Calculations were made to determine salmon temperature profiles and the amount of heat transferred. The results are discussed in Chapter 5 and Chapter 6 summarizes the main conclusions, as well as proposals for future research.

Part I - State of the Art

Chapter 1. Fish

1.1. Fish industry

Over the ages a huge variety of seafoods have been consumed and appreciated by humans and it is unanimously a great source of important nutritional components for a healthy diet (Rodriguez-Turienzo, et al., 2011). As stated by James (2013), the benefits of seafood consumption far outweigh the possible risks. Therefore fish consumption provides energy, protein and some important nutrients like the long-chain ω-3 polyunsaturated fatty acids and lowers the risk of mortality from coronary disease.

In 2013, according to the Food and Agriculture Organization of the United Nations (FAO), 135 million tons of fish (wild capture and aquaculture) were consumed by humans. Despite the increase of total fish production, the number of captured fish is stable over the last few years. Nevertheless, world´s fishery is still growing thanks to aquaculture, as can be seen in Figure 1 (Adapted from Fisheries –OECD-FAO Agricultural Outlook 2012-2021):

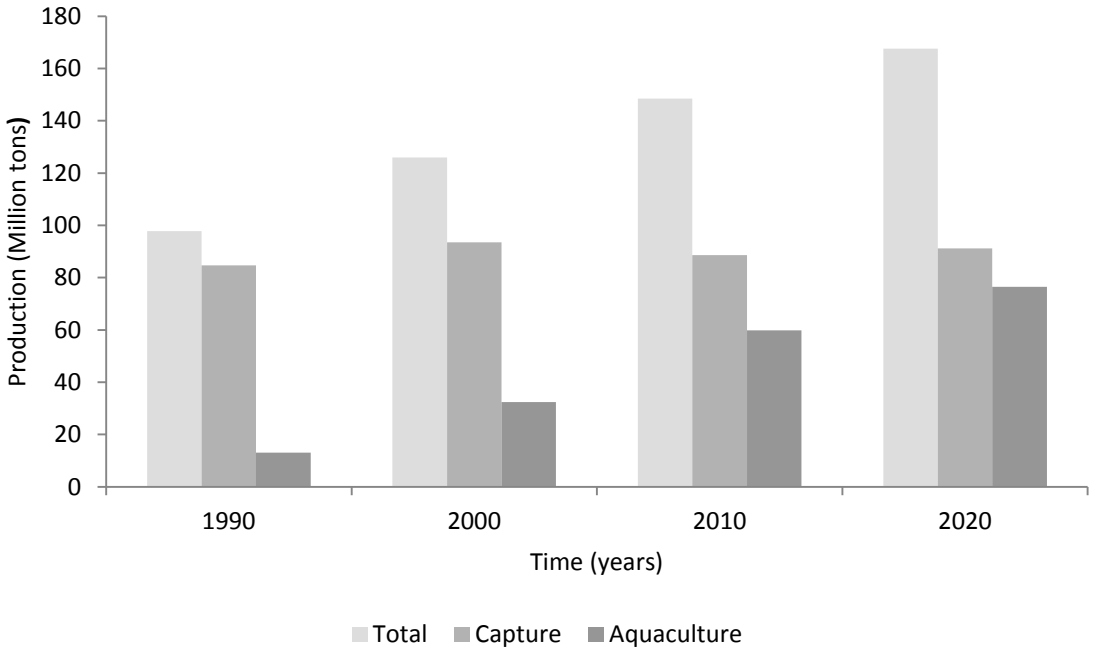

Figure 1. Evolution of captured fish, aquaculture and total fish production over the years.

FAO (2013) defines aquaculture as *"The farming of aquatic organisms in inland and coastal areas, involving intervention in the rearing process to enhance production and the individual or corporate ownership of the stock being cultivated"*. The aquaculture growth is reaching nearly 50 percent of the World´s food fish and it is probably the fastest growing food-producing sector. This industry is gaining prominence due to the fact that the maximum wild capture fisheries potential from the world's oceans has probably been reached.

1.2. Fish Preservation

Fish deterioration is most commonly a result of microbial growth, oxidation and enzymatic autolysis and can be prevented by using methods like freezing, salting, smoking, fermentation, drying and canning. According to Ghaly et al. (2010) 30% of landed fish are lost because of microbial activity. The increase of world's population and the need to store and transport the fish are factors that enhance this problem and make its preservation imperative in order to maintain its nutritional proprieties, flavor, color, texture and extend its shelf life (Ghaly, et al., 2010).

1.2.1. Freezing

Freezing is a technique widely used in fish preservation because it inhibits the enzyme activity and retards the growth of microorganisms, decreasing the microbial metabolism responsible for spoilage. This method requires the removal of heat from the fish´s body in order to lower the product temperature generally to -18 °C or below (Ghaly, et al., 2010). Depending on the species, fish is largely water, normally 60-80 percent. Freezing process converts most of this water into ice, lowering the water activity (a_w), and if properly carried out can assure a storage life of more than one year (Johnston, et al., 1994) (Fellows, 2000). It is noteworthy that freezing does not improve the quality of the product; final quality depends of the original product quality and of freezing conditions, storage, transport and distribution (Gonçalves, et al., 2008).

1.2.2. Glazing

In recent years the demand for frozen fish is growing in opposition to what is verified in fresh fish. The main reason is related with the high efficacy of frozen preservation in contrast with fresh fish, which is a very perishable product. However, traditional frozen storage of fish may lead to a progressive loss of intrinsic and sensory characteristics (Vanhaecke, et al., 2010). Glazing is a technique largely used to protect fish from this loss, and can be defined as the application of a layer of ice on frozen products' surface by spraying or brushing on water or by dipping in a water bath (Zoldos, et al., 2011).

During frozen or cold storage, marine products may suffer dehydration and surface drying resulting from the contact with very cold temperatures (freezer burn). So, seafood glazing acts as a barrier preventing the quality loss due to oxidation or rancidity, since the ice layer reduces the exposure to oxygen, disallowing the contact between product's surface and air (Vanhaecke, et al., 2010) (Johnston, et al., 1994).

A good glazing is essential in order to minimize quality loss resulting from exposure to factors like rate of freezing and thawing, temperature fluctuation, high storage temperature, incorrect transportation, distribution and consumption temperatures. However a poor glazing may jeopardize fish quality, doing more harm than good because of partial thawing of the fish and slow refreezing in cold storage. (Zoldos, et al., 2011). The glazing process must be closely controlled, to form a uniform and complete glaze on the fish's surface. The factors that condition the amount of glaze applied are the glazing time, the water temperature, the fish temperature and the size and shape of the product (Johnston, et al., 1994). These same factors will directly influence the thickness resulting from the coating application.

The phenomenon that allows a liquid to be kept in contact with a solid surface is known as wetting. A simpler way to define wetting, is the ability of a liquid to spread across the surface of a solid to produce a uniform and continuous surface (Ramiasa, et al., 2014). In glazing liquid coating spreads over the surface of the frozen fish and provides heat to the fish due temperature difference. This heat transfer causes the decrease of coating temperature and when it reaches the freezing point changes phase from liquid to solid. This phase transition is an exothermic process because releases energy from the system (Perry & Green, 2007).

The balance between adhesive and cohesive forces define the degree in which the coating solutions adhere to the solid surfaces (Casariego, et al., 2008). A good wettability indicates that the food and the adherend have a strong mutual affinity and will probably adhere well (Adhikari, et al., 2001).

According to Vanhaecke et al. (2010), a product ordinarily gets about 4% to 10% of glaze applied, being also normal a range of 2% to 20% for different products, reported as working well. In some extreme cases this percentage can reach 40%. The determination of the amount of glaze applied can be very important for the evaluation of its protective function and for economic reasons. So a low glaze application (<6%) may not ensure fish protection and can lead to a decrease of quality in the final product. From an economic perspective, an excessive glazing (over 12%) may assure high profits to sellers, because the consumers are paying water by the fish price. In both cases, the consumer is the most affected part (Vanhaecke, et al., 2010).

Chapter 2. Edible packaging

The quality of food is associated with its nutritional, organoleptic and hygienic characteristics that may change during storage and commercialization. In order to preserve the foods quality some physical and chemical processes are used (e.g. high pressure, sterilization, etc.), however packaging is the ultimate step of the preservation process (Debeaufort, et al., 1998).

Therefore, a great attention has been given to edible food packaging because when applied becomes an integral part of the food that could be eaten. The motivation for the increase of interest and research activity in edible packaging was due to the increasing consumer demand for safe, convenient, stable foods and also the awareness of the negative environmental impacts of nonbiodegradable waste resulting from packaging (Azeredo, 2012). Recently, from 2008 to 2012, over 400 patents have been published per year concerning the manufacture of edible packaging (Arancaanalysis, 2013).

2.1. Edible coatings and films

Although edible coatings and films have been used for centuries, for example as wax on some fruits, the term "edible film" only has been related to food applications in the past fifty years (Pavlath & Orts, 2009). In 1967, edible coatings were basically limited to wax layers on fruits, therefore had a very poor commercial use. However, during intervening years, this business grew exponentially (i.e. increase of 10 companies in 1986 to 600 companies in 1996) and nowadays, the edible films/coatings market expanded quickly for retaining quality of a variety of foods, exceeding the $100 million in annual revenue (Pavlath & Orts, 2009). Sometimes, the terms "edible films" and "edible coatings" appear as synonyms, but there is a difference: films are pre-formed separately and then applied on a food's surface or sealed into edible pouches, whereas coatings are formed directly onto food surfaces (Azeredo, 2012). The main goal of edible coatings and films is to extend fresh or processed foods shelf life, and their characteristics offer many advantages such as edibility, esthetic appearance, biocompatibility,

non-toxicity, gasses properties barrier, non-polluting and its low cost. They can also act as carriers of foods additives like antioxidants and antimicrobials (Elsabee & Abdou, 2013).

Edible coatings and films may be classified according to the kind of material from which they are derived. They can be classified into three main categories based on their origin and production (Figure 2) (Srinivasa & Tharanathan, 2007):

- Polymers directly extracted or recovered from biomass;
- Polymers produced by classical synthesis using renewable biobased monomers;
- Polymers produced by microorganisms.

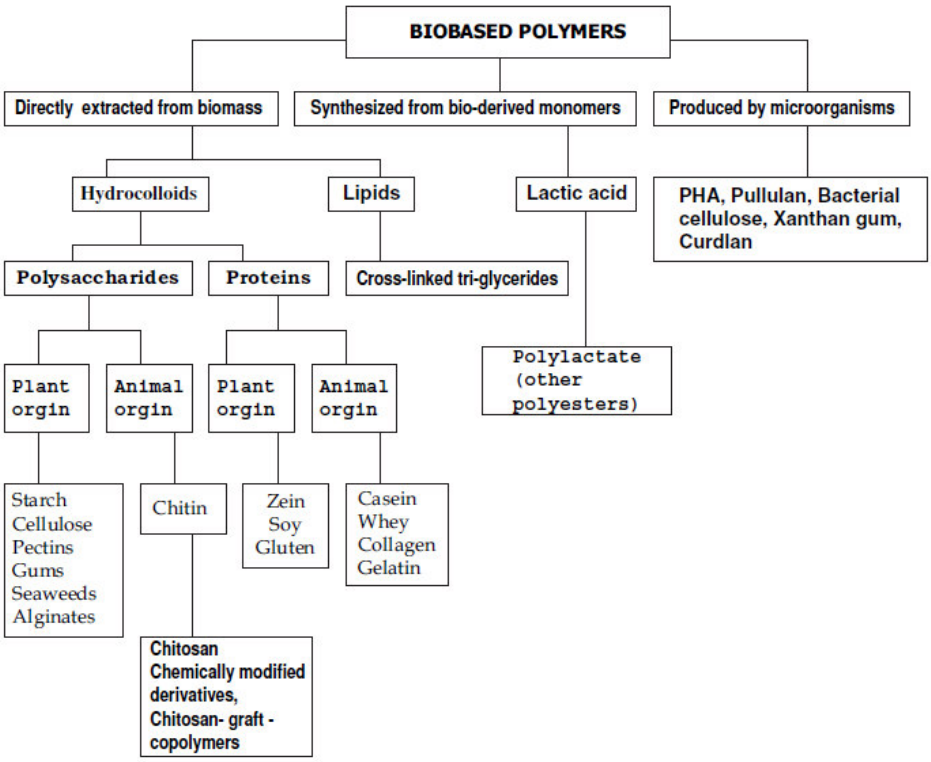

Figure 2. Types of biobased polymers used for biopackaging.

The most commonly used are polysaccharide-based coatings, protein-based coatings, lipid coatings and composite coatings. Polysaccharide-based coatings are long-chain biopolymers formed from mono- or disaccharide repeating units linked by glycosidic bonds. They are widely used because of their availability, low cost and hidrophilicity (good barrier to CO_2 and O_2). For

other side as inconvenient they form a poor barrier to water vapor and are very sensitive to moisture (Azeredo, 2012). Table 1 shows the inherent properties of selected polysaccharides and some of its advantages/disadvantages for coating/film use (Azeredo, 2012; Rabea, et al., 2003; Skurtys, et al., 2010). The main characteristics required for edible coatings/films depend on the type of food product. Therefore, for oxidation-sensitive products like polyunsatured fats, a low oxygen permeability is required (Debeaufort, et al., 1998).

Table 1. Characteristics of selected polysaccharides

Chemical class	Description	Advantages	Disadvantages
Starches	Polymers of D-glucopyranosyl, consisting of a mixture of the predominantly linear amylose and the highly branched amylopectin	→ forms relatively strong films	→ brittle and noncontinuos films → barrier properties decrease with relative humidity
Cellulose and its derivatives	Most abundant natural polymer, is an essentially linear natural polymer of (1→4)-β-D-glucopyranosyl units	→ very abundant → good film-forming properties (after etherification)	→ insoluble in water (requires etherification)
Anionic polysaccharides	Pectins: water-soluble anionic heteropolysaccharides composed majinly of 1→4)-α-D-galactopyranosyluronic acid units Alginates: linear copolymer of D-*mannuronic* and L-guluronic acid monomers, extracted from brown seaweeds	→ strength and permeability of films may be altered → alginate unique colloidal properties (thickening, stabilizing, gel producing, etc.)	→ alginate is indigestible → hydrophilic nature (poor water resistance)
Chitosan	(β-(1,4)-2-amino-2-deoxy-D-glucopyranose), majorly made from crustacean shells, is the second most abundant natural and non-toxic polymer in nature	→ abundance → cohesive and compact coatings/films → biomedical properties → selective permeability to gases	→ poor solubility in neutral solutions → low resistance to water vapor transfer → coagulate with proteins at high pH

2.1.1. Chitosan

The shells of crab, lobster and shrimp are rich in $CaCO_3$, protein and chitin. Chitin is a polysaccharide and its name derives from the Greek word "chiton", meaning a coat of mail or envelop (Srinivasa & Tharanathan, 2007). Chitin is the second most abundant naturally occurring biopolymer, after cellulose, and is found in the exoskeleton of crustaceans, in fungal cell walls and in other biological materials. Chitin is an insoluble linear mucopolysaccharide (Figure 3) consisting of N-acetyl-D-glucosamine (GlcNAc) repeat units, linked by β-(1→4) glycosidic bonds (Raafat & Sahl, 2009). It is structurally identical to cellulose, except that a secondary hydroxyl [-OH] on the second carbon atom of the hexose repeating unit is replaced by an acetamide [-$NHCOCH_3$] group (Elsabee & Abdou, 2013). Through a simple demineralization (treatment with hot diluted HCl) and deproteinization (treatment with hot diluted NaOH) steps, the amino polysaccharide chitin can be quantitatively recovered from crustacean wastes. Chitosan is the N-deacetylated derivative of chitin with its structure composed of 2-amino-2-deoxy-β-D-glucose (GlcN) in a β(1,4) linkage and with occasional N-acetyl glucosamine (GlcNAc) residues (Srinivasa & Tharanathan, 2007). Figure 3 represents both chemical structures of chitin and chitosan.

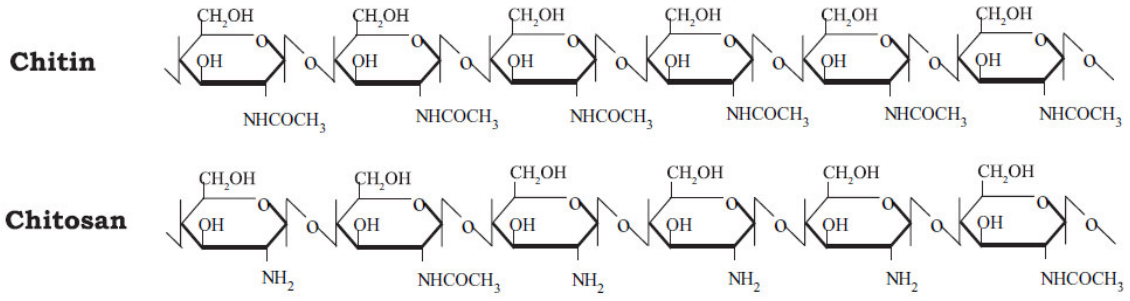

Figure 3. Chemical structures of chitin and chitosan

The process for chitin and chitosan isolation (Figure 4) (Srinivasa & Tharanathan, 2007) can be summarized in a few steps:

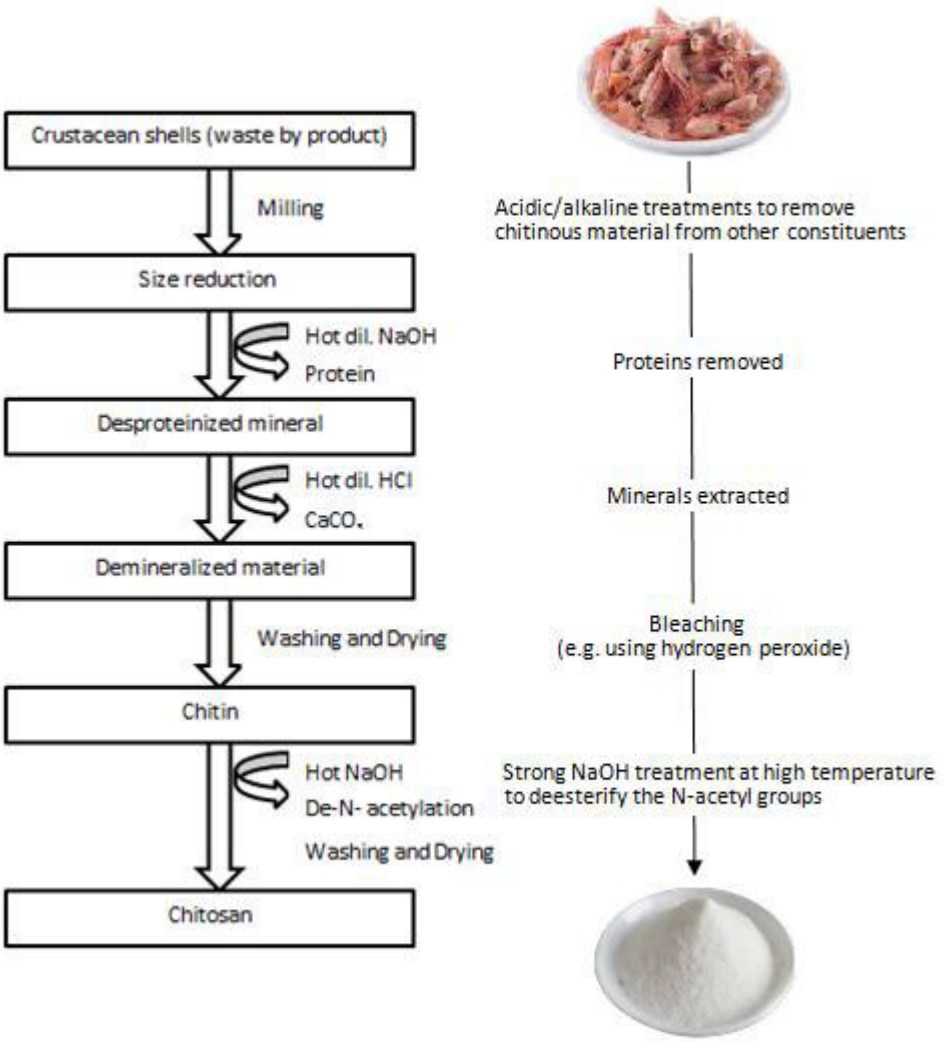

Figure 4. Isolation process of chitin and chitosan

Chitosan was first discovered in 1859 by Rouget and it is a highly basic polysaccharide, in contrast to most of the naturally occurring polysaccharides which are neutral or acidic (e.g. pectin and cellulose). The nitrogen content varies between 5% and 8% depending on the deacetylation level. The relative amount of the two monosaccharides in chitosan may be different, resulting in products with different degrees of deacetylation (75-95%), viscosities, molecular weights (50-2000 kDa), pK_a values (Raafat & Sahl, 2009). Although insoluble in most solvents, chitosan is soluble in dilute organic acids such as lactic acid, acetic acid, malic acid and formic

acid. Its use is limited due to chitosan insolubility in water, tendency to coagulate with proteins at high pH, high viscosity and relatively low resistance to water vapor transfer compared to lipid materials (Rabea, et al., 2003; Baldwin, 2007).

The conversion of chitin into chitosan lowers the molecular weight and changes the degree of N-acetylation, i.e. the ratio of GlcNAc to GlcN structural units, which influences its solubility and solution characteristics (Srinivasa & Tharanathan, 2007). Chitosan is a biomolecule with great potential because of its numerous physical-chemical and biomedical characteristics. Figure 5 shows some of them.

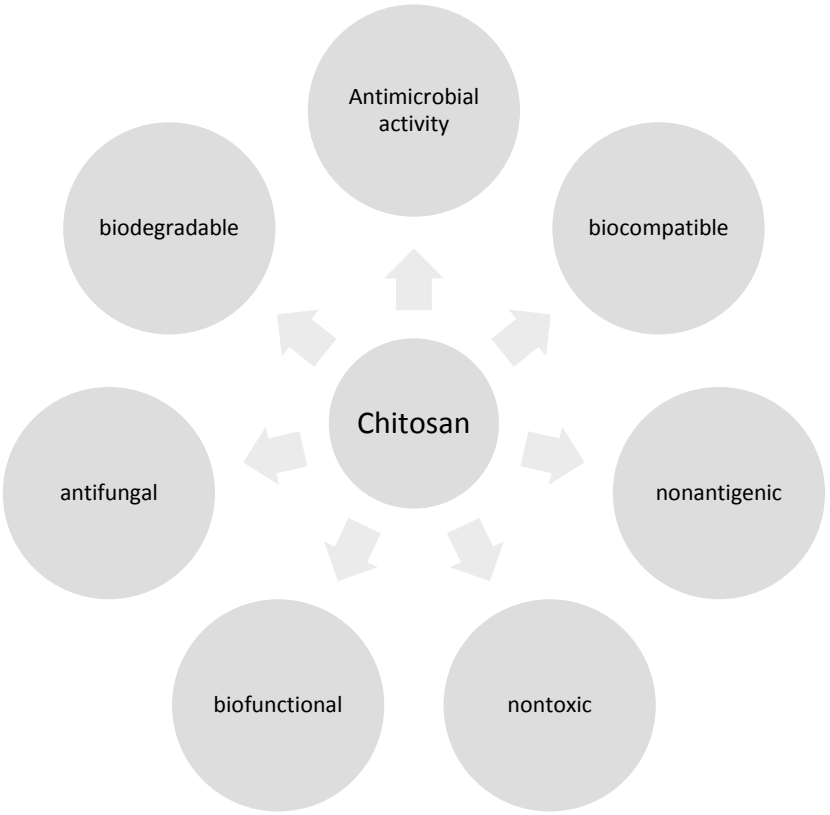

Figure 5. Physical-chemical and biomedical characteristics of chitosan

Chitosan stands out for its biodegradation properties and for the use of replenishable resources minimizing its impacts on the environment. Despite chitosan solutions are very stable over a long period of time, chitosan may suffer enzymatic degradation by non-specific enzymes,

as variable as chitinases, proteases, lipases, cellulases or hemicellulases (Raafat & Sahl, 2009; Srinivasa & Tharanathan, 2007). The antimicrobial and antifungal activity of chitosan seem to come from its polycationic nature (Elsabee & Abdou, 2013) and was found to increase with ionic strength but decreases with addition of metal ions (Baldwin, 2007). Chitosan is well tolerated by animal living tissues (e.g. ocular membranes, skin) and also plant tissues. This tolerance is granted by its biocompatibility, i.e. it´s not affected by the host and simultaneous don´t produce any undesirable local or systemic effects. The low toxicity of chitosan when compared with other natural polysaccharides is a favorable and attractive biological characteristic. It´s nontoxicity and biological functionality comes probably from its ability to exhibit polymorphism (Srinivasa & Tharanathan, 2007; Raafat & Sahl, 2009).

Chitosan was introduced to the market in the 90's of the XX century, and its use is growing since. Chitosan, its oligomers and many derivatives emerged as new biomaterials for a large range of industries as food and agriculture, medical, cosmetics, pharmaceutical and textile. Table 2 (Raafat & Sahl, 2009; Srinivasa & Tharanathan, 2007) lists examples of these applications.

Table 2. Industrial applications of chitosan

Applications	Advantages/benefits
Paper finishing	Imparts wet strength to paper
Fish, fruit and vegetables	Improve shelf life, delay fungal growth and prevent moisture loss
Medical	Increase glucose tolerance and insulin secretion
Effluent purification	Clarify waste and effluent water
Cosmetics	Used as active ingredients in hair shampoo and conditioner, gives softness to hair and skin

The use of chitosan for human consumption is shrouded in some controversy. Approved as supplement, chitosan has not received approval for food use by the Food and Drug Administration (FDA) in the United States, although it is approved in Canada (Baldwin, 2007). The term GRAS (Generally Recognized As Safe) is a FDA designation to indicate that a substance or a chemical added to foods and beverages is considered safe. Although FDA did not proclaim officially chitosan as GRAS, its use for medical uses (e.g. drug encapsulation and bandages) is approved (Raafat & Sahl, 2009). Primex ehf, an Iceland marine biotech company specialized in the manufacture and supply of chitosan and chitin derivatives, had submitted to FDA three GRAS claims for its shrimp-derived chitosan in three different occasions (January 2001, April 2005 and August 2012), but all submissions were cancelled before a FDA final verdict (FDA 2013). However, in 2001, Primex announced that its purified chitosan product named ChitoClear® has achieved a GRAS self-affirmed status in the US market (Raafat & Sahl, 2009).

The European Food Safety Authority (EFSA) panel confirmed that a cause-effect relationship has been established between the consumption, by adults, of 3 g of chitosan daily and the normal blood LDL-cholesterol concentrations (EFSA, 2011).

Chapter 3. Heat Transfer

From Thermodynamics it is known that energy exists in various forms and historically heat has always been perceived to be something that produces in us a sensation of warmth (Çengel, 1997). Heat is a form of energy that is transferred from one system to another as result of a temperature difference. This temperature difference is the driving force that allows the heat transfer (Azeredo, 2010).

The Principle of energy conservation (1st law of Thermodynamics) says that: "During the interaction between a system and the surroundings the amount of energy gained by the system is exactly the same lost by the neighborhood." That is, if a hotter solid comes into contact with a colder fluid, the heat lost by the solid is gained in the exactly same amount by the fluid and vice-versa. The amount of heat transferred may be estimated by Equation 1 under constant pressure conditions:

$$Q = m\, C_p\, \Delta T \qquad \text{Equation 1}$$

where: Q (J) = heat transferred, m (kg) = mass, Cp (J kg^{-1} K^{-1}) = heat capacity and ΔT (K) = temperature variation.

There are three different mechanisms through which heat can be transferred (Çengel, 1997):

- Conduction - energy transfer from the more energetic particles of a substance to the adjacent less energetic ones as a result of interactions between particles;
- Convection - energy transfer between a solid surface and the adjacent liquid or gas that is in motion, involving the combined effects of conduction and fluid motion;
- Radiation - energy emitted by matter in the form of electromagnetic waves (or photons) as a resust of the changes in the electronic configurations of the atoms or molecules.

3.1. Heat transfer in transient state

The processes of heat transfer are generally classified as being steady-state or transient (or unsteady). In steady-state the system temperature does not change; in transient state the system temperature varies with time and there may be heat accumulation or depletion (Azeredo, 2010). Heat transfer problems are also classified as being one-dimensional (temperature in the medium varies in one direction only and heat is transferred in one direction - heat transfer in other directions is negligible or zero); two-dimensional (temperature in a medium varies mainly in two primary directions - heat transfer in the third direction is negligible); three-dimensional (temperature varies along all three primary directions within the medium through the process of heat transfer) (Çengel, 1997). Figure 6 is a scheme of typical one-dimensional heat transfer geometries.

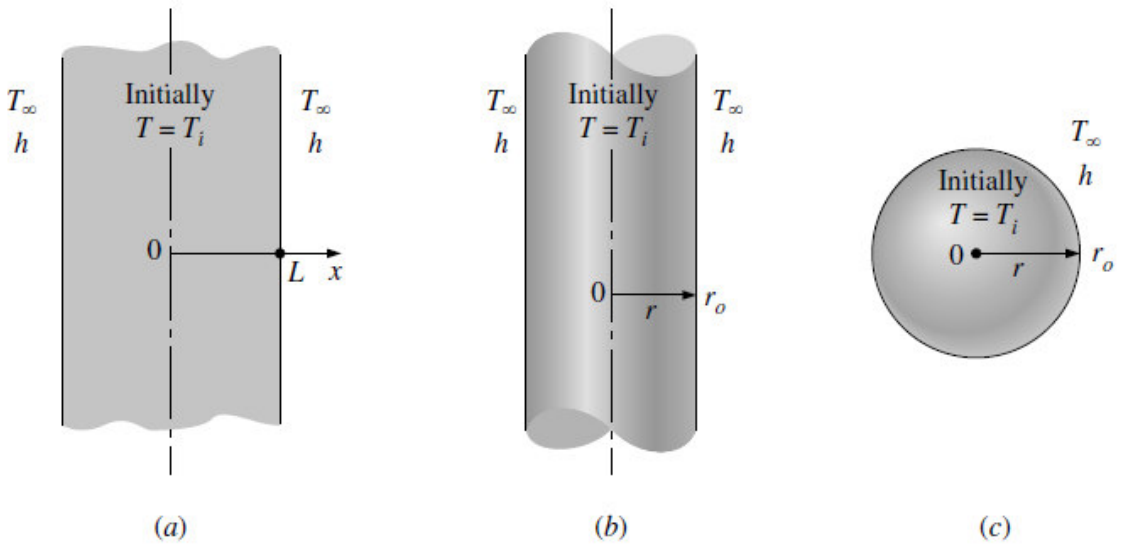

Figure 6. Representation of one-dimensional heat transfer in a large plane wall (a), a long cylinder (b) and a sphere (c).

As illustrated in Figure 7 in a plane wall with 2L thickness and geometric and thermal symmetry (center plane - x=0) heat transfer occurs mainly by convection between the plane wall and its environment. At time t=0 the entire wall is at its initial temperature (T_i) but as the time increases, the wall temperature and near the surfaces begins to drop because of the heat transfer from the wall to the surrounding medium ($T_i > T_\infty$). This results in a temperature profile (symmetric at all times about the center plane) that becomes flatter as time passes and eventually gets uniform at $T = T_\infty$. At this point, the heat transfer ends because there is no temperature difference, and the wall reached the thermal equilibrium with its neighborhood (Çengel, 1997).

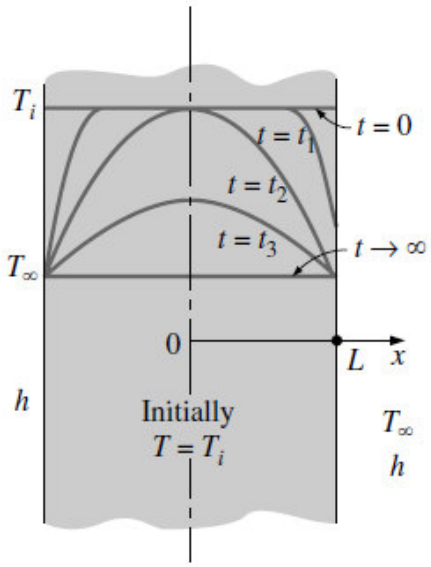

Figure 7. Schematic of transient temperature profile in a infinite and symmetric plane wall exposed to convection from its surfaces, with $T_i > T_\infty$.

One way to solve the problems of heat transfer in transient state is through the 2nd law of Fourier, using Equation 2:

$$\frac{\partial T}{\partial t} = \frac{1}{x^{(\gamma-1)}} \cdot \frac{\partial}{\partial x} \cdot \left[\alpha . \mathrm{x}^{(\gamma-1)} . \frac{\partial T}{\partial x}\right] \qquad \text{Equation 2}$$

In order to simplify this calculation there are solutions for the 2nd law of Fourier (Perry & Green, 2007). Thus, for the boundary conditions of an infinite plane wall (= 1) we have:

$$\frac{T-T_W}{T_0-T_W} = \frac{4}{\pi}\sum_{n=0}^{\infty}\frac{(-1)^n}{2n+1}\cos\left(\frac{(2n+1)\pi.x}{2L}\right)\exp\left(-\frac{\alpha.(2n+1)^2\pi^2 t}{4L^2}\right) \quad \text{Equation 3}$$

With:
$$\begin{cases} t = 0 & \forall, & T = T_0 \\ t > 0 & x = 0 & \frac{\partial T}{\partial x} = 0 \\ t > 0 & x = L & T = T_W \end{cases}$$

Where: T (K) = wall temperature at the coordinate x, T_w (K) = wall surface temperature, T_0 (K) = initial wall temperature, x (m) = wall depth, L (m) = wall semi-thickness and α ($m^2\ s^{-1}$) = thermal diffusivity.

And:

$$\alpha = \frac{k}{\rho.C_p} \quad \text{Equation 4}$$

Where: k ($W\ m^{-1}\ K^{-1}$) = thermal conductivity, ρ ($kg\ m^{-3}$) = density and C_p ($J\ kg^{-1}\ K^{-1}$) = heat capacity.

The thermal diffusivity (α) can be described as the quotient of the conducted heat and the stored heat and is defined as the speed that heat diffuses through a material (Azeredo, 2010).

3.1.1. Phase transition

The transition of a fluid from liquid to solid and back is called a phase transition and takes place isothermally at the phase transition temperature by absorption or release of latent heat. This phase change can be schematized by a phase diagram, as represented in Figure 8 (Fellows, 2000).

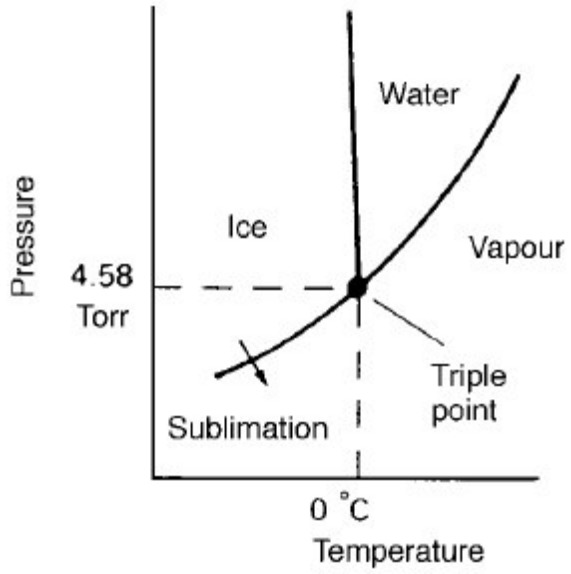

Figure 8. Representation of water phase diagram

Phase transition is important in many types of food processing as for example water to water vapour in distillation and evaporation and water to ice in freezing (Fellows, 2000). The same happens in glazing, which due to temperature differences between fish (coldest) and water/coating (hottest) leads to a phase transition of the coating from liquid to the solid state.

Part II - Experimental work

Chaper 4. Methodologies

4.1. Preparation of the samples

4.1.1. Fish preparation

At Vanibru - Comércio de Produtos Alimentares facilities (Braga, Portugal) frozen fillets of Atlantic salmon (*Salmo salar*) with proximally 1 kg, were carefully and evenly cut in a parallelepipedic shape with a vertical bone sawing machine (FK 32, BIZERBA, Germany) in a refrigerated room, where the temperature did not exceed 8 °C. The dimensions of the salmon pieces were approximately 6 cm x 2 cm x 2.5 cm (Figure 9) and presented an average weight of (26.4 ± 3.4) g for chitosan tests and (29.42 ± 2.01) g for water tests. These samples (Figure A.1) went for storage in a freezer with a pre-established and fixed temperature (Table 3), and stayed at least 24 hours in those conditions before any use in order to stabilize.

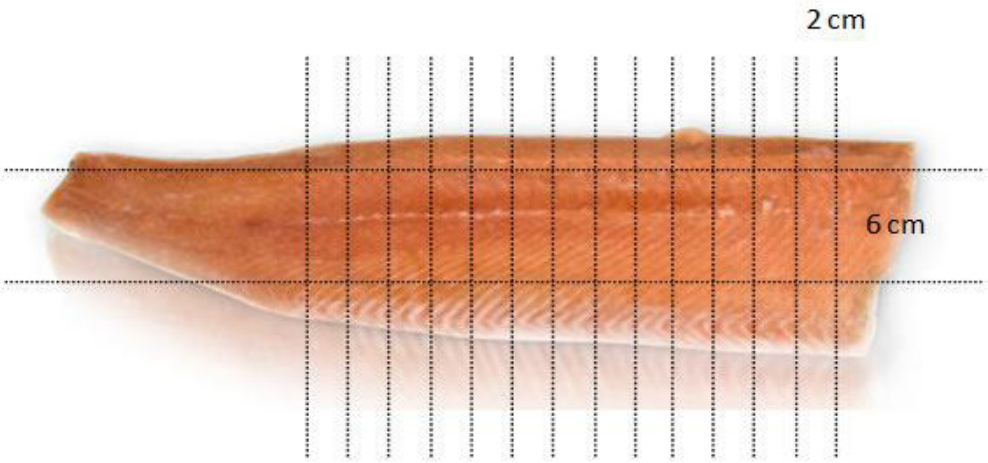

Figure 9. Representation of the scheme used to cut the salmon fillet (cuts along the dashed lines)

4.1.2. Coating solution preparation

The chitosan solution was prepared at 1.5% w/v. In a 2-L flask 22.2 mL of 1% v/v lactic acid (90% (w/w) purity) were added and the volume was completed with distilled water. Then the flask was placed in a heating plate at 45 °C under agitation. Slowly, 30±0.1 g of chitosan (from Golden-shell Biochemical Co. Ltd. China, with a 91% deacetylation degree) were added and stirred. This solution was left overnight under stirring to complete dissolution. After cooling, the chitosan solution was transferred to a closed glass container and stored in the refrigerator at the desired temperature (Table 3).

In the case of glazing with water no preparation was required. The dips were perfomed in a glazing bath equipment (HRG, GL3001, Spain) at the desired temperature (Table 3).

Table 3. Coating/water and salmon storage temperature

Coating	Coating temperatures (°C)	Salmon Temperature (°C)
Chitosan	2.5; 5; 8	-15 -20 -25
Water	0.5; 1.5; 2.5	

4.1.3. Salmon dipping in chitosan/water

Samples of frozen salmon were withdrawn from the freezer and weighed (RADWAG WLC 6/A2/C/2, Poland). They were subsequently immersed in the chitosan solution/water for 10, 20, 30, 40, 50 and 60 s and left to drain for about 180 s. Then salmon was reweighed and the amount of glazing was calculated using Eq. 5, where W_i is the weight of salmon before application of the coating and W_f is the weight of salmon after the application. All parameters were tested in triplicate. After coating application the salmon samples were packed in numerated zip-lock polyethylene bags and stored at -25 °C for at least 24 hours.

$$\%\ Glazing = \frac{W_f - W_i}{W_f}.100 \qquad \text{Equation 5}$$

Table 3 displays the various tested combinations of temperatures of salmon and temperatures of coatings/water. The solution temperature was monitored by an infrared Pronto Plus thermometer (HANNA Instruments, HI99556-10, Romania) with the respective probe (HANNA Instruments, HI765PW, Romania). The salmon temperature was monitored by a data logger (DS7922 1Wire® Thermochrom® iButton®, Dallas Semiconductor Inc., U.S.A.) stored in the industrial freezer together with the salmon samples.

Table 4. Coating/water and salmon temperatures of the tests carried out in the present work

	Coating temperatures (°C)	**Salmon temperature (°C)**
Chitosan	(2.4±0.11) ; (4.84±0.27) ; (7.75±0.49)	(-11.90±0.33) (-17.64±0.70) (-22.42±0.76)
Water	(0.51±0.06) ; (1.58±0.09) ; (2.53±0.07)	

4.1.5. Photos of salmon samples

Using the same cutting conditions described above, salmon pieces were cut with only a few millimeters wide. These samples were placed in numbered zip-lock polyethylene bags and placed back in the freezer at -25 °C to stabilize the temperature. Subsequently the samples were quickly transported to the laboratory and placed in a ultrafreezer (Cryocell DD86-750P) at -80 °C for at least 24 hours.

The salmon sample was placed in the center of the OLYMPUS magnifying glass plate (OLYMPUS SZ-CTV, Japan) and photographed with the 0.67 magnification[1] using the program "Image-Pro Plus" (op+I). To measure the coating thickness the picture was re-opened and the calibration "graph paper 0.67[1] has been chosen. The coating thickness was measured at various points (Figure 10 and 11). The process was repeated for the remaining samples.

[1] Previously carried out, graph paper was photographed and the relative distance of 1 millimeter was defined.

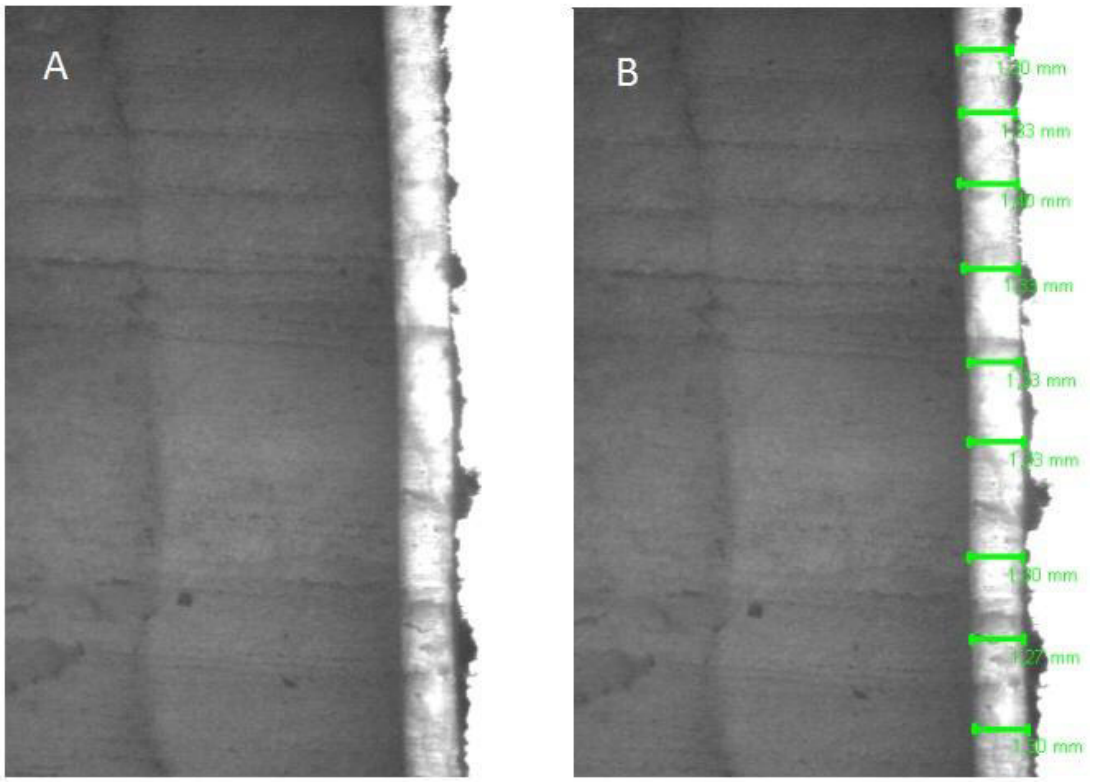

Figure 10. Example of photograph taken to salmon wall after coating with chitosan (A) and corresponding measurements (B).

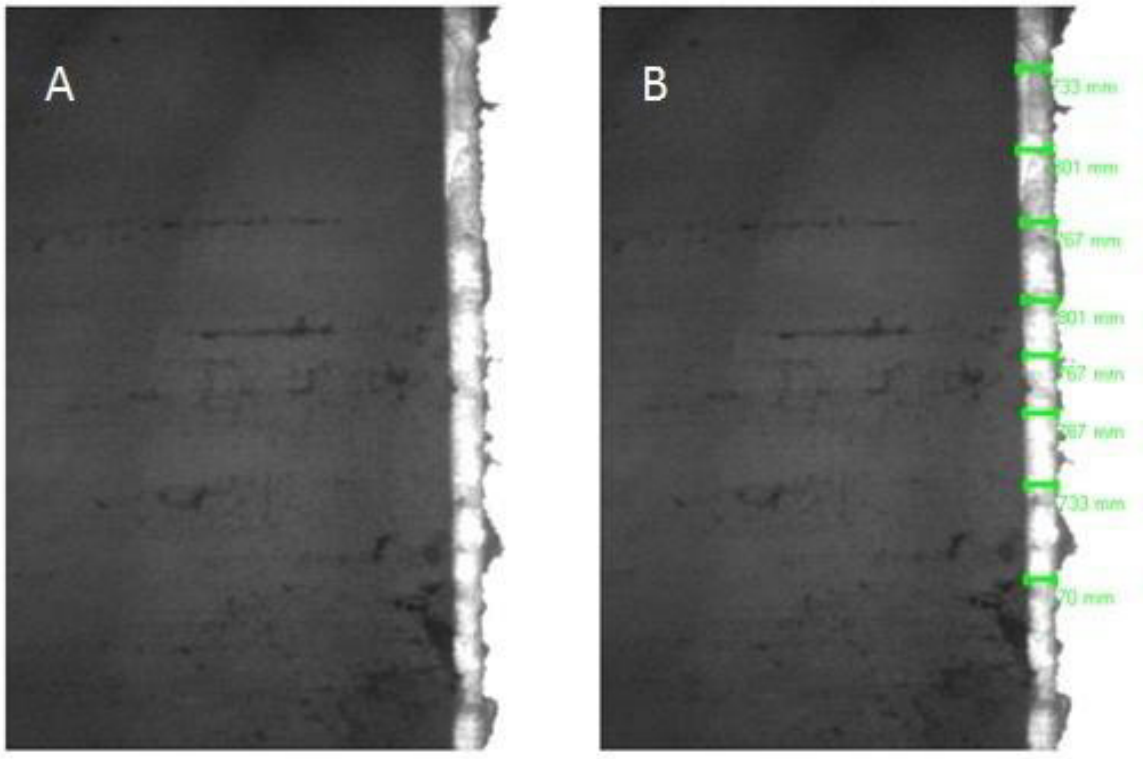

Figure 11. Example of photograph taken to salmon wall after glazed with water (A) and corresponding measurements (B).

4.2. Analytical and statistical analysis of the results

4.2.1. Differential scanning calorimetry (DSC)

DSC meauserements were performed with a PYRIS Diamond DSC (Perkin Elmer, USA). About 30 mg of 1.5% w/v chitosan solution was placed in aluminium DSC pans. The samples were heated from -30 to 50°C at a heating rate of 10 °C min^{-1} under a nitrogen atmosphere.

4.2.2. Determination of temperature profile

It was possible to set a temperature profile of salmon using Equations 3 and 4, representing how the salmon final temperature varies from the center to the surface. From this profile, it was possible to calculate the average temperature (T_{av}) of salmon for each dipping time, by calculating the area under each curve. Through a 2nd order polynomial fit it was possible to calculate the average temperature using Equation 6:

$$T_{av} = \frac{\int_{x_1}^{x_2} T\,dx}{\Delta x} \qquad \text{Equation 6}$$

4.2.3. Determination of heat transferred

The heat received by salmon samples upon immersion in the the coating was calculated using Equation 7:

$$Q_{salmon} = m_{salmon}\, C_{p\,salmon}\, (T_{av} - T_i) \qquad \text{Equation 7}$$

4.2.4. Statistical analyses

The tests conducted for each set of parameters were performed in triplicate. For each triplicate five measurements of thickness were made, which results in 15 thickness results for each condition. The data were subjected to one-way analysis of variance (ANOVA) using STATISTICA 10 software (StaSoft Inc. 2013), while means were compared by Tukey's test with the level of significance set at $p<0.05$. The same software was also used to perform a multivariate statistical analysis, using Principal Component Analysis (PCA).

Chapter 5. Results and Discussion

5.1. Differential scanning calorimetry (DSC)

Differential scanning calorimetry is a technique used for the measurement of the difference between the energy supplied to a sample and the energy supplied to a reference material, being both subjected to a controlled temperature programming (Souza, et al., 2010). DSC measures the amount of energy (heat) released or absorbed by a sample as it is cooled, heated or held at constant temperature, also performing precise temperature measurements.

The results of heat flow regarding a 1.5% w/v chitosan solution are presented in Figures 12. The thermal properties of 1.5% w/v chitosan solutions are summarized in Table 5 and compared with water properties.

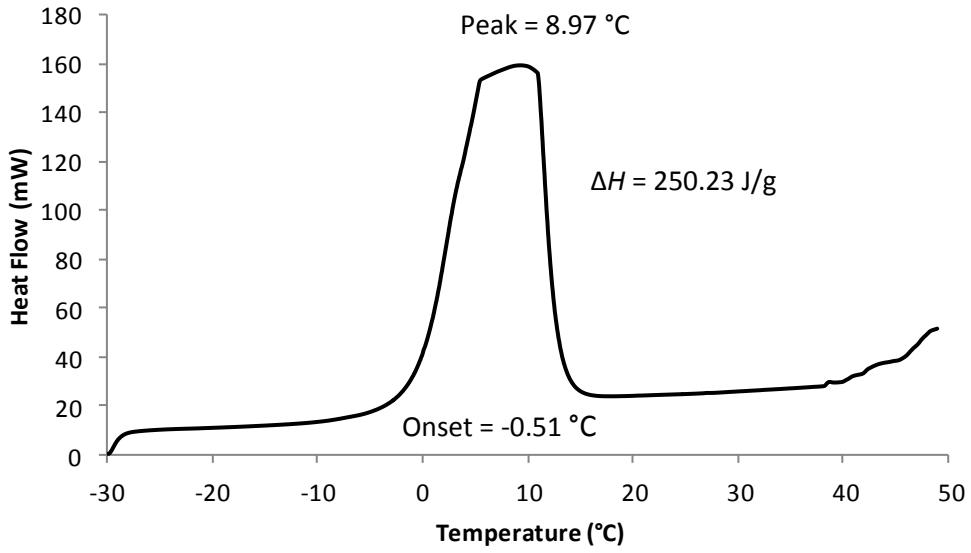

Figure 12. Heat flow variation of a sample of chitosan heated from -30 to 50 °C at a heating rate of 10 °C min^{-1} under a nitrogen atmosphere.

Table 5. Comparison of thermal properties of a 1.5% w/v chitosan solution and water

	ΔH (J g^{-1})	Freezing temperature (°C)
Water	334	0
Chitosan	250.23	-0.51

5.2. Thickness of coatings from chitosan solutions

The application of chitosan solutions at 2.5, 5 and 8 °C on frozen fish at -25, -20 and -15 °C with different dipping times (10, 20, 30, 40, 50, 60 s) results in different coating thicknesses. The final thickness for each set of parameters was measured in the pictures taken to the salmon. Figure 10 shows an example of a salmon picture with chitosan coating and the respective measurements.

The variation of coating thickness with dipping time is represented in Figures 13, 14 and 15.

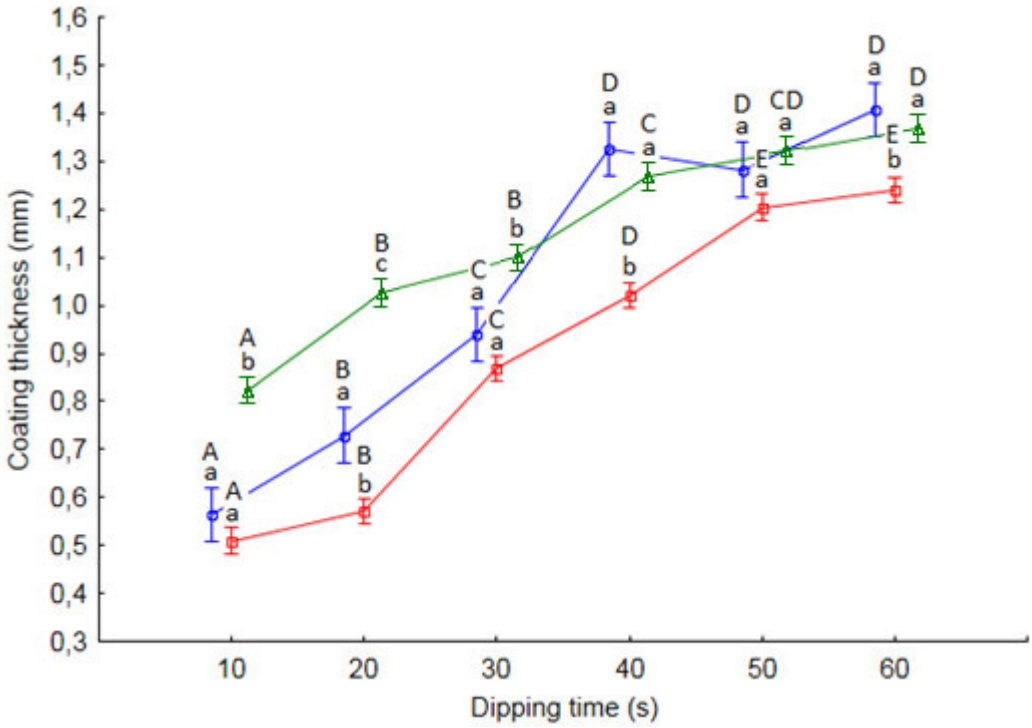

Figure 13. Coating thickness as a function of dipping time for salmon at -25 °C coated with chitosan at 2.5 °C (△), 5 °C (□) and 8 °C (○). Each point represents the mean ± standard deviation of fifteen replications. Different small letters in the same dipping time and different capital letters in points with the same color/marker indicate a statistically significant difference (Tukey test, $p<0.05$).

These results show that generally thickness increases with higher dipping time and lower coating temperature, except for the chitosan temperature of 8 °C that presents higher values of thickness than 5 °C. For short dipping times (10 to 30 s) the thickness for the temperatures of 8 and 5 °C are similar and did not compensate the energy spent to lower the chitosan solution temperature. However in the case for the temperature of 2.5 °C, the opposite is verified and the thickness for these conditions is consistently higher. This can be explaned by the lower temperature of the coating solution (2.5 °C), which is closer to chitosan's freezing point which therefore requires a lower quantity of energy for the phase change (liquid to solid). As chitosan temperature drops, specific heat also decreases (less heat is required to lower the temperature) which makes the temperature of 2.5 °C better able to exchange heat (Fellows, 2000). For longer dipping times (60 s) the coating thickness for 2.5 and 8 °C are statistically similar, reaching a maximum of (1.41 ± 0.05) mm, while at a temperature of 5 °C lower thickness values are attained (1.24 ± 0.03) mm.

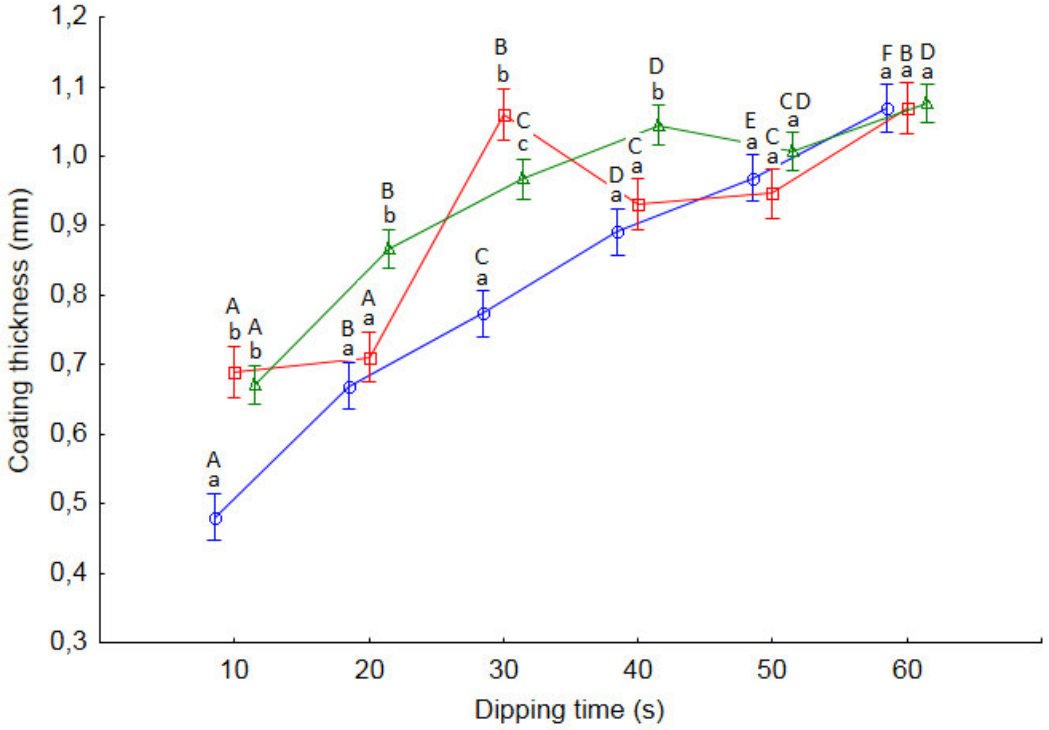

Figure 14. Coating thickness as a function of dipping time for salmon at -20 °C coated with chitosan at 2.5 °C (△), 5 °C (□) and 8 °C (○). Each point represents the mean ± standard deviation of fifteen replications. Different small letters in the same dipping time and different capital letters in points with the same color/marker indicate a statistically significant difference (Tukey test, $p<0.05$).

Using the same conditions of chitosan temperatures and dipping time, the increase of the salmon temperature in general decreases the final thickness. So, for a salmon temperature of -20 °C, the thickness for the higher coating temperature (8 °C) is the most affected. This can be explained by the lower capacity of the salmon to receive heat, transferred from the chitosan solution, because the difference between salmon and chitosan temperatures is smaller. However for longer dipping times there are lower thickness differences between the three chitosan temperatures, ending at 60 s to have all very similar results (being statistically equal). In these conditions, for the longgest dipping time (60 s) the maximum thickness obtained was (1.08 ± 0.03) mm, a lower result comparing with the salmon at -25 °C that was (1.41 ± 0.05) mm.

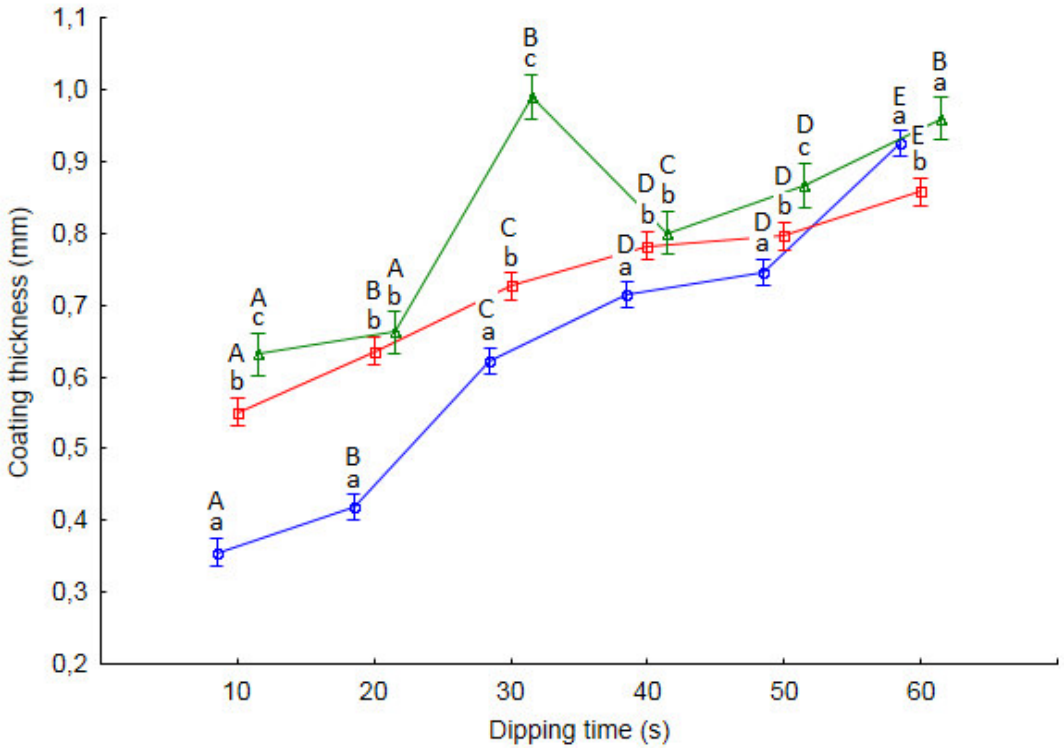

Figure 15. Coating thickness as a function of dipping time for salmon at -15 °C coated with chitosan at 2.5 °C (△), 5 °C (□) and 8 °C (○). Each point represents the mean ± standard deviation of fifteen replications. Different small letters in the same dipping time and different capital letters in points with the same color/marker indicate a statistically significant difference (Tukey test, p<0.05).

The results for salmon at -15 °C show that as long as the salmon temperature increases, the coating thickness for all chitosan solution temperatures will decrease. The most affected for this change is the chitosan temperature of 8 °C because of the same reasons mentioned above. Despite of this, for longer dipping times the differences between the three thicknesses are not significant. The highest thickness in these conditions is verified for a chitosan solution temperature of 2.5 °C, reaching (0.96 ± 0.03) mm. These results are the lowest in comparison with those obtained for the other salmon temperatures.

A study performed by El-hefian et al. (2010) showed that as chitosan solution temperature rises the correspondent viscosity falls. A higher viscosity can assure a superior adhesion between salmon and chitosan solution leading to a higher final thickness, since it has a greater resistance to movement. The thicknesses obtained for the various chitosan solution temperatures are in agreement with these results, since even though the temperature differences are relatively small, the chitosan solution temperature which presents higher thicknesses is the lowest (2.5 °C).

Analyzing the variation of salmon temperature by Equation 1 it can be seen that (keeping the other properties constant) the temperature variation (ΔT) is directly proportional to the heat transferred (Q), i.e. a larger temperature difference between the salmon and chitosan leads to a higher amount of heat transferred and consequently to a greater thickness of the chitosan coating.

The observation and analysis of all the results together indicates that cooling the salmon allows increasing the of thickness, being the salmon at -25 °C the one with a higher thickness. The temperature of the coating is more influent in short dipping times, being a little irrelevant for longer dipping times. However, in general, the lower the temperature the higher is the resulting coating thickness. It was also verified that under all tested conditions, higher dipping time results in greater thicknesses and 60 s does not limit the growth of thickness, i.e. in no case the thickness stabilizes/decreases. It was also noted that the colder the salmon, the more similar are the thicknesses of the various temperatures of chitosan.

5.2.1. Statistical analyses

The Multivariate Statistical Process Control enabled data analysis using the method of Principal Component Analysis (PCA), for the purpose of dimensionality reduction, diagnostics variables, and outlier detection. This follow-up analysis allowed finding which dipping conditions affected more significantly (positively or negatively) the thickness of the chitosan coating. In Figure 16 is shown an example of this analysis' results, where the condition in study is the salmon temperature. The remaining results are shown in Figures 17 and 18. The different dipping times and temperatures used affected differently the final thickness values, managing to display groups of results (same color corresponds to samples with the same conditions).

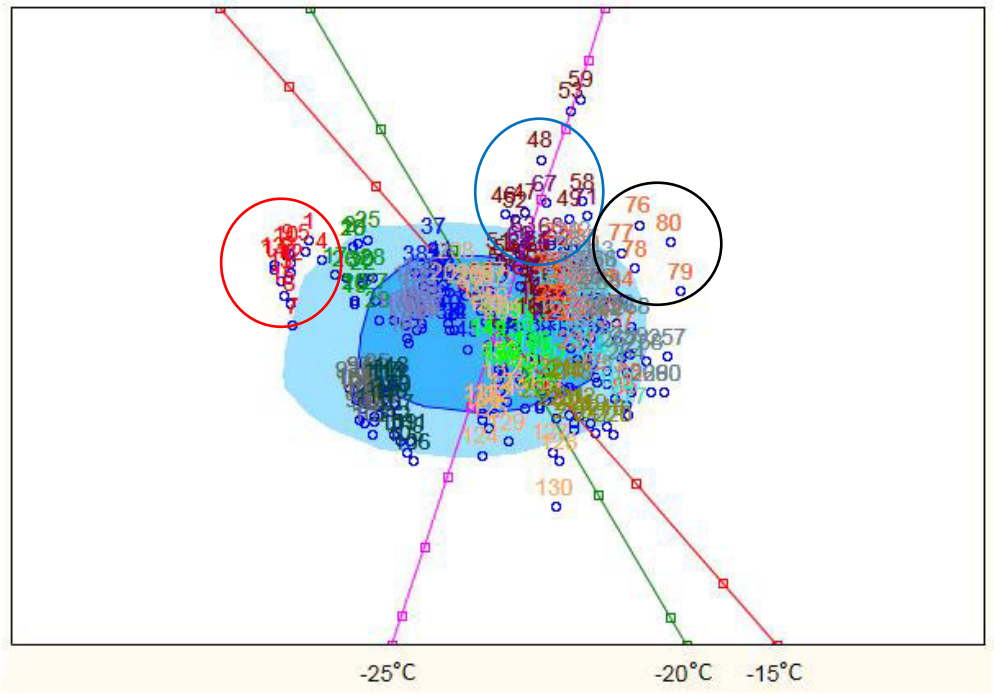

Figure 16. Principal component analysis for chitosan coatings in salmon. The variable in study is the salmon temperature and groups with the same color correspond to samples with the same conditions. Principal component 1 (PC1) corresponds mainly to dipping time and justifies 85.4% of variability.

In this analysis the samples that deviate from others indicate significant differences for the conditions in question, mainly due to the variation of the initial temperature of the salmon. In Figure 16 samples numerated from 1 to 15, corresponding to the coating temperature of 8 °C and 10 s of dipping time, separate from the others with significant differences in thickness (samples circled in Figure 16 by a red line). In these cases the temperature of the salmon greatly influences the thickness under these conditions. The same is true in samples 46 to 60 (excluding outliers 53 and 59, Figure A.2) corresponding to the coating temperature of 8 °C and dipping time of 40 s, that separate from the others seeming to have greater thickness (samples circled in Figure 16 by a blue line). Samples 76 to 80 also separate from the others with a greater thickness (coating temperature of 8 °C and dipping time of 60 s – samples circled in Figure 16 by a black line). These were the conditions of coating temperatures and dipping times for which salmon temperature appears to affect more the final thickness. This is in line with PC1 (which corresponds mainly to dipping time) justifying over 85% of the variability registered in these samples.

As illustrated in Figure 17, and being the variable in question the chitosan temperature, samples 1 to 15 (salmon at -25 °C and dipping time of 10 s), samples 16 to 30 (salmon at -25 °C and dipping time of 20 s), samples 91 to 105 (salmon at -20 °C and dipping time of 10 s), samples 181 to 195 (salmon at -15 °C and dipping time of 10 s) and samples 196 to 210 (salmon at -15 °C and dipping time of 20 s) appear to be more affected by chitosan temperature (these samples are circled in Figure 17 with a red line). This means that chitosan temperature influences mainly short dipping times, not being so significant for longer times.

Samples 46 to 90 correspond to the dipping time of 40, 50 and 60 s and salmon temperature of -25 °C (circled in Figure 17 with a blue line). These results deviate from the others, appearing to be the ones with the greatest thickness, which agrees with the fact that PC1 corresponds mainly to dipping time and accounts for almost 90% of the variability.

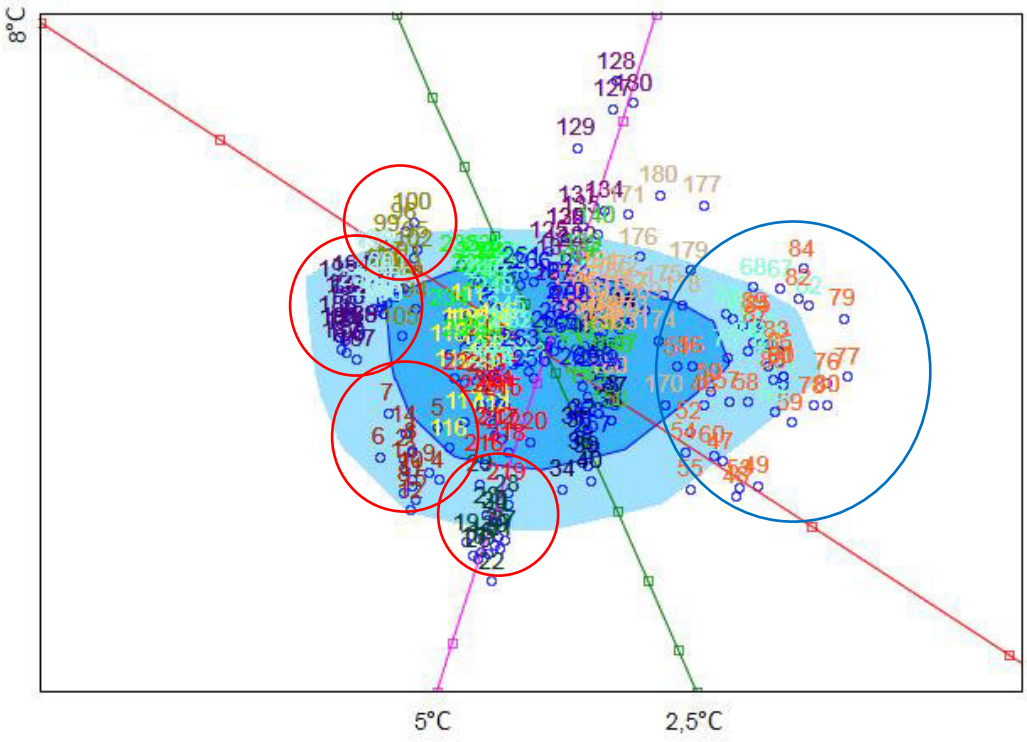

Figure 17. Principal component analysis for chitosan coatings in salmon. The variable in study is the chitosan temperature and groups with the same color correspond to samples with the same conditions. Principal component 1 (PC1) corresponds mainly to dipping time and justifies 88.6% of variability.

The same analysis performed now using as variable of study the dipping time is shown in Figure A.4. However, the presence of outliers at PC1 and PC2 values which are much larger than the remaining samples (cases 8, 14 and 99) does not allow taking any conclusion due to the viewing scale. These outliers were removed and the analysis was repeated (Figure 18) indicating that samples 91 to 105 (corresponding to a salmon temperature of -15 °C and chitosan temperature of 8 °C – samples circled red in Figure 18) have a smaller thickness, moving in the opposite direction from cases with greater thickness at a salmon temperature of -25 °C and chitosan temperature of 2.5 °C (from sample 31 to 45), confirming previous thickness results (circled in Figure 18 with a blue line). Salmon temperature and chitosan solution temperature are therefore the main influencing factors here, as corroborated by their influence in PC1 and PC2. This analysis showed that chitosan temperatures of 2.5 °C and 8 °C are the temperature limits

for the application of chitosan solutions because the samples under these conditions correspond to the border limits of the confidence intervals established for this analysis (95%).

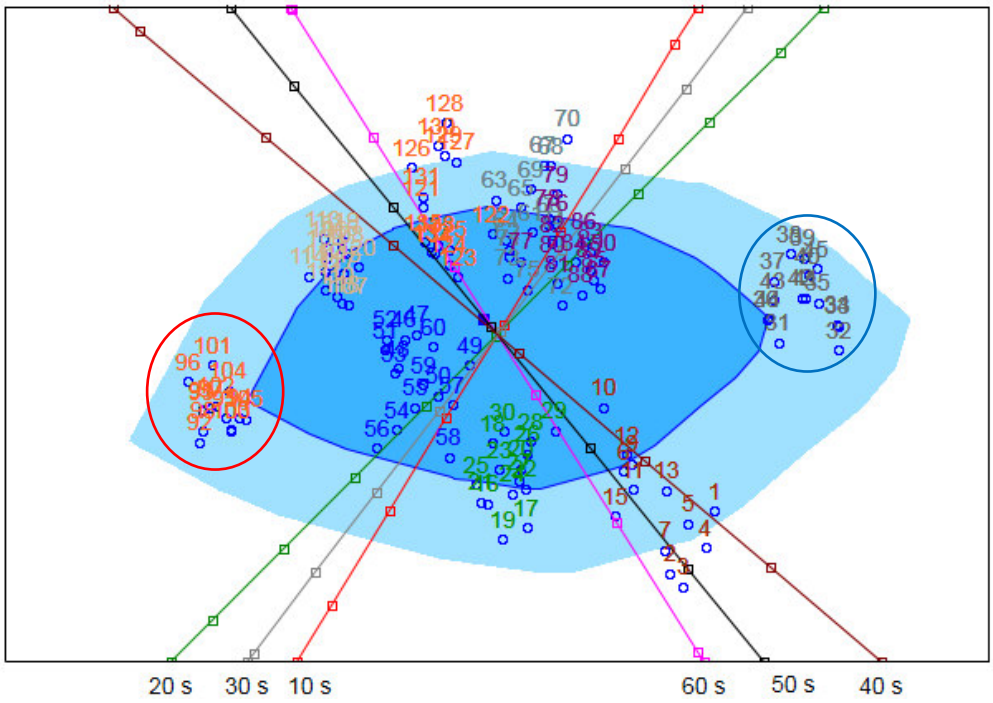

Figure 18. Principal component analysis without outliers for chitosan coating in salmon. The variable in study is dipping time and groups with the same color correspond to samples with the same conditions. Principal component 1 (PC1) corresponds to salmon temperature and chitosan temperature justifying 69.9% of variability; Principal component 2 (PC2) corresponds to salmon temperature and justifies 19.0% of variability.

5.3. Thickness of water glazing

The application of water at 0.5, 1.5 and 2.5 °C on frozen fish at -25, -20 and -15 °C with different dipping times (10, 20, 30, 40, 50, 60 s) results in different glazing thicknesses. The final thickness for each set of parameters was determined by the measuring of the pictures taken to the salmon wall. Figure 11 shows an example of salmon with water glazing and the respective measurements.

The variation of glazing thickness with dipping time is represented in Figures 19, 20 and 21.

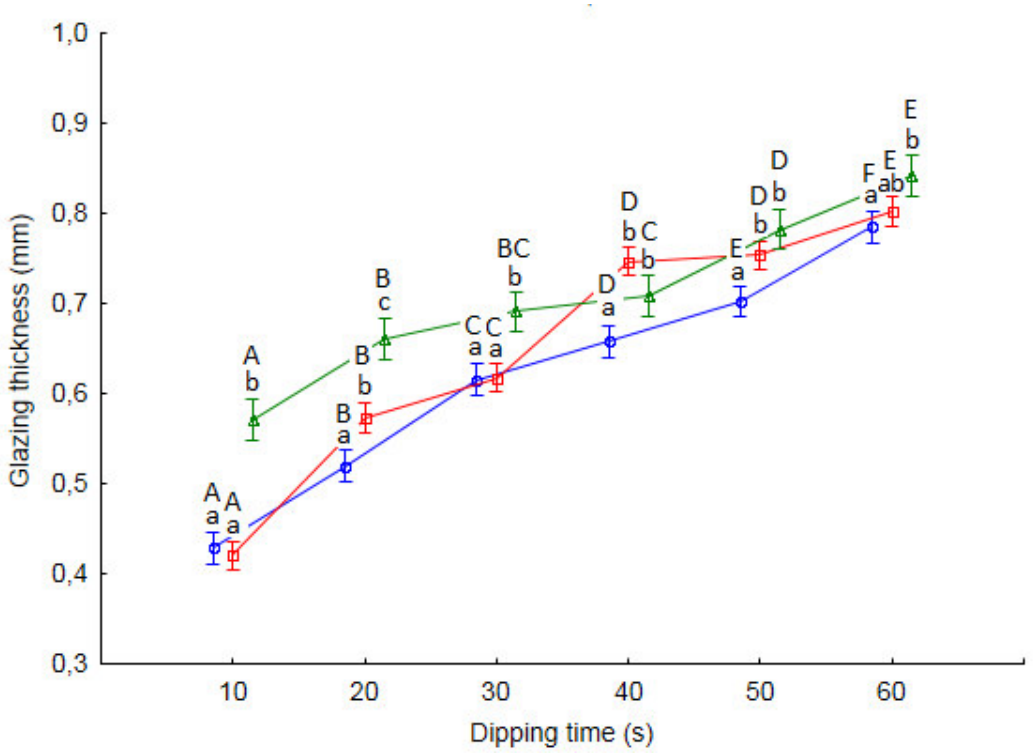

Figure 19. Coating thickness as a function of dipping time for salmon at -25 °C glazed with water at 0.5 °C (△), 1.5 °C (□) and 2.5 °C (○). Each point represents the mean ± standard deviation of fifteen replications. Different small letters in the same dipping time and different capital letters in points with the same color/marker indicate a statistically significant difference (Tukey test, $p<0.05$).

For salmon at -25 °C, the temperature of water at 0.5 °C shows the best overall results for short dipping times. However, for longer times, the difference tends to diminish and for 60 s of dipping time the glazing thickness values obtained at the three different temperatures are statistically similar, reaching a maximum of (0.84 ± 0.03) mm. It is also to be noted that in no case a stabilization or decrease in thickness was observed, therefore 60 s is not the limiting time for the increase of thickness. All the thickness values obtained with water glazing are below are lower than those obtained with chitosan coatings.

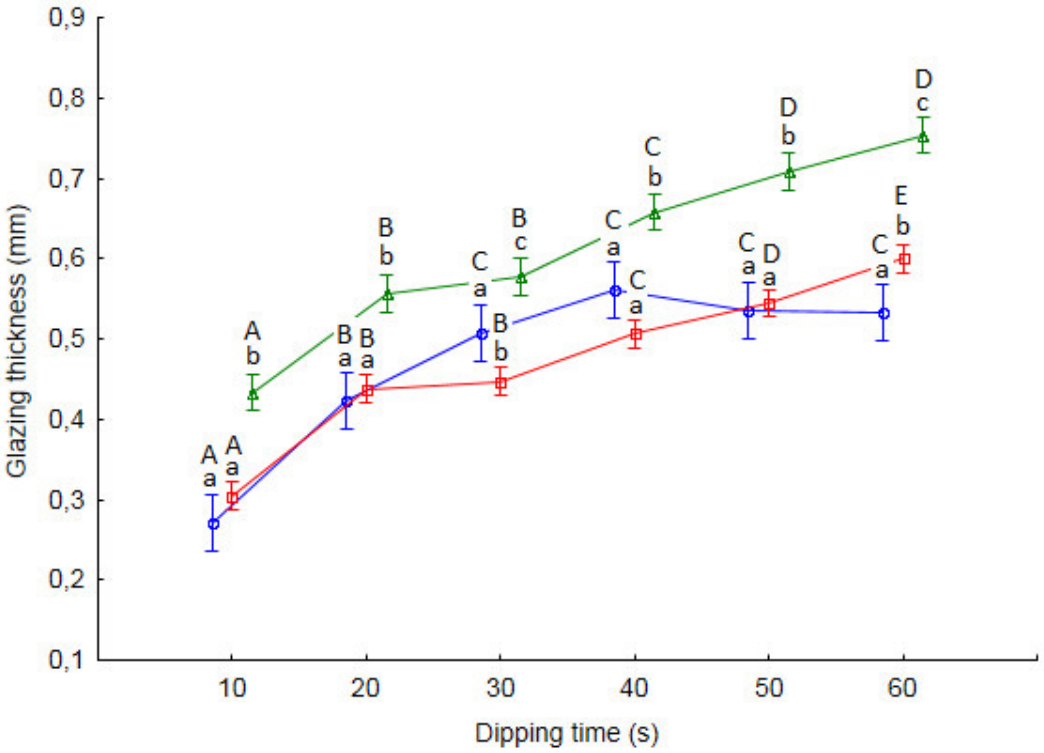

Figure 20. Coating thickness as a function of dipping time for salmon at -20 °C glazed with water at 0.5 °C (△), 1.5 °C (□) and 2.5 °C (○). Each point represents the mean ± standard deviation of fifteen replications. Different small letters in the same dipping time and different capital letters in points with the same color/marker indicate a statistically significant difference (Tukey test, p<0.05).

With the increase of the salmon temperature to -20 °C the thicknesses of the glazing obtained for various water temperatures decrease. As previously observed in chitosan the cases in which this effect is most clear are those of higher water temperatures (1.5 and 2.5 °C). The glazings obtained with water at these two temperatures have very similar thicknesses for all dipping times, with no statistically significant differences between the results. At the temperature of 0.5 °C, despite being the least affected, it is also observable a decrease in the final thickness of the glazing, and its maximum value is (0.75 ± 0.03) mm. At a water temperature of 2.5 °C the glazing thickness stabilizes after 40 s while at 1.5 and 0.5 °C a 60 s dipping time does not limit the thickness growth.

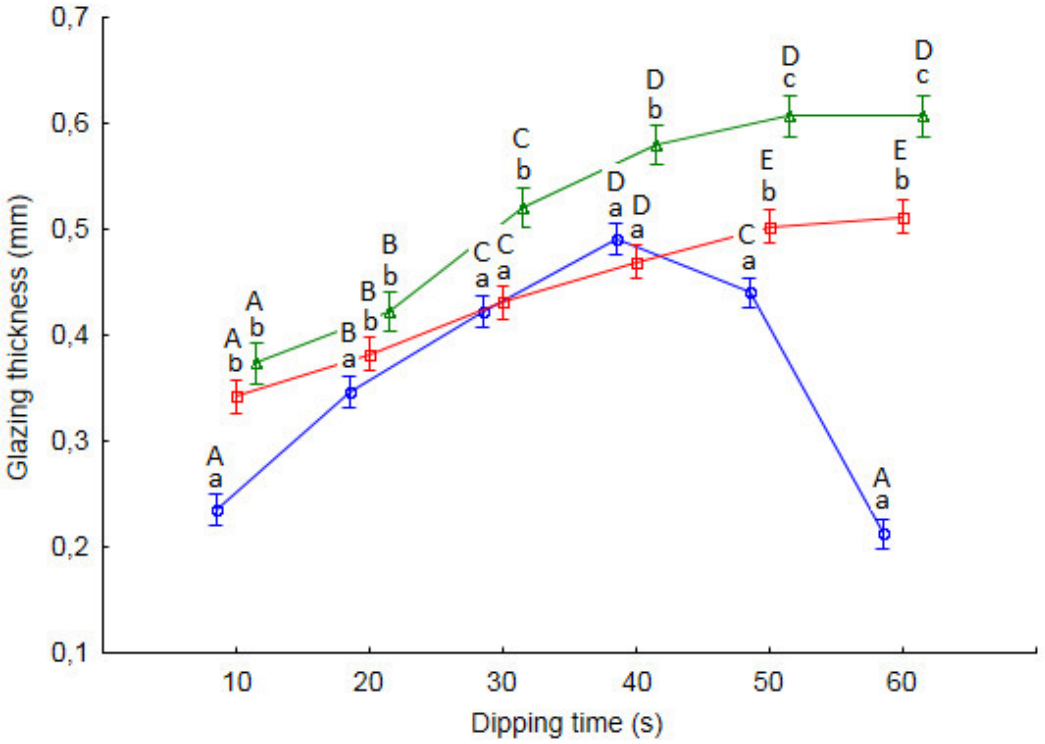

Figure 21. Coating thickness as a function of dipping time for salmon at -15 °C glazed with water at 0.5 °C (△), 1.5 °C (□) and 2.5 °C (○). Each point represents the mean ± standard deviation of fifteen replications. Different small letters in the same dipping time and different capital letters in points with the same color/marker indicate a statistically significant difference (Tukey test, p<0.05).

Using the same conditions of glazing water temperatures and dipping times, the increase of the salmon temperature consistently decreases the final glazing thickness. The maximum thickness on these conditions was achieved by water at 0.5 °C reaching a thickness of (0.61 ± 0.02) mm. It was possible to observe that after 40 s the thickness of glazing at 0.5 and 1.5°C appeared to stabilize, with no significant differences after this dipping time. At the higher temperature of 2.5 °C the thickness increases up to 40 s of dipping time, descending steeply for higher dipping times.

Despite the specific heat of water increases with decreasing water temperature, the differences are very slight and do not affect the final thickness. However, the lower the temperature the higher is the water viscosity, which makes colder water exhibit greater resistance to movement and therefore more easily adhere to salmon (ThermExcel, 2003).

In short, in comparison with chitosan, water has less ability to freeze on the product surface (phase change) even at lower temperatures. In water glazing the temperature of the salmon seems to be the most important since the colder the salmon the more similar are the thicknesses obtained for the various water temperatures. In general it was also possible to observe that after the dipping time of 40 s no major differences between thicknesses were recorded, thus not advising the use of higher dipping times.

Finally, the coating temperature of 2.5 °C may be considered as limiting to salmon temperatures higher than -25 °C because glazing thickness stabilizes after only 30-40 s. Also the temperature of -15 °C for salmon can be limiting because after 40 s the thickness is maintained or even lowers. Because it is not advisable the use of this conditions that do not allow an increase in thickness and may lead to an excessive increase in the salmon temperature, the concept of safe dipping time was introduced. Thus, for salmon at -20 °C and -15 °C, the safe dipping time would be 30-40 s.

5.3.1. Statistical analyses

The Multivariate Statistical Process Control used for chitosan coating data analysis was also performed for water glazing using the method of Principal Component Analysis (PCA). With this analysis it was found which dipping conditions affected more significantly (positively or negatively) the thickness of water glazing. Figure 22 shows an example of this analysis' results, where the condition in study is the salmon temperature. The remaining results are shown in Figure 23. The different dipping times and temperatures used affect differently the final thickness values, originating distinct groups of results (same color corresponds to samples treated under the same conditions).

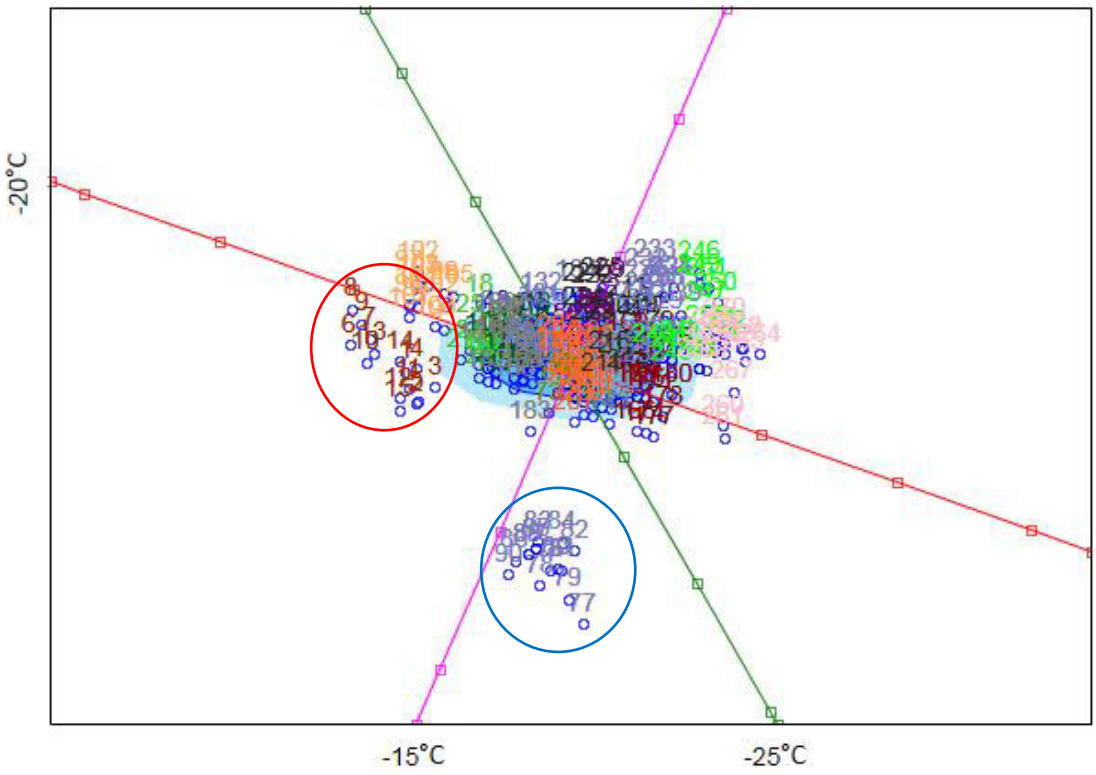

Figure 22. Principal component analysis for water glazing in salmon. The variable in study is the salmon temperature and groups with the same color correspond to samples with the same conditions. Principal component 1 (PC1) corresponds to glazing temperature and dipping time justifying 79.7% of variability; Principal component 2 (PC2) corresponds mainly to glazing temperature and justifies 14.2% of variability.

In this analysis the samples that deviate from each other indicate significant differences for the conditions in question, mainly due to the variation of the initial temperature of the salmon. The results illustrated in Figure 22 indicate that the change of salmon temperature affects especially two groups of samples. The position of samples 1 to 15 (with a water temperature of 2.5 °C and dipping time of 10 s – these samples are circled red in Figure 22) shows that the temperature of salmon significantly affects glazing thickness. The same is true for samples 76 to 90 (water temperature of 2.5 °C and dipping time of 60 s – these samples are circled blue in Figure 22) which deviate from the others, showing that the temperature of the salmon affects more these samples, resulting in a higher thickness of glazing for salmon at -25 °C. These results indicate that with the decrease of salmon temperature, variation of the water temperature is not very important and does not result in significantly different thickness values.

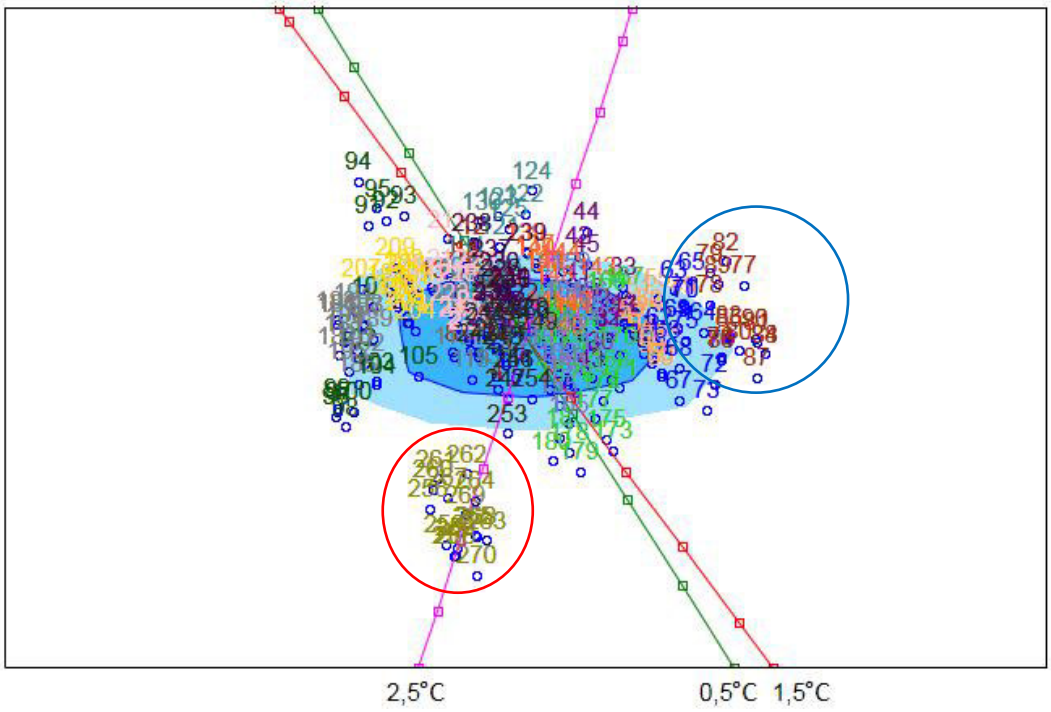

Figure 23. Principal component analysis for water glazing in salmon. The variable in study is the coating temperature and groups with the same color correspond to samples with the same conditions Principal component 1 (PC1) corresponds mainly to dipping time and justifies 87.5% of variability.

A similar analysis was performed now using as variable of study the water glazing temperature as shown in Figure 23. The temperature variation of the water glazing affected the samples numbered from 256 to 270 that correspond to a salmon temperature of -15 °C and dipping time of 60 s (these samples are circled red in Figure 23), separating them from the others and obtaining a smaller thickness. In the opposite direction, also the samples 76 to 90 (salmon temperature at -25 °C and dipping time of 60 s - these samples are circled blue in Figure 23) separate from the others and correspond to those which have a greater thickness. The PCA analysis allowed finding out which dipping conditions are most affected when one of these parameters changes.

5.4. Chitosan coating versus water glazing

The several tests carried out involved different temperatures of water and chitosan. However, for both coatings, were made dips and thickness measurements for a same temperature which was 2.5 °C. The thicknesses resulting from the application of chitosan and water at the same temperature in the same frozen salmon conditions were compared and analyzed. As an example of these results, Figure 24 shows the thicknesses of the two coatings for a salmon temperature of -25 °C. The results for the salmon temperatures of -20 and -15 °C are illustrated in Figures A.6 and A.7.

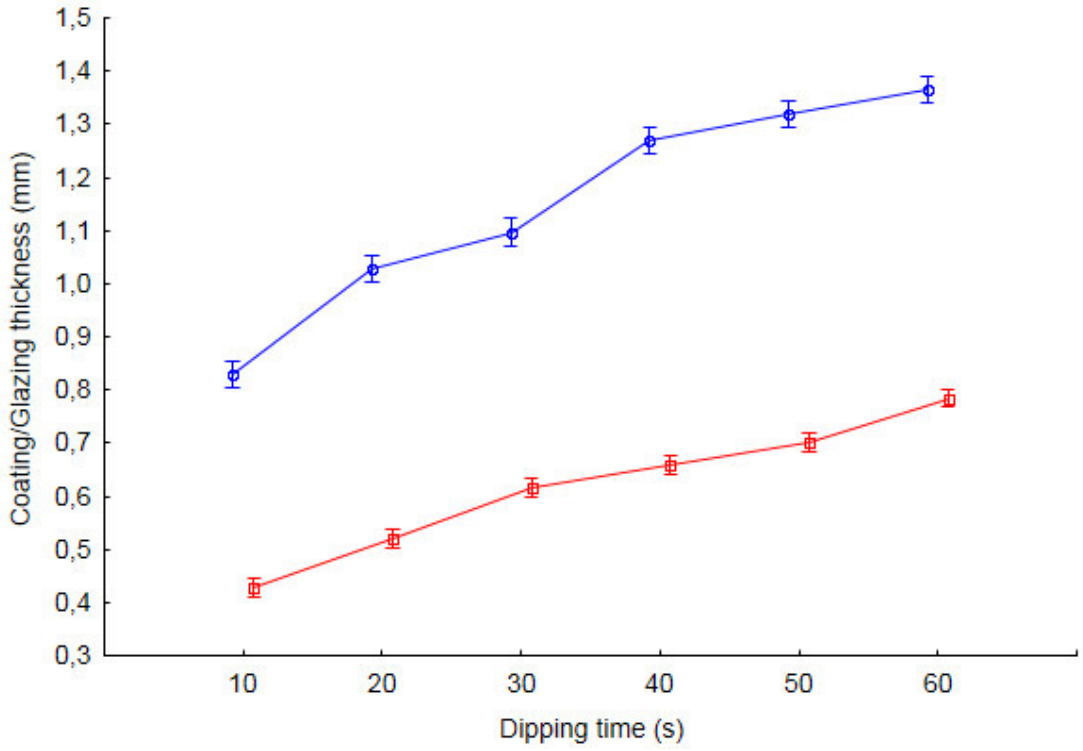

Figure 24. Comparison of coating/glazing thickness variation along dipping time for salmon at -25 °C glazed with water at 2.5 °C (□) and coated with chitosan at 2.5 °C (○). Each point represents the mean ± standard deviation of fifteen replications.

These results show that for all tested temperatures of salmon the thickness achieved by application of chitosan is always much higher than that obtained by water glazing. The main reasons are the higher viscosity of chitosan solutions in comparison with water and the lower heat of crystallization (Table 5). The heat of crystallization is the energy which is necessary to remove in order to solidify chitosan. As this energy is smaller for chitosan than for water, a larger amount of chitosan changes phase and adheres to the salmon. Looking at Figure 24 and comparing the maximum thickness reached by the water glazing at 60 s, it is possible to see that it is still smaller than the minimum thickness achieved by chitosan coating at 10 s. The same is true for salmon temperatures of -20 and -15 °C (Figure A.6 and A.7), where the maximum thickness of the glazing water is always lower than the minimum thickness of the chitosan coating. It can be clearly concluded that the chitosan has a greater ability to change phase and adhere to the frozen salmon.

A final analysis was carried out to compare the best results presented by water glazing (salmon temperature of -25 °C and water temperature of 0.5 °C) with the worst result presented by chitosan coating (salmon temperature of -15 °C and chitosan temperature of 8 °C). These results are presented in Figure 25 and show that despite until 30 s of dipping the water presents higher thicknesses, from that dipping time onwards both thicknesses are similar, with chitosan showing a better result for dipping times of 60 s.

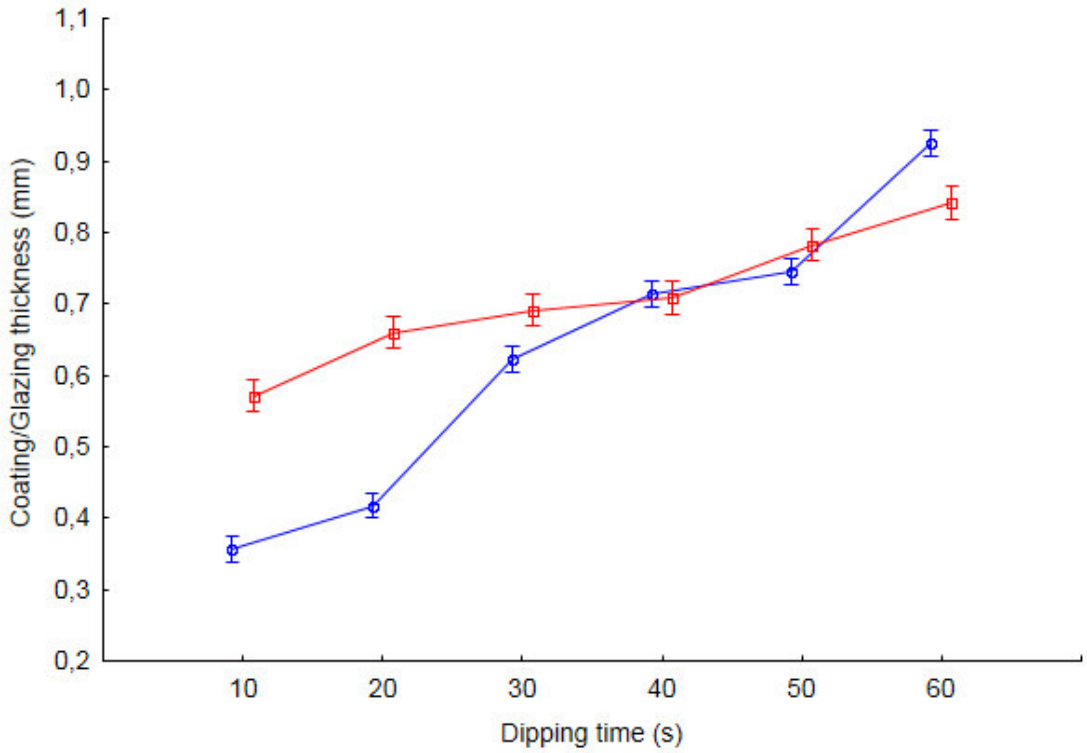

Figure 25. Comparison of coating/glazing thickness variation along dipping time for salmon at -25 °C glazed with water at 0.5 °C (□) and salmon at -15 °C coated with chitosan at 2.5 °C (○). Each point represents the mean ± standard deviation of fifteen replications.

Several authors, incuding Alishahi & Aider (2011) and No et al. (2007), related chitosan nontoxicity, antibacterial/antifungal properties, biocompabality and encapsulating capacity. Other authors, as Sathivel et al. (2007) and Soares et al. (2013) claim that chitosan coatings showed better results (for tests such as coating loss, pH values, thiobarbituric acid value (TBA), K value and total volatile basic nitrogen (TVB-N)) than water glazing, being a better option for frozen fish preservation. Despite the advantages of chitosan compared to water, the coatings may function only as "sacrifying" agents, wherein during freezing the coating sublimates before the product, thus delaying food deterioration (Mohan, et al., 2012). So, even when chitosan is compared with water only as a sacrifying agent, it also has better results once it has higher thickness values in all conditions tested, ensuring salmon protection during a greater period of time.

Frozen fish processing industry's biggest costs come from the use of electrical power required to maintain very low temperatures in glazing baths, cold chambers and equipments used to lower the product temperature before entering in the production line (e.g. cooling tunnels). Thus, by using water glazing the costs to maintain/place the salmon at -25 °C and glazing bath at 0.5 °C would be much higher than those needed to keep the salmon at -15 °C (regular freezers can be used for this temperature) and chitosan at 8 °C (usual room temperature in this type of industry).

5.5. Temperature profiles

According to DL nº 37/2004, frozen fish should be kept at a temperature of -18 °C or below in all its points. However, in terms of food safety, it is possible to see through Table 5 that the temperature of -5 °C is the minimum temperature necessary for growth of pathogenic bacteria associated with fish.

Table 6. Minimum temperature necessary for growth of pathogenic bacteria in fish

Microorganism	Temperature (°C) FDA (2011)	Temperature (°C) Jay et al. (2005)	Temperature (°C) Huss et al. (2004)
Clostridium botulinum	3.3	-	3.3
Clostridium perfringens	10	-	-
Vibrio cholerae	10	-	10
Vibrio parahaemolyticus	5	5	5
Vibrio vulnificus	8	-	8
Vibrio spp.	-	-5	-
Plesiominas shigelloides	-	-	8
Listeria monocytogenes	-0.4	1	0 - 2
Salmonella spp.	5.2	7	5
Shigella spp.	6.1	-	6
Escherichia coli	6.5	-	7
Staphylococcus aureus	7	6.7	7
Yersinia enterocolitica	-1.3	-2	-1.3
Bacillus cereus	4	7	-

In this context, the construction of temperature profiles for frozen salmon is very important in order to determine the salmon temperature at any point for different dipping times, verifying if during the glazing/coating process salmon does not exceed the critical temperature of -5 °C in most of its points. Nevertheless it is important to avoid temperature fluctuations, since recrystallisation causes the melting of ice crystals leading to surface dehydration and

consequently freezer burns. Every time the temperature drops again, the existing ice crystals increase their size, resulting in the loss of fish quality (Fellows, 2000).

Figure 26 shows an example of the temperature profile of salmon, in this case at -25 °C after the application of chitosan at 2.5 °C. Figures A.8, A.9 and A.10 show the temperature profiles for different initial temperatures of salmon after coating with chitosan; Figures A.11, A.12 and A.13 show the temperature profiles for different initial temperatures of salmon after glazing with water.

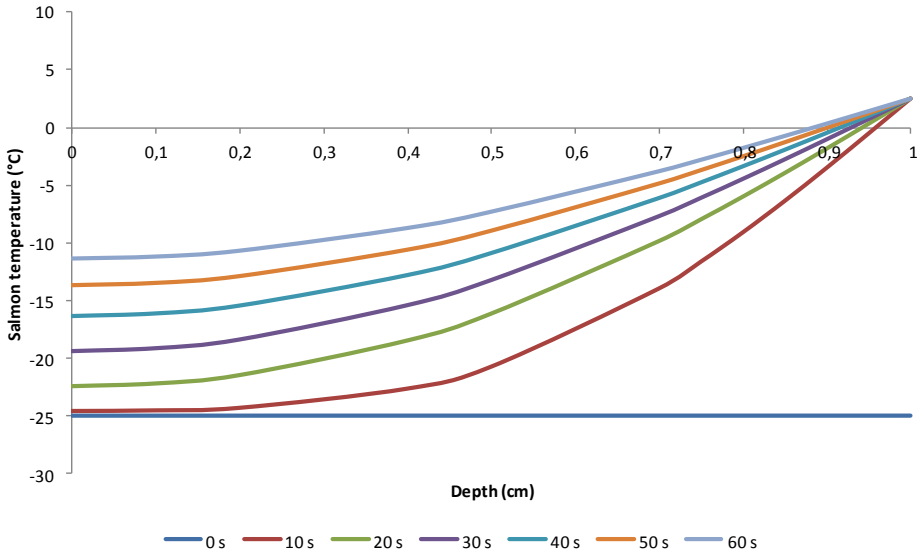

Figure 26. Temperature profile showing the temperature variation from the center (depth = 0) to the surface (depth = 1) of a sample of salmon initially at -25 °C after applying a chitosan coating at 2.5 °C. Each curve corresponds to a different dipping time.

In order to simplify the analysis of the salmon temperature profiles, Tables 7 and 8 summarize the theoretical "safe dipping time" for different salmon temperature and coating temperatures. This safe dipping time corresponds to the maximum time that salmon samples may be dipped in the coating until their temperature is above -5 °C in 80% of their volume, which was defined as the acceptable limit for this work.

Tables 7 and 8 show that, as expected, the maximum time that salmon may be dipped in both coatings increases with the decrease of salmon temperature and coating temperature. In general, as the temperatures used are lower in the water glazing than in chitosan coating, the safe dipping times are always higher in water.

The definition of safe dipping time for the various dipping conditions allowed the comparison of the maximum coating thickness where the salmon is still within the limits of food safety. Thus the glazing conditions to favor are those that present the longer dipping time and higher temperatures in order to reduce energy costs while continuing to be safe. When comparing the safe dipping time obtained for chitosan and water at the same glazing/coating temperature it can be concluded that in both cases this time is 30 s, but the resulting thickness for chitosan is much higher reaching (1.10 ± 0.02) mm while water glazing only reaches (0.61 ± 0.03) mm. The greatest thickness obtained for water glazing was (0.71 ± 0.02) mm for a salmon temperature of -25 °C, water temperature of 0.5 °C and dipping time of 40 s. The application of chitosan at 5 °C in salmon at -20 °C for 10 s leads to a thickness of (0.69 ± 0.04) mm. The thickness values obtained in both cases are quite similar, however in the case of chitosan coating both chitosan and salmon are at higher temperatures, resulting in energy savings. As for dipping time, a reduction from 40 to 10 seconds leads to obvious savings in processing time.

Table 7. Maximum theoretical time that salmon may be dipped in chitosan wherein its temperature is below -5 °C in 80% of its volume and resulting thickness.

Salmon temperature (°C)	Chitosan temperature (°C)	Safe dipping time (s)	Thickness (mm)
-25	2.5	30	1.10±0.02
	5	20	0.57±0.03
	8	10	0.56±0.06
-20	2.5	20	0.87±0.03
	5	10	0.69±0.04
	8	10	0.48±0.04
-15	2.5	10	0.63±0.03
	5	<10	0.55±0.02
	8	<10	0.36±0.02

Table 8. Maximum theoretical time that salmon may be dipped in water wherein its temperature is below -5 °C in 80% of its volume and resulting thickness.

Salmon temperature (°C)	Water temperature (°C)	Safe dipping time (s)	Thickness (mm)
-25	0.5	40	0.71±0.02
	1.5	30	0.62±0.02
	2.5	30	0.61±0.03
-20	0.5	30	0.58±0.02
	1.5	20	0.44±0.02
	2.5	20	0.42±0.04
-15	0.5	20	0.42±0.02
	1.5	10	0.34±0.02
	2.5	10	0.24±0.01

5.5.1. Glazing process at VANIBRU

In the specific case of the company VANIBRU, before the glazing process the frozen fish enters a cooling tunnel in order to decrease the temperature to about -35 °C. Only then it is dipped in glazing water that usually is at 0.5 °C. From Figure 27 it is possible to check the salmon temperature in each one of its points after being immersed in water at various temperatures for various dipping times. In the specific case in question, where salmon at -35 °C is immersed in water glazing at 0.5 °C, the safe dipping time is 60 s. This allows us to state that if dipping time does not exceed 60 s the salmon is always below the limit of -5 °C in 80% of its volume. This result is quite satisfactory since it allows a greater liberty in the glazing process by showing that only near the surface of the salmon temperature is above -5 °C, which makes the process effective regarding food safety, in order to prevent microbial growth.

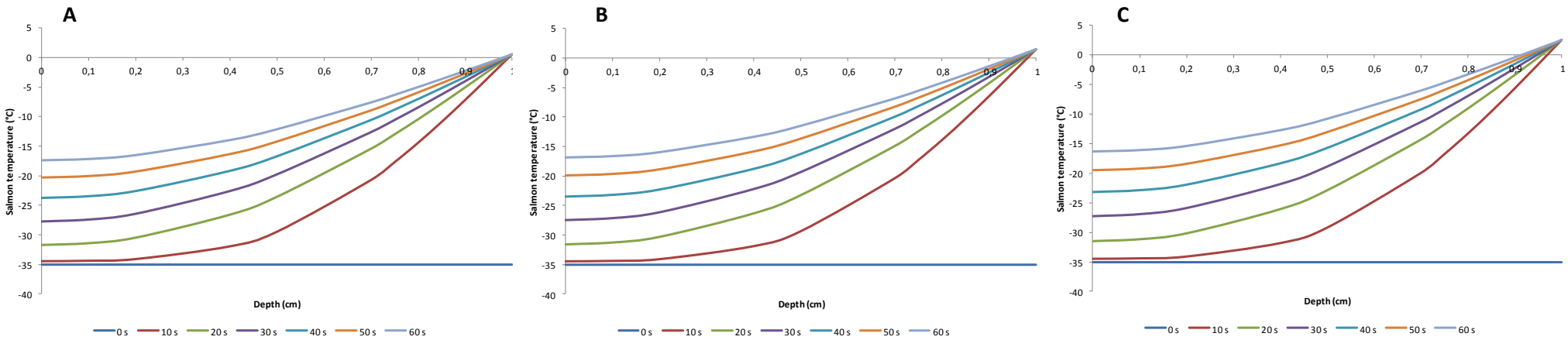

Figure 27. Temperature profile showing the temperature variation from the center (depth = 0) to the surface (depth = 1) of a sample of salmon initially at -35 °C after applying a water glazing at 0.5 °C (A), 1.5 °C (B) and 2.5 °C (C). Each curve corresponds to a different dipping time.

5.6. Heat transferred

The determination of salmon temperature profiles allows a direct observation of the salmon temperature at several points for different dipping conditions. In addition, from these profiles it was also possible to calculate the average temperature (T_{av}) of salmon during glazing. This average temperature is essential for determining the amount of heat transferred from the coating to the frozen salmon. With the knowledge that the amount of heat provided by the coating is equal in absolute value to the amount of heat acquired by the salmon, it should be possible to determine the amount of coating that changes phase (from liquid to solid) and adhere to the salmon surface. This would be important for a possible theoretical prediction of the mass that would change phase and adhere to salmon, forming a protective coating. However, to know this mass that changed phase, it would be necessary to know *a priori* its temperature profile. For example, although water freezes around 0 °C, it is likely that during dipping time ice temperature is lower due to contact with the colder salmon. Thus, to determine the temperature profile of the coating that changes phase it would be necessary a calculation similar to that used for the salmon profile, using Equations 3 and 4 for an infinite plane wall, and then calculate the average temperature of the frozen coating adhered, using Equation 6. However, this calculation is very complex, as it involves the joint use of the thermal characteristics of the salmon and coating. This calculation would be carried by an iterative method until the result converges. This step was not done though, as it departs from the proposed aims for this study.

Nevertheless, using the coating masses obtained experimentally in the various tests it was possible to calculate the average temperatures of the coating adhered to salmon for the several dipping times. Thus in Table 9 it is shown an example for salmon temperature of -25 °C and glazing water temperature of 0.5 °C. Table 9 displays, for each dipping time, the initial sample mass of frozen salmon, as well as the average temperature of salmon needed to calculate the heat acquired by the salmon, using Equation 7. Matching the equations of heat transferred by water glazing and heat gained by salmon, using the experimental amount of water frozen, it was possible to solve the equation in order of the average temperature. Table 9 thus shows the average temperatures obtained for the water that changed phase. Despite these temperatures seem credible it will be necessary to experimentally confirm these results.

Table 9. Thermal characteristics of salmon samples and glazing water that changed phase for different dipping times

Dipping time (s)	Initial salmon mass (g)	Water changed phase (g)	Salmon average temperature (°C)	Heat acquired by salmon (J)	Ice average temperature (°C)
10	29.0	2.4	-17.81	452.19	-1.4
20	27.2	3.0	-14.77	603.70	-1.7
30	28.1	3.4	-12.48	763.59	-1.9
40	26.4	3.8	-10.59	825.56	-2.1
50	28.7	4.2	-8.99	996.99	-2.3
60	26.9	4.4	-7.63	1014.09	-2.4

Chapter 6. Conclusions and future perspectives

Although water glazing is the most used coating, other coatings like chitosan have properties that can optimize this process. The purpose of using an ice layer on frozen fish is product protection but the value of glazing necessary to protect the fish is not set, which can lead to excessive application and consequently to commercial advantages. To master the process it is necessary to know how coating and product temperature, the superficial area and the dipping time affect the thickness of the water glazing and chitosan coating.

DSC analyses allowed determining the crystallization heat and the freezing temperature of 1.5% w/v chitosan solution. Both freezing point and the crystallization heat are lower than those of water, which allows a larger amount of chitosan to change phase and adhere to the salmon surface under comparable conditions to those of water glazing. This results in a greater thickness of the chitosan coating when compared with water glazing.

From the analysis of the thicknesses resulting from the application of chitosan it was concluded that the lower the salmon/chitosan temperature used, the higher the final coating thickness. It was also possible to conclude that the lower the salmon temperature, the less important is the dipping time for dipping times until 60 s. Finally, it was observed that thickness always increased throughout dipping time, being 60 s not a limit to the thickness increase.

The analysis of the thickness resulting from the application of water showed that, when compared with chitosan, water has less ability to freeze on salmon surface even at lower temperatures. The thickness analysis when the same temperature for salmon and coating was used, reinforces this conclusion. The decrease of both salmon temperature and water temperature favors the increase of the final thickness too. In general, after the dipping time of 40 s, no major differences between the thicknesses were recorded, thus not advising higher dipping times. The temperature of water at 2.5 °C will be near the upper limit for an effective glazing. Subsequently, a test with water at 5 °C was carried out and residual results were obtained for both thickness and glazing uptake.

The maximum time that salmon may be dipped in water/chitosan in order to continue safe, increases with the decrease of salmon temperature and coating temperature. This was also evidenced by the increase of safe dipping time (from 40 to 60 s) after lowering the salmon temperature from -25 °C to -35 °C. For the same dipping conditions, where safe dipping time of water glazing is equal to that of chitosan solution, the resulting thickness is higher for the chitosan coating.

It was possible to conclude that the average temperature of the coating that adheres to salmon decreases with time. In water glazing for 10 s the average temperature is -1.4 °C and after 60 s this value drops to -2.4 °C.

For future work it is suggested to test different chitosan concentrations, longer dipping times and the impact of different superficial areas in thickness. Although in theory a greater thickness results in greater protection of the frozen fish, it is proposed that, in a detailed study, a determination of how different coating thicknesses affect the various parameters that affect the quality of the fish during storage is performed. Parameters such as coating loss, weight loss, drip loss, TVC, TBA, TVB-N, K-value, pH and L*a*b* coordinates would be studied, providing information about the freshness of the salmon samples, indicating the state of surface dehydration, microbiological contamination, lipid oxidation, protein denaturation, and changes in odor and color.

It is also proposed that the average temperature of the coating that freezes and adheres to salmon is calculated analytically and compared with the experimental results. With the confirmation of these temperatures it would be possible to accurately discover *a priori* how much glazing/coating mass would adhere to frozen salmon.

From an economic point of view, the effective use of chitosan in replacement of glazing water brings initial costs for the concerned industry. It would be necessary to evaluate if adaptations of existing industrial equipment are necessary due to the high viscosity of chitosan and the need of a higher concentration. Compared to water, chitosan solutions have a higher cost of production however, according to Poeloengasih et al. (2008), this cost could be reduced eliminating the stages of deproteinization and demineralization from the chitosan isolation. The elimination of these phases would lead to a decrease in processing time and chemicals and power usage.

Also note that the use of chitosan allows the use of higher salmon temperatures, lower dipping time and virtually no cost to maintain the temperature of the chitosan (could be at the same temperature of the refrigerated room), achieving savings in energy and time.

Bibliography

Adhikari, B., Howes, T., Bhandari, B. & V., T., 2001. Stickiness in foods: a review of mechanisms and test methods. *International Journal of Food Properties,* Volume 4(1), pp. 1-33.

Alishahi, A. & Aider, M., 2012. Applications of Chitosan in the Seafood industry and Aquaculture: A Review. *Food Bioprocess Technol,* Volume 5, pp. 817-830.

Aranca analysis, 2013. *Edible Packaging.* [Online]
Available at: http://www.aranca.com/knowledge-center/patent-watch/18-edible-packaging
[Accessed 22 April 2014].

Azeredo, H., 2012. Edible Coatings. In: *Advances in Fruit Processing Technologies.* s.l.:s.n., pp. 345-361.

Azeredo, J., 2010. *Transferência de Calor e Massa.* Braga: Escola de Engenharia Biológica, Universidade do Minho.

Baldwin, E., 2007. Surface Treatments and Edible Coatings in Food Preservation. In: *Handbook of Food Preservation.* s.l.:CRC Press, pp. 477-507.

Casariego, A. et al., 2008. Chitosan coating surface properties as affected by plasticizer surfactant and polymer concentrations in relation to the surface properties of tomato and carrot. *Food Hydrocolloids,* pp. 1452-1459.

Debeaufort, F., Quezada-Gallo, J. & Voilley, A., 1998. Edible films and coatings: Tomorrow's Packagings: A Review. *Critical Reviews in Food Science and Nutrition,* Issue 38:4, pp. 299-313.

EFSA, 2011. *Scientific Opinion on the substantiation of health claims related to chitosan and reduction in body weight (ID 679, 1499), maintenance of normal blood LDL.cholesterol concentrations (ID 4663), reduction of intestinal transit time (ID 4664) and reduction o,* s.l.: s.n.

El-Hefian, E., Elgannoudi, E., Mainal, A. & Yahaya, A., 2010. Characterization of chitosan in acetic acid: Rheological and thermal studies. pp. 47-56.

Elsabee, M. & Abdou, E., 2013. Chitosan based edible films and coatings: A review. *Materials Science and Engineering C,* Issue 33, pp. 1819-1841.

Fellows, P., 2000. *Food Processing Technology.* Second Edition ed. Cambridge: Woodhead Publishing Limited.

Ghaly, A., Dave, D., Budge, S. & Brooks, M., 2010. Fish spoilage mechanisms and preservation techniques: review. *American Journal of Applied Sciences,* Issue 7, pp. 859-877.

Gonçalves, A., Rech, B., Rodrigues, P. & Pucci, D., 2008. Quality evaluation of frozen seafood (Genypterus brasiliensis, Prionotus punctatus, Pleoticus muelleri and Perna perna) previously treated with phosphates. *Pan-American Journal of Aquatics Sciences,* Issue 3, pp. 248-258.

Jacobsen, S. & Fossan, K.; 1999. The codex standard versus the enthalpy method: comparison of two techniques for determination of ice-glaze uptake on prawns. Journal of Food Engineering, 40, pp. 21-26.

James, D., 2013. *Risks and benefits of seafood consumption,* Rome: Food and Agriculture Organization.

Johnston, W., Nicholson, F., Roger, A. & Stroud, G., 1994. *Freezing and refrigerated storage in fisheries,* Rome.

Mohan, C., Ravishankar, C., Lalitha, K. & Gopal, T., 2012. Effect of chitosan edible coating on the quality of double filleted Indian oil sardine (Sardinella longiceps) during chilled storage. *Food Hydrocolloids,* Volume 26, pp. 167-174.

No, H., Meyers, S., Prinyawiwatkul, W. & Xu, Z., 2007. Applications of Chitosan for Improvement of Quality and Shelf Life of Foods: A Review. *Journal of Food Science,* Volume 72, pp. 87-100.

Pavlath, A. & Orts, W., 2009. Edible Films and Coatings: Why?, What, and How?. In: *Edible Films and Coatings for Food Applications.* New York: Springer Science, pp. 1-23.

Perry, R. & Green, D., 2007. *Perry's Chemical Engineer' Handbook.* s.l.:McGraw-Hill.

Raafat, D. & Sahl, H., 2009. Chitosan and its antimicrobial potential - a critical literature survey. *Microbial Biotechnology,* Issue 2, pp. 186-201.

Rabea, E. et al., 2003. Chitosan as Antimicrobial Agent: Applications and Mode of Action. *Biomacromolecules,* 4(6), pp. 1457-1465.

Rodriguez-Turienzo, L. et al., 2011. Whey protein-based coatings on frozen Atlantic salmon (Salmo salar): Influence of the plasticiser and the moment of coating on quality preservation. *Food Chemistry,* Issue 128, pp. 187-194.

Sathivel, S., Liu, Q., Huang, J. & Prinyawiwatkul, W., 2007. The influence of chitosan glazing on the quality of skinless pink salmon (Oncorhynchus gorbuscha) fillets during frozen storage. *Journal of Food Engineering,* Volume 83, pp. 366-373.

Skurtys, O. et al., 2010. *Food Hydrocolloid Edible Films and Coatings,* Santiago: s.n.

Soares, N., Mendes, T. & Vicente, A., 2013. Effect of chitosan-based solutions applied as edible coatings and water glazing on frozen salmon preservation - A pilot-sclase study. *Journal of Food Engineering,* Volume 119, pp. 316-323.

Srinivasa, P. & Tharanathan, R., 2007. Chitin/Chitosan - Safe, Ecofriendly Packaging Materials with Multiple Potential Uses. *Food Reviews International,* Issue 23:1, pp. 53-72.

ThermExcel, 2003. *Physical characteristics of Water.* [Online]
Available at: http://www.thermexcel.com/english/tables/eau_atm.htm
[Accessed 8 April 2014].

Vanhaecke, L., Verbeke, W. & Brabander, H., 2010. Glazing of frozen fish: Analytical and economic challenges. *Analytica Chimica Acta,* Issue 672, pp. 40-44.

Zoldos, P. et al., 2011. The effect of glaze on the quality of frozen stored Alaska pollack (Theragra chalcogramma) fillets under stable and unstable conditions. *ACTA VET. BRNO*, Issue 80, pp. 299-304.

Appendix A

Figure A.1. Samples of salmon used in the tests.

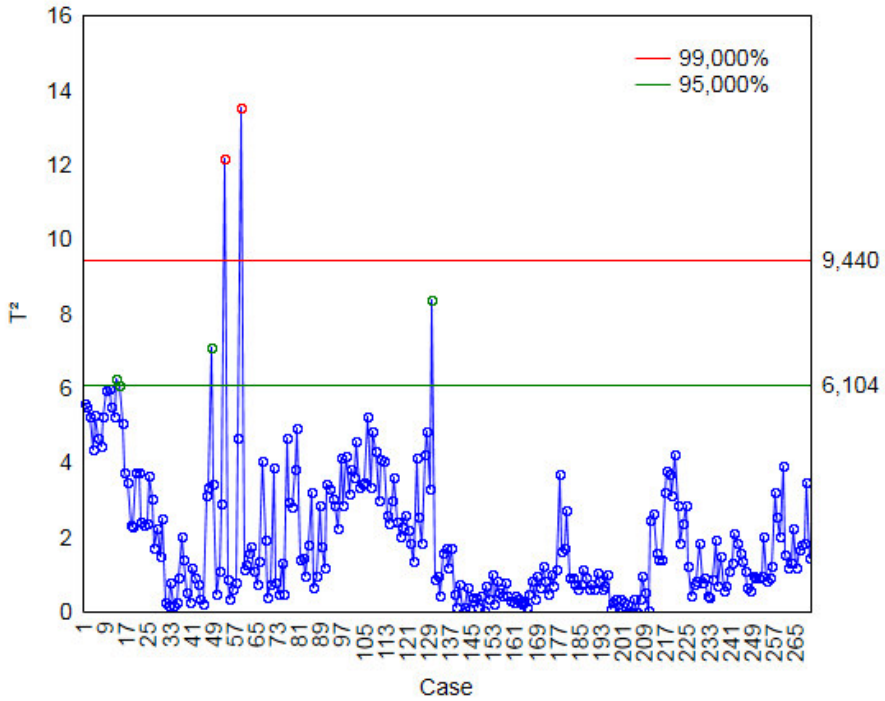

Figure A.2. Hotelling T^2 Control Chart for salmon temperature as variable after chitosan coating.

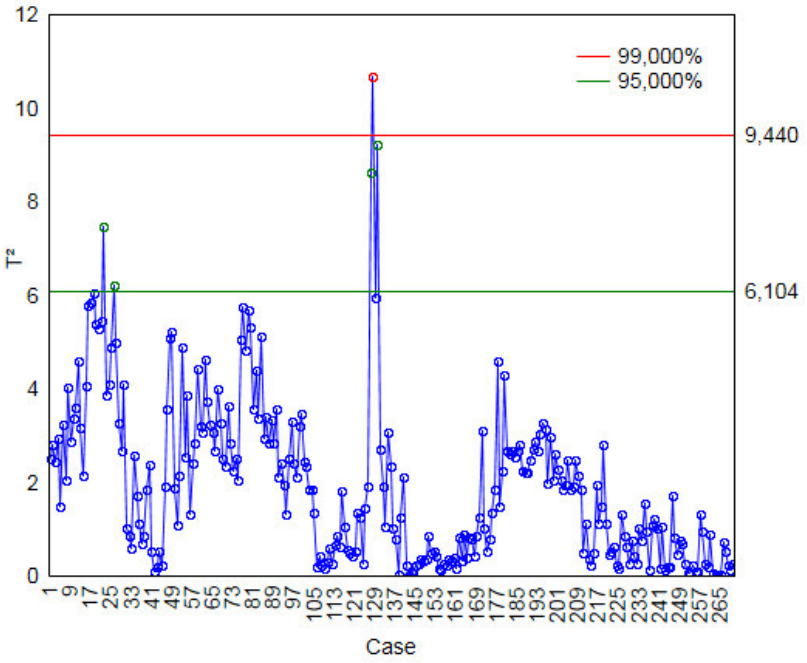

Figure A.3. Hotelling T^2 Control Chart for chitosan temperature as variable after chitosan coating.

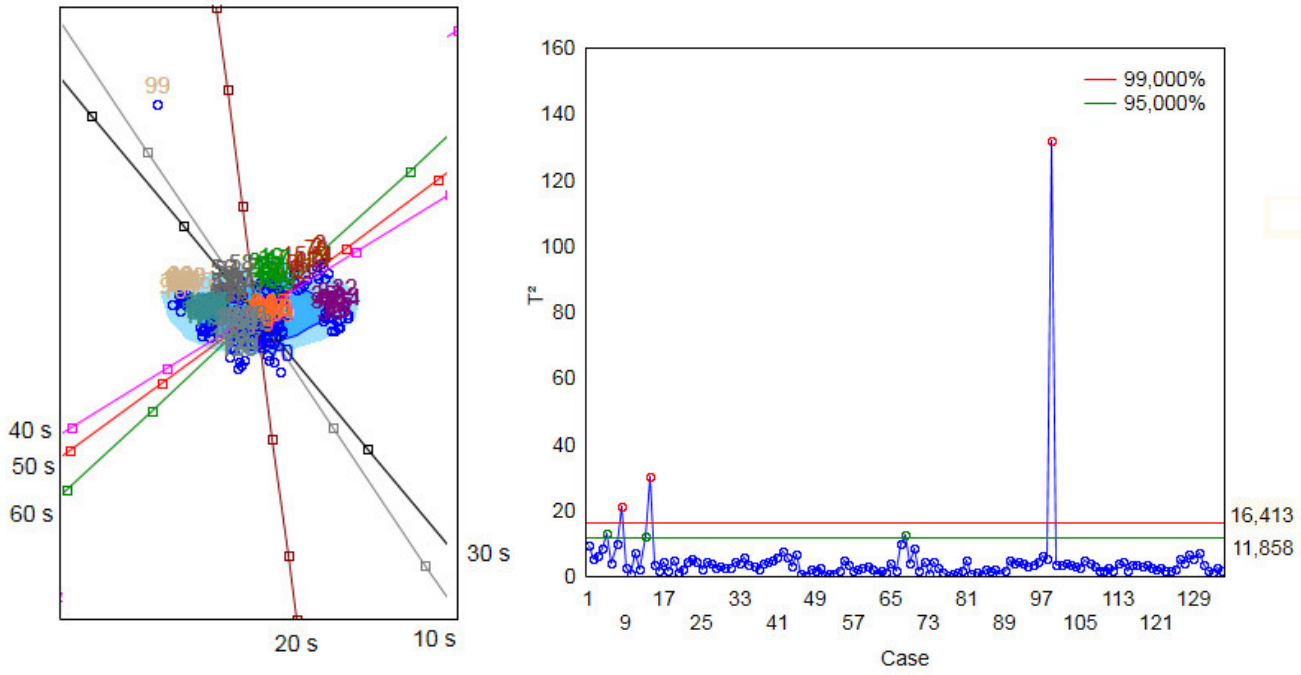

Figure A.4. Principal component analysis for chitosan coating in salmon. The variable in study is dipping time and groups with the same color correspond to samples with the same conditions (left). Hotelling T^2 Control Chart (right).

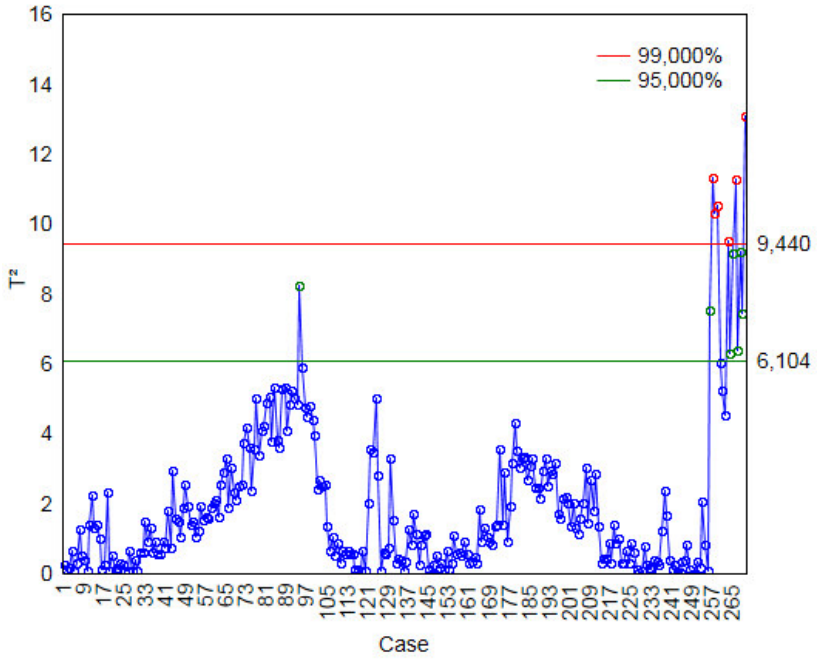

Figure A.5. Hotelling T^2 Control Chart for water temperature as variable after glazing.

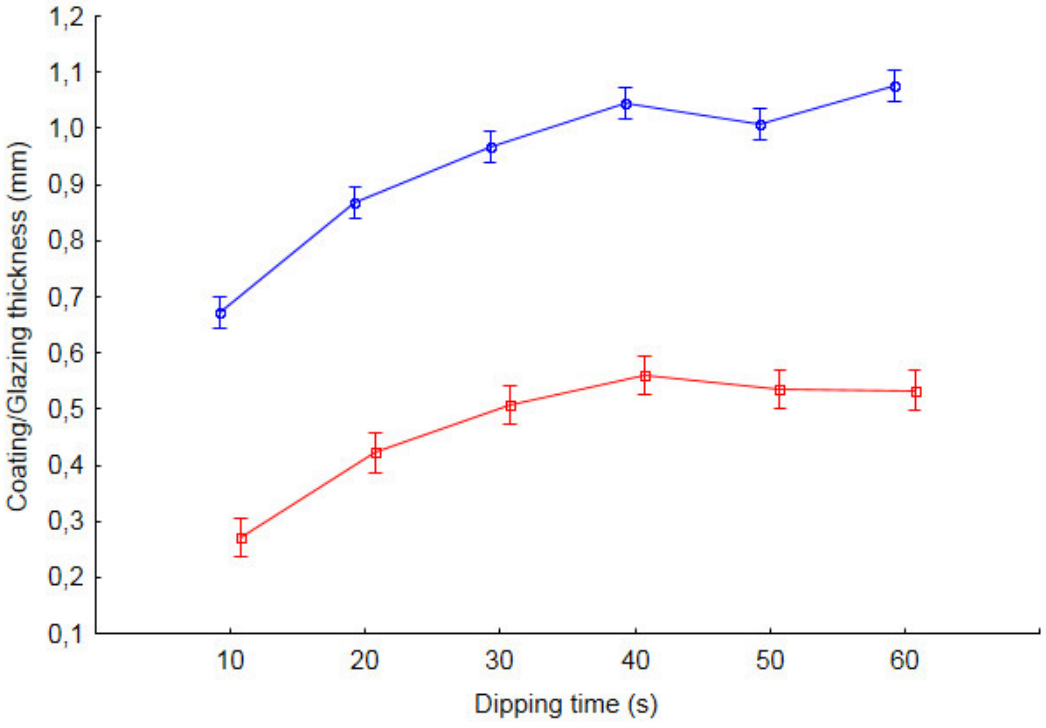

Figure A.6. Comparison of coating/glazing thickness variation along dipping time for salmon at -20 °C glazed with water at 2,5 °C (□) and coated with chitosan at 2.5 °C (○). Each point represents the mean ± standard deviation of fifteen replications.

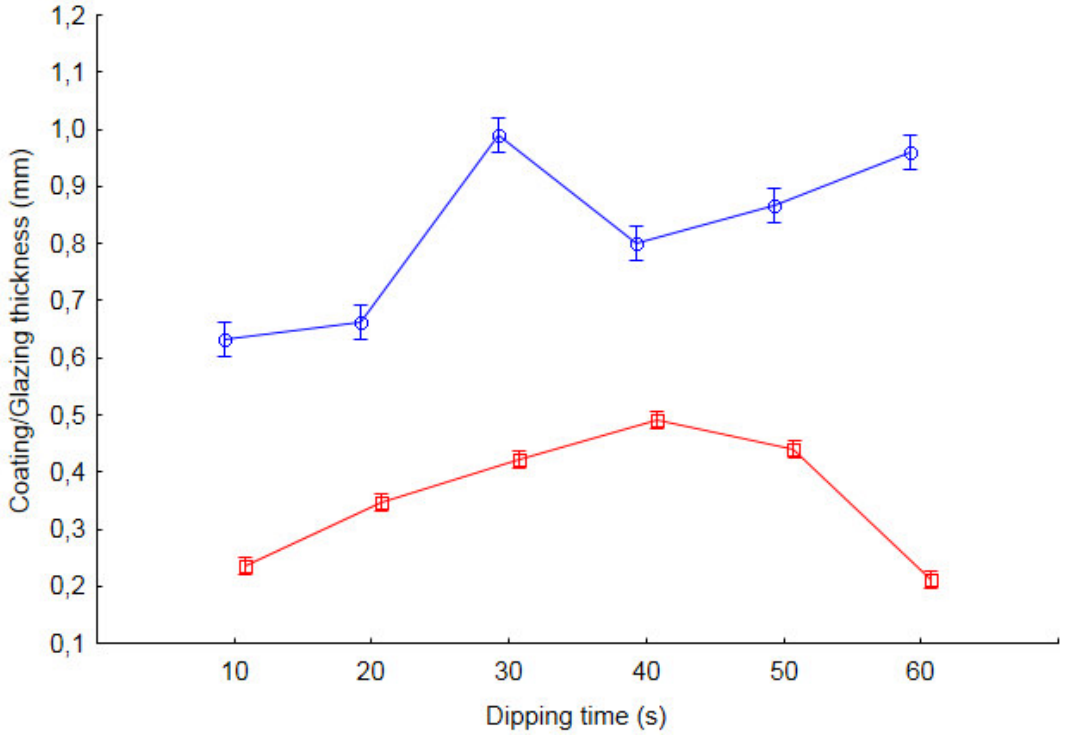

Figure A.7. Comparison of coating/glazing thickness variation along dipping time for salmon at -15 °C glazed with water at 2,5 °C (□) and coated with chitosan at 2.5 °C (○). Each point represents the mean ± standard deviation of fifteen replications.

Salmon temperature profile after coating with chitosan

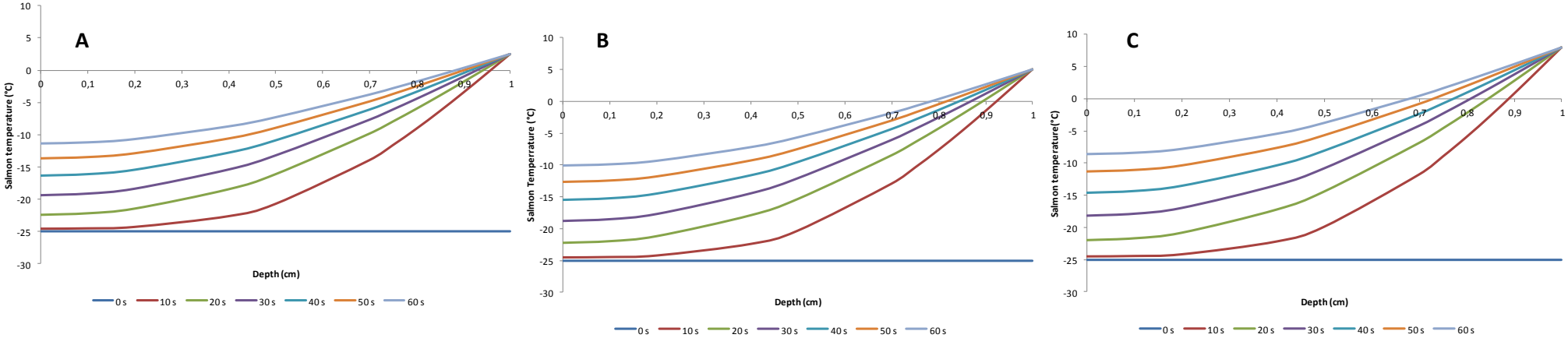

Figure A.8. Temperature profile showing the temperature variation from the center (depth = 0) to the surface (depth = 1) of a sample of salmon initially at -25 °C after applying a chitosan coating at 2.5 °C (A), 5 °C (B) and 8 °C (C). Each curve corresponds to a different dipping time.

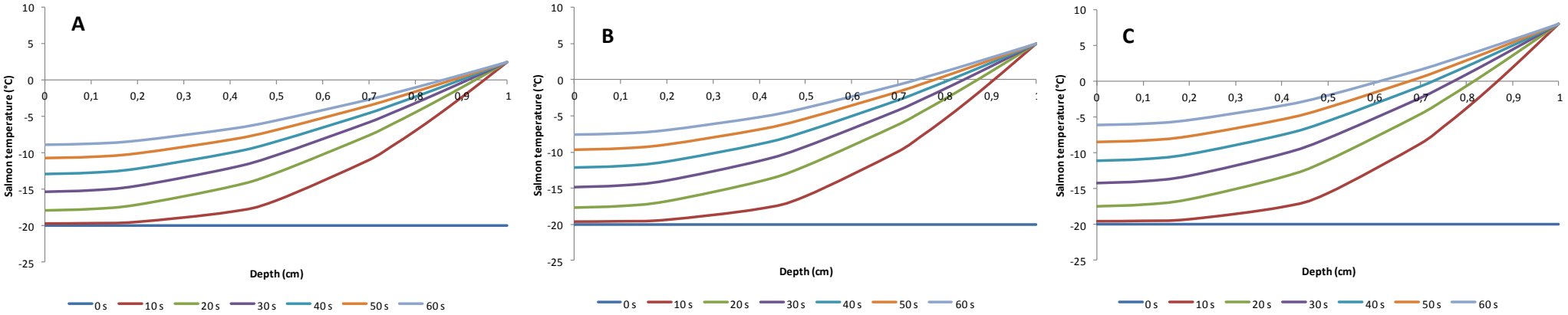

Figure A.9. Temperature profile showing the temperature variation from the center (depth = 0) to the surface (depth = 1) of a sample of salmon initially at -20 °C after applying a chitosan coating at 2.5 °C (A), 5 °C (B) and 8 °C (C). Each curve corresponds to a different dipping time.

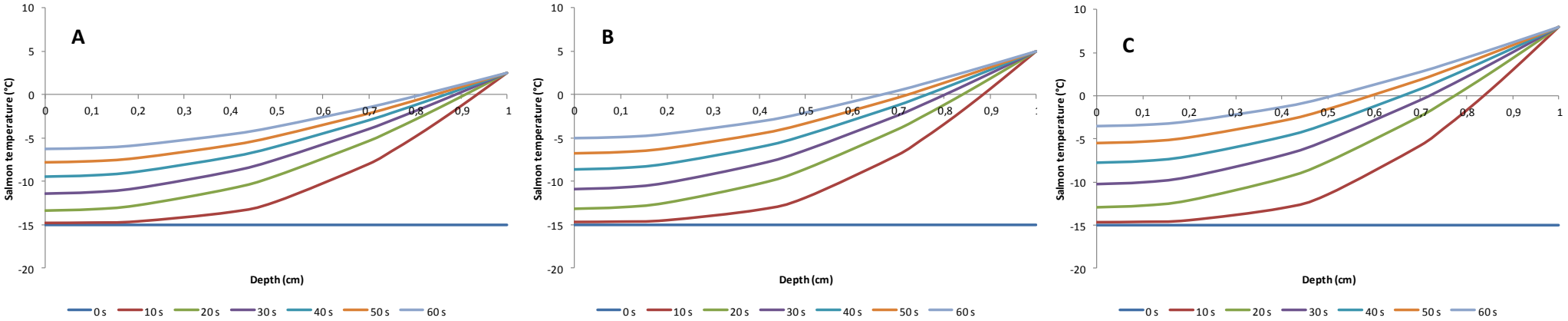

Figure A.10. Temperature profile showing the temperature variation from the center (depth = 0) to the surface (depth = 1) of a sample of salmon initially at -15 °C after applying a chitosan coating at 2.5 °C (A), 5 °C (B) and 8 °C (C). Each curve corresponds to a different dipping time.

Salmon temperature profile after glazing with water

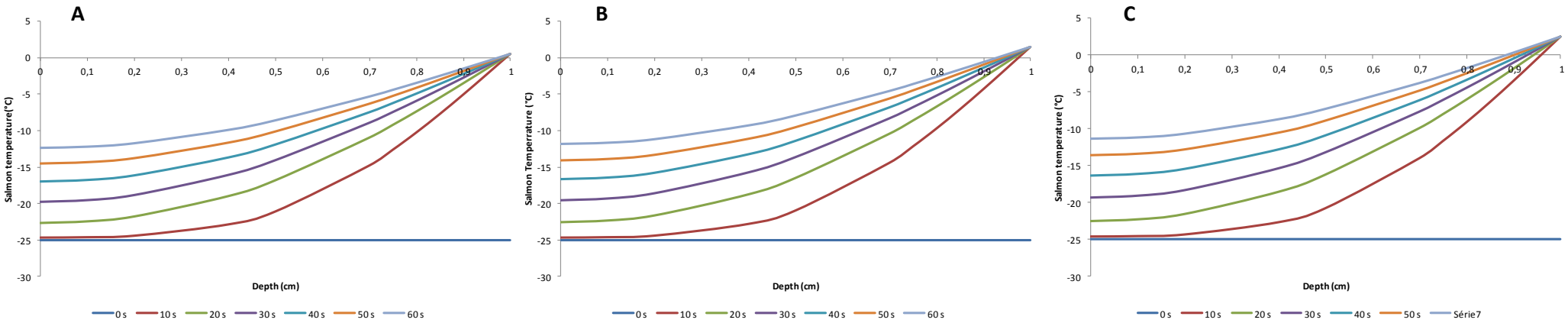

Figure A.11. Temperature profile showing the temperature variation from the center (depth = 0) to the surface (depth = 1) of a sample of salmon initially at -25 °C after applying a water glazing at 0.5 °C (A), 1.5 °C (B) and 2.5 °C (C). Each curve corresponds to a different dipping time.

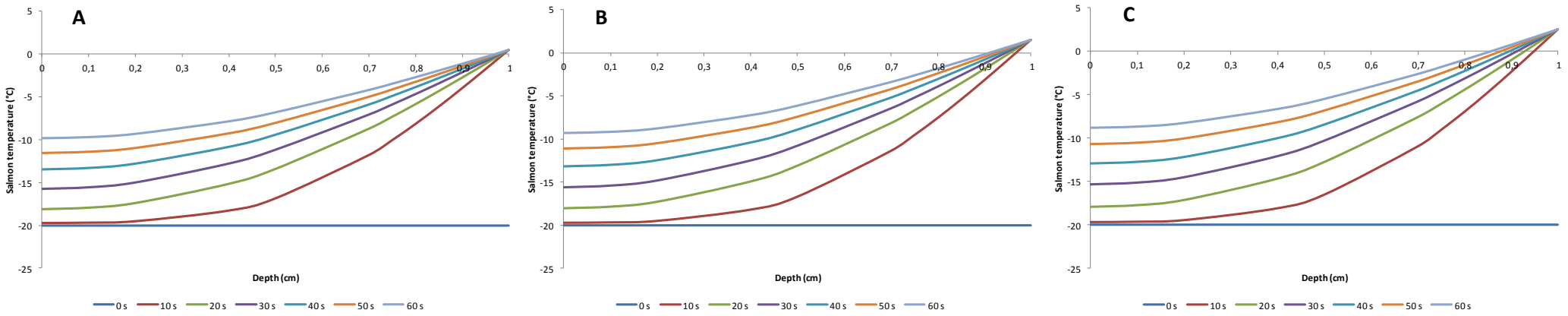

Figure A.12. Temperature profile showing the temperature variation from the center (depth = 0) to the surface (depth = 1) of a sample of salmon initially at -20 °C after applying a water glazing at 0.5 °C (A), 1.5 °C (B) and 2.5 °C (C). Each curve corresponds to a different dipping time.

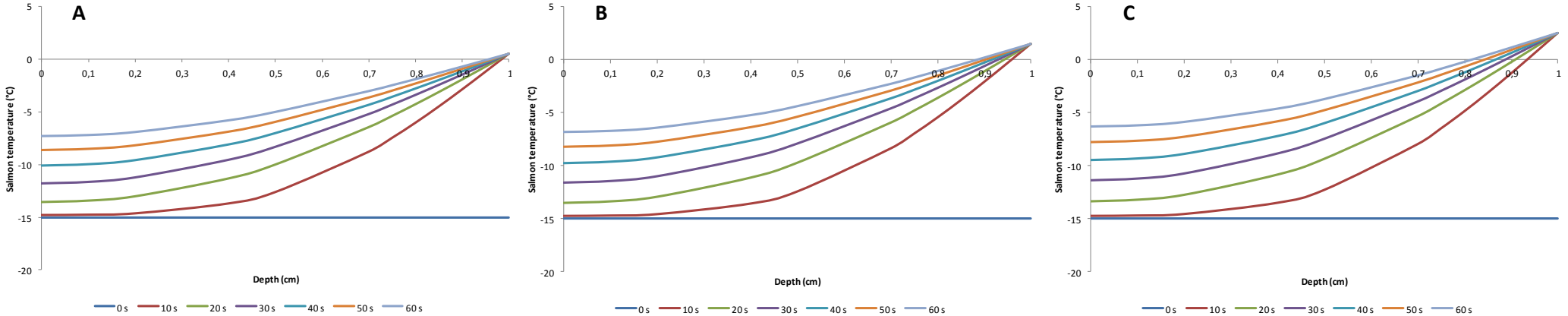

Figure A.13. Temperature profile showing the temperature variation from the center (depth = 0) to the surface (depth = 1) of a sample of salmon initially at -15 °C after applying a water glazing at 0.5 °C (A), 1.5 °C (B) and 2.5 °C (C). Each curve corresponds to a different dipping time.

Figure A.14. Air temperature registered every 3 minutes of industrial freezing chamber programmed at -15 °C by a data logger during frozen storage

Figure A.15. Air temperature registered every 3 minutes of industrial freezing chamber programmed at -20 °C by a data logger during frozen storage

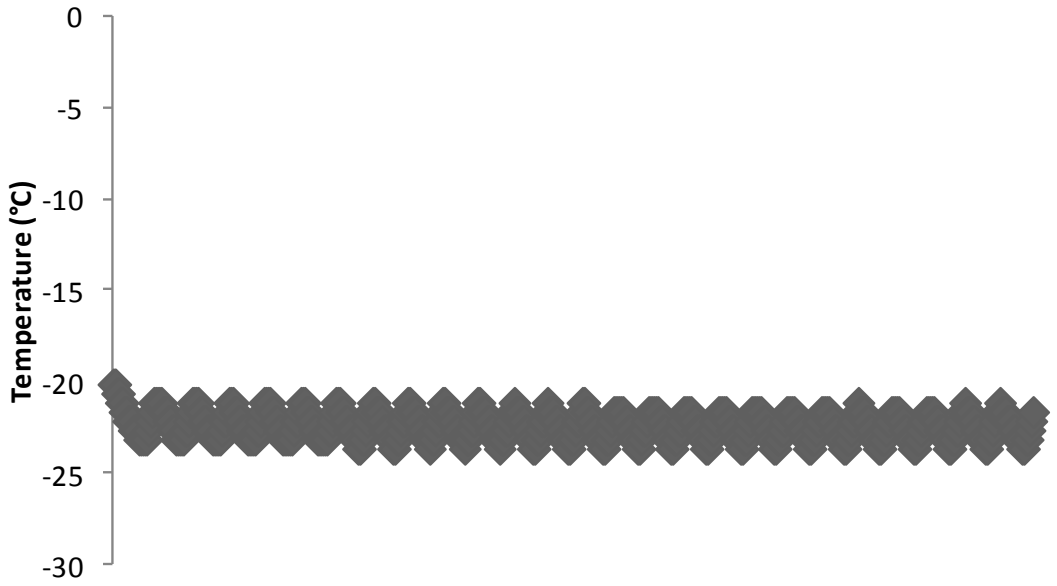

Figure A.16. Air temperature registered every 3 minutes of industrial freezing chamber programmed at -25 °C by a data logger during frozen storage