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Analysis of an edible coating of chitosan as an alternative to glazing of frozen salmon

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Trabalho realizado sob a orientação do
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Título

Análise de um revestimento edível de quitosano como alternativa para vidragem de salmão congelado

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Ano de conclusão

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"It is our failure to become our perceived ideal that ultimately defines us and makes us unique. It is not easy, but if you accept your misfortune and handle it right, your perceived failure can become a catalyst for profound re-invention. (…) Whether you fear it or not, disappointment will come. The beauty is that through disappointment you can gain clarity, and with clarity comes conviction and true originality."

Conan O'Brien

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Resumo

Análise de um revestimento edível de quitosano como alternativa para vidragem de salmão congelado

A natureza perecível do peixe, aliada ao aumento no seu consumo, tem levado à necessidade de melhoria das técnicas de preservação. A utilização de revestimentos de quitosano oferece vantagens em relação às técnicas tradicionais de conservação, no entanto existe a necessidade de avaliar o seu efeito nas propriedades sensoriais do salmão.

Neste trabalho as propriedades protetoras de uma solução de quitosano a 1.5 % foram estudadas e comparadas com as de amostras vidradas com água, e sem revestimento, sob *stress* térmico, com temperaturas a variar entre -15 ºC e -5 ºC, durante 70 dias; os parâmetros avaliados incluem os valores de pH, contagem de microrganismos a 30º C, Azoto Volátil Total, assim como cor e perda de revestimento. O efeito da solução de quitosano nas propriedades sensoriais, de salmão Atlântico (*Salmo salar*) também foi estudado, recorrendo a um colorímetro, um texturómetro e um painel sensorial treinado ao longo de seis meses. Os resultados mostram que sob *stress* térmico as amostras revestidas com quitosano obtiveram valores semelhantes aos obtidos com as amostras vidradas com água no que diz respeito a cor, valores de pH e Azoto Volátil Total, ao passo que oferecem melhores resultados em valores de contagem de microrganismos a 30 ºC e de perda de revestimento, mostrando que o quitosano pode ser uma melhor opção protetora que a vidragem.

Realizou-se uma análise sensorial para estudar e comparar os efeitos dos diferentes revestimentos nas propriedades organoléticas de amostras de salmão e os resultados mostraram que não existiram diferenças significativas entre os diferentes revestimentos no que diz respeito à cor e à textura. A análise sensorial realizada por um painel treinado demonstrou que o quitosano é uma melhor escolha após seis meses de conservação em amostras congeladas, enquanto para amostras descongeladas e cozidas não se verificaram diferenças significativas entre amostras vidradas com água e revestidas com quitosano, sendo que ambas apresentaram melhores resultados do que amostras sem revestimento. Houve um particular cuidado em determinar se teria ocorrido difusão de sabor dos revestimentos para as amostras de salmão, tendo a análise estatística dos resultados do painel treinado mostrado que não existiu nenhuma relação entre o tipo de revestimento e o sabor da amostra, indiciando que nenhuma difusão de sabor ocorreu.

Palavras-chave: salmão, quitosano, *stress* térmico, difusão de sabor, análise sensorial

Abstract

Analysis of an edible coating of chitosan as an alternative to glazing of frozen salmon

The perishable nature of fish, coupled with an increase in fish consumption in recent years, has led to the improvement of fish preservation techniques. Chitosan coatings offer several advantages over more traditional freezing techniques, however there is a need to assess their effect on the sensory properties of salmon.

In this work the protective properties of a chitosan solution at 1.5 % (w/v) were studied and compared to those of uncoated and water glazed samples, under thermal stress conditions, with temperature varying between -15 ºC and -5 ºC, during 70 days. Assessed parameters included pH, Total Volatile Basic Nitrogen (TVB-N), Total Viable Count (TVC) values, as well as coating loss and color parameters. The effect of the chitosan solution on the sensory properties, especially flavor, of Atlantic salmon (*Salmo salar*) was also studied through the use of a colorimeter, a texturometer and a trained sensory panel over six months of storage.

Results show that under thermal stress conditions the chitosan coated samples presented similar values regarding color, pH and TVB-N values while offering better results in terms of TVC and coating loss values, proving chitosan a better protective option than water glazing.

Sensory analysis was conducted to study and compare the effects of different coatings, and the results show that no significant differences were found between different coatings regarding color and texture. Sensory analysis by a trained panel of judges demonstrated that chitosan was a better choice after six months in frozen samples, while in thawed and cooked samples no significant differences were present between chitosan coated and water glazed samples, while both were better than uncoated samples, after six months of storage. In particular flavor was assessed in order to determine if flavor diffusion from the chitosan coating had occurred, and statistical analysis of the results of the trained panel of judges showed no relation between coating type and sample flavor, indicating that no flavor diffusion had occurred.

Keywords: salmon, chitosan, thermal stress, flavor diffusion, sensory analysis

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List of Nomenclature

Abbreviations

CSIRO - Commonwealth Scientific and Industrial Research Organization

DD - Degree of Deacetylation

EC - European Comission

EEC - European Economic Community

EU - European Union

FAO - Department of Food and Agriculture Organization of the United Nations

FDA - Food and Drug Administration

GRAS - Generally Recognized as Safe

MIC - Minimum Growth Inhibitory Concentration

MRD - Maximum Recovery Diluent

Mw - Molecular Weight

OECD - Organisation for Economic Co-operation and Development

Op+I - Olympus pro plus

PCA - Principal Component Analysis

QDA - Quantitative Descriptive Analysis

QI - Quality index

QIM - Quality Index Method

TFRU - Tasmanian Food Research Unit

TVB-N - Total Volatile Basic Nitrogen

TVC - Total viable count

TPA - Texture Profile Analysis

List of Symbols

a_W **-** Water activity

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Introduction

In today's society, the search for better and more valuable products, as well as a growing concern relating to the health implications of the consumers' diet has led to changes in the frozen fish industry.

Fish attracts the consumers attention as a source of important components in a nutritional and healthy diet (Rodriguez-Turienzo et al., 2011), which leads to an increase in fish consumption. Taking in consideration that fish is a very perishable product improving its preservation is a very important issue in the fish industry. The most used method in the preservation of fish is freezing. However, even glazing has its limitations and the search for improved performance has led to the proposal to use a chitosan coating in order to improve microbiological safety and extend the shelf-life of fish (Soares, Oliveira, & Vicente, 2015). However it is necessary to know if the use of a chitosan coating has indeed an effect that can be perceived at the time of consumption. In this context this work intends to analyze the effects, from microbiological to sensory effects of a chitosan coating in frozen salmon at the moment of consumption. This thesis is organized in two parts, Part I – State of the Art and Part II – Experimental Work. Part I is composed by four chapters, and Part II is constituted by an additional three chapters.

Chapter 1 provides an overview on the importance of fish in our daily life and diet, and on the growing industry. This chapter also reflects on the changes happening in the industry, and also refers to the current legislation, the importance of fish preservation and its main methods. The concept of fish quality and our perception of it, as well as the methods that allow us to assess it, are presented in Chapter 2. Chapter 3 introduces the new methods for fish preservation, such as edible packaging, films and coatings, where several compounds with the ability to be used in edible packaging, films or coatings are presented, with a greater focus on chitosan, the compound of choice for this work and its physicochemical and biomedical characteristics that make it a solution for the preservation of fish in the frozen fish industry. Chapter 4 addresses the question of flavor diffusion from the chitosan coatings.

Part II is initiated with Chapter 5, which introduces the methods used in this work, such as the preparation of salmon samples for coating and glazing, the determination of the values of TVC, TVB-N, glazing percentages, coating loss, pH, texture and color parameters, as well as microscopic and sensory analyses. In Chapter 6 the results of the performed tests are presented and discussed, and Chapter 7 shows the main conclusions of this thesis and suggests future work and enhancements.

Part I – State of the Art

Chapter 1. Fish

1.1. Fish Industry and consumption

The consumption of fish has been steadily increasing over the last few years, due to its nutritional characteristics as well as for its benefits to the health of the consumers.

According to the latest publication of State of the World Fisheries and Aquaculture (SOFIA), from the department of Food and Agriculture Organization of the United Nations (FAO), the total amount of world fisheries has been steadily increasing over the past few decades, as shown in Figure 1-1, with the use of fish for food purposes increasing at an average annual rate of 3.2 % (FAO, 2014).

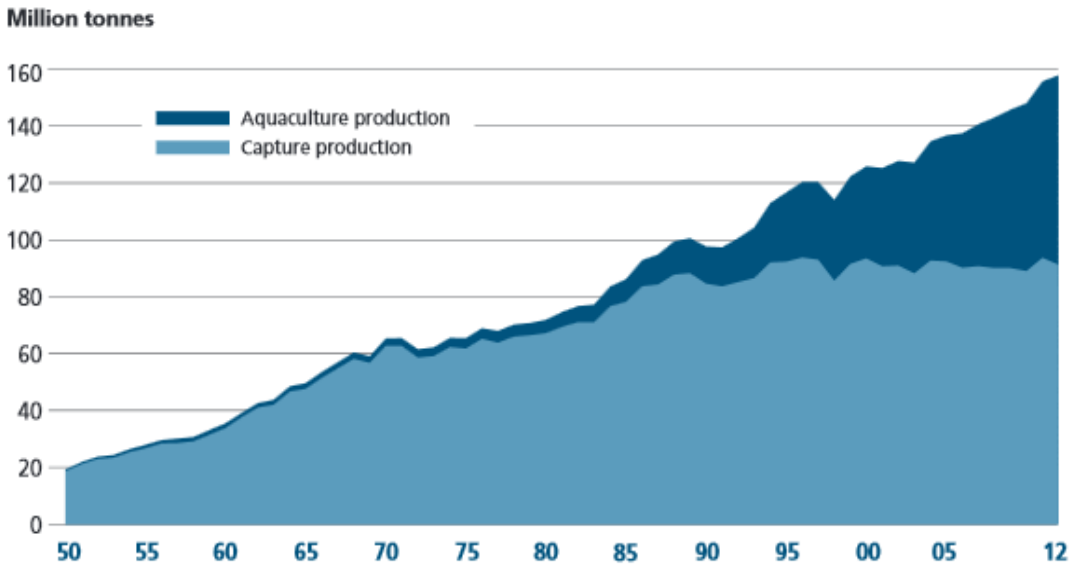

Figure 1-1 World capture fisheries and aquaculture production (adapted from (FAO, 2014)).

The consumption of fish *per capita* increased from an average of 9.9 kg in 1960, to 19.2 kg in 2012, as shown in Table 1-1 (FAO, 2014).

Table 1-1 World fisheries and aquaculture production and utilization (adapted from (FAO, 2014))

	2007	2008	2009	2010	2011	2012
			(millions of tonnes)			
Production						
Capture						
Inland	10.1	10.3	10.5	11.3	11.1	11.6
Marine	80.7	79.9	79.6	77.8	82.6	79.7
Total Capture	90.8	90.1	90.1	89.1	93.7	91.3
Aquaculture						
Inland	29.9	32.4	34.3	36.8	38.7	41.9
Marine	20.0	20.5	21.4	22.3	23.3	24.7
Total Aquaculture	49.9	52.9	55.7	59.0	62.0	66.6
Total World fisheries	140.7	143.1	145.8	148.1	155.7	158.0
Utilization						
Human consumption	117.3	120.9	123.7	128.2	131.2	136.2
Non-food uses	23.4	22.2	22.1	19.9	24.5	21.7
Population (*billions*)	6.7	6.8	6.8	6.9	7.0	7.1
Per capita food fish supply (*kg*)	17.6	17.9	18.1	18.5	18.7	19.2

Table 1-1 and Figure 1-2 also show that most of the capture is marine while aquaculture is carried out mostly inland. It is also showed that fish utilization is mostly for human consumption and has been increasing over the last few years, as well as the *per capita* food fish supply, reaching a new high in the year of 2012 (FAO, 2014).

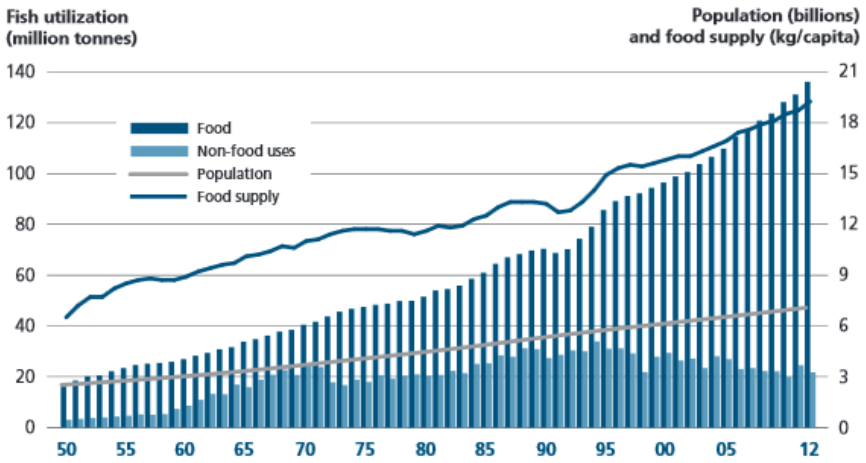

Figure 1-2 World fish utilization and supply (adapted from (FAO, 2014))

However, fish besides being a healthy food product is also as a source of proteins. A daily portion of 150 g of fish can provide about 50 % to 60 % of the protein daily needs for an adult. In 2010, fish was responsible for 16.7 % of the world population consumption of animal protein and of 6.5 % of all consumed protein. Furthermore, fish provided more than 2.9 billion of people with almost 20 % of their animal protein intake,

and 4.3 billion of people with around 15 % of their animal protein intake. Fish proteins can represent an essential nutritional component in some densely populated countries, where the total levels of protein consumption can be low, which demonstrates the great importance that this industry has in society, both in developed and developing countries (FAO, 2014).

It is also possible to verify in Table 1-1 and in Figure 1-2, that while the world capture of fish has remained constant, the production in aquiculture has been increasing over the last few years, which leads to an overall increase in total production of fish (FAO, 2014).

According to the 2014 OECD-FAO Agricultural Outlook report, fish and fishery products are expected to continue to be highly traded, but overall trade is projected to grow at a slightly slower rate than it did the past, mainly due to higher transportation costs, slower output growth and a decreased demand in selected importing countries (OECD/FAO, 2014)

According to forecasts for the time period between 2014 and 2023, an increase by 17 % in total world fisheries production is expected, despite the recent instability of prices. This increase in production will be mainly caused by aquiculture production, which is predicted to reach approximately 49 % of the total world fisheries by 2023. While currently aquaculture production and capture fisheries are equal in terms of human consumption volume, aquaculture production has already surpass in 2014 capture fisheries in terms of human consumption, and by 2023 the difference is expected to be quite significant, as shown in Figure 1-3 (OECD/FAO, 2014).

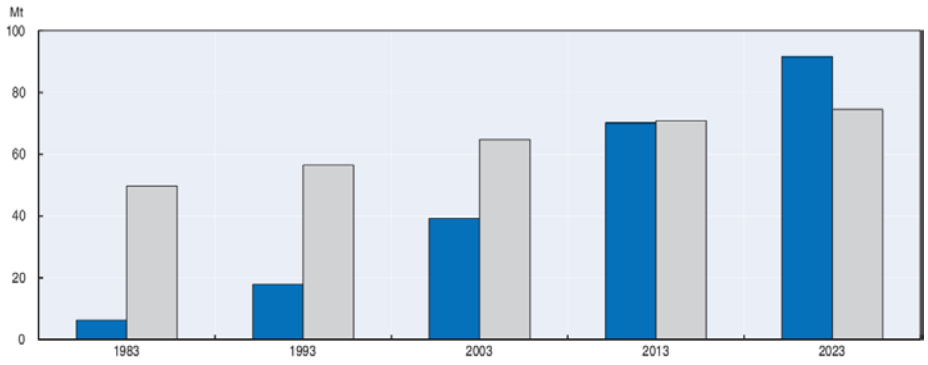

Figure 1-3 Fishery production in live weight equivalent Aquaculture Capture for food

1.1.1 Legislation

Fresh fish is among the most perishable foods due to some intrinsic characteristics of fish, such as its lipid content and its consequent oxidation, due to microbiological changes that occur in the fish, and also due to external factors such as temperature, exposure time before preservation methods are applied, handling, physical condition and fish size (Huss, 1995).

In order to respect food legislation and be fit for human consumption the fish product has to comply with certain microbiological levels. The European Regulation EC Nº1441/2007 defines the microbiological standard regarding foodstuffs. However, the only standard directly applicable to frozen fish regards the presence of histamine, which is limited to 200 mg/kg of fish; nevertheless it is common in the frozen fish industry to assume limits regarding fresh fish in its control, such as the standard regarding *Salmonella*, or the one regarding *E. coli* (Official Journal of the European Union, 2007). The *Codex Alimentarius* defines several other standards such as microbiological ones, presence of additives and method of preparation (Codex Alimentarius, 1966, 2012)

Thereby the improvement in preservation techniques in order to bring the fish product in a safely manner to the consumer, while maintaining its organoleptic characteristics, are a major concern of this industry.

1.2. *Post Mortem* changes

1.2.1. Sensory changes

Sensory changes can be defined as those sensed with the senses, such as appearance, odor, texture and flavor (Huss, 1995).

The first sensory changes of fish during storage are related with appearance and texture. The taste of the species is usually developed after the first couple of days during storage in ice (Huss, 1995).

A characteristic pattern of the deterioration of fish stored in ice can be divided into four phases, which can be seen below (Huss, 1995).

- Phase 1: The fish is very fresh and has a sweet, seaweedy and delicate taste. The taste can be very slightly metallic;
- Phase 2: There is a loss of the characteristic odor and taste. The flesh becomes neutral but has no off-flavors. The texture is still pleasant;
- Phase 3: There is sign of spoilage and a variety of volatile, unpleasant-smelling substances are produced depending on the fish species and type of spoilage.
- Phase 4: The fish can be characterized as spoiled and putrid.

1.2.2. Microbiological changes

In live and newly caught fish, microorganisms can only be found on the surface, and in the intestines. The flesh is sterile as the immune system of the fish prevents the bacteria from growing in the flesh. When the fish dies, the immune system collapses and bacteria are allowed to proliferate freely. During storage, bacteria invade the flesh by moving between the muscle fibers (Huss, 1995).

Because microbiological growth is the main cause of fish spoilage, factors such as temperature, moisture, and oxygen must be controlled in order to delay fish spoilage (Johnston, Nicholson, Roger, & Stroud, 1994).

1.3. Preservation of fish

Fresh fish is one of the most perishable foodstuffs. Fish deterioration is a common result of microbial growth or oxidation, and can be prevented by using methods such as freezing. The increase of world population and the need to store and transport fish are factors that intensify this problem and make its preservation, in order to maintain its nutritional proprieties, flavor, color, texture and extend its shelf life, one of the industry greatest concerns (Ghaly, Dave, Budge, & Brooks, 2010).

Fish preservation can be accomplished by several methods. In the fishing industry the most widely used are freezing and glazing.

1.3.1. Freezing

Freezing represents the main method of fish processing for human consumption. Freezing inhibits enzymatic activities, which allows to slow down the growth of microorganisms, reducing the microbial metabolism responsible for the deterioration

(González-Méndez, Alemán-Escobedo, Zamorano-García, & Camou-Arriola, 2004; Nielsen & Jessen, 2007)

The fish products are constituted by a great percentage of water, up to 80 %, most of which is transformed to ice during the freezing process, which allows to decrease the water activity (a_W), and if the process is conducted correctly it allows to guarantee a shelf life of over a year (Johnston et al., 1994). However the freezing process does not guarantee that the final product quality after one year of storage is the same that the initial product quality, because freezing cannot inhibit completely the chemical and microbial reactions, such as lipid oxidation, protein denaturation, as well as surface dehydration resulting in fish deterioration during prolonged storage, resulting in undesirable flavors, rancidity, dehydration and autolysis and microbial spoilage (Gonçalves & Gindri Junior, 2009; Rodriguez-Turienzo et al., 2011; Sathivel, Liu, Huang, & Prinyawiwatkul, 2007)

The extent of loss of quality depends on many factors, including freezing and thawing speed, storage temperature, temperature fluctuations, overuse of freezing-thawing processes during storage, transportation, exposure and consumption. It should also be noted that freezing does not improve the quality of the product; the final quality depends essentially on the quality of the product at the moment of freezing and of the freezing conditions, storage and distribution (Gonçalves & Gindri Junior, 2009).

The Council directive 89/208/EEC regulates the freezing process and establishes that the temperature for quick-frozen food products should be maintained below -18 ^{o}C, as this is the temperature that inhibits microbiological activity capable of deteriorating the quality of food products. However some temperature fluctuations are inevitable in handling and storage of the product, prior to the sale to the consumer, so fluctuations up to 3 ^{o}C are accepted during transportation (Ghaly et al., 2010; Jiang & Lee, 2004; Official Journal of the European Communities, 1989).

1.3.2. Glazing

In the last few years the demand for frozen fish has been growing, as opposed to fresh fish. One of the main reasons is the efficiency of the preservation of the frozen fish. However, the traditional storage process of frozen fish can lead to a progressive

deterioration of its intrinsic and sensory characteristics (Vanhaecke, Verbeke, & De Brabander, 2010). Glazing is largely used in the fish industry to protect fish from the deterioration of these characteristics, and can be defined as the application of a layer of ice in frozen products surface by means of a dipping process, or by spraying in a water bath (Zoldos et al., 2011). Glazing is still considered the less expensive protection technology, having thus became a widely used process in the fish industry; Nevertheless new alternitives have arisen such as packaging materials that are impermeable to humidity and oxygen and can provide an effective protection during the storage period (Noomhorm & Vongsawasdi, 2004).

During frozen storage, sea products may suffer from dehydration and surface drying, in result of contact with very cold temperatures (freeze burn). Glazing will delay the dehydration of the surface of the product, as it will be the glazing to be sublimated instead of the water of the fish tissue; glazing will also reduce the oxidation rate, through air exclusion from the surface of the product, also serving as a protective barrier regarding temperature fluctuation. The amount of glazing, and consequently the thickness of the glazing, obtained depends on factors such as the size and shape of the fish product, the water and product temperatures and also with the glazing time. (Gonçalves & Gindri Junior, 2009; Johnston et al., 1994; Vanhaecke et al., 2010)

It is intended that the entire product surface is completely and uniformly glazed, typically with a percentage of glazing between 4 % and 10 %, although it may vary between 2 % and 20 % depending on the product in question (Vanhaecke et al., 2010).

The amount of glazing to be used in a fish product, as mentioned before, does not have a specific legislation, and it can be a very important factor for guaranteeing consumer satisfaction, for assessing its protective function, and also for economic reasons. Thus, a low percentage of glazing (below 6 %) may not assure the protection of the fish, and can lead to a diminished quality of the final product. From an economic perspective, an excessive percentage of glazing (over 12 %) can guarantee higher profits for the sellers, since the consumers will be paying water for the price of fish, although some efforts are being made in this area in the European Community. In any of these circumstances the consumer is always the most affected party (Vanhaecke et al., 2010).

Chapter 2. Fish Quality

Raw seafood is a highly perishable product. In order to maximize the value of fish, regarding both taste and economic value, freshness quality must be maintained (Sea Fish, 2011).

Quality is defined as the degree to which a set of inherent characteristics fulfill specified requirements (ISO/IEC, 2005). Regarding fish products, it involves aspects associated with gastronomic delights, purity, nutrition, safety, consistency, and product excellence. In international fish trade, two of the most important aspects taken in consideration are safety and sensory quality (regarding the level of spoilage) (FAO, 2005).

Freshness is one of the most important parameters for the quality of the final product. Freshness can be translated by some sensory, (bio)chemical, physical and microbiological parameters (Olafsdóttir et al., 1997).

In the majority of cases "quality" refers to the visual appearance and freshness or the deterioration which the fish has endured. It can also involve safety characteristics such as lack of harmful bacteria, parasites or chemicals present in the fish. It is important to remember that "quality'' involves different things to different people involving a certain degree of subjectivity (Huss, H. H, 1995).

The principal methods for the assessment of fresh fish quality can be split into two categories: sensory and instrumental (or non-sensory). Considering that the consumer is the final evaluator of quality, most chemical or instrumental methods should be linked to a sensory evaluation before being used in the laboratory. Nevertheless, sensory methods should be executed scientifically under carefully controlled conditions so that the effects of test environment or personal bias can be reduced (Huss, H. H, 1995). The instrumental methods comprise chemical, physical and microbiological methods (FAO, 2005).

The several methods for the assessment of fish quality can be seen in Figure 2-1 (Alasalvar, Grigor, & Ali, 2010).

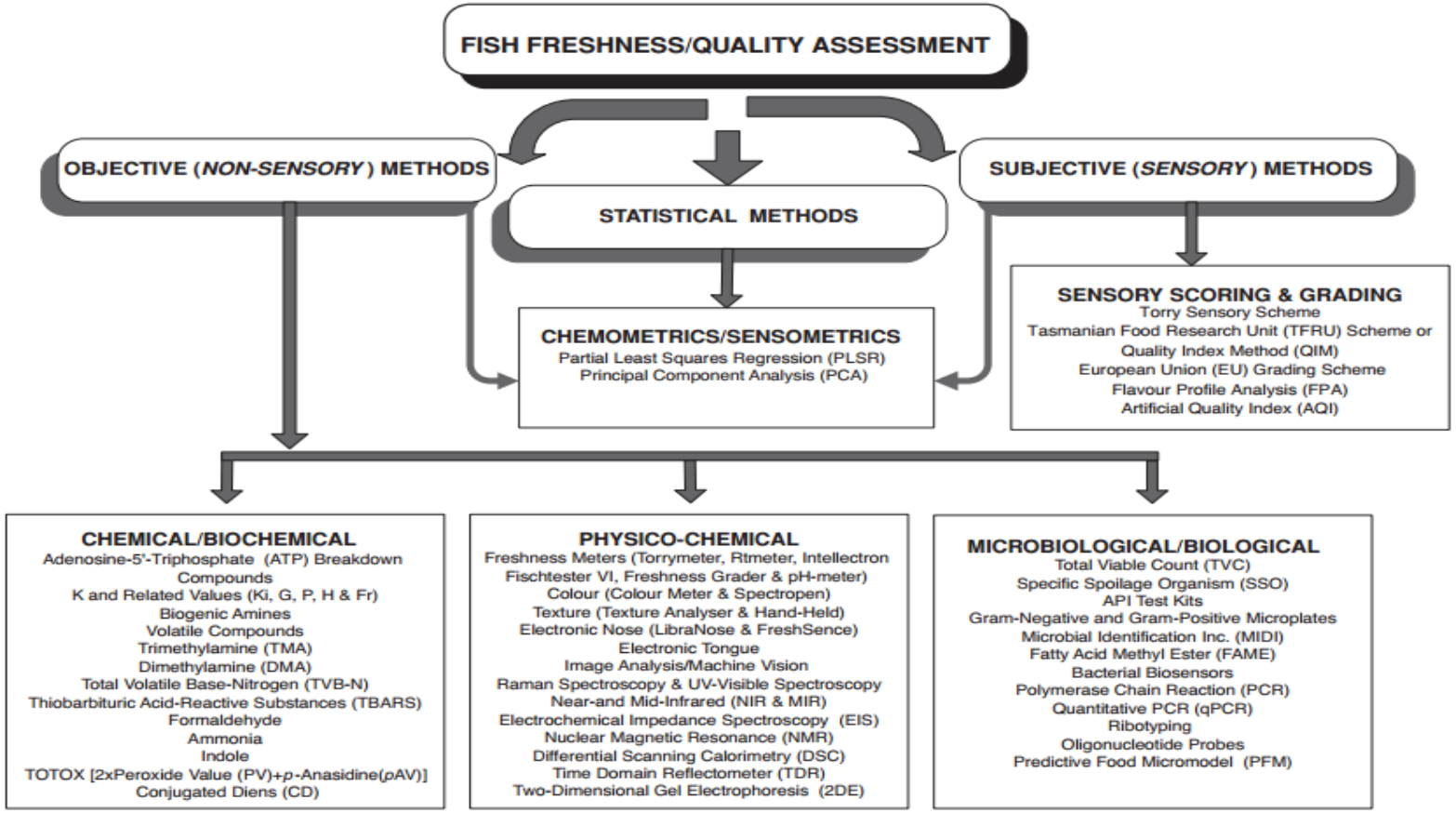

Figure 2-1 Methods used for fish freshness and quality assessment (adapted from (Alasalvar et al., 2010).

2.1. Non-sensory methods

The need for processing in order to perform a sensory evaluation, using instrumental methods, led to the search for alternative non-sensory instrumental methods, such as chemical, physical, and microbiological methods (Alasalvar et al., 2010).

2.1.1. Microbiological methods

The activity of microorganisms is the main factor limiting the shelf life of fresh fish.

The purpose of microbiological exams of fish products is to assess the possible presence of bacteria or organisms of public health importance and to give an impression of the hygienic quality of the fish including temperature abuse and hygiene during handling and processing (FAO, 2005; Huss, H. H, 1995)

2.1.1.1. Total Viable Counts (TVC)

The total viable count represents, the total number of bacteria that are capable of forming visible colonies on a culture media at a given temperature (Huss, H. H, 1995).

An estimate of the total viable counts is used as an acceptability index in standards, guidelines and specifications (ISO, 2013; Olafsdóttir et al., 1997).

If a count is performed after systematic sampling and a detailed knowledge of the handling of the fish before sampling, temperature conditions and packaging, the results of the count can provide a comparative measure of the overall degree of bacterial contamination and the hygiene utilized (Huss, H. H, 1995).

Higher incubation temperatures (above 30 °C) are considered inappropriate when performing an examination to seafood products held at chill temperatures (Huss, H. H, 1995).

2.1.2. Chemical methods

The interest in the use of chemical methods for the assessment of fish quality is tied to the ability to establish quantitative standards. The establishment of tolerance levels of chemical spoilage indicators helps eliminate the necessity of making decisions regarding product quality based on personal opinions. In general, sensory methods are

great for assessing products of very good or poor quality. On the other hand, chemical methods may best be applied regarding products of marginal quality (Huss, H. H, 1995).

Chemical methods rely on the measurement of metabolites produced during fish storage or distribution to obtain a quantitative fish index, so the chemical compound to be analyzed and measured should increase or decrease with the level of microbial spoilage or autolysis (FAO, 2005; Huss, H. H, 1995). One of the most widely used methods is the Total Volatile Basic Nitrogen (TVB-N).

2.1.2.1. Total Volatile Basic Nitrogen

A TVB-N test measures the content of trimethylamine, dimethylamine, ammonia and other basic nitrogenous compounds that generally associated with fish spoilage (FAO, 2005; Huss, H. H, 1995).

Despite TVB-N analyses being considered simple to execute, they have the disadvantage of only reflecting in the later stages of fish spoilage, and are normally unreliable for measurements in the first few days of storage. This methods also presents the disadvantage of not giving any information about the type of spoilage (Huss, H. H, 1995).

The Directive 95/149/EC establishes limits for TVB-N values, while also imposing the methods of analysis. Some of this limits can be seen in Table 2-1 (Official Journal of the European Communities, 1995).

Table 2-1 Fish categories and respective TVB-N limit (adapted from (Official Journal of the European Communities, 1995)**)**

Fish category	TVB-N limit (mg nitrogen/100 g of fish)
Sebastes sp.	25
Helicolenus dactylopterus	
Sebastichthys capensis	
Pleuronectidae (except *Hippoglossus sp.*)	30
Salmo salar	35
Merluccidae	
Gadidae	

2.1.3. Physical methods

The use of physical methods generally involves the measurement of fish pH, its texture and/or color (FAO, 2005).

2.1.3.1. Color

Color is one of the major attributes that impact the consumer perception of quality. The degree of acceptability of a product is conditioned by how much the color deviates from the expected range for food acceptance by the consumer (Francis, 1995; HunterLab, 2008).

A color space is a useful conceptual tool that helps to understand the color capabilities of a particular device or digital file. There are several color spaces, such as CIE xyz (1931), CIE L*a*b*, and CIE L u'v' (1976).

Color space (CIE L*a*b*)

The L*a*b* color space (also referred to as the CIELAB space) is one of the uniform color spaces defined by the CIE in 1976 (Minolta, 2007).

The structure of the L*a*b* color space derives from the theory that a color cannot be both green and red at the same time, neither it can be blue and yellow at the same time. This way, single values are used to describe the red/green and the yellow/blue attributes. When a color is expressed in CIE L*a*b*, L* stands for lightness, a* and b* are the chromatically coordinates. +a* represents red direction, while -a* is the green direction, +b* is the yellow direction, and -b* represents the blue direction (Minolta, 2007; X-Rite, 2004).

Color differences

Color can be measured numerically trough the help of a colorimeter in an easy and accurate manner, complying with international standards.

In the CIE L*a*b* color space, color difference are expressed through a single value, ΔE*ab, that provides the value of the difference between colors, but does not gives any information regarding how the colors are different from one another. ΔE*ab for the CIE L*a*b* color space can be calculated through Equation 2-1.

$$\Delta E^*ab = \sqrt{(\Delta L)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad \text{Equation 2-1}$$

In which the parameters ΔL*, Δa*, Δb* regard the difference in the L*, a* and b* values between two different colors (Minolta, 2007).

The color of a product can be critical to its acceptability by the consumers. Considering that, gauging the difference between two samples is very important. However not all differences can be seen by the normal consumer.

ΔE*ab values lesser than 1 are normally invisible to the naked eye, while values between 1 and 2 represent a small difference that may be detected by a trained observer. Values greater than 2 and less than 3.5 represent medium differences that can be obvious even to untrained observers. Values above 3.5 are very obvious to all observers (Cruse, 2015; EFI, n.d.).

2.1.3.2. pH

Knowledge about the pH of fish can give important information about the fish condition. Measurements are performed with a pH-meter that can be placed directly into the fish muscle or into a suspension of fish muscle and distilled water (Huss, H. H, 1995).

Normal pH values for salmon samples are usually between a minimum limit of 6.0 and a maximum limit of 6.5. At a normal condition the salmon's pH is close to a neutrl value, but as the post mortem changes occur, the decomposition of nitrogenous compounds leads to the increase in pH of the fish fillet. This increase in pH has an altering effect on the quality of the product during storage; especially, the sensorial characteristics such as odor, color, and texture which are affected negatively (Kilincceker, Dogan, & Kucukoner, 2009).

2.1.3.3. Texture

Texture is an important property of fish muscle. Fish muscle can suffer changes either resulting from frozen storage or resulting from autolytic degradation. Texture can be monitored organoleptically but there was a need for the development of an unbiased rheological test which could truthfully reflect the subjective assessment of a well-trained

panel of judges (Huss, H. H, 1995). The initial developments in the creation of a procedure to measure the texture of foods, were made by Friedman, Whitney and Szczesniak, at the General Foods Corporation, in 1963, when they published a procedure for texture measurement. This method was later adapted and improved by Dr. Malcom Bourne in 1968, and more changes have been made since then, leading to the current state of the Texture Profile Analysis (TPA) test (Rosenthal, 2010).

TPA has become a widely used double compression test that helps to determine the textural properties of foods. In this test, samples are compressed twice using a texture analyzer to provide information into how samples behave when chewed. Due to the procedure, this test as also become known as the two bite test, as it tries to mimic the mouth biting on food. One of the great advantages of this test is that it can measure multiple parameters with just one experiment, such as the ones seen in Table 2-2 (Rosenthal, 2010; Texture Technologies Corporation, 2015a).

Table 2-2. Some of the parameters obtained in a TPA and their meaning (adapted from (Texture Technologies Corporation, 2015a))

Parameter	Meaning
Hardness	Hardness is the value of the peak force that occurs during the first compression
Fracturability	Fracturability occurs when the TPA plot has its first significant peak during the first compression
Cohesiveness	The ability of a product to withstand a second deformation regarding its resistance under the first deformation.
Springiness	Represents how well a product physically springs back after it has been deformed during the first compression

These parameters have evolved and changed during the last few decades, incorporating suggestions from consumers, and aiming to produce more reliable results and data on the textural properties of foods.

2.2. Sensory Analysis

Sensory assessment of fish freshness is still one of the most important assessment methods used by the seafood industry. It is mostly utilized in the determination of product specification or standards in quality control. Thus, sensory assessments are gaining importance in market development and are regularly correlated with non-

sensory methods such as chemical, microbial, and physical assessment techniques (Green, 2010).

Sensory assessment of fish quality can be defined as the scientific discipline used to evoke, measure, analyze and interpret reactions to characteristics of food as perceived through the use of one or more of the five senses to judge (FAO, 2005; Huss, 1995).

Most of the sensory characteristics of fish can only be measured reliably by humans. Nonetheless, developments are being made in the research and development of instruments that can measure individual quality changes (Huss, 1995).

In sensory analysis the characteristics of appearance, odor, flavor and texture are assessed using the human senses. In the scientific approach, the process can be separated into three distinct steps. The first one consists of the detection of a stimulus by the human senses; the second one consists of an evaluation and interpretation by a mental process; and lastly the third step consists of the response of the assessor to the stimuli (Huss, H. H, 1995).

The use of sensory assessment arises from the necessities such as to define quality control parameters, and conduct market research. Its possible do divide the sensory assessment in to an objective assessment and a subjective assessment (Torry Research Station, 2001).

Objective sensory assessment is utilized for two main objectives. The first one is frequently met when it is necessary to describe specific aspects of quality that are significant. The second one is the use of an objective assessment to create a distinction between two or more products (Torry Research Station, 2001).

Subjective assessment is utilized in product development and market research, and is largely used to discover what the normal consumers thinks about fish products. Thus this type of assessment is more often used in the industry and it is a vital part of it (Torry Research Station, 2001).

It is quite important to be conscious of the differences that exist between different individuals, and their sensitivity and perceptions relating to different products characteristics when selecting and training judges for sensory analysis. Interpretation of

the stimulus and the respective response should be trained very carefully in order to guarantee an objective response (Huss, 1995).

2.2.1. Sensory methods

There are two principal kinds of sensory testing methods, objective and subjective, which are used to assess fish freshness. Objective tests are subdivided in descriptive and discriminative sensory methods (Green, 2010; Huss, 1995).

Discriminative tests are applied in determining whether a difference exists between samples, while descriptive tests are utilized to determine the type and the intensity of the differences. The subjective test method is a test that is based on measurements of the preference or acceptance of the product by the consumer and they are especially important in market research studies (Huss, H. H, 1995).

2.2.1.1. Discriminative tests

Discriminative tests used in the sensory evaluation of fish include tests such as triangle and ranking tests. The triangle test is one of the most used tests in the sensory assessment of fish, it is implemented and described in ISO 4120:2004 (ISO, 2004). Triangle testes allow determining if a significant difference exists between two samples. Judges are given three coded samples, and are asked to determine which one differs from the other two (Huss, H. H, 1995).

In a ranking test, several samples are given to the panel of judges, and they are asked to organize them. Normally this test is quicker and is often applied in preliminary screening (Huss, H. H, 1995).

2.2.1.2. Descriptive tests

Descriptive tests used in the sensory evaluation of fish include methods such as structured scaling and profiling. Structured scaling provides the panel of judges with an actual scale, which presents several degrees of intensity. A few descriptive attributes are selected frequently centered on work from a fully trained descriptive panel. Descriptive words should be carefully selected, and the panel of judges trained so that they approve the used terms and objective terms are preferred and should be selected instead of subjective terms (Huss, H. H, 1995).

Profiling allows for a complete description of the product being assessed, and it is an excellent way to describe a product, using for example flavor profiling. Quantitative Descriptive Analysis (QDA) allows obtaining a detailed description of all flavor characteristics present in the product assessed in a qualitative and quantitative way. Judges are given a wide selection of reference samples and use the samples in order to define a terminology that accurately describes the product in question

The use of advanced multivariate analysis allows for a statistic analysis and allows to possibly correlate single attributes to a change in the sensory properties of a product. The results can be seen in a "spiders web", which is possible to see in Figure 2-2 (Huss, H. H, 1995).

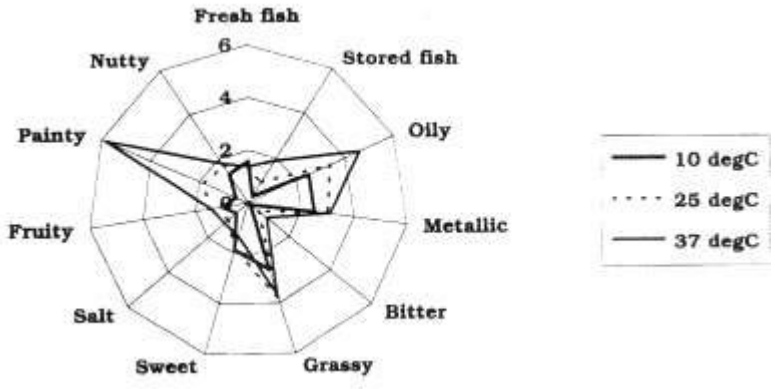

Figure 2-2 Flavor profiles of several components of a fish oil (adapted from Huss, H. H. (1995)).

2.2.1.3. Scoring methods

During the last half of century several schemes for sensory assessment of fish have been developed. The first modern and detailed method is considered to be developed by Torry Research Station in the United Kingdom (Huss, H. H, 1995), while more recent methods include the European Union Scheme and the QIM method.

i) Torry Scale

The fundamental idea behind the development of the Torry scale was that each quality parameter can be considered independent of the other parameters. After the development of this method, the sensory analysis changed, starting to collect a group of distinctive features that would be expressed in a score (Huss, H. H, 1995).

Scores vary between 10 and 3. Scores under a 3 are considered needless, considering that at that point the fish is not fit for human consumption. An average score of 5.5 can be used to function as the limit for acceptability towards consumption. The spoilage attributes can be detected in both the thawed and the cooked fish and adequate scoring systems exist for both forms (Green, 2010).

ii) European Union Scheme

Nowadays in Europe, the method generally used for quality assessment is the EU scheme, which was introduced in the council decision No. 103/76 January 1976 (Huss, H. H, 1995; Official Journal of the European Communities, 1976). This method was updated in 1996, by the council regulation (EC) No. 2406/96, that established the EU scheme used by fish inspectors today (Green, 2010; Official Journal of the European Communities, 1996).

This scheme provides three quality levels: E (Extra), which is the highest quality; A, which is considered an acceptable quality; and B, which is the threshold level beyond which fish is not admitted for human consumption (Green, 2010).

There are still, however, some inconsistencies as this scheme does not take in account the differences between species only making use of general parameters, and mixes both subjective and objective sensory methods (Green, 2010; Huss, H. H, 1995).

Studies show that the more recent QIM scheme is more trustworthy in sensory assessment when compared to the EU grading scheme (Green, 2010).

iii) QIM Method

The QIM method was created and developed at the Tasmanian Food Research Unit (TFRU) of the Commonwealth Scientific and Industrial Research Organization (CSIRO), in Australia between the late 1970s and early 1980s. The QIM scheme answers some of the natural restrictions in the EU grading scheme (Green, 2010).

The implementation of QIM method is based on parameters such as accuracy, precision, and robustness within different user groups and also accounts for the ability to adapt to changing circumstances in order to meet future requirements. Its ease of

use, cost, and probability of adoption in several countries are other important characteristics (Green, 2010).

The QIM method does not measure quality itself or freshness but instead it measures the degree or rate of deterioration or change in the important criteria that are used to describe these qualities. The sum of these changes or deteriorations can then be construed into corresponding days of storage and remaining shelf-life (Green, 2010).

Taking in consideration that all fish have their own characteristic spoilage patterns and sensory characteristics, QIM schemes are developed for individual species. Each characteristic is scored from 0 to 3 demerit points by assessors, with low scores indicating the best quality, and higher scores indicating a higher degree of deterioration. The description of how to asses each parameter is written in guidelines (Green, 2010; Huss, H. H, 1995).

The sum of all characteristics is called QIM index points. The value of the QIM index points increases linearly with the increase in storage time in ice of a given fish. Through the use of the QIM system, a linear relationship between the quality index (QI) and storage time on ice can be created, making it easier to gauge the remaining shelf-life of fish (Green, 2010).

2.2.2. Training of judges

Training of judges for sensory assessment is needed in almost all sensory methods. A laboratory panel must consist of 8 to 10 members, and the training and testing of panel members should be held regularly (Huss, H. H, 1995).

Some of the advantages and disadvantages of using a panel of judges for sensory assessment can be seen in Table 2-3 (CAMO Software AS, 2015).

Table 2-3 Advantages and disadvantages of using a panel of judges for sensory assessment (adapted from (CAMO Software AS, 2015))

Advantages	Disadvantages
Help manufacturers, scientists, food technologists etc. gain a clear perception of what ordinary consumers may experience	Can become fatigued with the entire process of testing and assessing descriptive data
Testing can be much more rapid than most non-sensory methods	May be subject to biases e.g. from loss of interest or from distractions
Use of more than one sense, making them more flexible instruments	To ensure precision in the analysis and interpretation of the descriptive data, several assessors may be required, making it an expensive proposition
Very sensitive and good at detecting minute differences in product characteristics	Recruiting and training sensory panelists can be a time-consuming and costly process
Acceptable for writing into specifications for quality	It may not be easy to replace assessors quickly, as the incoming assessor will have to be given intensive training to develop requisite expertise of the job
Not required to conduct the descriptive analysis of a product. This makes sensory panels a feasible proposition to study products	Can be more expensive than some non-sensory methods
	The panelists may not be good at quantifying perceptions
	Interpretation of results may get problematic and be open to dispute

Chapter 3. Edible Packaging

The quality of food product is dependent on the organoleptic, nutritional, and hygienic characteristics, but these change and evolve during storage time. Most of these changes are mainly related to interactions between foods and surrounding media, or migrations that can occur between the different components in a composite food (Debeaufort, Quezada-Gallo, & Voilley, 1998)

Several physical and chemical processes, such as sterilization or high pressure, have been developed in order to try to stabilize foods and thus allow to better preserve and maintain food quality. Nonetheless, the use of a performing package is needed in the ultimate step of the preservation process (Debeaufort et al., 1998).

3.1. Edible coatings and films

An edible coating or film can be defined as primary packaging prepared from edible components. In this type of packaging a thin layer of edible material can be directly applied to a food or formed into a film and used as a food wrap without altering the original ingredients or the processing method. Edible coatings and films can be used to improve gas and moisture barriers, as well as mechanical properties, sensory perceptions, and microbial protection while extending the shelf life of several products (Pascall & Lin, 2012).

Edible coatings and films can be produced using several biodegradable polymers, such as lipids, proteins, resins and polysaccharides, with or without the addition of plasticizers or surfactants (Pinheiro et al., 2010). They can be classified according to the components that they are made of, or also regarding the type of material from which they are derived. In the latter case they can be divided in three main categories, which can be seen in Figure 3-1 (Srinivasa & Tharanathan, 2007).

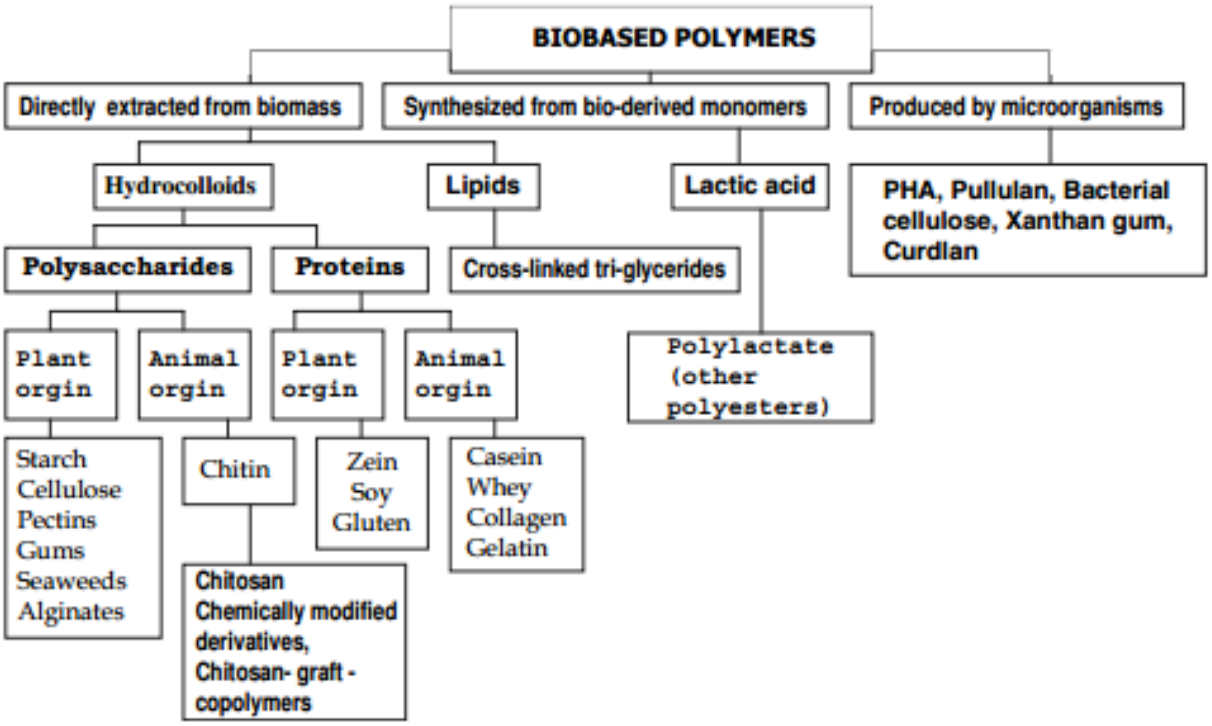

Figure 3-1 Types of biobased polymers used for biopackaging categorized by type of material from which they are derived (adapted from (Srinivasa & Tharanathan, 2007)).

The three most used polymeric ingredients to produce edible films and coatings are polysaccharides, proteins, and lipids. It is also possible to combine two or all of these ingredients in order to produce composite edible films and coatings. Of these polymeric ingredients, chitosan, a polysaccharide, has the most interest for this study. Polysaccharide based edible films or coatings are hydrophilic and are able to have a good oxygen barrier however they present a poor moisture barrier (Pascall & Lin, 2012).

The production and the use of composite films should be done in a way that helps to minimize the disadvantages of the individual components, and at the same time takes advantage of the strength in their properties (Pascall & Lin, 2012).

The behaviour and functionality of edible coatings and films are highly dependable of their mechanical and transport properties. These properties are dependable of parameters such as the coating or film composition, their method of formation and application (Pinheiro et al., 2010).

Edible packaging has several properties that allow protecting products in different ways. Some of those properties are barrier, carrier and enhancement properties.

Barrier properties are the ones that are more relevant in chitosan coatings, and this property allows the edible packaging to function as a barrier that protects the products from being exposed directly to the environment, preventing microbial contamination from pathogenic bacteria, offering a barrier from moisture, oxygen and other gases, as well as from fats and oils (Pascall & Lin, 2012).

The necessity for edible coatings and films arises from changes in the way product arrives to the consumers. It now travels longer distances, which implies more time of transportation and storage, and with this comes the need for a longer shelf-life. During time wasting steps of the processes of handling, storage and transportation, the products start to suffer dehydration, deterioration, loses appearance, flavor and also nutritional value. Damages to the product can occur quite quickly if no special protection is provided, even if this damage is not immediately visible (Pavlath & Orts, 2009).

Edible films and coatings have to be functionally and organoleptically compatible with foods, as they are considered food components. They normally have to be as tasteless as possible, so that they are not detected by the consumer. In the cases in which the films or coatings have a particular flavor or odor, their characteristics should be compatible to the product that they are protecting (Debeaufort et al., 1998).

Taking in consideration that edible films and coatings are considered both a packaging and a food component, they have to fulfill some specific requirements, which can be seen in Table 3-1 (Debeaufort et al., 1998).

Table 3-1 Specific requirements for coatings and films (adapted from (Debeaufort et al., 1998))

Requirements	Good sensory qualities
	High barrier and mechanical efficiencies
	Enough biochemical, physico-chemical and microbial stability
	Free of toxics and safe for health
	Simple technology
	Nonpolluting
	Low cost of raw materials and process

A coating must meet several requirements for legality, safety, and performance (Baldwin & Hagenmaier, 2012). Regarding the requirement safe for health, items that

are supposed to be edible or that are in contact with food normally should be recognized by a group of qualified experts as being safe under the conditions of its intended use, and produced under good manufacturing practices (Pavlath & Orts, 2009). These products are referred as Generally Recognized As Safe (GRAS).

For the last few years, research on edible films and coatings in foods has been driven by food engineers trying to respond to the high demand of consumers for a longer shelf–life and better quality of fresh foods. Between 1967 and today, the business of edible films and coatings grew, reaching around 600 companies in the market by 1996. By 2009 the total annual revenue exceeded 100 million dollars (Pavlath & Orts, 2009).

This growing investment in edible films and coating can also be seen in the research area, with in the last few years (2008-2012) an average of around 450 patents being submitted yearly, has it is possible to see in Figure 3-2 (Aranca, 2013).

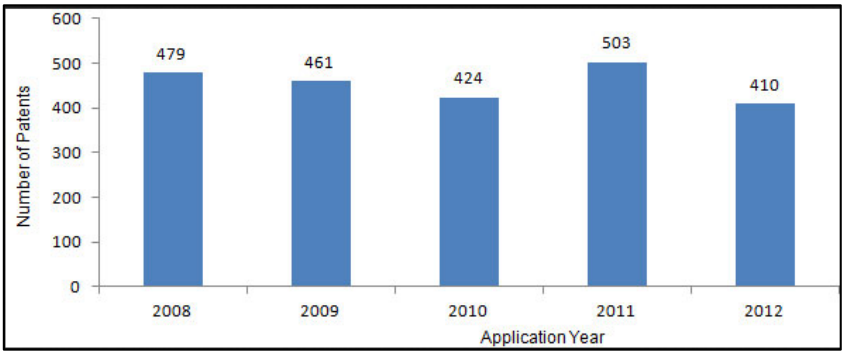

Figure 3-2 Patents submission trends related to edible coatings and films (adapted from (Aranca, 2013)).

3.1.1. Chitin and Chitosan

3.1.1.1. Chitin

Chitin is the second most abundant natural biopolymer after cellulose, and can be found as a main structural constituent of the exoskeleton of invertebrates, insects, yeast, and cell walls of fungi (Srinivasa & Tharanathan, 2007).

Chitin is a water insoluble polymer and a structural polysaccharide composed of a β-1,4-linked N-acetylglucosamine residue and a cellulose-like biopolymer (Y. C. Chung, Tsai, & Li, 2006; Elsabee & Abdou, 2013). The structure of chitin can be seen in Figure 3-3 (Shiekh, Malik, Al-Thabaiti, & Shiekh, 2013).

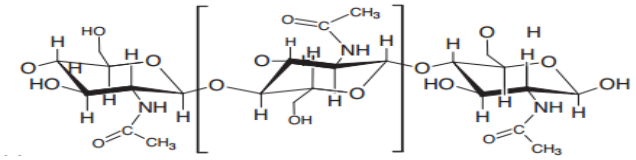

Figure 3-3 Structure of chitin (adapted from (Shiekh et al., 2013))

Chitin can be extracted by a chemical or an enzymatic method (Castro & Paulín, 2012). The three traditional steps for the isolation of chitin, through the chemical method, the most common one, usually are demineralization, deproteinization and decolorization, which can be seen in Figure 3-4 with an extra step of deacetylation to transform chitin to chitosan (Shiekh et al., 2013).

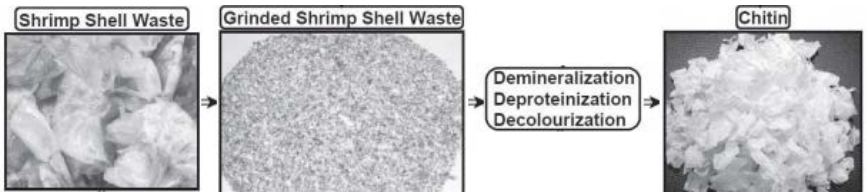

Figure 3-4 Steps for the isolation of chitin (adapted from (Shiekh et al., 2013))

3.1.1.2. Chitosan

Chitosan is one of the most important derivatives of chitin. Chitosan can be defined as a copolymer that is composed by N-acetyl-D-glucosamine and D-glucosamine units, which can be distributed throughout the biopolymer either randomly or in blocks, these units are combined by ß-(1,4) glucosidic linkages thus forming a long chain linear polymer (Castro & Paulín, 2012; Chen, 2008; Singh & Kumari, 2012). The chemical structure of chitosan can be seen in Figure 3-5 (Shiekh et al., 2013).

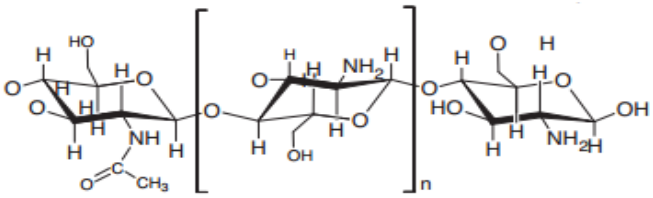

Figure 3-5 Chemical structure of chitosan (adapted from (Shiekh et al., 2013))

Chitosan can be obtained in three different methods, the first is through a thermochemical deacetylation of chitin in the presence of alkali, secondly through an enzymatic hydrolysis in the presence of a chitin deacetylase, and lastly it can be naturally found in certain fungi as a component of their structure (Castro & Paulín, 2012).

The production of chitosan from chitin mainly occurs through a thorough alkaline deacetylation, in which chitin is boiled in concentrated alkali for several hours, in a process that is represented in Figure 3-6 (Raafat & Sahl, 2009).

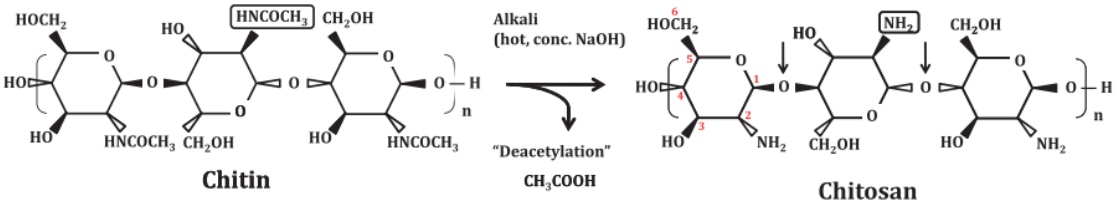

Figure 3-6 Alkaline deacetylation process, transforming chitin to chitosan (adapted from (Raafat & Sahl, 2009))

Chitosan can be described by its degree of deacetylation (DD) and molecular weight (Mw) (Elsabee & Abdou, 2013). These properties along with the positive charge, the nature of chemical modifications of chitosan molecules, chain lengths, charge densities and charge distributions, salt-forms, viscosities, and water retention values strongly affect its physicochemical characteristics, which in turn affect almost all of its applications. Thus, the selection of the most suitable chitosan for use is linked to the intended application (Castro & Paulín, 2012; Raafat & Sahl, 2009).

DD and the Mw can heavily affect the solubility, physical and rheological properties, affecting also the performance of the chitosan. Nevertheless, both the DD and the Mw can be modified, or example lowering of the DD can be achieved through reacetylation, and the lowering of the Mw can be achieved through acidic or enzymatic depolymerisation (Castro & Paulín, 2012).

In addition to these properties, depolymerization of chitosan is also useful in the adjustment of properties such as viscosity, solubility and biological activity (Castro & Paulín, 2012).

Although there are not known in detail the chemical and physical process that compose some applications of chitosan, there is considerable indications that most of their physiological activities and functional properties are linked to the chitosan molecular weight (Raafat & Sahl, 2009).

The DD, the ratio between N-Acetylglucosamine to glucosamine structural units, is another important property that can affect the final function of chitosan. The DD is influenced by the preparation procedure, among other conditions longer treatment times provide chitosan with a higher DD. The value of DD has influence in moisture absorption, charge distribution, intrinsic viscosity, and chitosan solubility in aqueous solutions (Raafat & Sahl, 2009).

3.1.1.2.1. Biological properties of chitosan

Due to the fact that chitosan combines several advantageous characteristics, such as biodegradability, biocompatibility, non-toxicity, and activities such as anti-viral, anti-fungal and anti-microbial, it has gained a lot of interest in industrial, and especially pharmaceutical and biomedical applications (Raafat & Sahl, 2009).

Anti-fungal activity

Chitosan has been proved to have anti-fungal activity (Ing, Zin, Sarwar, & Katas, 2012). From studies conducted it was possible to conclude that antifungal activity of chitosan was altered by factors such as molecular weight, concentration, degree of substitution, types of fungus, and types of functional groups in chitosan derivatives chains (Ing et al., 2012).

In Table 3-2 it is possible to see the minimum growth inhibitory concentration (MIC) of chitosan against several fungi (Rabea, Badawy, Stevens, Smagghe, & Steurbaut, 2003)).

Table 3-2 MIC of Native Chitosan against fungi (adapted from (Rabea et al., 2003))

fungi	MIC (ppm)
Botrytis cinerea	10
Fusarium oxysporum	100
Drechstera sorokiana	10
Micronectriella nivalis	10
Piricularia oryzae	5000
Rhizoctonia solani	1000
Trichophyton equinum	2500

Anti-bacterial activity

Chitosan as also shown to have an anti-bacterial activity, which is suspected to have its origin from a reaction between chitosan and cell walls altering their permeability (Y. Chung et al., 2004).

The anti-bacterial activity can be influenced by several parameters, such as the chitosan type, degree of polymerization and other intrinsic physicochemical properties. Other factors that affect the activity are the molecular weight, the solvent used, and the value of the pH, with a higher activity for low pH values. The activity is also different in Gram positive and Gram negative bacteria, with higher activity values against Gram positive bacteria (Qi, Xu, Jiang, Hu, & Zou, 2004).

Other mechanisms for the anti-microbial activity have been suggested, such as chelation with essential nutrients or trace elements resulting in bacteria inhibition, or chitosan interaction with anionic groups on the surface of the cell, forming polyelectrolyte complexes with bacterial surface compounds, creating an impermeable layer around the cell (Qi et al., 2004). In a general form there is a strong link between the presence of cationic amino groups (NH_3^+) and the anti-bacterial activity (Y. C. Chung, Yeh, & Tsai, 2011).

Some of the advantages that make chitosan more attractive than other disinfectants are the low toxicity level towards mammals, a higher level of anti-bacteria activity and killing rate, and possessing a broad spectrum of bacteria to whom chitosan presents activity, some of which can be seen in Table 3-3 (Rabea et al., 2003).

Table 3-3 MIC of Native Chitosan against fungi (adapted from (Rabea et al., 2003))

Bacteria	MIC (ppm)
Agrobacterium tumefaciens	100
Bacillus cereus	1000
Corinebacterium michiganence	10
Erwinia sp.	500
Erwinia carotovora subsp.	200
Escherichia coli	20
Klebsiella pneumoniae	700
Micrococcus luteus	20
Pseudomonas fluorescens	500
Staphylococcus aureus	20
Xanthomonas campestris	500

Anti-viral activity

Chitosan presents anti-viral activity, having the ability to induce resistance to viral infections in plants, while also inhibiting viral infections in animal cells, and preventing the growth of phage infections in infected microbial cultures (Chirkov, 2002; Rabea et al., 2003).

In phage infection, the addition of chitosan helps to prevent the reproduction of infectious phages in infected cultures of Gram-negative and Gram-positive organisms. This effect is directly related to the chitosan concentration, its molecular structure and its polymerization degree, with higher polymerization degrees being the most effective. It was also reported that the positive charge of chitosan is also important for the inhibitory properties (Chirkov, 2002; Rabea et al., 2003).

Several mechanisms of inhibition of phages replication by chitosan have been suggested. Chitosan can act decreasing the viability of cultured bacterial cells, it can also neutralize the infection of mature or daughter phage particles in the inoculum, and lastly block the replication of the viral phage (Chirkov, 2002).

In respect to the effects of chitosan on viral infection in animals, studies have shown that chitosan acts regulating the activity of the cells involved in immune responses to a viral infection. Macrophages, which are one of the cells regulated by chitosan, are very important in the immune systems response, as they release immune response mediators. Another effect that chitosan has is the ability to induce interferon synthesis, that helps suppress virus replication (Chirkov, 2002; Rabea et al., 2003).

Anti-microbial activity

While a definitive mechanism for the anti-microbial action of chitosan has not yet been defined, some possible mechanisms have been postulated, and a strong link with the polycationic nature of chitosan has been proposed. Three of those mechanism are the positive amino groups interaction with the cell membranes which are negatively charged, changing the barrier properties, reducing the cell viability; another suggested mechanism relates to the ability of chitosan to activate a defense response in plants, which allows for the inhibition of microbial growth due to the chelation of metal ions;

other mechanism involves chitosan binding to DNA and RNA and protein synthesis inhibition, through the penetration of low molecular weight chitosan into the cell (Castro & Paulín, 2012; Raafat & Sahl, 2009).

Although these are some of the proposed mechanisms, it is not believed that any of them act by themselves to explain the antimicrobial activity of chitosan, the anti-microbial activity is believed to be a result of a sequence of molecular processes (Raafat & Sahl, 2009).

An overview of some of the biological properties of chitosan can be seen in Figure 3-7 (Kim, 2014).

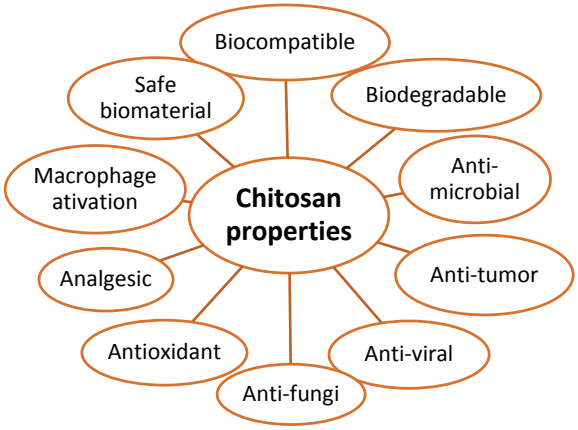

Figure 3-7 Overview of some biological properties of chitosan (adapted from (Kim, 2014)).

As seen before, the intrinsic physicochemical properties of chitosan influence its activity. Such activities and which parameters they are influenced by are represented in Table 3-4 (Kim, 2014).

Table 3-4 Relationship between chitosan biological activities and their characteristics (adapted from (Kim, 2014))

Property	Characteristic
Biodegradability	DD, distribution of acetyl groups, Mw
Biocompatibility	DD
Mucoadhesion	DD, Mw
Hemostatic	DD, Mw
Analgesic	DD
Adsorption enhancer	DD
Antimicrobial	Mw
Anticholesterolemic	DD, Mw, viscosity
Antioxidant	DD, Mw

3.1.1.2.2. Economic and regulatory aspects

Considering that one of the main sources of chitin is the exoskeleton of crustaceans, there is a considerable amount of raw material available at a low cost, meaning that the production of chitosan on a large scale, in a renewable fashion, is economically feasible. Chitosan is produced in several countries all over the world, and used in many more. Another positive aspect is the fact the production of chitosan offers an alternative to the use of the wastes created from crustaceans (Raafat & Sahl, 2009).

Regarding the safety of chitosan, it has been approved for use in biomedical applications by the FDA, such applications include wound bandages and drug encapsulation, although it was not yet been granted GRAS status, a Norwegian company reported in 2001 that their product, a purified chitosan product, had obtained self-affirmed GRAS status in the United States (Raafat & Sahl, 2009).

3.1.1.2.3. Chitosan applications

The interest and potential for chitosan applications is growing as discoveries about chitosan properties, and new ways to explore its increase. Some of the reasons for the widespread use of chitosan is its capacity to have a diversified and wide range of applications, as chitosan is a biomolecule with great potential, and also the cationic nature of chitosan that differentiates chitosan from other polymers (Ravi Kumar, 2000; Rinaudo, 2006; Srinivasa & Tharanathan, 2007).

Several applications have been created in the last decade, overcoming one of the main problems or limitations that arises against the use of chitosan applications, which is the low solubility of chitin (Dutta, Duta, & Tripathi, 2004; Ravi Kumar, 2000). Another limitation, but one that will become easier to overcome, is the lack of approval in some countries, such as FDA approval in the USA.

Some of applications of chitosan, including some of the most relevant regarding coating of food products, in varied fields can be seen in Figure 3-8 (Srinivasa & Tharanathan, 2007).

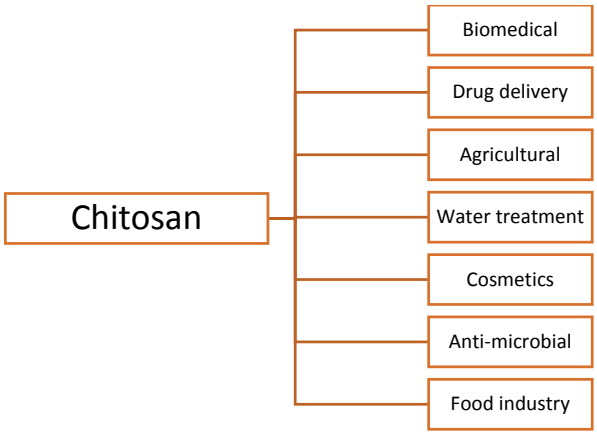

Figure 3-8 Applications of chitosan (adapted from (Srinivasa & Tharanathan, 2007)).

Agricultural

Chitosan and its derivatives have shown the ability to induce the stimulation of plant protection through the triggering of defence mechanisms against fungal and viral attacks. The main application of this effect is used in the form of a coating that is applied to seeds or to leafs of the plants. The use of chitosan also showed improvements in plant growth, both in terms of accelerating its growth and enhancing it. Another application of chitosan regarding the agricultural field is its use as a fertilizer, with its effect connected to the high nitrogen content and molecular structure (Castro & Paulín, 2012; Dutta et al., 2004; Rinaudo, 2006; Srinivasa & Tharanathan, 2007).

Anti-microbial

In recent times, the demand for products without chemical preservatives as led to an intensification in the search for new antimicrobial agents from natural origin, that help inhibit the growth of several pathogenic and spoilage mechanisms. The capability of chitosan as an anti-microbial and anti-fungal agent has been under investigation, and the research seems to point to the presence of free NH^{2+} groups at the C-2 position as the responsible for that particular ability, although the exact mechanism is not yet known (Srinivasa & Tharanathan, 2007).

Food industry

One of the most common applications of chitosan in the food industry in recent years is in its use as edible packaging, especially as coatings or films. The advantages presented by chitosan include the barrier properties that it embodies (mainly to

oxygen), as well as the possibility of reducing the environmental impact of food packaging, either through better recyclability or by direct reduction of environmental pollution created by food packages. Some of the food products that can benefit from the use of a chitosan coating include bread, eggs, fruits and vegetables, as well as in seafood products and their derivatives. In fruits, vegetables and seafood products the use of chitosan is of special interest, due to the perishability of these products. Chitosan can offer a protective barrier reducing respiration and transpiration rates, also retarding microbial growth, in the case of fruits and vegetables, and in the case of seafood products retarding the quality deterioration from lipid oxidation due to the antioxidant properties provided by the chitosan coating (Castro & Paulín, 2012; Srinivasa & Tharanathan, 2007).

Chitosan is also used in other food products, due to its strong anionic charge, acting as a clarification and deacidification agent, in fruit juices, and as an emulsifier in mayonnaise (Castro & Paulín, 2012; Raafat & Sahl, 2009).

Tests have also been made regarding the decrease in food consumption ratio in animals, with the results showing that animals fed with a diet contemplating chitin decreased the food consumption and increased the body weight (Dutta et al., 2004).

Chapter 4. Diffusion

Diffusion is the process by which matter is transported from a location of a system to another, resulting from random molecular motions. Diffusion processes are known to be dependent on factors such as temperature, pressure, solute size, molecular weight and viscosity. Diffusion velocities change according to the medium, in gases diffusions processes are generally fast (10 cm/min) whereas they are much slower in liquids (0.05 cm/min). Besides diffusion in gases and liquids, diffusion also occurs in polymers (Cranck, 1975; Masaro & Zhu, 1999).

The fundamental concepts of mass transfer are similar to those of heat conduction which was adapted for the first time by Fick to cover quantitative diffusion in an isotropic medium (Karimi, 2006).

Fick created two laws (or equations) for diffusion. One for a steady state diffusion, Fick's First Law, and a second one for diffusion under unsteady circumstances, Fick's Second Law, which can be seen respectively in Equation 4-1 and Equation 4-2 (Cranck, 1975).

$$\mathrm{F} = -\mathrm{D}\frac{\partial \mathrm{C}}{\partial \mathrm{x}} \qquad \text{Equation 4-1}$$

In which *F* is the rate of transfer per unit area of section, *C* is the concentration of the diffusing substance, *x* is the space coordinate measured normal to the section, and *D* is the diffusion coefficient (Cranck, 1975).

$$\frac{\partial \mathrm{C}}{\partial \mathrm{t}} = \mathrm{D}\frac{\partial^2 \mathrm{C}}{\partial \mathrm{x}^2} \qquad \text{Equation 4-2}$$

In which *C* is the concentration of the diffusing substance, *x* is the space coordinate measured normal to the section, and *D* is the diffusion coefficient (Cranck, 1975).

Diffusion can be divided and classified in three different categories, regarding the rate of diffusion and polymer relaxation (Cranck, 1975):

- Case I Or Fickian diffusion in which the rate of diffusion is much less than that of relaxation;
- Case II diffusion, the other extreme in which diffusion is very rapid compared with the relaxation processes;
- Non-Fickian or anomalous diffusion, which occurs when the diffusion and relaxation rates are comparable.

The driving force, for diffusion, across the interface of multiphase systems is of chemical potential-base (Karimi, 2006).

4.1. Fickian Diffusion

Fickian diffusion, also known as Brownian transport, is often observed in polymer networks in cases when the temperature is well above the glass transition temperature of the polymer (T_g). When the polymer is in the rubbery state, the polymer chains have a higher mobility that allows an easier penetration of the solvent (Masaro & Zhu, 1999).

Therefore, Fickian diffusion is characterized by a solvent diffusion rate, R_{diff}, slower than the polymer relaxation rate, R_{relax} ($R_{diff}<R_{relax}$). A large gradient of solvent penetration is observed in the system. The solvent concentration profile shows an exponential decrease from the completely swollen region to the core of the polymer (Masaro & Zhu, 1999).

4.2. Non-Fickian Diffusion

Non-Fickian diffusion processes are generally observed in glassy polymers, i.e. when the temperature of study is below the glass transition temperature of the polymer. At a specific temperature below T_g, the polymer chains are not sufficiently mobile to permit immediate penetration of the solvent in the polymer core (Masaro & Zhu, 1999).

Two types of non-Fickian diffusion were defined and can be classified as such: Case II diffusion and anomalous diffusion. The Case II diffusion is a process of moving boundaries and a linear sorption kinetics, which is opposed to the processes of Fickian diffusion (Karimi, 2006; Masaro & Zhu, 1999).

The biggest difference between these two diffusion categories regards the solvent diffusion rate. For the Case II diffusion, the solvent diffusion rate is the opposite of what happen in Fickian diffusion, so it is faster than the polymer relaxation process ($R_{diff}>R_{relax}$), whereas in the case of anomalous diffusion the solvent diffusion rate and the polymer relaxation are considered to be relatively of the same order of magnitude ($R_{diff}\approx R_{relax}$) (Masaro & Zhu, 1999).

4.3. Diffusion in Polymers and gels

Diffusion in polymers and gels has been studied for the past few decades, and the results gave a better knowledge of the transfer phenomena in these situation, and led to the creation of several theories to help explain these results (Masaro & Zhu, 1999).

Transport properties are affected by the free volume within the polymer and by the segmental mobility of the polymer chains. The segmental mobility of the polymer chains is affected by parameters such as the extent of unsaturation, the degree of crosslinking, the degree of crystallinity and the nature of substituents. The glass transition temperature of polymers also has a very important influence on the transport properties. Polymers with low glass transition temperatures possess greater segmental mobility and will have higher diffusivity (George & Thomas, 2001).

When it comes to polymers which are in direct contact with foods, the phenomena of swelling of the polymer due to water uptake can be considered negligible (Quintas, Bourbon, Martins, Quintas, & Pinheiro, 2011)

4.3.1. Diffusion in rubbery polymers

Diffusion in rubbery polymers usually means that the temperature of the polymer is higher than the glass transition temperature of the polymer ($T>T_g$). The rubbery state represents a liquid-like structure with high segmental motion resulting an increase of free volume with temperature (Karimi, 2006).

Some of the important characteristics of rubbery polymers are the unsaturation, the segmental mobility and the large amount of free volume between molecules, which allow for the observation of a smooth and easy diffusion of small molecules through the rubbery polymers (George & Thomas, 2001).

4.3.2. Diffusion in glassy polymers

Contrary to rubbery polymers, diffusion in glassy polymers generally has the temperature of the polymer lower than the glass transition temperature of the polymer ($T<T_g$) (Karimi, 2006).

Glassy polymers are characterized by a hard, highly viscous and brittle structure, which has restricted chain mobility. Motion within the structure is mainly due to vibration within a frozen quasi-lattice. This means that these dense structures have very little void space (0.2–10 %), which leads to the diffusion in glassy polymers being much more complex compared to that in rubbery polymers (George & Thomas, 2001; Karimi, 2006).

4.4. Diffusion theories and physical models

There are several theories that involve physical concepts such as obstruction effects, hydrodynamic effects, free volume effects and the Arrhenius' theory (regarding the temperature effect), each of which have several models that try to explain the different diffusion theories and concepts (Masaro & Zhu, 1999).

4.4.1. Obstruction effects

Diffusion models that are based upon obstruction effects, regard polymer chains as motionless when in comparison to the diffusing molecules. This approximation assumes that the polymer self-diffusion coefficient is much smaller in comparison to that of the diffusant. This way, the polymer is represented as fixed and impenetrable segments that are immersed in a solution. The presence of the motionless polymer chains leads to an increase in the mean path length of the diffusing molecules between two points in the system (Masaro & Zhu, 1999). Some of the models that support this theory can be seen below.

4.4.1.1. The Maxwell–Fricke model

The obstruction concept was introduced for the first time by Fricke in 1924, who created several studies, based on the electric conductivity and capacitance of spheroids dispersed in dog blood medium. For the purpose of this study, the author considered

different geometries of spheroids in what lead to the development of the Maxell-Fricke model (Fricke, 1925; Masaro & Zhu, 1999).

4.4.1.2. The Mackie and Meares model

Mackie and Meares, in 1955, used the physical concept proposed by Fricke in order to describe how diffusion of electrolytes behaved in a resin membrane, under the assumption that the polymer mobility is not as important as the mobility of ions or water, so that sites occupied by the polymer are permanently unavailable to ions or water. Taking that consideration into account, it leads to the notion that the motionless polymer chains imposes a tortuosity or an increase in the path length for the molecules in motion (Mackie & Meares, 1955; Masaro & Zhu, 1999).

4.4.1.3. The Ogston *et al*. model

To offer a theoretical response for the empirical equation of Laurent and coworkers, which relates the sedimentation of proteins in hyaluronic acid solutions, Ogston and coworkers tried to develop a new approach for the diffusion of larger molecules. The authors assumed that the polymer acted as a barrier formed by a random distribution of long molecular fibers, and doing so, the self-diffusion coefficient for a given diffusant molecule will depend both on the size of the obstacle present in the solution and on the size of the molecule itself (Masaro & Zhu, 1999; Ogston, Preston, & Wells, 1973).

4.4.2. Hydrodynamic theories

The hydrodynamic theories include the effect of the hydrodynamic interactions that exist in the whole system. These interactions include, among other, frictional interactions between the solute and the polymer, which can be considered the most important interaction, between the solute and the solvent, and also between the solvent and the polymer. These considerations allow for the description of the diffusion in regimes with higher concentration when the polymer chains start to overlap, which was harder to obtain with the obstruction models (Masaro & Zhu, 1999).

4.4.2.1. Cukier's model

Cukier, in 1984, developed an equation to support and describe the diffusion of Brownian spheres in semi-dilute polymer solutions which took into account the existing

hydrodynamic interactions. In this theory, the semi-dilute solution was considered to be a homogeneous monomer unit environment as the polymer coils overlap. This semi-dilute solution of the polymer was considered to be motionless when in comparison to the diffusing solvent, and was represented by randomly distributed spheres immersed in an incompressible Navier–Stokes fluid. This way, the diffusant was considered to undergo screening effects due to the overlapping of the polymer chain (Cukier, 1984; Masaro & Zhu, 1999).

4.4.2.2. Altenberger *et al.* model

This model describes the rigid body of the polymer, considering it as immobilized points randomly distributed in a solution. The solvent is considered an incompressible Newtonian fluid, which fills the space between these points. A small molecule present will interact with these points, leading to the hydrodynamic interactions being represented by the friction with the stationary points. The mobility of a diffusant will be affected by the concentration of the polymer. At low concentrations (dilute or semi-dilute regimes) the interactions are considered weak (Altenberger & Tirrell, 1986; Masaro & Zhu, 1999).

4.4.2.3. Phillies' model

This model uses a more phenomenological approach in order to describe the self-diffusion behavior of macromolecules (such as polymers and proteins) in a wider range of concentrations. One of the conclusions of this model is that the polymers' self-diffusion coefficient obeys a scaling law (Masaro & Zhu, 1999; Phillies, 1987).

4.4.3. Free volume theory

Free volume can be defined as the volume of a given system at his current temperature minus the volume of the same system at the temperature of 0 K. As a result of this difference, the rearrangement of the free volume creates holes through which diffusing particles are capable to pass through. The free volume is constituted by all of the species present in the system, solvent, solute and polymer. The free volume theories work under the assumption that the free volume is the major parameter controlling the diffusion rate of molecules (Masaro & Zhu, 1999).

4.4.3.1. Fujita's model

Fujita's model was the first diffusion model based on the free volume theory. The application of this model and theory offered successful correlations between the model and the data in the case of the diffusion of small molecules in semi-crystalline polymers (Fujita & Kishimoto, 1958; Masaro & Zhu, 1999).

4.4.3.2. Vrentas and Duda's model

Vrentas and Duda and coworkers gave major contributions to the development of free volume theory along the years by re-examining and improving the free volume model since it was first modelled by Fujita. Vrentas and Duda extended the free volume theory to a wider range of temperatures and polymer concentrations; they also took into account the free volume contributions from both the solvent and the polymer. As a result, the Fujita's free volume model is considered as a special case of the Vrentas and Duda's model. The free volume theory of Vrentas and Duda takes into consideration several physical parameters among which are included the temperature, the activation energy, the polymer concentration, the solvent size, and the molecular weight of the diffusant (Masaro & Zhu, 1999; Vrentas & Duda, 1977).

4.4.4. Arrhenius' theory

The Arrhenius equation describes the temperature dependence of a chemical reaction rate as can be seen in Equation 4-3 (Masaro & Zhu, 1999).

$$k = A \exp\left(-\frac{E_a}{RT}\right) \qquad \text{Equation 4-3}$$

In which *k* represents the kinetic rate of a chemical reaction, *A* a pre-exponential factor, *T* the temperature, *R* is the gas constant and E_a the activation energy (Masaro & Zhu, 1999).

Recent works reported several diffusion experiments using different temperatures leading to the assessment of the activation energy of diffusants in polymer systems with the Arrhenius equation. The variation of diffusivity can be described as a relationship with the Arrhenius equation, which can be seen in Equation 4-4 (George & Thomas, 2001; Masaro & Zhu, 1999).

$$D = D_0 \exp\left(-\frac{E_D}{RT}\right)$$ Equation 4-4

In which D_0 is a pre-exponential factor, E_D is the activation energy of diffusion (George & Thomas, 2001).

Part II – Experimental Work

Chapter 5. Materials and Methods

5.1. Salmon Preparation

Frozen Atlantic salmon (*Salmo salar*) supplied by the company Vanibru – Comércio de produtos alimentares, Braga, Portugal) was used. Each salmon was cut in several pieces, with about two cm of thickness, using a vertical bone-sawing machine (FK 32, BIZERBA, Germany). This process was carried out in a refrigerated room (with temperature between 5 ^{o}C and 8 ^{o}C) in order to reduce the temperature uptake and fluctuation. The samples were separated according to the intended use and intended coating and stored in plastic bags in an industrial freezing chamber (-25 ^{o}C) until further use or transportation.

5.2. Preparation of coating

The chitosan solutions used in this project were prepared using chitosan from Golden-shell Biochemical Co. Ltd. (China) with a 91 % degree of deacetylation. In a 5 L Erlenmeyer a 2 L solution of chitosan (1.5 % w/v) was prepared dissolving 30 g ± 0.01 with 22.2 mL of a 1 % lactic acid solution (90 % (w/w) purity) and the volume was completed up to 2 L with distilled water. The solution was stirred with a magnetic stirrer in a heating plate (VWR; Model: VMS-C7 Advanced) at 70 ^{o}C, until complete dissolution of the chitosan. The temperature was then turned off and the solution remained in agitation overnight. The solution was then transferred to a closed glass container and stored at 8 ^{o}C.

5.3. Preparation of the samples

5.3.1 Preparation of the samples with a chitosan coating

Samples of frozen salmon were removed from the industrial freezing chamber and were weighed (RADWAG WLC 6/A2/C/2, Poland), and dipped in a 1.5 % (w/v) chitosan solution at 8 ^{o}C (measured using an infrared Pronto Plus thermometer (HANNA Instruments, HI99556-10, Romania) with the respective probe (HANNA Instruments, HI765PW, Romania)) during 10 s and then drained for two min, before being weighed again and stored in the industrial freezing chamber until further use. The dipping process

was performed with a pilot-scale glazing tank, previously built for this effect an with a stainless steel mesh.

5.3.2 Preparation of the samples with water glazing

A similar process was followed in order to proceed to the glazing of salmon with water. The salmon samples were weighed before dipping in water for 40 s and then drained for 1 min, before being weighed again and stored in an industrial freezing chamber until further use. The dipping process was performed with the pilot-scale glazing tank and mesh mentioned above.

5.3.3 Preparation of the control samples

The control samples did not require any additional treatment other than the cutting of the salmon and storage in an industrial freezing chamber.

5.4. Storage and transport of the samples

The salmon samples were stored in plastic bags, in different corrugated boxes depending on intended use. Samples were separated by test (sensory analysis, physical tests and microbiological tests) and by coating. The samples used for sensory analysis were transported to the Instituto Politécnico de Viana de Castelo – Escola Superior de Tecnologia e Gestão facilities, by a freezer truck, where they were stored at -20 ^{o}C in an industrial freezing chamber. The samples used for the microbiological tests were kept in the same industrial freezing chamber at -25 ^{o}C until they were sent to the contracted laboratory for analysis. The samples used for the thermal stress test were stored in a different industrial freezing chamber, in individual zip-lock polyethylene bags, which had temperature fluctuations between -15 ^{o}C and -5 ^{o}C, until further use. The storage temperature of these samples was recorded using a data logger (DS7922 1Wire® Thermochrom® iButton®, Dallas Semiconductor Inc., U.S.A.) stored inside the industrial freezing chamber. The samples analyzed in the Universidade do Minho facilities (Laboratory of Industry and Processes) were quickly transported by car with an appropriate quantity of ice accumulators.

5.5. Samples analysis

5.5.1 Microscopic analysis

For the realization of the thermal stress experiment, one of the analyses performed was discovering how the thickness of the water glazing and the chitosan coating varied during the length of the experiment.

Using the same vertical bone-sawing machine as mentioned above, coated and glazed salmon slices were cut with just a few millimeters wide. These samples were then placed in individual zip-lock bags polyethylene bags and kept at -25 ^{o}C until further use. When necesseray the samples were quickly taken to the laboratory, where they were stored at -20 ^{o}C during 24 h before being measured.

In order to measure the thickness of the glazing or coating of the salmon samples, an OLYMPUS magnifying glass (OLYMPUS SZ-CTV, Japan) was used. The salmon samples were photographed with a magnification of 0.67 using the program "Image-Pro Plus" (op+I), light position, contrast and brightness values were also defined. The pictures were then opened and the calibration graph paper 0.67 chosen. The coating or glazing thickness was measured ten times at different points in the samples. This process was then repeated for all of the samples obtained with different coatings.

5.5.2 Percentage of glazing or coating

In order to calculate the percentage of glazing or coating, salmon pieces were weighed before being dipped (W_1) and after draining were weighed again (W_2). Percentage of glazing or coating was then calculated using Equation 5-1.

$$\% \text{ Glazing} = \frac{W_2 - W_1}{W_1} * 100 \qquad \text{Equation 5-1}$$

5.5.3 Coating and glazing loss

For the thermal stress experiment, another of the analysis performed was verifying the coating or glazing loss of coated and glazed salmon in response to the temperature fluctuation that the salmon was suffering. Measurements were performed every two weeks during the first month of the experiment, and every three weeks after the first month, for a total of ten weeks.

Before every measurement the salmon samples were inspected for ice buildup, and if necessary, that ice was removed. The samples were then weighed (W_3), and coating or glazing loss calculated according to Equation 5-2.

$$\%\text{Coating loss} = \frac{W_2 - W_3}{W_2 - W_1} * 100 \qquad \text{Equation 5-2}$$

5.5.4 Weight loss

During the thermal stress experiment, weight loss was controlled, in order to verify its variation with the temperature fluctuation.

In order to accomplish this, the salmon control samples (without any coating), were weighed in the beginning of the experiment (W_4).

Then in every controlled moment, initially from two to two weeks, and after a month from three to three weeks, the samples were weighed again (W_5), and the weight loss was calculated according to Equation 5-3.

$$\%\ \text{Weight Loss} = \frac{W_4 - W_5}{W_4} * 100 \qquad \text{Equation 5-3}$$

5.5.5 Sensory analysis

5.4.5.1. Preparation of samples

The samples used for the sensory analysis are initially removed from the industrial freezing chamber and evaluated by the panel of judges, then they are left to thaw during 19 h, in two distinct ways. One of them is a 'traditional' way, leaving the samples to thaw inside a freezer after removal of the coating. In the other one, the samples are left to thaw inside individually marked zip-lock bags, without removal of the coating, inside the same freezer, and the coating is removed after thawing.

After thawing and analysis by the panel of judges, the samples are boiled in 2 L of water at a temperature near 100 ^{o}C for 5 min, before being placed to cool down for 30 min, after which they are served to the panel of judges.

5.4.5.2. Procedure of analysis

The first sensory analysis that is conducted is of the samples in the frozen state, where each member of the panel of judges assesses three types of samples, all of them frozen, glazed with water, coated with chitosan and uncoated samples, regarding three parameters, color, odor and overall appearance, rating each parameter in a scale that goes from very bad to great.

After that analysis the samples are left to thaw, as mentioned above, and after 19h they are assessed again, this time already thawed, and the panel assesses five types of samples, all of them thawed, chitosan coated samples, water glazed samples and uncoated samples, that were thawed according to a 'traditional' procedure, and chitosan coated and water glazed samples that were left thawing inside individually marked zip-lock bags. The samples are evaluated according to four parameters, color, odor, texture and general appearance, and are rated in a scale that goes from very bad to great.

After all of the judges completed their assessment of all of the samples, the samples go through the preparation process that was described above and are asses in the cooked state. They assess five types of samples, chitosan coated, water glazed and uncoated samples, traditionally thawed, and chitosan coated and water glazed samples that were thawed inside individually marked zip-lock bags. The panel of judges assesses the samples regarding four parameters, odor, texture, flavor and general appearance, and rate them in a scale from very bad to great.

The assessment sheet used by the panel of judges for the assessment of the samples, was developed by the trained panel of the Instituto Politécnico de Viana do Castelo – Escola Superior de Tecnologia e Gestão, and offers guidelines for the evaluation of the salmon samples, as can be seen in Appendix A, Appendix B, and Appendix C for frozen, thawed and cooked samples, respectively.

5.5.6 Determination of TVC

The determination of Total Viable Count was estimated and performed according to the procedure based on the ISO 4833-1:2013 standard (ISO, 2013).

The procedure was performed in quadruplicated, with four different salmon samples, repeated for the differently coated samples, or uncoated samples.

The salmon samples were transported to the laboratory in a hard cooler with an appropriate amount of ice accumulators. The samples were then left to thaw inside a refrigerator, before being analyzed.

The 1 g samples necessary for testing were obtained randomly, selecting them from the salmon samples mentioned above.

The 1 g samples were added to a stomacher bag containing 9 mL of maximum recovery diluent (MRD) and stomached for 1 min. Using a sterile pipette, 1 mL of the initial inoculum was transferred to 9 mL of MRD, and successive dilution were performed, as many as necessary, of which an example can be seen in Figure 5-1.

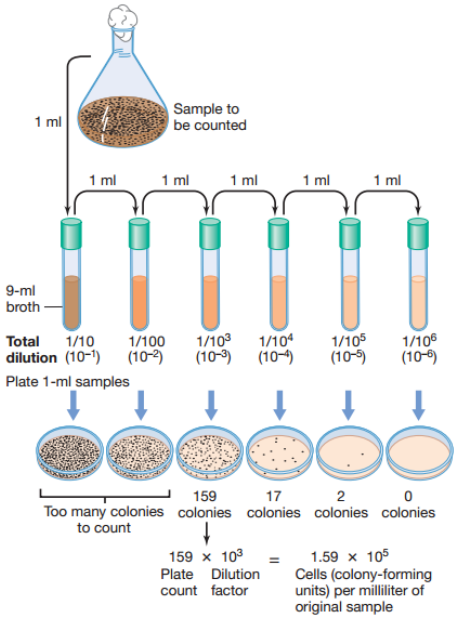

Figure 5-1 Example of serial dilution from an initial sample (adapted from (Madigan, Clark, Stahl, & Martinko, 2010)).

These dilution were then, mixed by use of a vortex, and then 1 mL was aseptically inoculated in a labeled and sterile Petri dish, and 15 mL of plate count agar at a temperature of 44 ^{o}C to 47 ^{o}C, prepared simultaneously, were added.

The Petri dishes containing the inocula and the medium were rotated in order to allow for the inocula and the medium to mix, and after solidifying were inverted and incubated at 30 ^{o}C for 72 h.

Each Petri dish containing more than 15 and fewer than 300 colonies were then counted and the number of microorganisms (N) in the test sample was calculated using Equation 5-4, in which $\sum C$ is the sum of the colonies counted on the two dishes retained from two successive dilutions, at least one of which contains a minimum of 10 colonies; *V* is the volume of inoculum placed in each dish, in milliliters; and *d* is the dilution corresponding to the first dilution retained.

$$N = \frac{\sum C}{V*1.1*d} \qquad \text{Equation 5-4}$$

The results were reported as the number of microorganisms per gram of sample.

5.5.7 Determination of TVB-N

The TVB-N values for coated and uncoated samples, were determined by the Conway method, as referenced in the NP 2930:2009 standard (IPQ, 2009). A 50 g sample of salmon (*m*) was homogenized with 100 mL of 5 % trichloroacetic acid (w/v) and, after waiting for 2 min, the mixture was filtered through gauze. 1 mL of boric acid (H_3BO_3) was then transferred to the center of the Conway cell (Figure 5-2), and in the periphery of the cell, 1 mL of filtrate (V_3), 0.5 mL distilled water and 1 mL of potassium carbonate (K_2CO_3) saturated solution were added. The Conway cell was then carefully closed avoiding mixing the solutions and placed into an incubator at a temperature of 40 °C for 90 min. After that period, the boric acid solution was titrated with 0.02 mol/L hydrochloric acid until a pink coloration was achieved. A blank and a diffusion control were also performed, replacing the volume of extract by an equal volume of distilled water and 0.1 % (w/v) ammonium sulfate respectively.

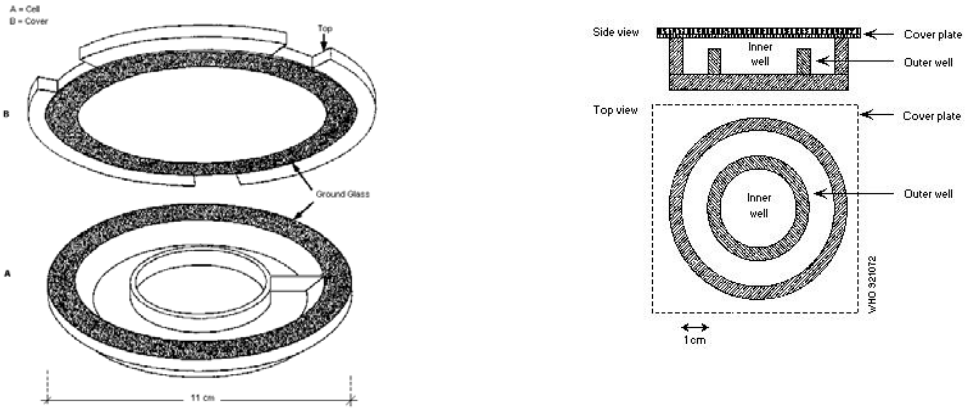

Figure 5-2 Representation of a Conway Cell, with a side and top view of the cell (adapted from http://www.ufrgs.br/imunovet/molecular_immunology/invitrocellfree.html and http://www.inchem.org/documents/antidote/antidote/ant02.htm)

The amount of TVB-N was calculated using the Equation 5-5, where V_0, V_1, and V_2 represent the volumes of hydrochloric acid (mL) added in the blank test, in the diffusion control test, and in the extract test, respectively, and F_c is a volume correction factor (moisture of sample).

$$\text{TVB-N Value} = \frac{21*(V_2-V_0)}{(V_1-V_0)*V_3*m}*(100+F_c) \qquad \text{Equation 5-5}$$

The results for all salmon samples, coated or uncoated, were expressed in mg of nitrogen per 100 g of sample.

5.5.8 Determination of color

In order to assess the effects of thermal stress in the salmon color, and the effect of the glazing and chitosan coating in relation to the uncoated samples, instrumental measures of the color of the samples were made, using a colorimeter (CHROMA METER CR-400/410, AQUATEKNICA, SA, Konica Minolta, Japan) in the University of Minho's Laboratory for Industry and Processes.

In order to assess the effects prolonged storage in the salmon color, and the corresponding effect of the glazing and chitosan coating in relation to the uncoated samples, instrumental measurements of the color of the samples, both thawed and cooked, using a colorimeter (CHROMA METER CR-300, AQUATEKNICA, SA, Konica Minolta, Japan) in the facilities of the Instituto Politécnico de Viana de Castelo – Escola Superior de Tecnologia e Gestão.

In both cases the procedure was similar, samples with 2 cm of thickness were left thawing inside a refrigerator for 19 h, and then evaluated, and in Viana also after being cooked, by measuring six points of the sample, three on each side of the sample in a total of three samples for each coating or glazing.

The results were obtained in the CIE L*a*b* system, in which the parameters observed were L* for luminosity L* (L*=0 corresponds to black and L*=100 corresponds to white) and a* (-a* for green and +a* for red) and b* (-b* for blue and +b* for yellow) for the color coordinates.

The uncoated samples in the initial moment were used as a control, allowing for the calculation of the ΔE*ab for the remaining coatings and moments.

After obtaining the results in the CIE L*a*b* system, those values were transformed into 8-bit encoded RGB codes, using the MATLAB's function lab2rgb (example of complete function: lab2rgb([70 5 10],'OutputType','uint8')). After obtaining the RGB codes, those were converted in color codes and patterns.

5.5.9 Determination of texture

Simultaneously to the sensory analysis by the panel of judges samples of samples were evaluated regarding their texture, using a texturometer (TA.XT *plus* Texture Analyser, Stable Micro Systems Ltd.) in the facilities of the Instituto Politécnico de Viana de Castelo – Escola Superior de Tecnologia e Gestão.

The performed test was a texture profile analysis (TPA), in which through the use of a 10 mm diameter cylinder DELRIN probe, the samples were compressed two times, in order to simulate the bite of a person.

The data obtained was observed and treated with the texturometer *exponent* software allowed for the attainment of the parameters of interest, one of them, the distance, was obtained by manually marking in the software the points from the beginning to the top of a peak.

The raw parameters, of interest, obtained with this test were the peak positive force of the first cycles, the area to positive peak of the first and second cycles, and the distance (from the beginning to the maximum peak) of the first and second cycles, which were used to calculate the parameters Hardness, Cohesiveness and Springiness, which in turn were used to determine the value of the parameter Chewiness, the calculation of these four parameter can be seen in Equation 5-6, Equation 5-7, Equation 5-8 and Equation 5-9, the raw parameters can be seen in Figure 5-3(Texture Technologies Corporation, 2015a).

$$\text{Hardness} = \text{Peak positive force of the first cycle} \qquad \text{Equation 5-6}$$

$$\text{Cohesiveness} = \frac{\text{Area to positive peak of } 2^{nd} \text{ cycle}}{\text{Area to positive peak of } 1^{st} \text{ cycle}} \qquad \text{Equation 5-7}$$

$$\text{Springiness} = \frac{\text{Distance of second cycle}}{\text{Distance of first cycle}} \qquad \text{Equation 5-8}$$

$$\text{Chewiness} = \text{Hardness} * \text{Cohesiveness} * \text{Springiness} \qquad \text{Equation 5-9}$$

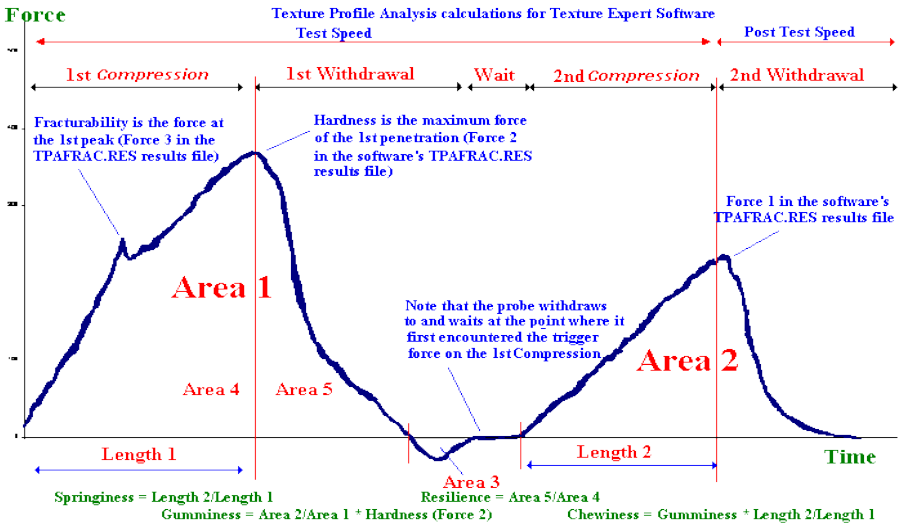

Figure 5-3 Generic example of a TPA (adapted from (Texture Technologies Corporation, 2015b)).

The samples were analysed after thawing and after cooking, the same as the samples used for the sensory assessment. The samples analysed were chitosan coated, and water glazed samples, three of each type, which were thawed in individually marked zip-lock bags inside a freezer for 19 h.

The thawed samples were placed in the texturometer and at least six points in each samples were taken, for a minimum of 18 test points for each coating or glazing. The same minimum number of points was taken in the tests for the cooked samples.

The TPA for the thawed samples and for the cooked samples are very similar, with the only difference being the distance after impact that the probe travels, with the thawed samples having a distance of 15 mm, and the cooked samples a distance of 10 mm. All of the settings for both the thawed and cooked samples can be seen in Table 5-1.

Table 5-1 Settings for the tests performed with the thawed samples

Caption	Value (Thawed samples)	Value (cooked samples)	Units
Pre-Test Speed	1.00	1.00	mm/s
Test Speed	1.00	1.00	mm/s
Post-Test Speed	2.00	2.00	mm/s
Target Mode	Distance	Distance	
Distance	15.00	10.00	mm
Time	2.00	2.00	s
Trigger Type	Auto (Force)	Auto (Force)	
Trigger Force	0.04903	0.04903	N
Break Mode	Off	Off	
Tare Mode	Auto	Auto	
Advanced Options	On	On	
Control Oven	Disabled	Disabled	
Frame deflection Correction	Off (XT2 Compatibility)	Off (XT2 Compatibility)	

5.5.10 Determination of pH

During the duration of the thermal stress experiment, measurements of the salmon pH were taken. In order to do so the coating/glazing was removed from the samples, and the samples were left in the refrigerator thawing during 18 h. After that time period, 5 g of the sample were taken, and grinded in a coffee grinder (Tristar, Netherland). Then in a sample cup, with was added 50 ml, *per* 5 g of sample, of Mili-Q purified distilled water. That solution was then shaken in an orbital shaker (Edmund Bühler, Germany) for 30 min, after which the solution pH was measured using a pH meter (Metrohm 620 pH meter, Swiss made).

5.6. Statistical analyses

Experiments were performed at least in triplicate, and in some cases more. The mean values of those independent determinations were calculated for each treatment at every moment. The statistical significance of differences among treatment was evaluated by a factorial ANOVA test followed by the Tukey HSD test with significance at $p<0.05$. Data were evaluated statistically using the software STATISTICA version 10.0 (StatSoft Inc. 2011). For samples assed for organoleptic changes due to coatings, a Principal Component analysis (PCA) was performed.

Chapter 6. Results and Discussion

6.1. Thermal stress samples analysis

During frozen storage, frozen fish should be maintained below the temperature of -18 ^{o}C at all times, however, it is also know that during processing temperature fluctuations exist, no matter how well the process is conducted, and these temperatures can get as high as -5 ^{o}C (Ministério da Agricultura, Desenvolvimento Rural e Pescas, 2004). -5 ^{o}C is also the minimum temperature necessary for growth of pathogenic bacteria associated with fish (Jay, Loessner, & Golden, 2008). For this reason a thermal stress test was conducted.

6.1.1 Glazing and Coating uptake

The percentages of glazing and coating uptake obtained were 9.9 ± 0.7 % and 13.6 ± 0.8 % respectively for glazing and chitosan coating uptake, with an average weight before dipping of 124.250 ± 19.962 g and an average weight after dipping of 137.900 ± 22.149 g for the water glazed samples and with an average weight before dipping of 134.270 ± 11.492 g and an average weight after dipping of 155.305 ± 13.031 g for the chitosan coated samples, the graphical representation of the glazing and coating uptake percentages can be seen in Figure 6-1.

This data was calculated using Equation 5-1.

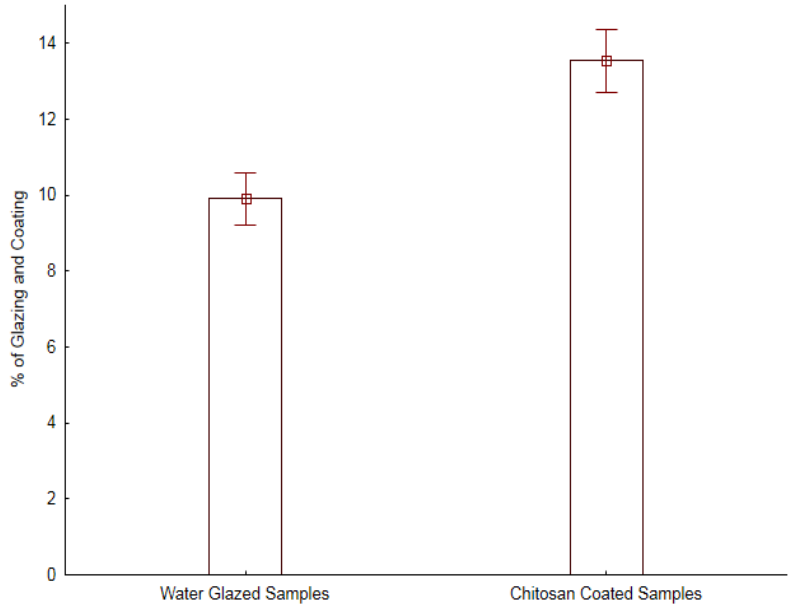

Figure 6-1 Glazing and Coating uptake (%) for salmon samples glazed with water and coated with 1.5% chitosan. Each bar represents the mean ± standard deviation of twenty replications.

These values for glazing and coating percentages, are in line with those reported in previous works under the same conditions, although slightly higher percentages for both water and chitosan were obtained (Soares et al., 2015).

6.1.2 Temperature profile

The temperature profile of the thermal stress conducted can be seen in Figure 6-2. In order to collect this data a data logger (DS7922 1Wire® Thermochrom® iButton®, Dallas Semiconductor Inc., U.S.A.) was used, stored inside the industrial freezing chamber containing the frozen fish. The temperature fluctuations were created using an automatic power switch.

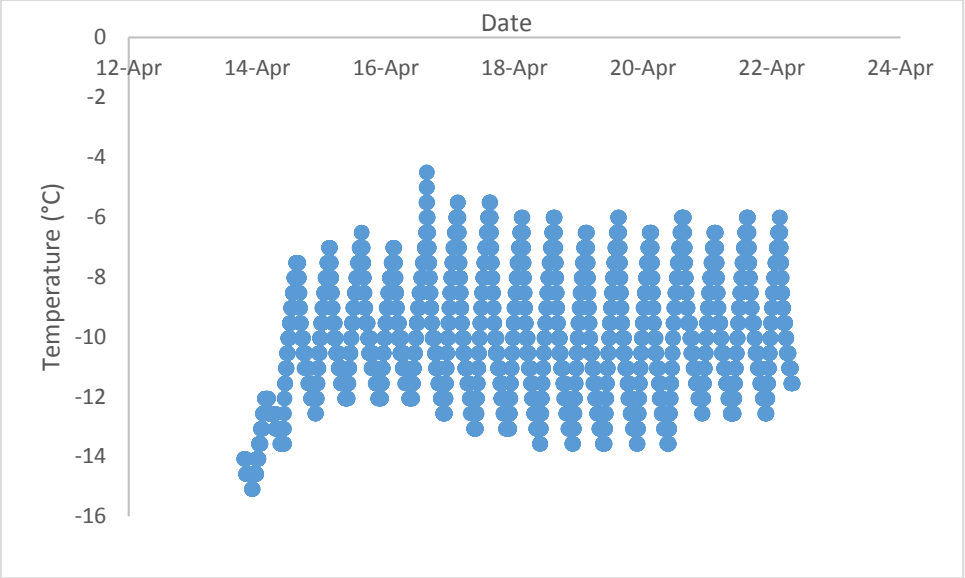

Figure 6-2 Temperature profile for the first two weeks inside the freezing chamber during the thermal stress test.

As shown in Figure 6-1, temperature fluctuated generally between -15 ºC and -5 ºC, in the desired interval to conduct the thermal stress test.

6.1.3 TVC

The TVC values of frozen salmon samples for the thermal stress test for 70 days of storage between -15 ºC and -5 ºC are presented in Table 6-1.

It is possible to see in Table 6-1, that as expected, the TVC values of the uncoated samples increase with storage time, achieving the highest value of 1333 CFU/g, with similar behavior for the water glazed samples with a lesser value of 920 CFU/g. All of the values are greatly influenced by the natural variation of TVC values for fish.

It is also possible to see that the chitosan coated samples clearly present the best results at all moments, with almost all of the samples being below the detectable value of the test (<10 CFU/g). These results on chitosan confirms the ability by chitosan coatings to reduce, inhibit or prevent growth of microorganisms on food surfaces that has been referenced by several authors over past years (Castro & Paulín, 2012; Raafat & Sahl, 2009; Rabea et al., 2003).

Nonetheless freezing still seems to be effective since all of the values are well below both the maximum limit of 10E+7 CFU/g for sensory detection and rejection (Olafsdóttir et al., 1997) and the microbiological limit of 5E+5 CFU/g for quality frozen fish (International Commission on Microbiological Specifications for Foods, 1986).

Table 6-1 TVC values for frozen salmon samples uncoated, glazed with water and coated with chitosan during 70 days of storage between -15 ºC and -5 ºC; standard deviation corresponds to four replications

TVC -15 ºC to -5 ºC	Storage Time (days)	Sample 1 (CFU/g)	Sample 2 (CFU/g)	Sample 3 (CFU/g)	Sample 4 (CFU/g)	Mean (CFU/g)	SD
Uncoated Samples	0	460	650	560	840	628	140
	14	810	600	1100	560	768	214
	28	950	840	940	450	795	204
	49	600	1400	520	1500	1005	447
	70	2100	1300	980	950	1333	464
Water Glazed Samples	0	-	-	-	-	-	-
	14	670	730	870	790	765	74
	28	250	560	840	1200	712	350
	49	190	350	180	430	287	106
	70	980	820	970	910	920	64
Chitosan Coated Samples	0	-	-	-	-	-	-
	14	<10	<10	180	<10	-	-
	28	<10	120	<10	<10	-	-
	49	<10	<10	<10	<10	-	-
	70	<10	<10	<10	<10	-	-

6.1.4 TVB-N

The TVB-N values for uncoated, water glazed, and chitosan coated frozen salmon samples during storage can be seen in Figure 6-3. The initial value, used as a control, of an uncoated sample at 0 days was 11.223 ± 1.334 mg of nitrogen/100 g. Throughout the duration of the test, the TVB-N values of all of the samples tested do not appear to suffer great changes, which is supported by the lack of statistically significant differences.

Uncoated samples present after 70 days a decreased value of 9.863 ± 0.531 mg of nitrogen/100 g, while water glazed samples have an initial value after 14 days of storage of 11.078 ± 0.933 mg of nitrogen/100 g, decreasing slightly to a value of 8.540 ± 0.760 mg of nitrogen/100 g after 70 days, and chitosan coated samples, at 14 days of storage present a value of 9.628 ± 0.917 mg of nitrogen/100 g that decreases to 9.453 ± 0.888 mg of nitrogen/100 g after 70 days of storage, and all of these value are well below the 35 mg nitrogen/100 g fish established as the acceptable limit for salmon by EU Directive 95/149 (Official Journal of the European Communities, 1995).

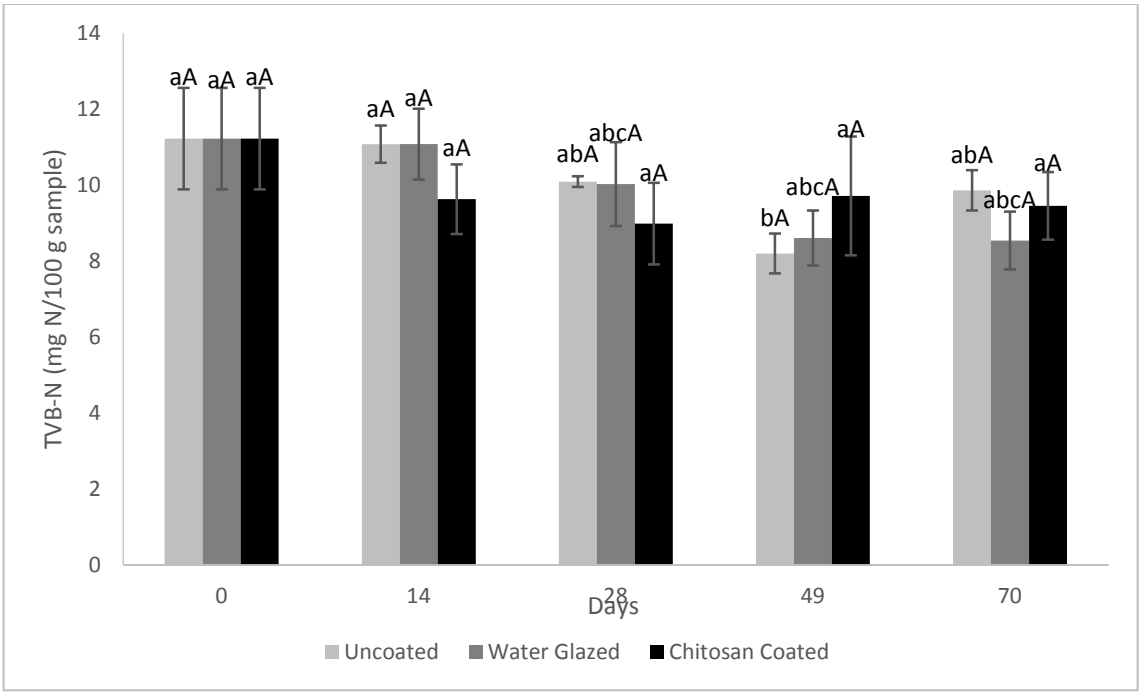

Figure 6-3 TVB-N values for salmon samples during 70 days weeks of storage between -15 ºC and -5 ºC; standard deviation corresponds to four replications; different small letters in the same sample type, and different capital letters in the same time moment indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).

The inexistent statistically significant differences do not allow for a detection of any influence by the different coatings.

Previous studies have shown that an increase in the TVB-N values only seems to happen after 90 days of storage, which can explain the lack of differences among the various coatings since the activity of spoilage bacteria and enzymes is slowed down at lower temperatures (Gonçalves & Gindri Junior, 2009). Another factor contributing to the lack of differences is that the salmon used in this study can be considered to be in good condition, as the low TVC values found seem to indicate.

6.1.5 pH value

The pH values obtained during frozen storage for 70 days can be seen in Figure 6-4. The initial values of pH were 6.410 ± 0.010 for the uncoated samples, 6.430 ± 0.069 for the water glazed samples, and 6.493 ± 0.015 for the chitosan coated samples. After 70 days of frozen storage the pH values had little variation with final values of 6.400 ± 0.030 for the uncoated samples, 6.503 ± 0.015 for the water glazed samples and 6.513 ± 0.006 for the chitosan coated samples.

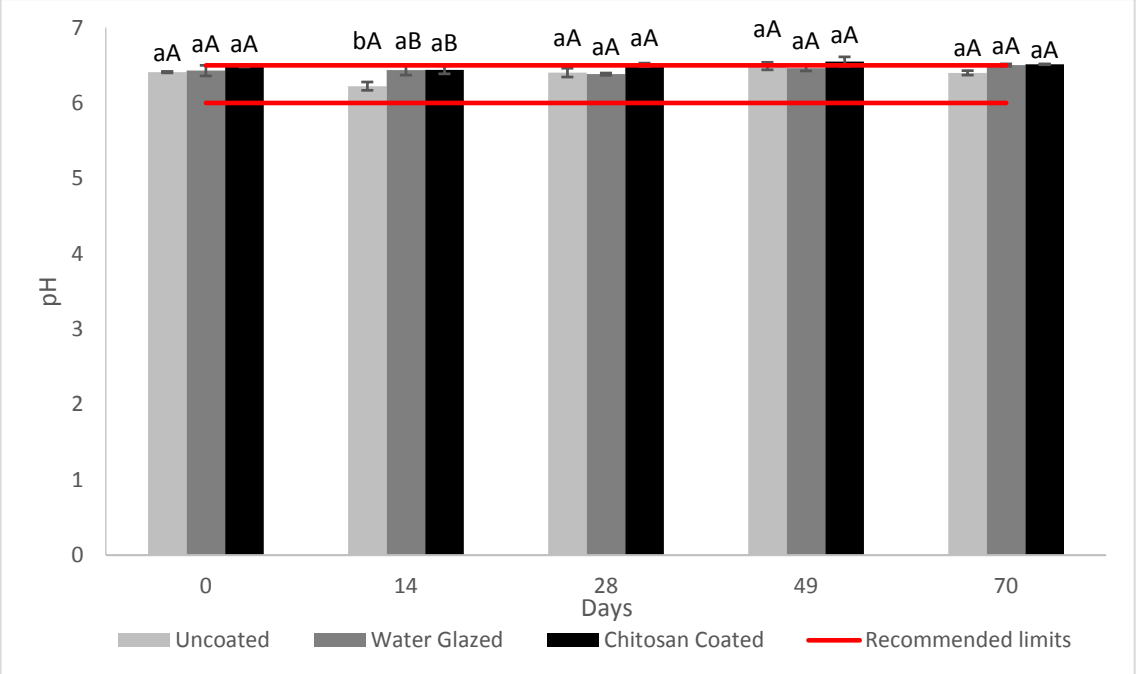

Figure 6-4 pH values for salmon samples during 70 days of storage between -15 ºC and -5 ºC; standard deviation corresponds to three replications; different small letters in the same sample type, and different capital letters in the same time moment indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).

It is possible to see that the chitosan coated samples had higher pH values throughout all of the thermal stress storage when compared with uncoated and water glazed samples although there were almost no statistically significant differences with the exception of the exception of the uncoated samples after 14 days of storage, and the chitosan coated samples after 49 days of storage, which represent the lowest and highest value of pH recorded in all of the samples, respectively. The type of treatment applied does not seem to show a significant influence on the evolution of pH.

The lack of variation of the pH value can possible be attributed to the duration of the thermal stress test, as in previous works a significant change in the value of pH only

occurs after 14 weeks of storage, although in slightly different conditions (Soares, Mendes, & Vicente, 2013). The results also show that the thermal stress endured by the salmon samples has not accelerated changes in their pH values.

It is also possible to see that, with the exception of the mean pH value of chitosan coated samples after 49 days of storage, which is slightly above, all of the samples are within the minimum and maximum recommend limits of 6 and 6.5, found in previous works (Kilincceker et al., 2009).

6.1.6 Color

In order to try to reduce the normal color variation in salmon, the same type of salmon was used for all of the samples, despite that, variation among samples is still present, as it is possible to see when observing the ΔE*ab value between control sample with uncoated sample at the initial moment, since both suffered the same treatment, and color differences were already present, this is represented in graphs by a dotted line, which represents the natural differences between samples, which can be seen that it is quite high, with a value similar to those of the perceived differences to a untrained assessor. Nevertheless a comparison between different types of coatings was tested, in addition to the separate assessment of the color parameters variation during storage for the different groups of samples.

The color parameters L*, a* and b*, during 70 days of storage of the control, uncoated, water glazed, and chitosan coated samples can be seen in Figure A. 4, Figure A. 5 and Figure A. 6 respectively found in Appendix D.

Regarding the results for the L*a*b* parameters obtained during the thermal stress test present some statistical significant differences especially in the later moments of the thermal stress test, but with the exception of the lightness no tendency in these parameters was found; regarding lightness, it appears that as the thermal stress was conducted, the lightness values increased in all of the different treatments applied to the salmon samples.

These values of L*a*b* parameters were transformed to RGB codes, and a visual representation of this codes, for uncoated, water glazed, and chitosan coated samples can be seen in Figure 6-5, Figure 6-6, and Figure 6-7 respectively.

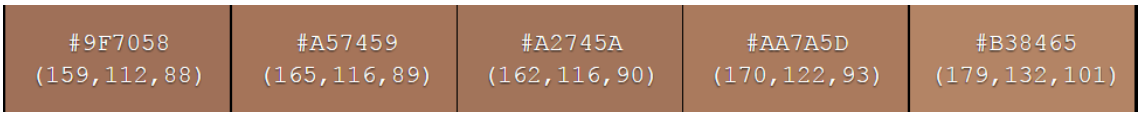

Figure 6-5 Visual representation, in RGB, of the color parameters L*a*b* for uncoated salmon samples during 70 days of storage between -15 ºC and -5 ºC; From left to right is possible to see from the initial moment to the last one.

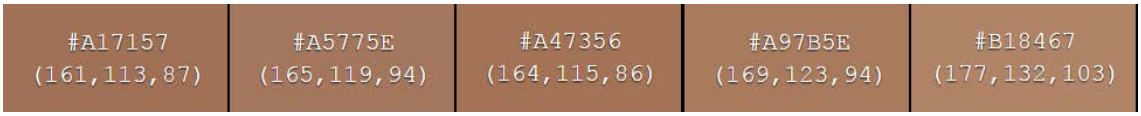

Figure 6-6 Visual representation, in RGB, of the color parameters L*a*b* for water glazed salmon samples during 70 days of storage between -15 ºC and -5 ºC; From left to right is possible to see from the initial moment to the last one.

Figure 6-7 Visual representation, in RGB, of the color parameters L*a*b* for chitosan coated salmon samples during 70 days of storage between -15 ºC and -5 ºC; From left to right is possible to see from the initial moment to the last one.

Each image represents the visual representation of the L*a*b* measurements made during the thermal stress tests, and also includes the RGB code and numerical value.

It is possible to see that between each moment in each of the different treatments, and between different treatments, there does not seem to be an impactful visual difference, although it is possible to perceive some differences between the samples, especially in the later stages of the thermal stress test. It is also possible to see that, as indicated by the L*a*b* parameters, there seems to be a tendency for the RGB colors to become lighter as the thermal stress test progresses.

Regarding perceived color differences, which were calculated as the difference between the assessed sample and a samples with the same coating at the initial moment of assessment, it is possible to see in Figure 6-8 how that value varied during the 70 days of storage for the different samples, and it is also possible to see the ΔE*ab value

between two identical samples (control and uncoated samples at day 0), which represents the natural color different between samples.

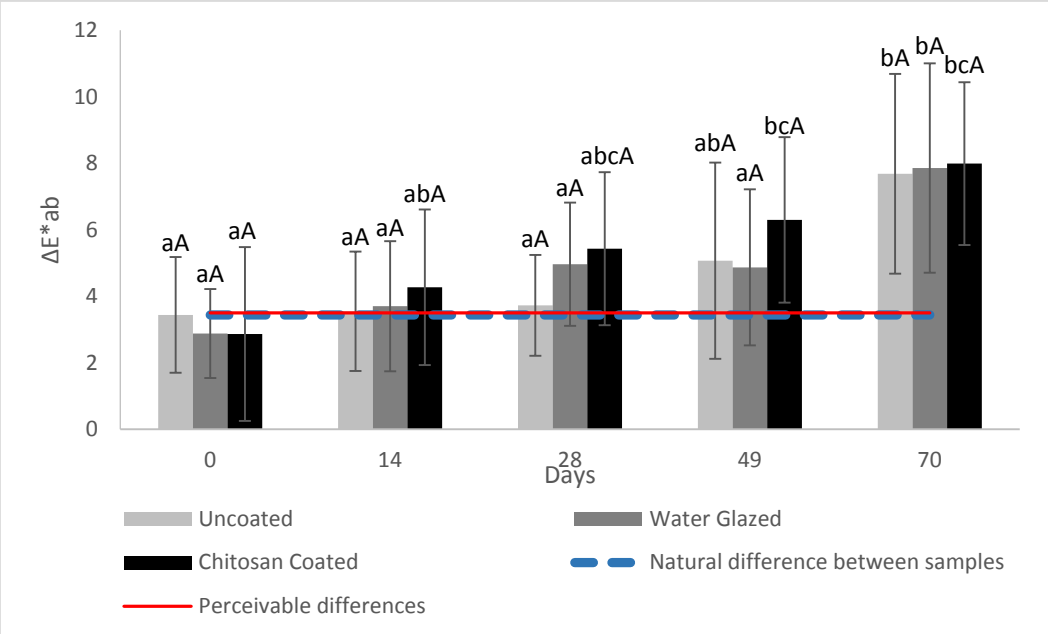

Figure 6-8 ΔE*ab values for salmon samples during 70 days of storage between -15 ºC and -5 ºC; standard deviation corresponds to eighteen replications; different small letters in the same sample type, and different capital letters in the same time moment indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).

As shown in Figure 6-8, the perceived color differences, represented by the ΔE*ab value, follow an increasing tendency in almost all of the samples analyzed, which is to be expected as more time suffering the temperature fluctuation will lead to bigger differences in color values.

In most of the samples there were no statistically significant differences, especially in the initial stages, while later on some statistically significant differences begin to appear. With the exception of the initial values in terms of differences that can be seen by humans, nearly all of the samples present differences that would be obvious to even an untrained observer (represented by a value of ΔE*ab greater than 3.5) (Cruse, 2015; EFI, n.d.). Between different coated samples, there are also no statistically significant differences.

It is also possible to see the natural color variation between samples, represented by the ΔE*ab value between a control sample, and the uncoated sample at 0 days, both that had not suffered any treatment or thermal stress, and therefore are expected to be

in the same condition, other than the natural color variation represented by the dotted line mentioned above.

At the end of the 70 days the treatment that presented the lower value of ΔE*ab was that of uncoated samples, although by a small margin and one that does not represent a statistical significant difference between coatings, a result that is contrary to that of a previous study that indicated chitosan as a better color preservation agent (Soares et al., 2015). It is also known that chitosan coatings help protect against oxidation and protein denaturation, both of which have an influence in the color preservation of samples (Castro & Paulín, 2012; Ojagh, Núñez-Flores, López-Caballero, Montero, & Gómez-Guillén, 2011; Rodriguez-Turienzo et al., 2011).

This may be due to the fact that all of ΔE*ab values are calculated using a control sample as the standard for the evaluation. An error in the initial reading of that group of samples can affect the other results, leading to inconsistent results. It is also worth noting that while ΔE*ab calculates the color differences it does not give information if those differences are positive or negative in the customers perspective. It can also be due to the natural variation of color between salmon samples (although the salmon species is the same, the samples come from different salmons).

It is also possible to see that between the last two moments of evaluation the highest difference in the ΔE*ab was obtained, as the ΔE*ab values went from 5.1 ± 3.0 to 7.7 ± 3.0, for the uncoated samples, for the water glazed samples it changed from 4.9 ± 2.3 to 7.9± 3.2, and for the chitosan coated samples from 6.3 ± 2.5 to 8.0 ± 2.5. It is also noticeable that, while chitosan still represents the biggest color difference, the leap in the value of ΔE*ab for 49 days to the value of ΔE*ab for 70 days is lower in the chitosan coated samples than in the other samples. It seems so that chitosan would perform better as the storage conditions continued to worsen, but with 70 days being the duration of the thermal stress test, it was not possible to see if this was a tendency that would continue in the consequent moments.

6.1.7 Coating loss

The percentage of water glazing or chitosan coating lost by the salmon samples stored between -15 ^{o}C to -5 ^{o}C during 70 days, is represented in Figure 6-9. As it is

possible to see, the loss of coating or glazing follows a steadily increasing trend, as it is to be expected based on previous works, although the order of the values are extremely different due to the temperature fluctuation in this test (Soares et al., 2013, 2015).

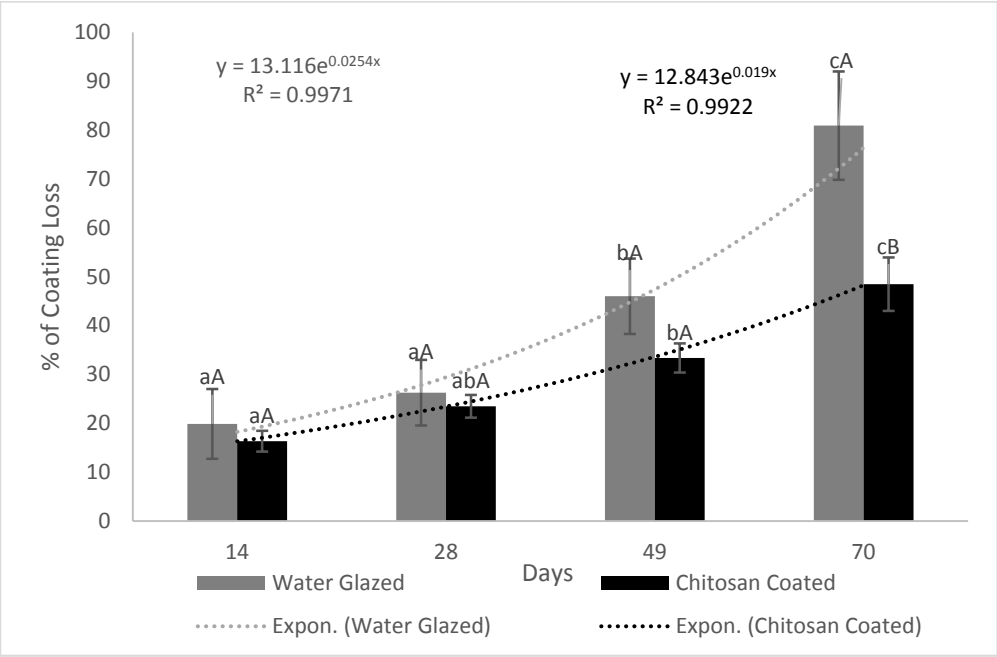

Figure 6-9 Water glazing and chitosan coating losses of salmon samples during 70 days storage between -15 ºC and -5 ºC; standard deviation corresponds to four replications; different small letters in the same sample type, and different capital letters in the same time moment indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).

For the first 49 days, although stable there was a higher loss in the water glazed samples than in the chitosan coated samples, and no statistical differences were found. In the last time period, however there was a significant difference between chitosan coated and water glazed samples in favor, as in all of the other moments, of the chitosan coated samples.

In the last moment of testing, after 70 days of storage, the water glazed samples had lost 81 ± 11 % of their initial glazing, compared to a loss of 48 ± 5 % of the initial coating of the chitosan coated samples. Such a big difference, especially taking in consideration that almost all of the glazing was lost, indicates that under thermal stress conditions the chitosan coating proved to be more effective than the water glazing in protecting the salmon from exposure and increasing its protection.

The inclusion of an exponential trend line allows for the determination of the moment in which the coatings would completely disappear. With the exponential trend

lines adjusted to the obtained data and their equations shown in Figure 6-9 it is possible to determine that the water glazed samples (left trend line equation) would lose all of their coating after 80 days of storage under thermal stress, while the chitosan coated samples (right trend line equation) would last around 108 days, 28 more than the water glazed samples, which would represent a 26 % increase in shelf life time under thermal stress conditions in a 70 days test. It is thus reasonable to assume that under normal conditions and normal storage times the chitosan coating would provide an even greater increase in shelf life, taking in consideration that in normal storage conditions the weight loss tends to follow a more linear progress (Soares et al., 2013). With a normal shelf life time for frozen salmon normally around one year, with an increase of at least 26 %, the chitosan coated salmon samples would have a shelf life time of one year and 3 months, 3 months more than the water glazed samples.

One of the possible reasons for the lesser loss in the chitosan coated samples, may be related to the rheological properties of the chitosan, the viscosity of chitosan is higher than that of water, and increases with chitosan concentration, which may result in a higher resistance to the temperature fluctuation (Hwang & Shin, 2001; Sathivel et al., 2007).

It is also worth noting that while chitosan clearly resists better than water glazing, it may not completely protect the entire salmon, due to the fact that the edges and corners are more easily dehydrated, in this sense the coating of salmon does not eliminate fish dehydration, but it does help to retard it (Johnston et al., 1994).

The method in how chitosan protects the product it is coating is also not clear, with some authors defending that chitosan creates a barrier to external exposure, allowing for the tissue water preservation, while others considering chitosan as sacrificing agent simply delaying the dehydration of the tissue water of the salmon (Kilincceker et al., 2009; Rodriguez-Turienzo et al., 2011; Sathivel et al., 2007).

6.1.8 Weight loss

When fish is not protected by a glazing or coating, the sublimated water will be the tissue water, leading to a reduction in weight.

Figure 6-10 shows the weight lost, in percentage, of the control samples (the uncoated samples) during the 90 days of the thermal stress test. After 90 days of storage, the control samples lost 3.671 ± 0.997 % of its initial weight.

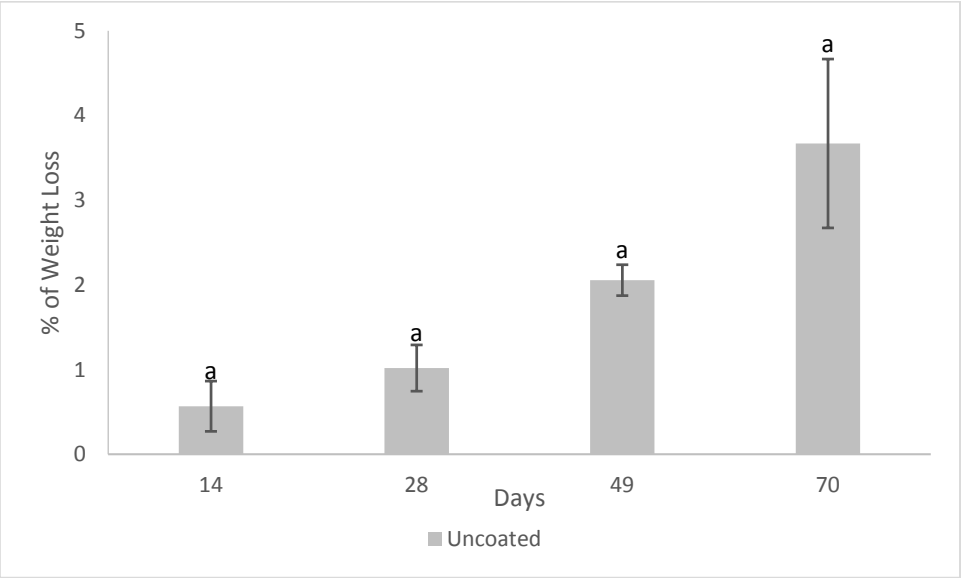

Figure 6-10 Weight loss (%) of salmon samples from the control group during 70 days of storage between -15 ºC and -5 ºC. Each bar represents the mean ± standard deviation of three replications. Different letters indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).

This value is higher than those found in previous works (Soares et al., 2013, 2015) but still presents an increasing tendency in all moments. The difference in values can be in part explained by the temperature fluctuation that is present in this thermal stress test, which is one of the factors that can influence weight lost (Johnston et al., 1994). Other studies also support this, as normally moisture loss increases with the presence of temperature fluctuations, and as it is possible to see in Figure 6-2 a temperature fluctuation of 10 ºC is present during the thermal stress test (Gonçalves & Gindri Junior, 2009).

6.1.9 Microscopic photos

In addition to seeing how the coating and glazing losses behaved during the thermal stress tests, thickness measurements were also made, in the initial and final moments of this test, which can be seen in Table 6-2.

Table 6-2 Water glazing and chitosan coating thickness measurements of salmon samples before and after 70 days of storage between -15 ºC and -5 ºC; standard deviation corresponds to ten replications; different small letters in the same sample type, and different capital letters in the same time moment indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).

	Thickness (mm)	
Sample	0 days	70 days
Water Glazed	1.068 ± 0.140 ^{aA}	0.394 ± 0.312 ^{bA}
Chitosan Coated	1.953 ± 0.175 ^{aB}	1.944 ± 0.673 ^{bA}

While the water glazed samples presented initial values similar to those found in a previous work, the chitosan coated samples presented a significantly higher thickness than those reported (Fernandes, 2014). This may be due to small differences in the coating solution temperature, the salmon temperature, dipping times and especially draining time, as well as the size, shape and weight of the salmon samples, which was quite different than those used in this study, and can affect the coating percentages of the sample, due to the need for higher amounts of chitosan to coat a bigger sample, possibly leading to the higher thickness values obtained due to a possible heterogeneous coating. Another possible explanation is related to the ability of the coating to change from a liquid state to a frozen state (Fernandes, 2014).

Using a higher temperature of the coating solution leads to a chitosan solution of higher viscosity which in its turn adheres better to the surface of the salmon, creating a higher coating percentage and in turn a higher value of thickness (El-Hefian, Elgannoudi, Mainal, & Yahaya, 2010).

As shown in Table 6-2, the thickness results substantiate the results observed though the glazing and coating loss. The water glazed samples, which suffered a loss of 81 ± 11 % of their initial glazing have a final thickness a lot thinner than the initial value of 1.068 ± 0.140 mm with a final value of 0.394 ± 0.088 mm, something that was to be expected considering the thermal stress the samples went through, while the chitosan coated samples that suffered a loss of 48 ± 5 % of their initial coating only has its thickness slightly affected, going from an initial value of 1.953 ± 0.175 mm to a final value of 1.944 ± 0.673 mm, showing that chitosan coatings are able to protect the product better and longer than the water glazing, forming a tougher barrier to deteriorate; although that considering the loss of almost 50 % of their initial coating it would be expected that a lower final value of thickness would be obtained.

This high value can be a result of a non-homogenous coating of the sample, which may introduce an error in the thickness values, as well as the possibility of the thin slices collected to analyze the final thickness of chitosan were obtained from samples that had different, higher, coating percentages, leading to a final thickness higher than expected.

An example of the photographs taken to the water glazed and chitosan coated samples, before and after measurements can be seen in Figure 6-11 and Figure 6-12 respectively.

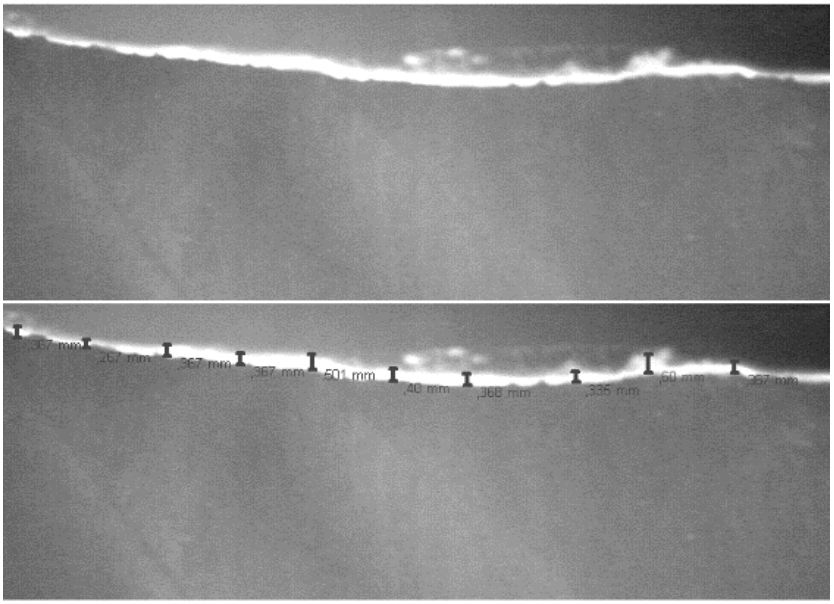

Figure 6-11 Water glazed samples, before (top photo) and after measurements (bottom photo).

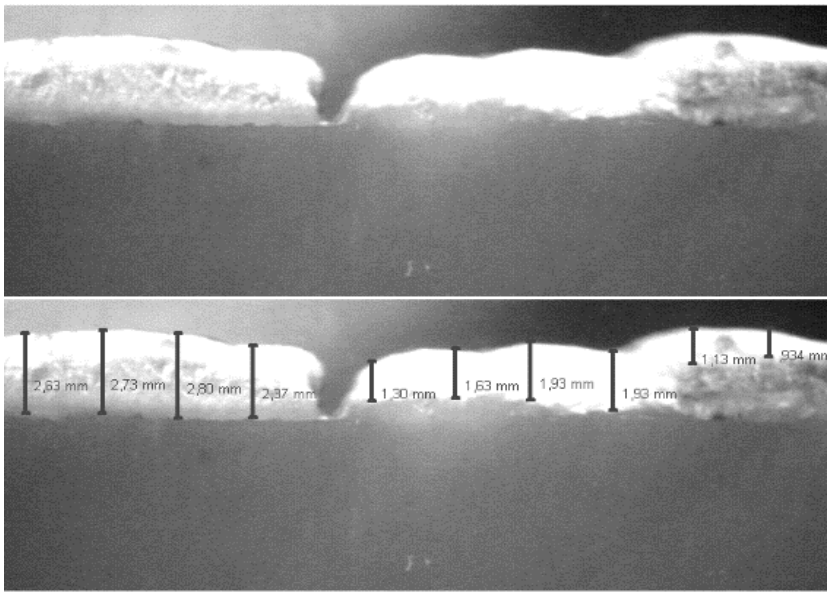

Figure 6-12 Chitosan coated samples, before (top photo) and after measurements (bottom photo).

6.2. General samples used in sensory analysis

6.2.1 Percentage of glazing and coating uptake

The percentages of glazing and coating uptake obtained for the samples used in the sensory analysis were 9.6 ± 0.7 % and 12.0 ± 0.7 % respectively for glazing and chitosan coating uptake, with an average weight before dipping of 130.344 ± 17.576 g and an average weight after dipping of 144.100 ± 19.149 g for the water glazed samples and with an average weight before dipping of 140.275 ± 20.537 g and an average weight after dipping of 159.404 ± 23.114 g for the chitosan coated samples, the graphical representation of the glazing and coating uptake percentages can be seen in Figure 6-13.

This data was calculated using Equation 5-1.

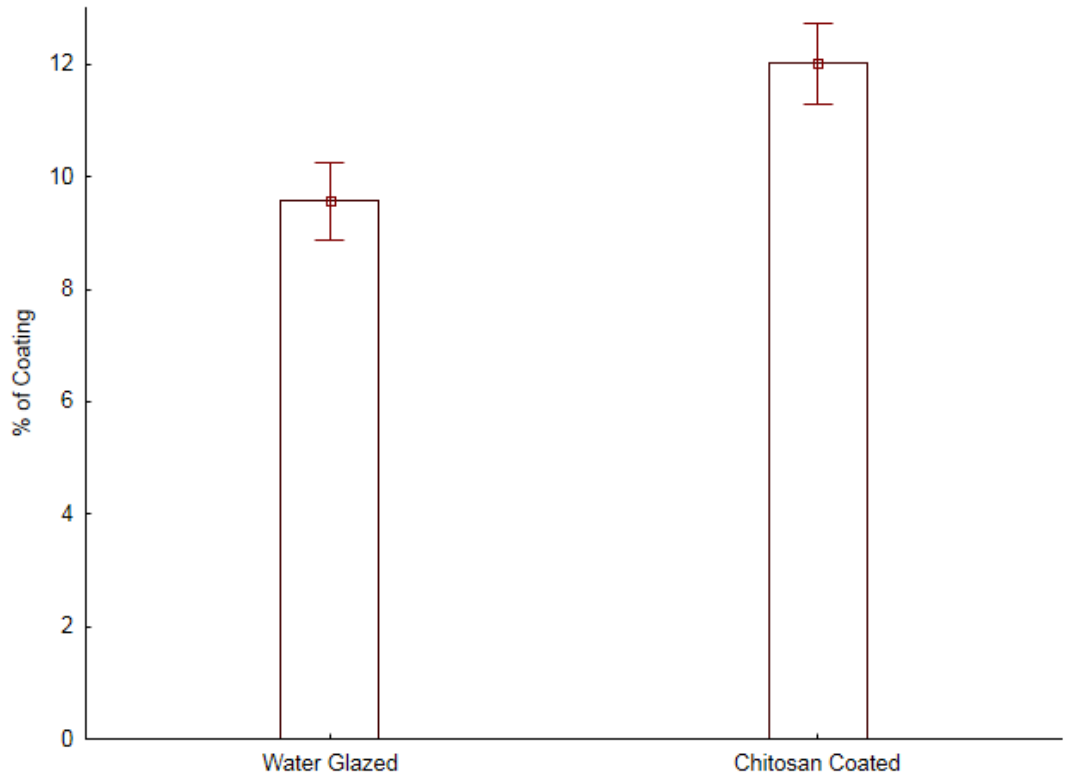

Figure 6-13 Glazing and Coating uptake (%) for salmon samples glazed with water and coated with 1.5% chitosan used for sensory analysis. Each bar represents the mean ± standard deviation of one hundred and five replications.

The values for glazing and coating percentages, found in Figure 6-13, are extremely similar to those found in the thermal stress sample test, seen in 6.1.1, and are in line with those reported in previous works, although higher percentages especially for chitosan were found (Soares et al., 2015).

6.2.2 TVC

The TVC values for frozen salmon samples for the samples used in sensory analysis stored at -20 ºC during six months can be seen in Table 6-3.

Analyzing Table 6-3, it is possible to see that as expected the TVC values, with the exception of the final value of the water glazed samples increases with storage time, remaining at all times clearly higher than the TVC values of the chitosan coated samples, presenting the same tendency seen in 6.1.3, with several of the chitosan coated samples not being detectable by the test. The results of this test, particularly the chitosan coated samples confirms the microbial protection and the ability to reduce and inhibit growth of microorganisms by the chitosan coating, in normal storage conditions and over a longer period of time than that of the thermal stress test, seen before. This ability of chitosan has been referenced by several authors over past years (Castro & Paulín, 2012; Raafat & Sahl, 2009; Rabea et al., 2003).

Nevertheless, such as in the case of the thermal stress test, all of the tested samples were well below the defined limits for sensory detection and rejection (Olafsdóttir et al., 1997) and the microbiological limit for quality frozen fish (International Commission on Microbiological Specifications for Foods, 1986).

Table 6-3 TVC values for frozen uncoated, glazed with water and coated with chitosan salmon samples during 6 months of storage at -20 ºC; standard deviation corresponds to four replications

TVC -20 ºC	Storage Time (months)	Sample 1 (CFU/g)	Sample 2 (CFU/g)	Sample 3 (CFU/g)	Sample 4 (CFU/g)	Mean (CFU/g)	SD
Control Samples	0	140	270	440	750	400	264
Water Glazed Samples	2	810	880	480	950	780	208
	4	1200	1000	1200	1500	1225	206
	6	640	570	530	890	658	162
Chitosan Coated Samples	2	460	230	140	<10	277	190
	4	<10	110	<10	<10	-	-
	6	<10	120	100	<10	-	-

6.2.3 TVB-N

The TVB-N values for water glazed and chitosan coated frozen salmon samples during six months of storage at -20 ºC can be seen in Figure 6-14. At the initial moment of the test, the TVB-N value of an uncoated sample was measured and used as a control for the remaining samples. The control sample presented a value of 10.768 ± 0.886 mg of nitrogen/100 g. During the test, the TVB-N values of all of the samples did not vary greatly, as seen by the lack of statistically significant differences in almost all of the samples. This lack of statistical significant differences also contributes to the inability to detect any influence by the different coatings.

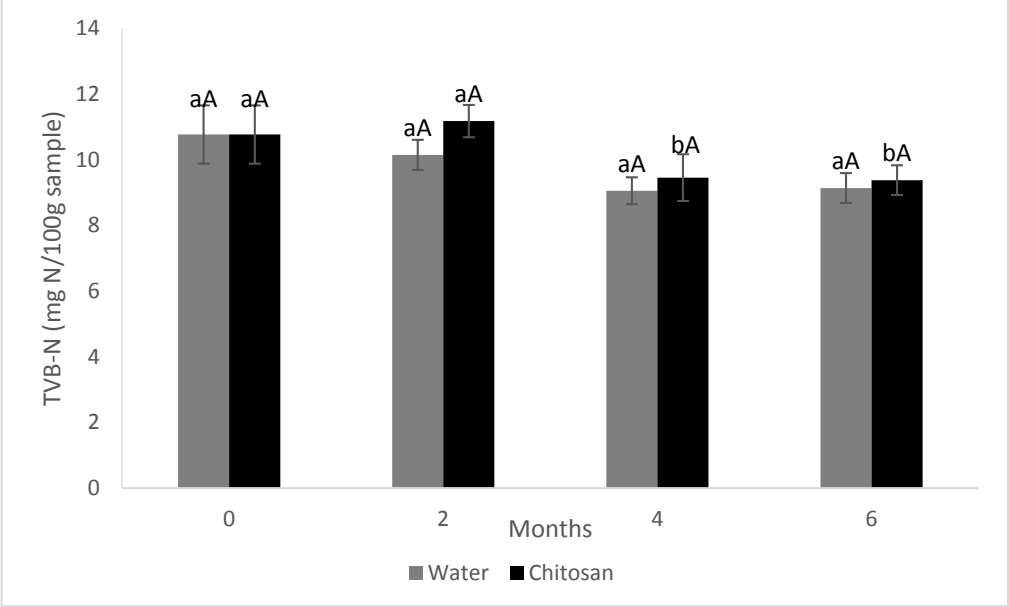

Figure 6-14 TVB-N values for salmon samples during six months of storage at -20 ºC; standard deviation corresponds to four replications; different small letters in the same sample type, and different capital letters in the same time moment indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).

After six months of storage water glazed samples present a value of 9.138 ± 0,454 mg of nitrogen/100 g, while chitosan coated samples present a value of storage of 9.378 ± 0.453 mg of nitrogen/100 g, both lower than the control sample and in line with those found in the thermal stress test, and well below the 35 mg nitrogen/100 g fish established as the acceptable limit for salmon by EU Directive 95/149 (Official Journal of the European Communities, 1995). The lack of variation in the TVB-B values, and the absence of an expected increase after 3 months of storage can be a result of the low temperature used in the test, and the initial quality and good condition of the salmon,

which is supported by the low TVC values found in 6.2.1 (Gonçalves & Gindri Junior, 2009).

6.3. Analysis of frozen samples

6.3.1 Sensory analysis

In Figure 6-15, the sensory profiles of uncoated, water glazed and chitosan-coated samples in frozen state are shown for all moments of testing.

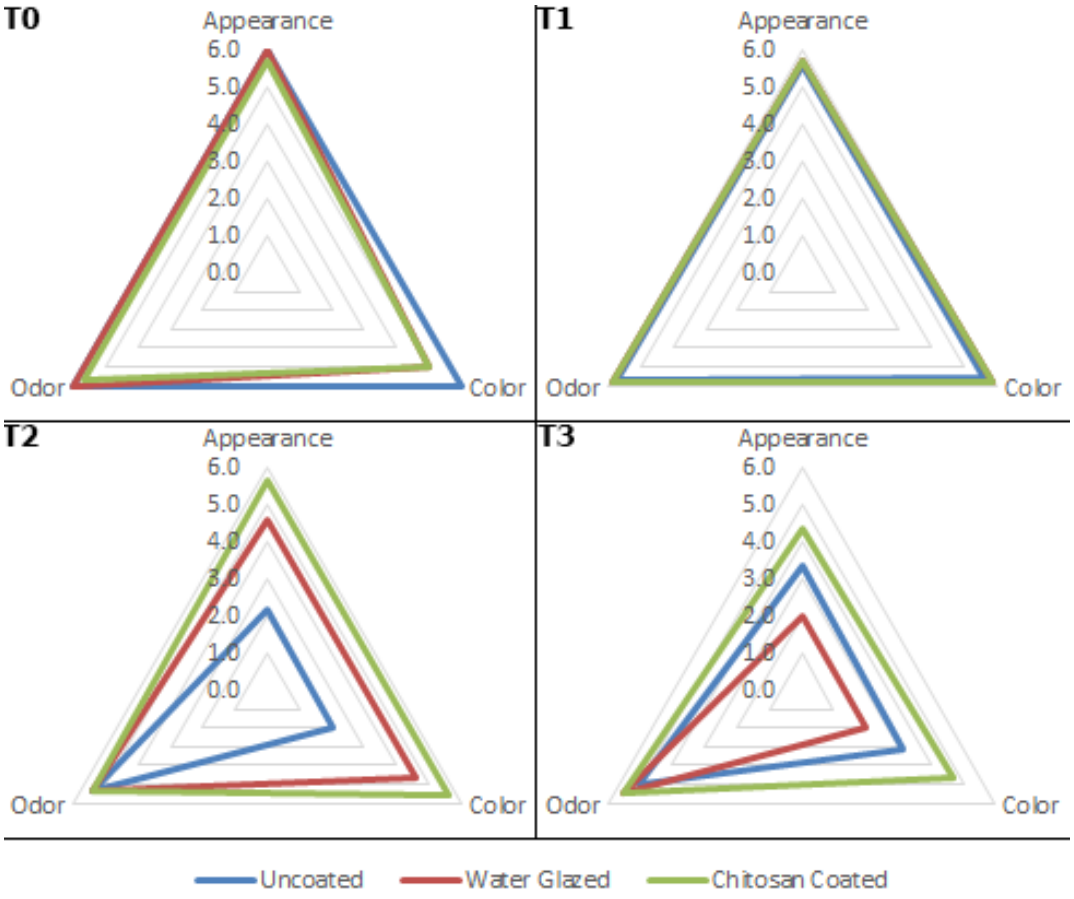

Figure 6-15 Sensory profile of uncoated, water glazed, and chitosan coated frozen salmon samples, at the beginning of storage (top left), after two months of storage (top right), four months of storage (bottom left) and six months of storage (bottom right) at -20 ºC.

It is possible to see in Figure 6-15 that for the initial moment and for the first two months of storage at -20 ºC differences between the different types of samples does not appear to be notable, as the overall values for the parameters evaluated, appearance, odor and color, were similar between them, with no clear distinction between samples.

On the other hand for the time periods of four and six months it is possible to see in Figure 6-15 differences becoming clearer and significant, clearly indicating that chitosan

acts as a better preservation agent, while water glazed and uncoated samples are less rated in all parameters evaluated.

6.3.2 Statistical analysis

Principal component analysis (PCA) and canonical analysis were performed in order to determine which parameters greater influence the differences between samples, and how those same parameters are related between them, in frozen salmon samples. The parameters in question were odor, color and appearance.

The results of the principal component analysis in the frozen samples show that 92.59 % of the variation is represented by Factor 1 and Factor 2, with Factor 1 being responsible for 67.39 % of the samples variation, and Factor 2 for 25.20 %. In Figure 6-16 it is possible to see variables projection after component reduction.

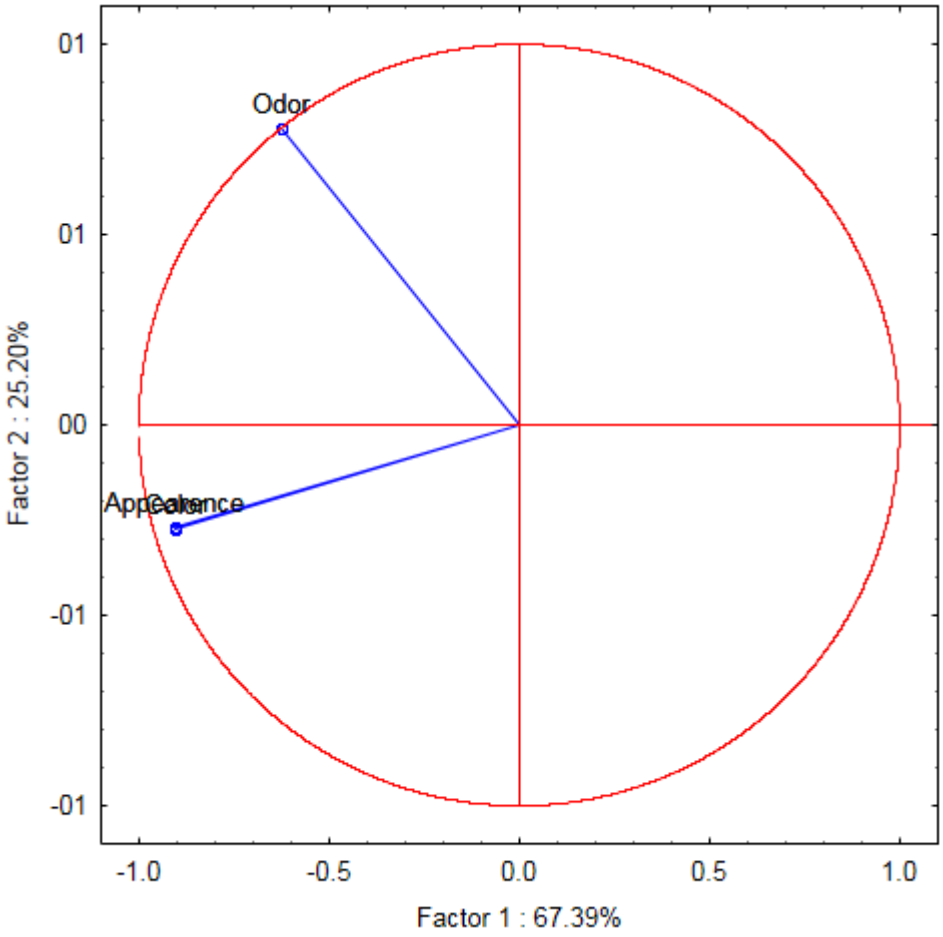

Figure 6-16 Variable projection after PCA analysis for the frozen salmon samples.

It is possible to see that the parameters that influence the most Factor 1 are appearance and color, while Factor 2 is mainly influenced by odor.

These results are supported by Table 6-4 where it is possible to see the variable contribution based on correlations within the different factors.

Table 6-4 Variable contribution within reduced factors after PCA analysis for the frozen salmon samples

Variable	Factor 1	Factor 2
Odor	-0.624	0.781
Color	-0.903	-0.273
Appearance	-0.904	-0.267

Through PCA it was also possible to obtain a case projection after the analysis, which is shown in Figure 6-17.

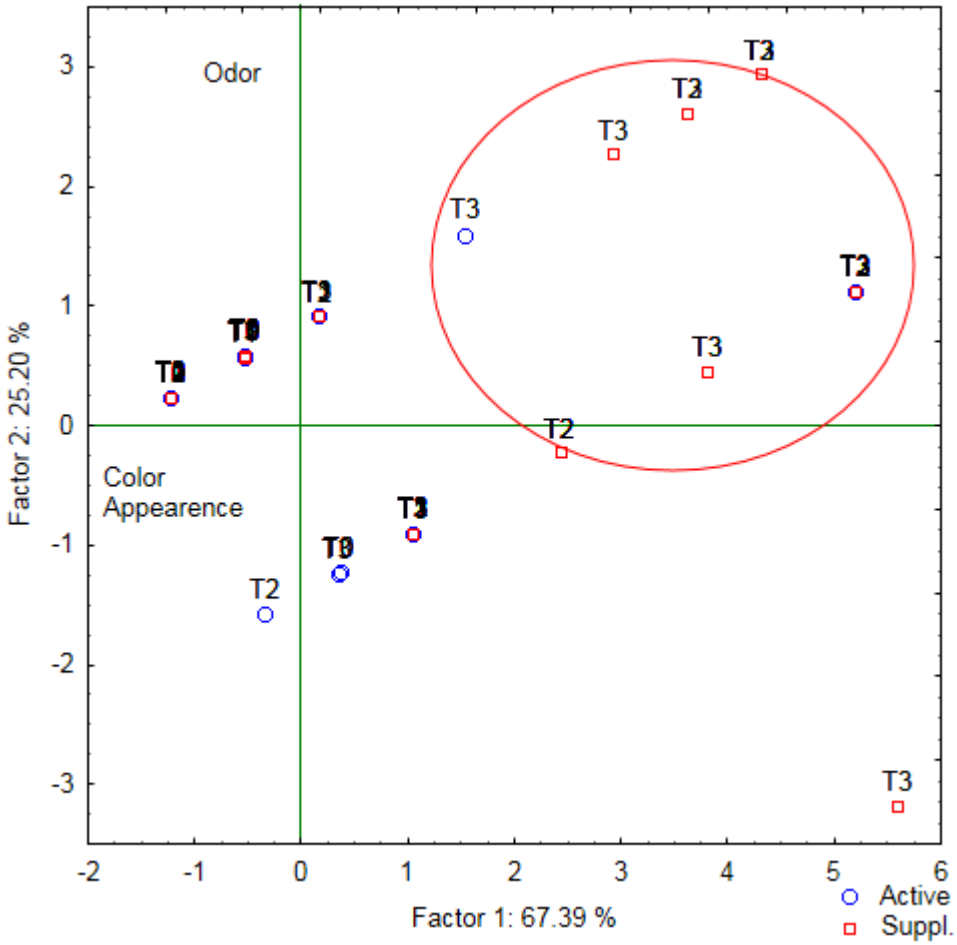

Figure 6-17 Case projection after PCA analysis for the frozen salmon samples.

In Figure 6-17 all assessed samples are displayed, red samples are water glazed and uncoated samples, while blue samples represent the chitosan-coated samples, for a

better interpretation of the results, since these are the ones of interest for this study. The samples are also labeled by time, in order to provide a better distinction.

It is possible to see that the chitosan coated samples follow the same distribution as the other samples, which indicates that no changes occurred due to the type of coating used in the samples. A clear pattern that is seen is that the samples labeled T3, meaning that they are the samples assessed after six months, are clearly distanced, and thus cause more variation, from the other samples, both in terms of Factor 1 and Factor 2, indicating that they are clearly different, and in this case worse, than the samples assessed at other times. This is supported by the findings in the sensory analysis, were the six month samples present overall lower scores when compared to samples from earlier moments of assessment.

Table 6-5 Eigenvalues of frozen salmon samples

	Eigenvalue	% Total Variance
Factor 1	2.022	67.394
Factor 2	0.756	25.202
Factor 3	0.222	7.404

As seen in Table 6-5 Factor 1 is responsible for the most part of the variation, as it is the only Eigenvalue superior to 1, meaning that the color and appearance parameters are the ones causing higher differences between samples (Barbosa, Alves, & Oliveira, 2016).

6.4. Analysis of samples after thawing process

6.4.1 Sensory analysis

In Figure 6-18 the sensory profiles of uncoated, water glazed, chitosan-coated, water thawed and chitosan thawed samples after thawing are shown for all moments of testing, at zero months, two months, four months and six months respectively. The sensory profiles of samples after thawing are evaluated in four parameters, appearance, texture, odor and color.

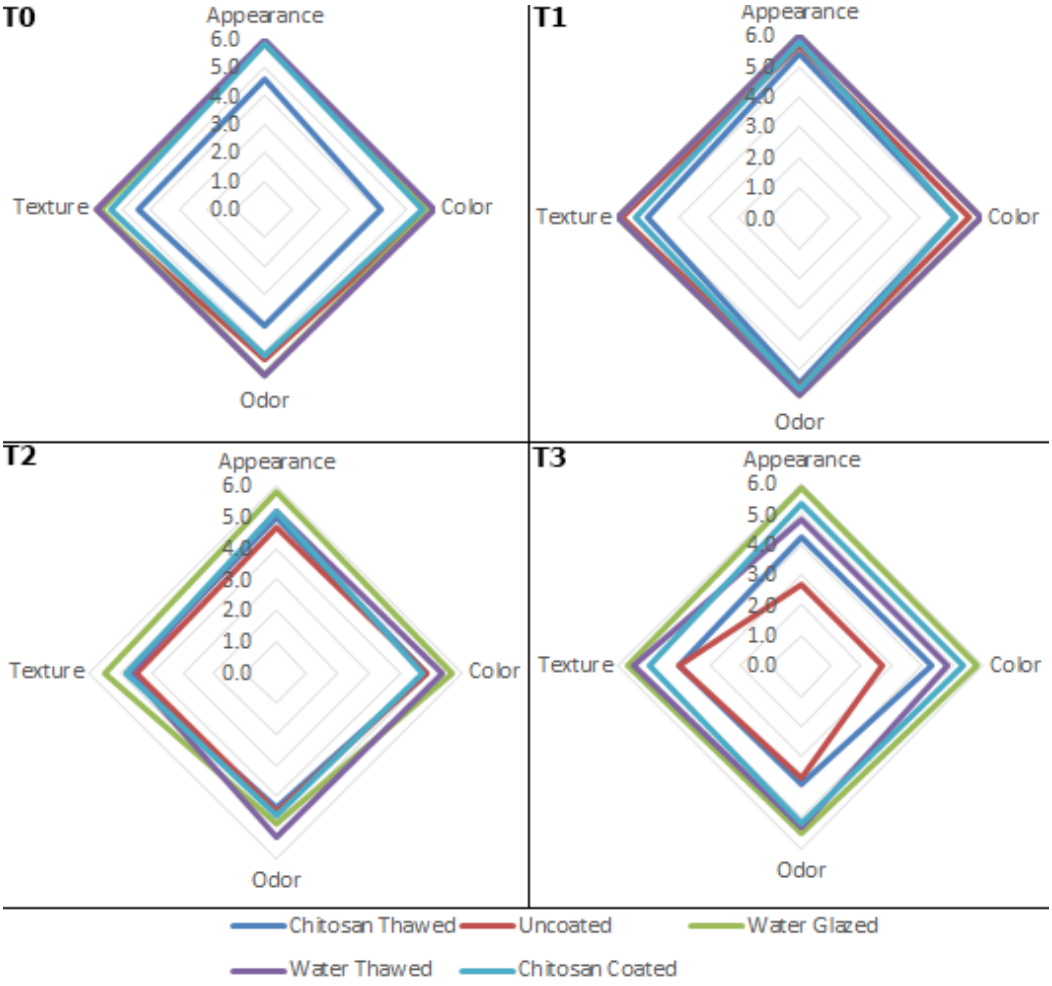

Figure 6-18 Sensory profile of uncoated, water glazed, chitosan coated, water thawed and chitosan thawed, salmon samples after thawing, at the beginning of storage (top left), after two months of storage (top right), four months of storage (bottom left) and six months of storage (bottom right) at -20 ºC.

As it happened in the frozen samples, for the first two moments of evaluation differences between samples are not clearly noted, although it is possible to see that in the first moment, the chitosan thawed samples are clearly different and less rated than the other, which are quite similar. This may be due to the processing of the removal of chitosan coating of the samples, which differs from the normal procedure of removal, possibly causing the reported differences.

For sample evaluation at four and six months, it is possible to see more differences between samples, especially for the six month evaluation, were it becomes clearer that the water glazed and the chitosan coated samples are better rated than the remaining samples, with the uncoated samples receiving the worst classification in all of the parameters evaluated.

6.4.2 Statistical analysis

Principal component analysis (PCA) and canonical analysis were performed in order to determine which parameters greater influence the differences between samples, and how those same parameters are related between them, in thawed salmon samples. The parameters in question were appearance odor, color and texture.

The results of the principal component analysis for the thawed samples, show that 84.06 % of the variation is represented by factor 1 and Factor 2, with Factor 1 being responsible for 72.82 % of the sample variation and Factor 2 corresponding to 11.24 %. In Figure 6-19 it is possible to see the projected variables after component reduction.

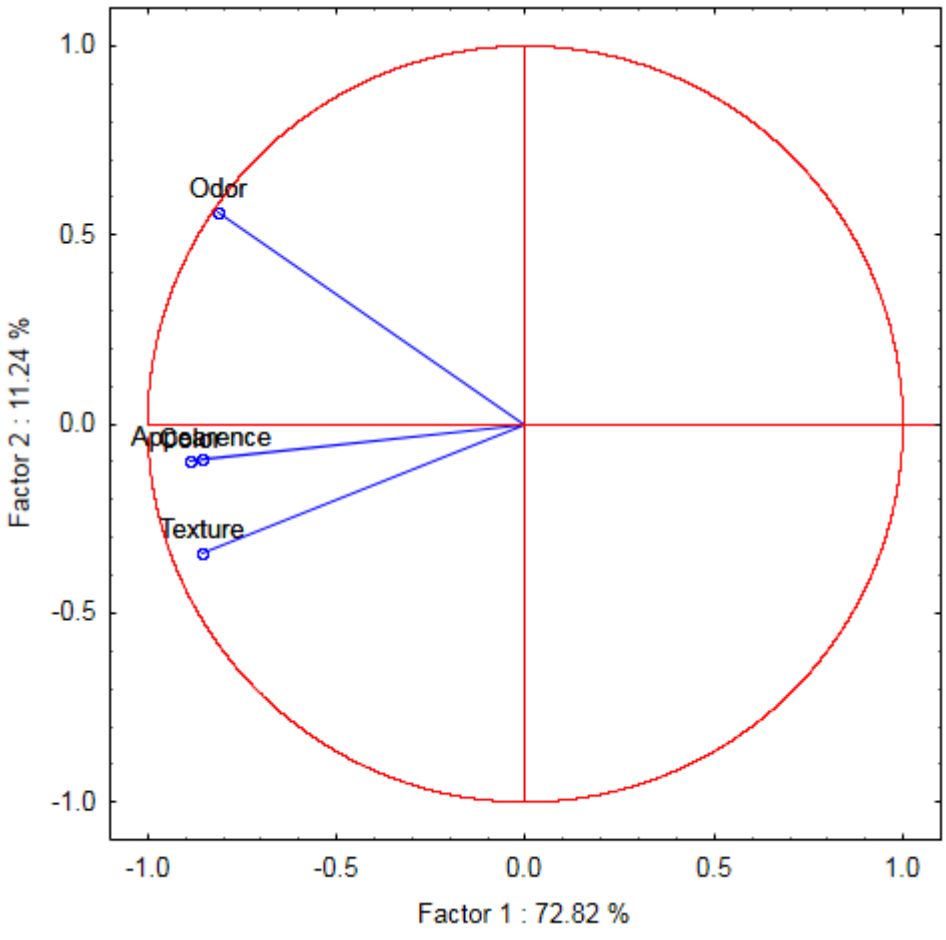

Figure 6-19 Variable projection after PCA analysis for the thawed salmon samples.

It is possible to see that all parameters influence Factor 1 in a similar manner, while Factor 2 is mainly influenced by odor, and to a less extent by texture.

Table 6-6 Variable contribution within reduced factors after PCA analysis for the thawed salmon samples

Variable	Factor 1	Factor 2
Texture	-0.854351	-0.340246
Odor	-0.813745	0.561804
Color	-0.886336	-0.097675
Appearance	-0.857297	-0.093203

Through PCA it was also possible to obtain a case projection after the analysis, which can be seen in Figure 6-20.

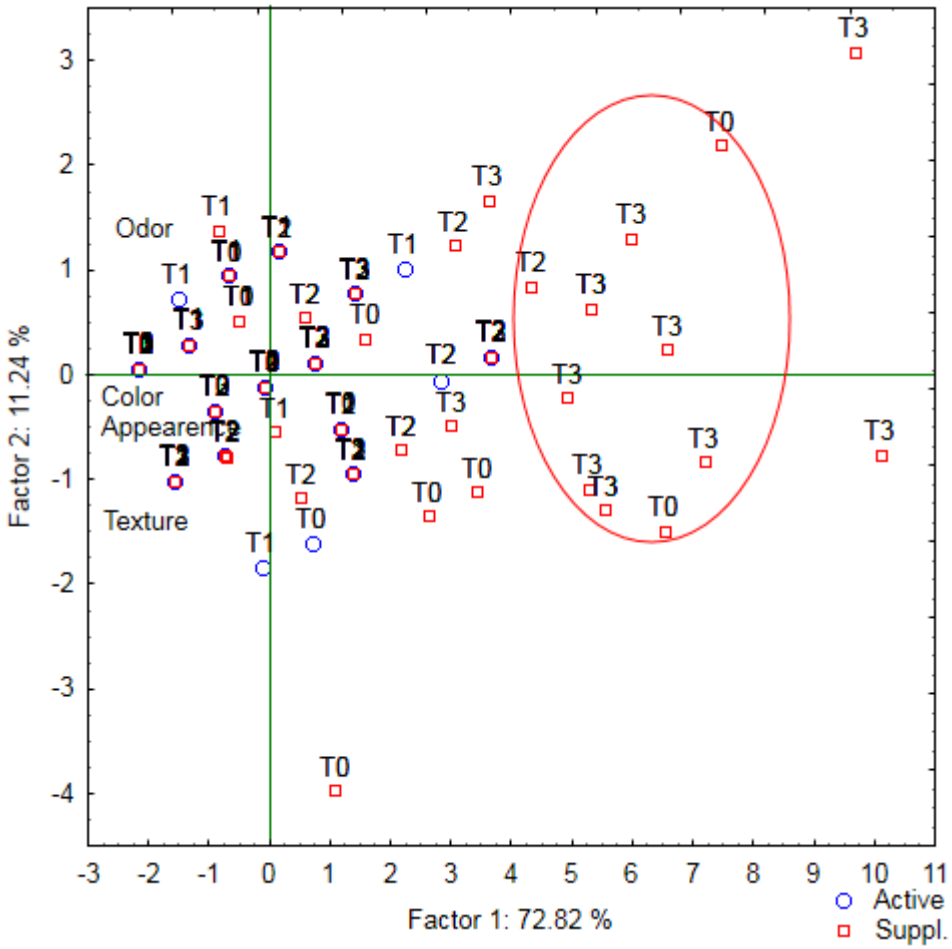

Figure 6-20 Case projection after PCA analysis for the thawed salmon samples.

In Figure 6-20 all assessed samples are displayed: red samples are water glazed, uncoated, water thawed and chitosan thawed samples, while blue samples represent the chitosan-coated samples; this was done for a better interpretation of the results, since these are the ones of interest for this study. The samples are also labeled by time, in order to provide a better distinction.

It is possible to see that the chitosan coated samples follow the same distribution as the other samples, except for the assessment after six months (T3), where chitosan coated samples present less variation and closer to rest of the samples, which indicates that at the very least no changes occurred due to the type of coating used in the samples, and that it is possible that chitosan helped in improving the score given to those samples, as they have less variance than the rest of the samples from T3. A clear pattern that is seen is that the samples labeled with T3, the samples assessed after six months, are clearly distanced, and thus cause more variation, from the other samples, especially regarding Factor 1 indicating that they are clearly different, and worse, than the samples assessed at other times, which is supported by the findings in the sensory analysis, where the six month samples presented overall lower scores when compared to samples from earlier moments of assessment.

Table 6-7 Eigenvalues of the thawed salmon samples

	Eigenvalue	% Total Variance
Factor 1	2.912647	72.81617
Factor 2	0.449618	11.24046
Factor 3	0.353356	8.83389
Factor 4	0.284379	7.10948

As seen in Table 6-7, Factor 1 is responsible for the most part of the variation, as it is the only Eigenvalue superior to 1, meaning all of the assessed parameters contribute in a similar same manner for the samples variation (Barbosa et al., 2016).

6.4.3 Texture

The textural properties of thawed salmon were assessed by a texture profile analysis, which allowed for the determination of the four parameters: hardness, cohesiveness, springiness and chewiness. The results for the thawed samples for these four parameters, during six months of storage at -20 ^{o}C can be seen in Figure 6-21, Figure 6-22, Figure 6-23 and Figure 6-24. Differences in process and handling between the water glazed and chitosan-coated samples occurred due to difficulties in removing the chitosan coating from the samples, which may influence the results.

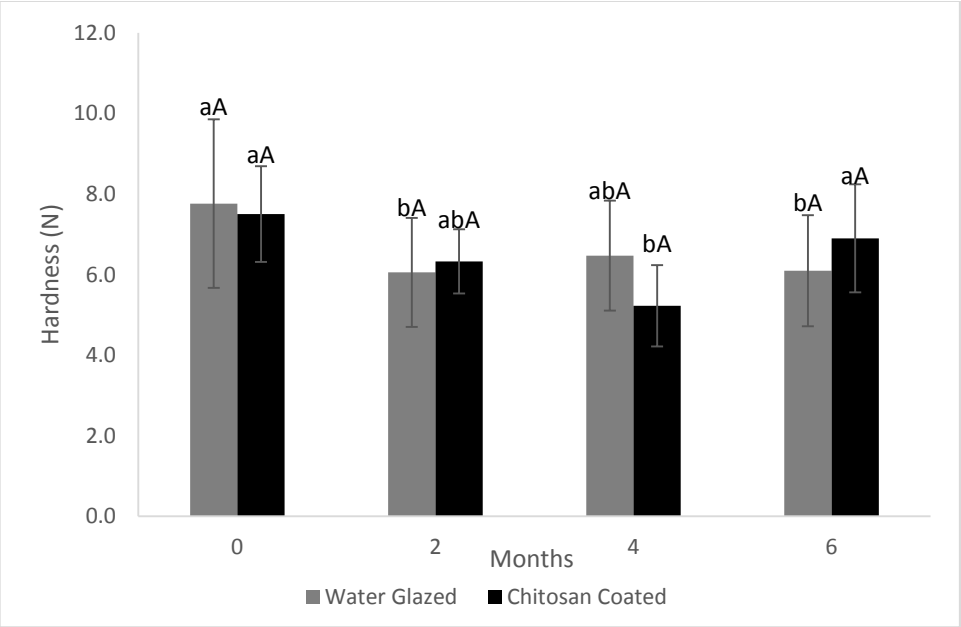

Figure 6-21 Hardness values for thawed salmon samples during six months of storage at -20 ºC; standard deviation corresponds to four replications; different small letters in the same sample type, and different capital letters in the same time moment indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).

Regarding the hardness parameter, shown in Figure 6-21, it is possible to see that there do not seem to be many significant statistically significant differences in the same type of sample during the several months of storage, and that between different coatings applied at the same moment of storage no differences were present, suggesting that the presence of different coatings did not affect the hardness values of the samples. Regarding the variation of the hardness values during storage, it appears to be a decreasing tendency in both the water glazed samples and the chitosan coated samples, with initial values of 7.762 ± 2.094 N, and 7.503 ± 1.188 N, and final values of 6.094 ± 1.379 N and 6.900 ± 1.342 N for water glazed and chitosan coated samples, respectively. These results are similar to those found in other studies for thawed salmon with similar conditions, although slightly higher for all moments of evaluation, suggesting that that tendency is related to the samples, rather than the coatings applied (Casas, Martinez, Guillen, Pin, & Salmeron, 2006; Hultmann & Rustad, 2004; Martinez, Salmerón, Guillén, & Casas, 2007)

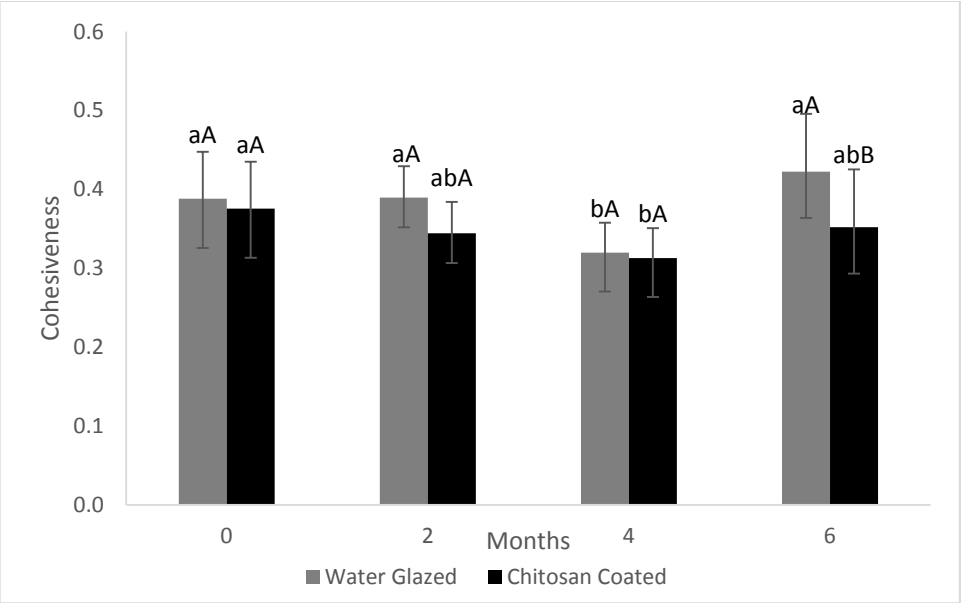

Figure 6-22 Cohesiveness values for thawed salmon samples during six months of storage at -20 ºC; standard deviation corresponds to four replications; different small letters in the same sample type, and different capital letters in the same time moment indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).

When it comes to the cohesiveness parameter, shown in Figure 6-22, it is possible to see that there does not seem to be many significant statistical differences in the same type of sample during the several months of storage, and that between different coatings applied at the same moment of storage there is only a significant statistical difference in the last moment of evaluation at six months, making it hard to assess if it was a trend to continue and thus implying differences caused by the coatings, or an exception which would suggest that the presence of different coatings did not affect the cohesiveness values of the samples. Regarding the variation of the cohesiveness values during storage, it appears to be a decreasing tendency in both the water glazed samples and the chitosan coated samples, with the exception of the last moment of evaluation, especially for the water glazed samples, where there is a significant rise in the cohesiveness value, which causes the differences between the coatings.

The initial values of cohesiveness are 0.388 ± 0.059 and 0.376 ± 0.062, with final values of 0.423 ± 0.073 and 0.352 ± 0.059, respectively for water glazed and chitosan coated samples. Nevertheless overall there was not much variation, as the final values are statistical similar to the initial values, for both the water glazed and the chitosan coated samples. The obtained results are comparable to those found in previous studies

for thawed salmon tests under similar conditions (Casas, Martinez, Guillen, Pin, & Salmeron, 2006; Martinez, Salmerón, Guillén, & Casas, 2007).

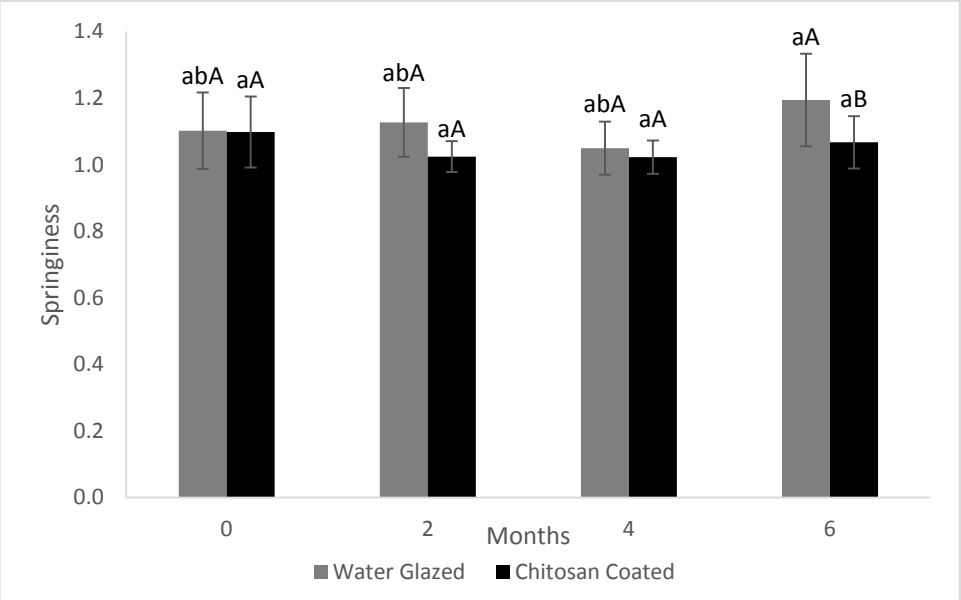

Figure 6-23 Springiness values for thawed salmon samples during six months of storage at -20 ºC; standard deviation corresponds to four replications; different small letters in the same sample type, and different capital letters in the same time moment indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).

The springiness values, are shown in Figure 6-23, and it is possible to see that there are not many significant statistical differences in the same type of sample during the several months of storage, and that between different coatings applied at the same moment of storage there is only a significant statistical difference in the last moment of evaluation at six months, making it difficult to determine if it is a trend that was going to continue indicating differences caused by the different coatings, or an exception that would mean that the different coatings did not affect the springiness values. Regarding the variation of the springiness values during six months of storage, it does not appear to be a dominant tendency in both the water glazed samples and the chitosan coated samples, as the values are similar throughout the duration of the test.

The initial values of springiness are 1.102 ± 0.115 and 1.098 ± 0.107, with final values of 1.194 ± 0.139 and 1.067 ± 0.079, respectively for water glazed and chitosan coated samples. Overall there was not much variation, as the final values are statistical similar to the initial values, for both types of coating. The obtained results are comparable, although slightly higher than to those found in previous studies for thawed salmon tests under similar conditions (Casas, Martinez, Guillen, Pin, & Salmeron, 2006; Martinez,

Salmerón, Guillén, & Casas, 2007). The higher values for springiness can be a result of the samples used, since the values are higher in both coatings, representing a better ability to bounce back between compressions (Texture Technologies Corporation, 2015a).

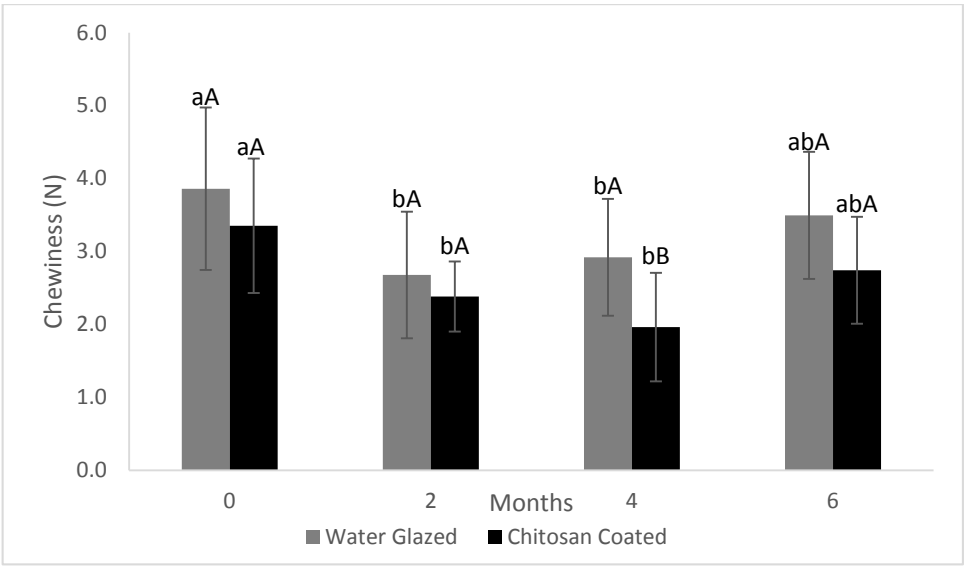

Figure 6-24 Chewiness values for thawed salmon samples during six months of storage at -20 ºC; standard deviation corresponds to four replications; different small letters in the same sample type, and different capital letters in the same time moment indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).

The chewiness values, are seen in Figure 6-24, and it is shown there are some significant statistical differences in the same type of sample during the several months of storage, especially during the two and four month mark, while between different coatings applied at the same moment of storage there is only a significant statistical difference at four months, while in the last moment of evaluation there is no significant statistical difference between coatings indicating that the different coatings did not affect the chewiness values. Regarding the variation of the chewiness values during six months of storage, with the exception of the last moment, a decreasing tendency appears to be present especially in the chitosan coated samples. Nevertheless the final values statistically similar to the other moments of evaluation, but lower than the initial ones.

The initial values of chewiness are 3.860 ± 1.113 N and 3.353 ± 0.922 N, with final values of 3.494 ± 0.871 N and 2.742 ± 0.733 N, respectively for water glazed and chitosan coated samples. The final values obtained are statistical similar to those of the other

moments of evaluation, for both types of coating. The obtained results are comparable, although slightly higher than to those reported in previous works for thawed salmon tests under similar conditions, which is to be expected since chewiness values are dependent of the hardness, cohesiveness and springiness values, which are also slightly higher than those reported, thus causing slightly higher values of chewiness (Casas, Martinez, Guillen, Pin, & Salmeron, 2006; Martinez, Salmerón, Guillén, & Casas, 2007).

6.4.4 Color

The color parameters L*, a* and b*, during six months storage of the water glazed and chitosan coated uncooked samples can be seen in Figure A. 7, Figure A. 8, and Figure A. 9 respectively, found in Appendix E.

Regarding the results for the L*a*b* parameters during six months of storage some statistical significant differences are present especially in the later moments of storage, but with the exception of the lightness parameter no difference between coating was found; Regarding the lightness parameter, chitosan samples present higher lightness values than the water glazed samples.

The L*a*b* parameters were transformed to RGB codes, and a visual representation for water glazed and chitosan coated samples can be seen in Figure 6-25 and Figure 6-26.

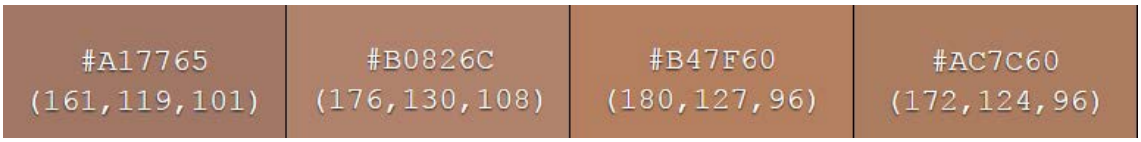

Figure 6-25 Visual representation, in RGB, of the color parameters L*a*b* for uncooked water glazed salmon samples during six months of storage at -20 ºC; From left to right is possible to see from the initial moment to the last one.

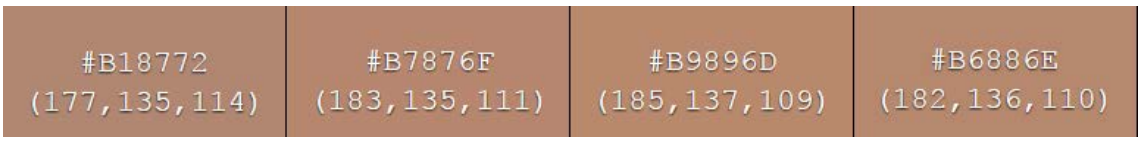

Figure 6-26 Visual representation, in RGB, of the color parameters L*a*b* for uncooked chitosan coated salmon samples during six months of storage at -20 ºC; From left to right is possible to see from the initial moment to the last one.

It is possible to see that between each moment in each of the different treatments, visually there seem to be some visual difference, although they do not appear to vary

greatly. As indicated by the L* parameter, there seems to be a tendency for the RGB colors to be lighter in the chitosan coated samples than in the water glazed samples.

Regarding perceived color differences, which were calculated as the difference between the assessed sample and a samples with the same coating at the initial moment of assessment, it is possible to see in Figure 6-27 how that value changed during the six months of storage for the different samples, it is also possible to see that when observing the ΔE*ab value between the control sample with uncoated sample at the initial moment, color differences were already present, this is represented in graphs by a dotted line, which represents the natural differences between samples, which can be seen that it is quite high, with a value similar to those of the perceived differences to a untrained assessor.

In terms of perceived color differences all of the samples present differences that would be obvious to even an untrained observer, with the exception of the initial moment (represented by a value of ΔE*ab greater than 3.5) (Cruse, 2015; EFI, n.d.).

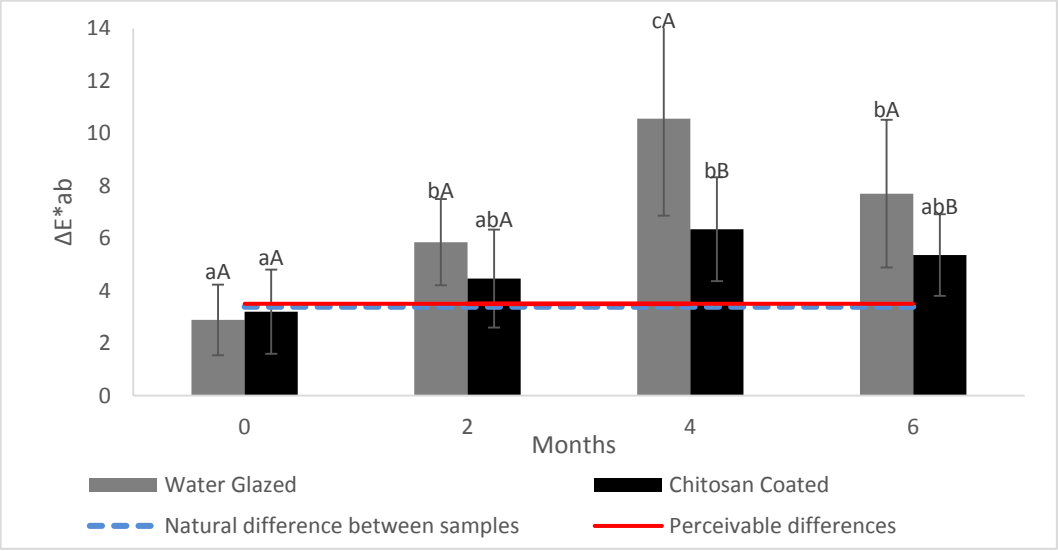

Figure 6-27 ΔE*ab values for thawed salmon samples during six months of storage at -20 ºC; standard deviation corresponds to eighteen replications; different small letters in the same sample type, and different capital letters in the same time moment indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).

As shown in Figure 6-27, the perceived color differences, represented by the ΔE*ab value, tend to follow an increasing tendency in almost all of the samples analyzed, with the exception of the last moment of analysis, which is to be expected as the storage time increases, leading to higher differences in the color values.

During the six month period of storage the treatment that presented the lower value of ΔE*ab was that of chitosan coated samples, with the exception of the initial sample, a result also found in a previous study that indicated chitosan as a better color preservation agent (Soares et al., 2015), and an expected result due to the ability of chitosan coatings to help protect against oxidation and protein denaturation, both of which have an influence in color preservation (Castro & Paulín, 2012; Ojagh et al., 2011; Rodriguez-Turienzo et al., 2011).

6.5. Analysis of cooked samples

6.5.1 Sensory analysis

In Figure 6-28 the sensory profiles of uncoated, water glazed, chitosan coated, water thawed and chitosan thawed samples after cooking are shown for all moments of testing, at zero months, two months, four months and six months respectively. The sensory profile of samples after thawing are evaluated in four parameters, appearance, texture, odor and flavor.

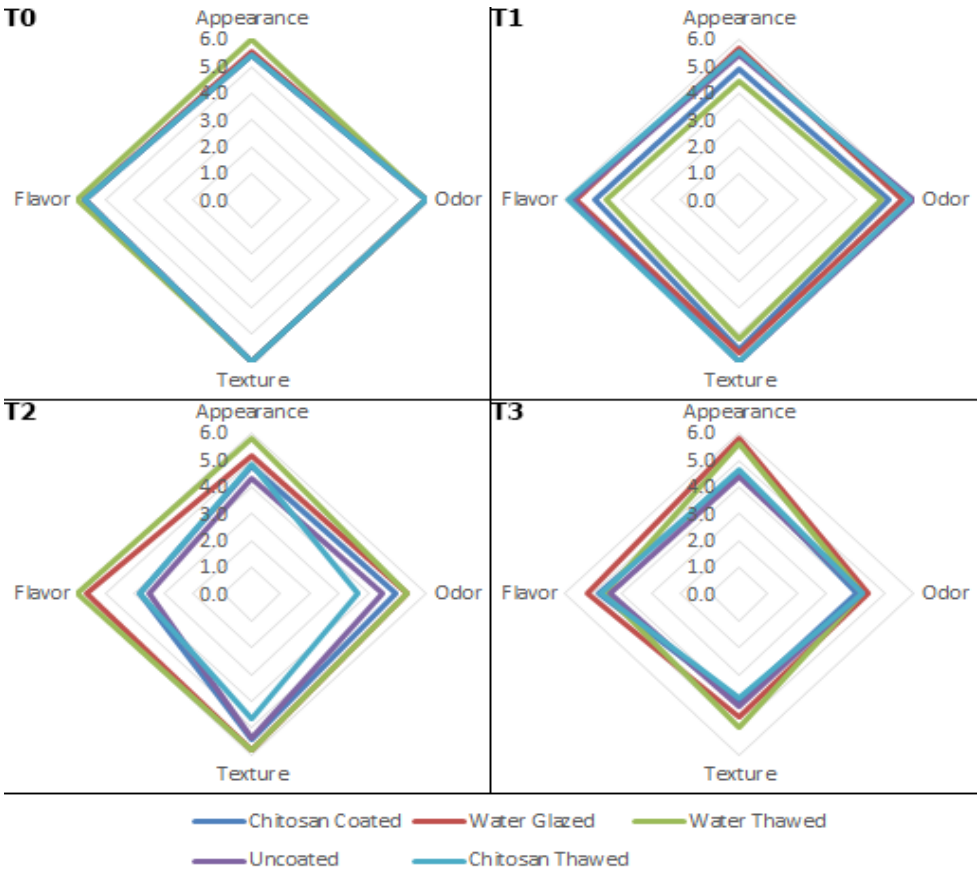

Figure 6-28 Sensory profile of uncoated, water glazed, and chitosan coated frozen salmon samples, at the beginning of storage (top left), after two months of storage (top right), four months of storage (bottom left) and six months of storage (bottom right) at -20 ºC.

The same pattern as the frozen and thawed samples is present in the cooked samples, as for the initial evaluation and for the two month evaluation no significant differences are present, especially for the initial moment, were all of the samples are quite similar. For the two month mark, some differences are noticeable, with the best samples being the ones with and water glazing and the chitosan thawed samples, and the lower rated ones being the water thawed samples.

For the last two moments of evaluation clearer differences are present, as well as lower ratings for the majority of the samples, especially for the six month evaluation, as shown in Figure 6-28. It is possible to see that regarding overall assessment of all parameters the water glazed and the water thawed samples are the highest rated ones, especially regarding the appearance of the samples. The chitosan coated and chitosan thawed samples although, due to the present difference in appearance, having a slight overall lower rating, in the flavor and odor parameters have an extremely similar rating than those of the water glazed and water thawed samples, suggesting that no aroma diffusion occurred during the six months of storage and evaluation.

Nevertheless after cooking, the differences between samples appear to be smaller in the last moment of evaluation.

6.5.2 Statistical analysis

Principal component analysis (PCA) and canonical analysis were performed in order to determine which parameters greater influence the differences between samples, and how those same parameters are related between them, in cooked salmon samples. The parameters in question were odor, color and appearance.

The results of the principal component analysis for the cooked samples, show that 83.75 % of the variation is represented by Factor 1 and Factor 2, with Factor 1 responsible for 61.77 % of the sample variation and Factor 2 corresponding to 21.98 %. In Figure 6-29 it is possible to see the projected variables after component reduction.

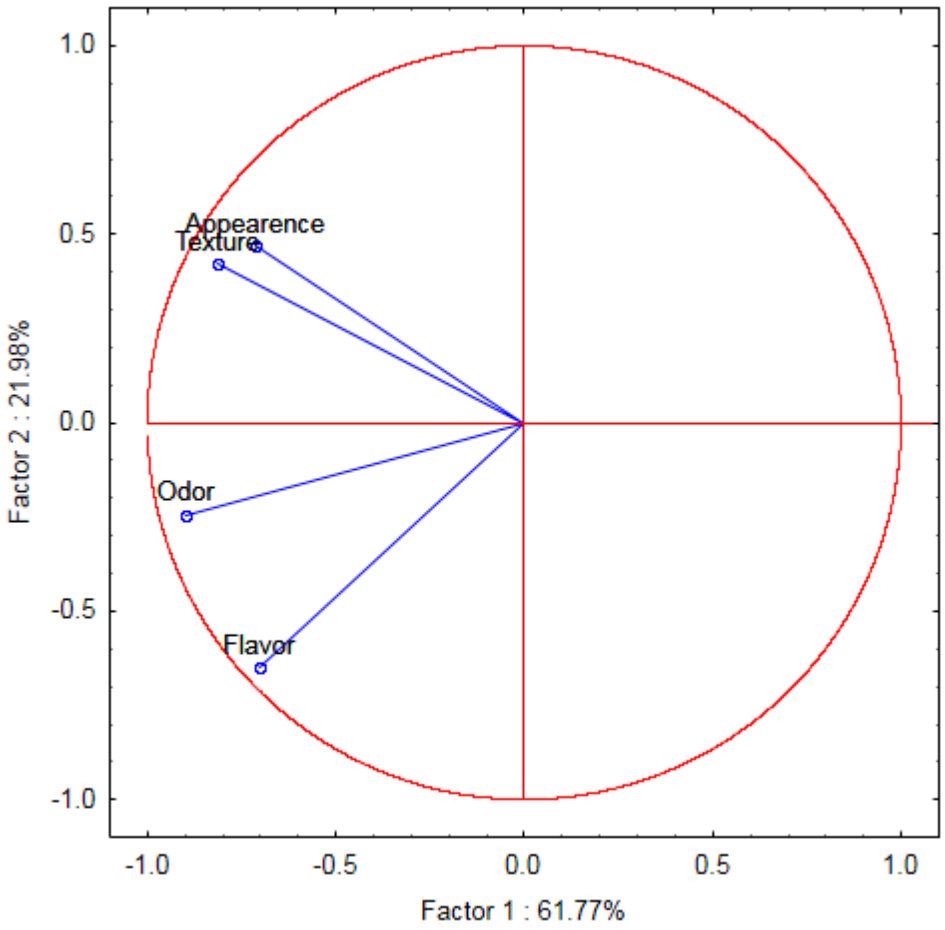

Figure 6-29 Variable projection after PCA analysis for the cooked salmon samples.

It is possible to see that texture and odor influence Factor 1 slightly higher than flavor and appearance, while in Factor 2 the opposite occurs, with appearance and especially flavor influencing sample variation more than texture and odor.

These results are supported by Table 6-8 where it is possible to see the variable contribution based on correlations within the different factors.

Table 6-8 Variable contribution within reduced factors after PCA analysis for the cooked salmon samples

Variable	Factor 1	Factor 2
Appearance	-0.711	0.469
Odor	-0.900	-0.246
Texture	-0.814	0.422
Flavor	-0.703	-0.649

Through PCA it was also possible to obtain a case projection after the analysis which can be seen in Figure 6-30.

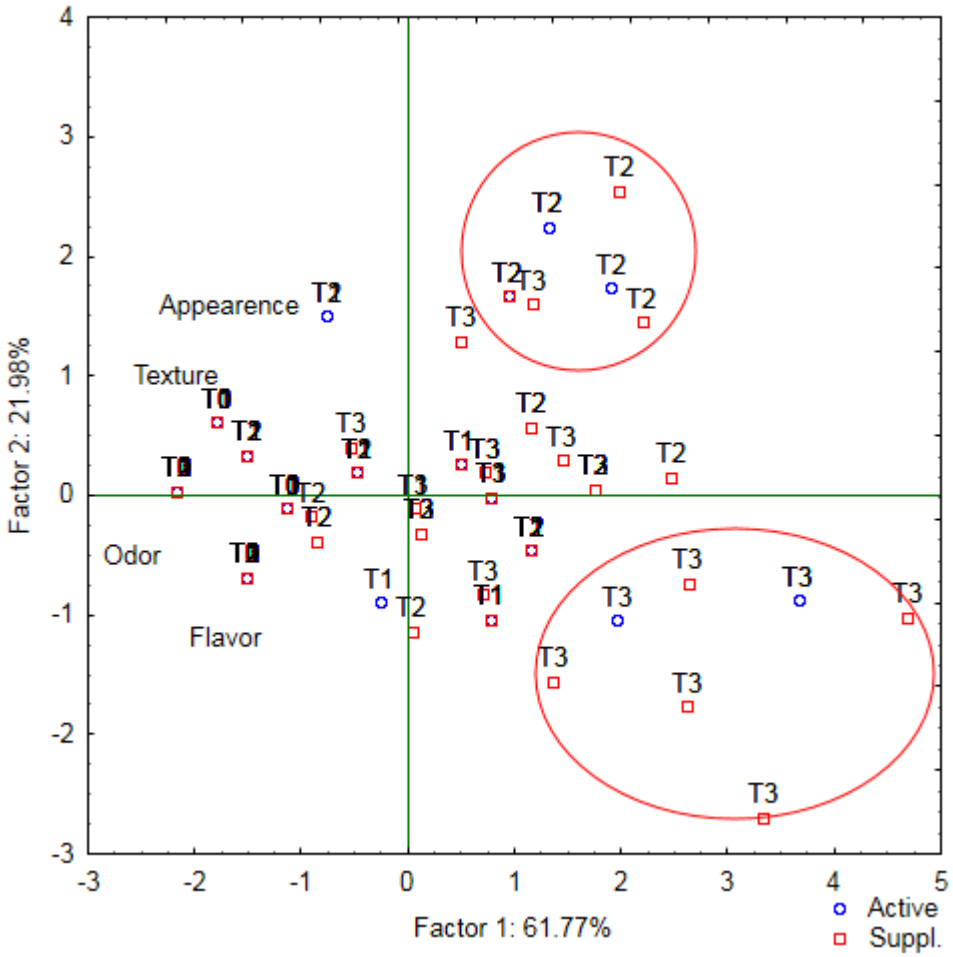

Figure 6-30 Case projection after PCA analysis for the cooked salmon samples.

In Figure 6-30 all assessed samples are displayed, red samples are water glazed, uncoated, water thawed and chitosan thawed samples, while blue samples represent the chitosan coated samples, for a better interpretation of the results, since these are the ones of interest for this study. The samples are also labelled by time, in order to provide a better distinction.

It is possible to see that the chitosan coated samples follow the same distribution as the other samples, which indicates that no changes occurred due to the type of coating used in the samples. A clear pattern that is seen is that the samples labeled with T2 and T3, meaning that they are the samples assessed after four and six months respectively, are clearly distanced are clearly distanced, and thus cause more variation, from the other samples, both in terms of Factor 1 and Factor 2, indicating that they are clearly different, and worse, than the samples assessed at other times, which is supported by

the findings in the sensory analysis, were the six month samples present overall lower scores when compared to samples from earlier moments of assessment.

Table 6-9 Eigenvalues of cooked salmon samples

	Eigenvalue	% Total Variance
Factor 1	2.470875	61.77187
Factor 2	0.879149	21.97873
Factor 3	0.540951	13.52377
Factor 4	0.109025	2.72564

As seen in Table 6-9 Factor 1 is responsible for the most part of the variation, as it is the only Eigenvalue superior to 1, so the odor and texture parameters are the ones causing higher differences between samples, while flavor causes the least variation of the assessed parameters (Barbosa et al., 2016). These results seem to point that chitosan coated samples are not different from those with an water glazing or from uncoated samples, and along with flavor being the least important parameter from those sensory assessed, it indicates that no flavor diffusion seem to have happened from the chitosan coated samples, at least not to a point that is perceivable by the panel of judges.

6.5.3 Texture

The textural properties of cooked salmon are normally assessed through a sensory analysis by a trained panel, nevertheless a texture profile analysis was conducted, allowing for the determination of the following four parameters: hardness, cohesiveness, springiness and chewiness. The results for the cooked samples for these parameters, during six months of storage at -20 ^{o}C are shown in Figure 6-31, Figure 6-32, Figure 6-33 and Figure 6-34. Differences in process and handling between the water glazed and chitosan coated samples occurred due to difficulties in removing the chitosan coating from the samples, which may influence the results.

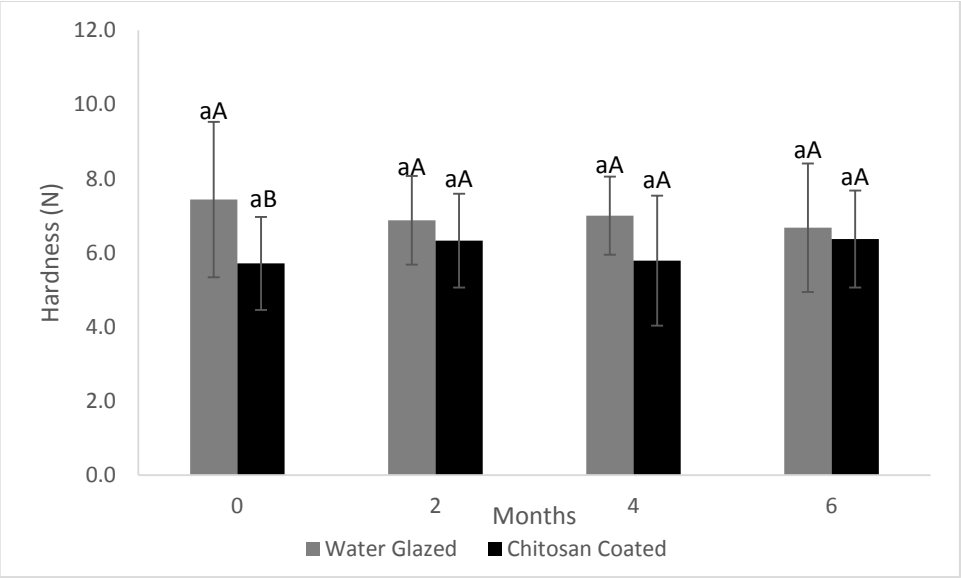

Figure 6-31 Hardness values for cooked salmon samples during six months of storage at -20 ºC; standard deviation corresponds to four replications; different small letters in the same sample type, and different capital letters in the same time moment indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).

Regarding the hardness parameter, shown in Figure 6-31, it is possible to see that rarely are any significant statistical differences in the same type of sample during the several months of storage, and that between different coatings applied at the same moment of storage no differences were present, indicating that the presence of different coatings did not affect the hardness values. Regarding the variation of the hardness values during storage, it appears to be a decreasing tendency for the water glazed samples and an increasing tendency for the chitosan coated samples, with initial values of 7.434 ± 2.096 N, and 5.711 ± 1.254 N, and final values of 6.675 ± 1.734 N and 6.368 ± 1.309 N for water glazed and chitosan coated samples, respectively, although all values are statically similar. These results are similar to those found in studies for thawed salmon, with small differences, indicating that the difference in this parameter between thawed and uncooked samples is not significant. It is also possible that the difference in the perforation distance of the different tests for the thawed and cooked samples (15 mm for the thawed samples, and 10 mm for the cooked samples) (Casas et al., 2006; Hultmann & Rustad, 2004; Martinez et al., 2007)

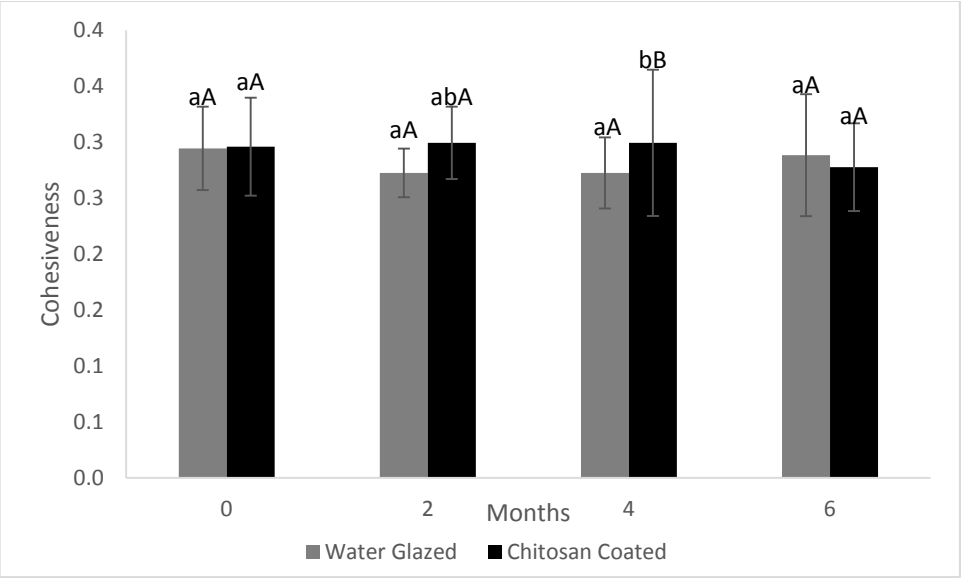

Figure 6-32 Cohesiveness values for cooked salmon samples during six months of storage at -20 ºC; standard deviation corresponds to four replications; different small letters in the same sample type, and different capital letters in the same time moment indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).

The cohesiveness values are shown in Figure 6-32, and it is possible to see that there are not many significant statistical differences in the same type of sample during the several months of storage, and that between different coatings applied at the same moment of storage there is only a significant statistical difference in the four month mark, while in the last moment of evaluation at six months there are no significant statistical differences between water glazed and chitosan-coated samples, suggesting that different coatings do not affect the cohesiveness values of the samples. Regarding the variation of the cohesiveness values during storage, there is not a dominant tendency in both the water glazed samples and the chitosan-coated samples, with the cohesiveness values remaining relatively stable.

The initial values of cohesiveness are 0.294 ± 0.037 and 0.296 ± 0.044, with final values of 0.288 ± 0.055 and 0.278 ± 0.039, respectively for water glazed and chitosan coated samples. The obtained results are comparable to those found in previous studies for thawed salmon (Casas, Martinez, Guillen, Pin, & Salmeron, 2006; Martinez, Salmerón, Guillén, & Casas, 2007). These values are and comparable to those found in this study for thawed salmon samples, although for all samples the cohesiveness values are lower for the cooked salmon samples, indicating that the cooked samples are less resistant to a second deformation, which is to be expected due to the effect of

temperature in the muscle fibers of the salmon (Texture Technologies Corporation, 2015a).

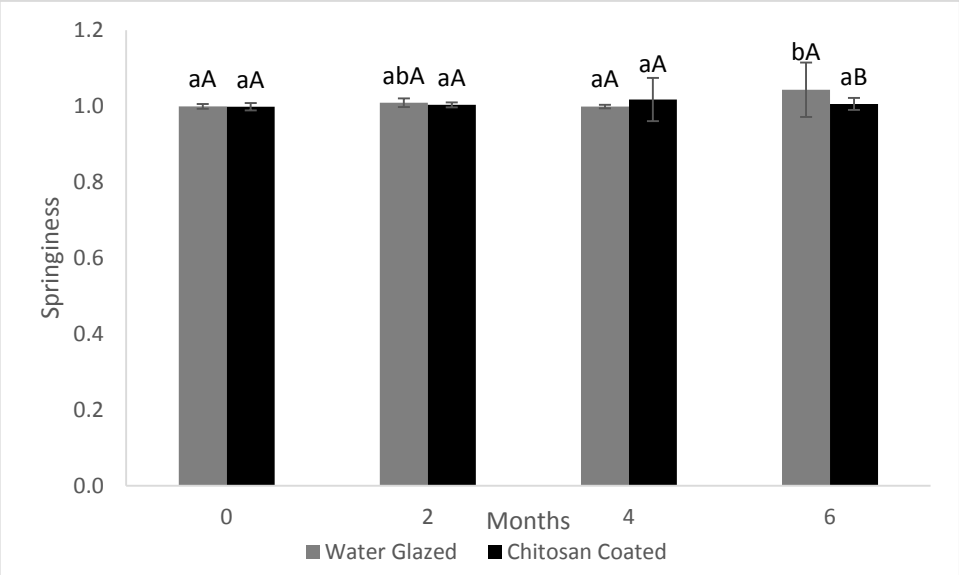

Figure 6-33 Springiness values for cooked salmon samples during six months of storage at -20 ºC; standard deviation corresponds to four replications; different small letters in the same sample type, and different capital letters in the same time moment indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).

The springiness values are shown in Figure 6-33, and it is possible to see that there are barely any significant statistical differences in the same type of sample during the several months of storage, and that between different coatings applied at the same moment of storage there is only a significant statistical difference in the last moment of evaluation at six months, making it difficult to conclude if it is a trend that was going to continue indicating differences caused by the different coatings, or an outlier which would mean that the different coatings did not affect the springiness values, which considering the values in the previous months may be the most reasonable expectation. Regarding the variation of the springiness values during six months of storage, it seem to be a slight increasing tendency in both the water glazed samples and the chitosan coated samples, although the values are quite similar throughout the duration of the test, as the lack of significant statistical differences indicates.

The initial values of springiness are 0.999 ± 0.006 and 0.999 ± 0.010, with final values of 1.043 ± 0.072 and 1.006 ± 0.016, respectively for water glazed and chitosan coated samples. Overall there was not much variation, as the final values are statistical similar to the initial values. The obtained results are comparable, while slightly higher, to those

found in previous studies for thawed salmon tests under similar conditions, but lower than those found for the thawed samples performed in this study (Casas, Martinez, Guillen, Pin, & Salmeron, 2006; Martinez, Salmerón, Guillén, & Casas, 2007). The lower values for springiness of the cooked samples can be a result of the cooking process, as temperature affects the muscle fibers, such as happened with the cohesiveness values, representing a slightly worse ability to bounce back between compressions for the cooked samples (Texture Technologies Corporation, 2015a).

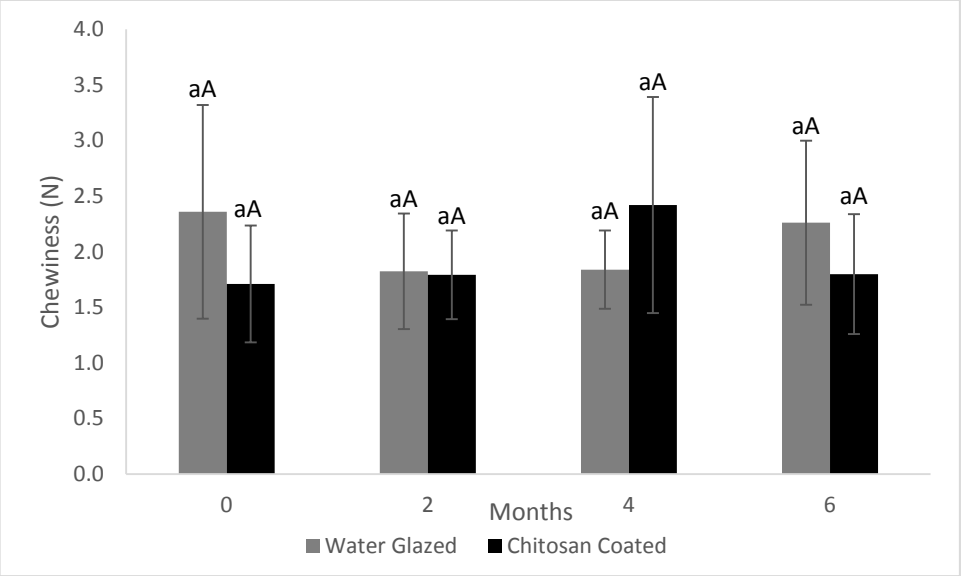

Figure 6-34 Chewiness values for cooked salmon samples during six months of storage at -20 ºC; standard deviation corresponds to four replications; different small letters in the same sample type, and different capital letters in the same time moment indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).

The chewiness values are shown in Figure 6-34, and it is seen there not any significant statistical differences both in the same type of sample during the several months of storage, and between different coatings applied at the same moment of storage suggesting that the elapsed time as well as the different coatings did not affect the chewiness values. Regarding the variation of the chewiness values during storage, it does not appear to be a clear tendency for both the water glazed and the chitosan coated samples.

The initial values of chewiness are 2.359 ± 0.961 N and 1.710 ± 0.525 N, with final values of 2.261 ± 0.738 N and 1.798 ± 0.539 N, respectively for water glazed and chitosan coated samples. The final values obtained are statistical similar to those of the other moments of evaluation, for both types of coating. The obtained results are comparable,

than to those reported in previous works for thawed salmon tests but lower than those found for thawed salmon tests performed during this study, which is to be expected since chewiness values are dependent of the hardness, cohesiveness and springiness values, and the cohesiveness and springiness values of the cooked samples are lower than those found for the thawed samples, thus causing lower values of chewiness for the cooked samples (Casas, Martinez, Guillen, Pin, & Salmeron, 2006; Martinez, Salmerón, Guillén, & Casas, 2007).

6.5.4 Color

The color parameters L*, a* and b*, during six months storage of the water glazed and chitosan coated cooked samples can be seen in Figure A. 10, Figure A. 11, and Figure A. 12 respectively, found in Appendix E.

Regarding the results for the L*a*b* parameters during six months of storage some statistical significant differences are present especially in the later moments of storage, but no tendency in the variation of the L*a*b* parameters was found, and no difference between coatings was present.

The L*a*b* parameters were transformed to RGB codes, and a visual representation for water glazed and chitosan coated samples can be seen in Figure 6-35 and Figure 6-36.

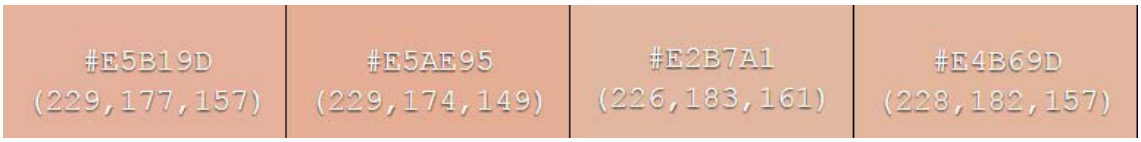

Figure 6-35 Visual representation, in RGB, of the color parameters L*a*b* for cooked water glazed salmon samples during six months of storage at -20 ºC; From left to right is possible to see from the initial moment to the last one.

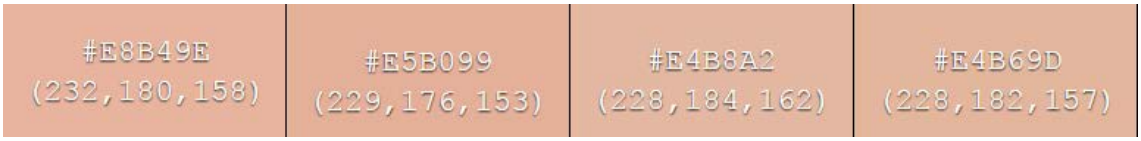

Figure 6-36 Visual representation, in RGB, of the color parameters L*a*b* for cooked chitosan coated salmon samples during six months of storage at -20 ºC; From left to right is possible to see from the initial moment to the last one.

It is possible to see that between each moment in each of the different treatments and between treatments, visually it does not seem to be great visual differences, although slight differences between colors can be noted.

Regarding perceived color differences, which were calculated as the difference between the assessed sample and a samples with the same coating at the initial moment of assessment, it is possible to see in Figure 6-37 how that value changed during the six months of storage for the different samples, it is also possible to see that when observing the ΔE*ab value between the control sample with uncoated sample at the initial moment, color differences were already present, this is represented in graphs by a dotted line, which represents the natural differences between samples, which can be seen that it is quite high, with a value similar to those of the perceived differences to a untrained assessor. In terms of perceived color differences, most of the samples present differences that would be obvious to even an untrained observer, with the exception of the initial water glazing sample, and the initial and the two month sample of the chitosan coated samples (represented by a value of ΔE*ab greater than 3.5) (Cruse, 2015; EFI, n.d.).

As shown in Figure 6-37 the perceived color differences, represented by the ΔE*ab value, for both the water glazed and the chitosan coated samples tend to follow an increasing tendency, with the exception of the last moment of analysis which is to be expected as the storage time increases, leading to higher differences in the color values.

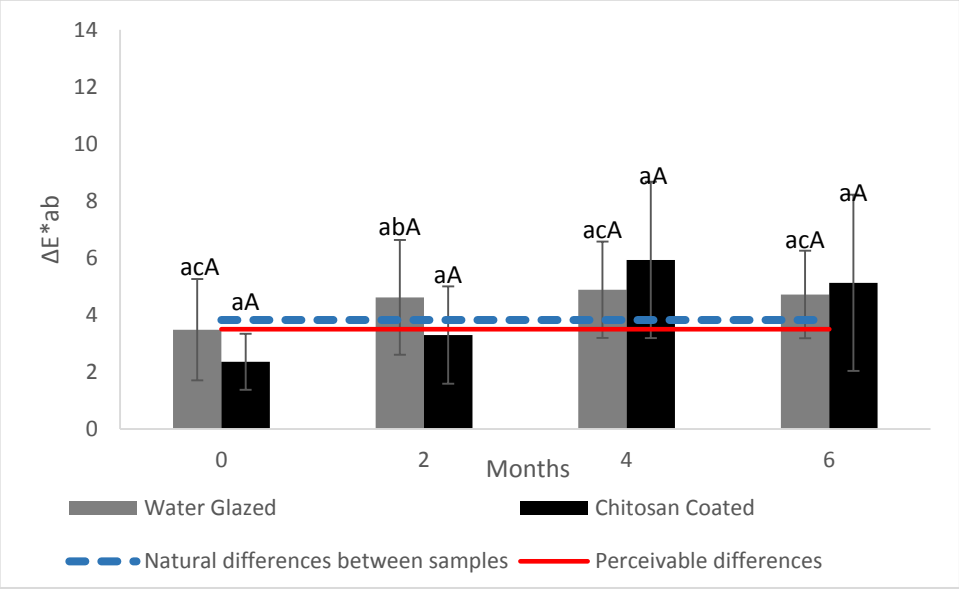

Figure 6-37 ΔE*ab values for cooked salmon samples during six months of storage at -20 ºC; standard deviation corresponds to eighteen replications; different small letters in the same sample type, and different capital letters in the same time moment indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).

During the six month period of storage the treatment that presented the lower value of ΔE*ab was that the water glazed samples, although by a small margin and one that is not statistically different than the chitosan coated samples, an unexpected result according to other studies (Soares et al., 2015), and to the ability of chitosan coatings to help protect against oxidation and protein denaturation, both of which have an influence in color preservation (Castro & Paulín, 2012; Ojagh et al., 2011; Rodriguez-Turienzo et al., 2011). This may due to the fact, that the chitosan coated samples go to a process of coating removal more extensive than that of the water glazed samples, which are easier to remove the glazing. Nevertheless is possible to see that between the last two moments of testing the ΔE*ab value of chitosan has a higher decrease in value than the water glazed samples.

It is also possible to see that the final ΔE*ab values of both the water glazed and chitosan coated cooked samples are lower than the uncooked samples. This may be due to the degradation of carotenoids (mainly astaxanthin and canthaxanthin) under high temperatures, which along with haem proteins are responsible for color of salmon. Moreover, carotenoids are bound to some myofibrillar proteins, and with the increase of temperature resulting in an increase in the degree of protein denaturation it will lead to influences in color values (Borsarelli & Mercadante, 2009; Rodriguez-Turienzo et al., 2011).

Chapter 7. Conclusions and Future Work

Although water glazing is currently the most used coating, other options have emerged, such as chitosan coatings, which have properties that can add value to the product they are protecting, other than the protection through sacrifice of the coating. The main goal in using an ice layer on frozen fish is to protect the product, but no value is added to it; the chitosan coating offers the possibility to add value other than the protection, such as a better microbiological protection, ensuring a longer shelf life. However it is necessary to know how the chitosan coating and the product combine and if there are any changes in terms of flavor diffusion, and other sensory properties.

Thermal stress tests allowed for the evaluation of the response of different coatings when under less than ideal temperature circumstances, with temperature fluctuating between -15 ^{o}C and -5 ^{o}C. Ideally all frozen products would be kept under -18 ^{o}C, but with transport between storage and retail, with opening and closing of the storage facilities were fish is kept this is not always possible: Those temperatures were thus chosen to mimic the fluctuations that could happen in a normal storage of fish. Results show that the chitosan coating presented better results in almost all of the categories assessed in which there was a significant variation from the norm. TVB-N results were within the normal range, and similar for uncoated, glazed and chitosan coated samples. No influence of the different coatings was observed regarding the pH values of the samples.

In terms of color, there seems to be more consistency, through visual assessment, of the chitosan-coated samples. Regarding the ΔE*ab values, although the final difference was higher for chitosan coatings, the differences between the assessed moments was smaller in the chitosan coatings, especially in the later stages of the test, showing promising results for a better conservation of color when using a chitosan coating. When it comes to the protection of the frozen fish, the chitosan coating offered better results than the water glazed or the uncoated samples, in both the coating loss and the microbiological tests performed. Chitosan coated samples had lower losses of coating than the water glazed samples, with the water glazed samples losing over 80 % of their initial glazing at the end of the test, while the chitosan coated samples only lost less than 50 % of their initial coating, proving that chitosan coating would be better fitted

for protection of the samples under these more extreme conditions. Microbiological evidence further supports this statement, as the chitosan coated samples had much lower values of TVC, with most of them being undetectable by the performed test, than the uncoated and water glazed samples.

For the assessment of sensory properties and the chitosan coating effect on them, several parameters were analyzed for frozen, thawed and cooked samples. Color parameters were similar for all coatings, for both the thawed and cooked samples, although ΔE*ab values were slightly lower in the cooked samples, as expected, due to changes suffered in the cooking process. Textural parameters showed no significant differences between water glazed and chitosan coated samples, while between thawed and cooked samples, slight changes were seen, which were expected given the different conditions of the samples at the moment of testing. As for the sensory analysis, sensory profiles and statistical analysis were conducted with the results of both of them indicating that there was no noteworthy change in the relevant parameters assessed, in the frozen, thawed and cooked samples, while in some parameters, such as appearance and color, the presence of the chitosan coating was beneficial. The flavor parameter was observed with special interest, as it is the one that can provide the most important information of whether flavor diffusion had or not occurred, and results show that no significant differences in flavor occurred between chitosan coated and water glazed samples, leading to the conclusion that no flavor diffusion from the chitosan coating was present in the assessed samples.

With no evidence of flavor diffusion from chitosan coatings to the salmon samples, opportunities arise for the use of chitosan coatings, with flavor encapsulation and release being one of the most significant ones. Considering that while chitosan molecules will not diffuse from the coating, smaller molecules may diffuse, leading to an opportunity to assess the viability of encapsulation and release of an added flavor to the chitosan coating that can offer added value to the product, aside from the microbiological protection already offered.

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Appendixes

Appendix A – Sensory evaluation sheet for frozen salmon samples

Panelist: _____	Date: ___ / ___ / _____
Product: **Frozen Salmon**	Code: _____

Mr(s) panelist, first judge the overall appearance of the product, then its color and finally judge its odor, following this list as presented.

Attribute	**Great**	**Good**	**Average**	**Acceptable**	**Poor**	**Bad**	**Very Bad**
	6	**5**	**4**	**3**	**2**	**1**	**0**
APPEARENCE	Absence of freezer burns and dehydration		Dehydration in less than 25% of the surface Slight freezer burns		Dehydration between 25% and 50% of the surface (extensive and profound freezer burns)		Strong dehydration in over 50% of the surface (extensive and profound freezer burns in all of the surface)
COLOR	Normal, characteristic of the species		Slight changes Visible in less than 25% of the surface		Changes between 25% and 50% of the surface		Abnormal Very visible and profound, affecting all of the surface
ODOR	Characteristic of the species		Neutral Identical to fresh fish preserved in a refrigerator		Characteristic odor almost imperceptible Strange odors, unpleasant, sour		Musty odor Rancid odor, unpleasant

Figure A. 1 Sensory evaluation sheet for frozen salmon samples.

Appendix B – Sensory evaluation sheet for thawed salmon samples

Panelist: _____ Date: ___ / ___ / _____

Product: **Thawed Salmon** Code: _____

Mr(s) panelist, first judge the overall appearance of the product, then its color, its odor and finally judge its texture, following this list as presented.

Attribute	Great	Good	Average	Acceptable	Poor	Bad	Very Bad
	6	5	4	3	2	1	0
APPEARANCE	Characteristic of the species		Slight visual change of the surface (less than 20%) Without evidence of freezer burns		Slight visible changes in over 50% of the surface		Total change, darkening of all of the surface
COLOR	Normal, Characteristic of the species (without changes of the initial color while fresh)		Slight changes Visible in less than 50% of the surface and flesh (slight discoloration)		Opaque flesh, without glare Discoloration of the abdominal wall		Dark flesh of purple or very brown color
ODOR	Characteristic of the species		Neutral Identical to fresh fish preserved in a refrigerator		Characteristic odor almost imperceptible Strange odors, unpleasant Uncharacteristic smell arises		Musty odor Rancid odor, unpleasant
TEXTURE	Flesh with firm consistency		Firm flesh with slight exudate		Rigid or hard flesh with exudate		Elastic or soft flesh with abundant exudate Fibrous and dry

Figure A. 2 Sensory evaluation sheet for thawed salmon samples.

Appendix C – Sensory evaluation sheet for cooked salmon samples

Panelist: _____ Date: ___ / ___ / _____

Product: **Cooked Salmon** Code: _____

Mr(s) panelist, first judge the overall appearance of the product, then its odor, texture and finally judge its flavor, following this list as presented.

Attribute	Great	Good	Average	Acceptable	Poor	Bad	Very Bad
	6	5	4	3	2	1	0
APPEARANCE	Flesh with a tonality characteristic of the species		Flesh with a lighter tonality (lighter pink, yellowish)		Flesh with a lightly colored tone, uncharacteristic		Flesh with an intense dark colored tone, (blackened tones, brownish)
TEXTURE FLESH COHESION	Cohesive musculature		Muscle parts stay together, but they show separation "lines" (they separate with careful manipulation)		Musculature still stays together, but separates easily		Muscles separate extremely easily Muscle don't stay together and crumble
ODOR	Fresh, normal Characteristic of the species		Slight loss, identical to fresh fish preserved in a refrigerator		Uncharacteristic smell arises		Oxidized odor, to chemical substances, acid milk, acetic acid, ammonia, oxidized fish oil and polyphosphates
FLAVOR	Fresh, normal Characteristic of the species		Loss of characteristic flavor		Uncharacteristic flavor arises		Flavor to caramel, condensed milk, metal, boiled milk

Figure A. 3 Sensory evaluation sheet for cooked salmon samples.

Appendix D – Graphic representation of the color parameters L*, a* and b*, of the control, uncoated, water glazed, and chitosan coated samples for the thermal stress test

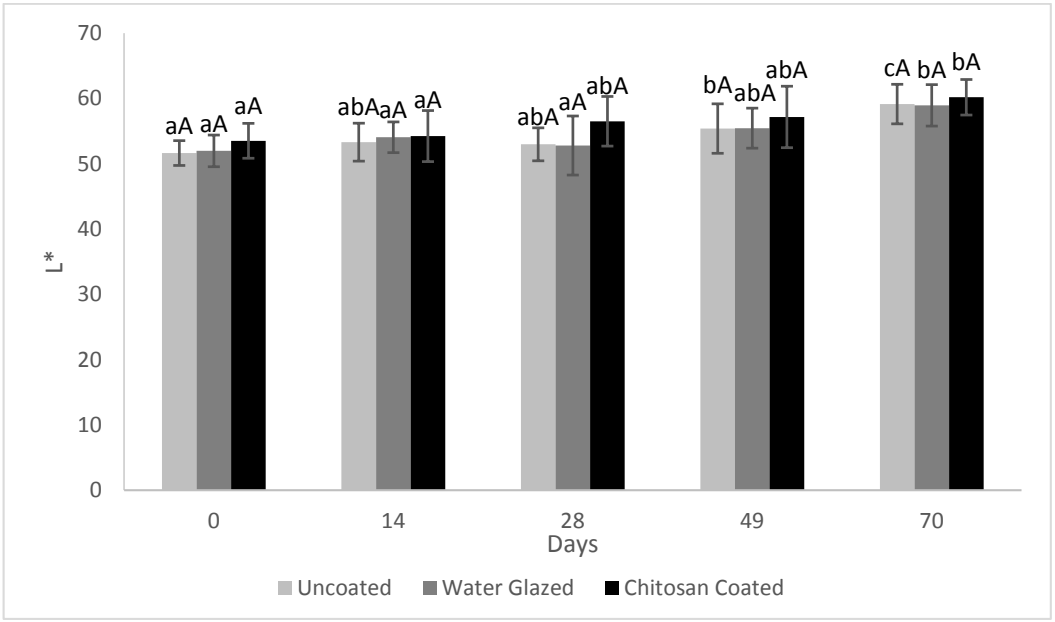

Figure A. 4 Values of the color parameter L* for uncoated, water glazed, and chitosan coated salmon samples during 70 day storage between -15 ºC and -5 ºC; standard deviation corresponds to eighteen replications; different small letters in the same sample type, and different capital letters in the same time moment indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).

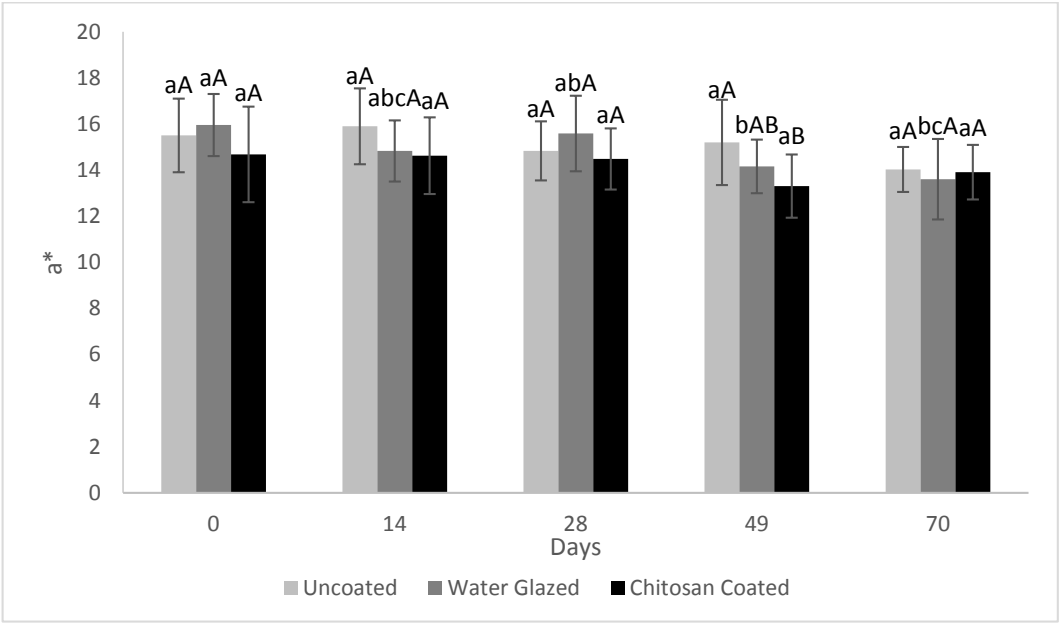

Figure A. 5 Values of the color parameter a* for uncoated, water glazed, and chitosan coated salmon samples during 70 day storage between -15 ºC and -5 ºC; standard deviation corresponds to eighteen replications; different small letters in the same sample type, and different capital letters in the same time moment indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).

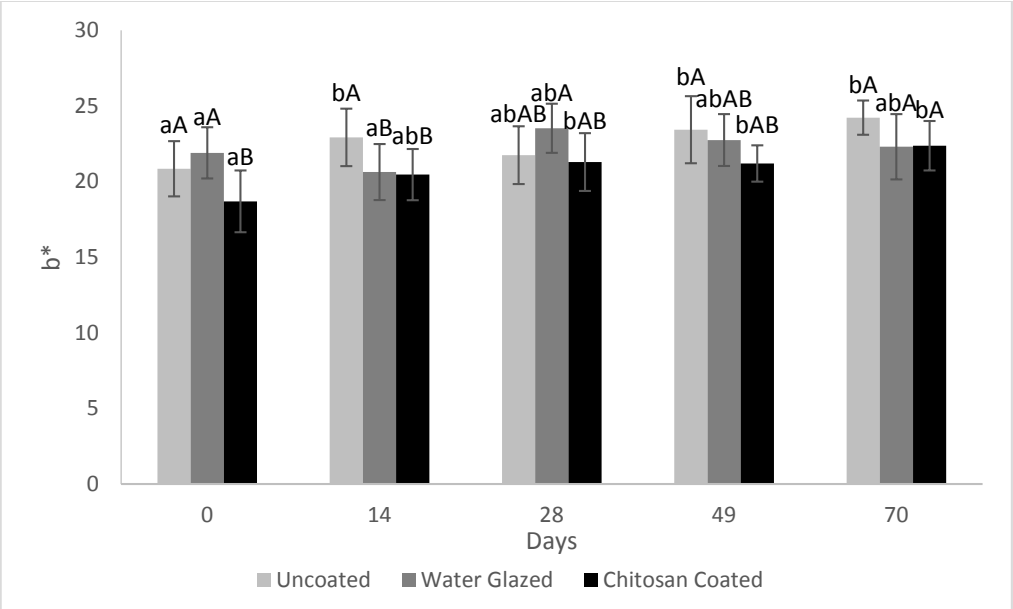

Figure A. 6 Values of the color parameter b* for uncoated, water glazed, and chitosan coated salmon samples during 70 day storage between -15 ºC and -5 ºC; standard deviation corresponds to eighteen replications; different small letters in the same sample type, and different capital letters in the same time moment indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).

Appendix E – Graphic representation of the color parameters L*, a* and b*, of the water glazed and chitosan coated samples used in the sensory analysis

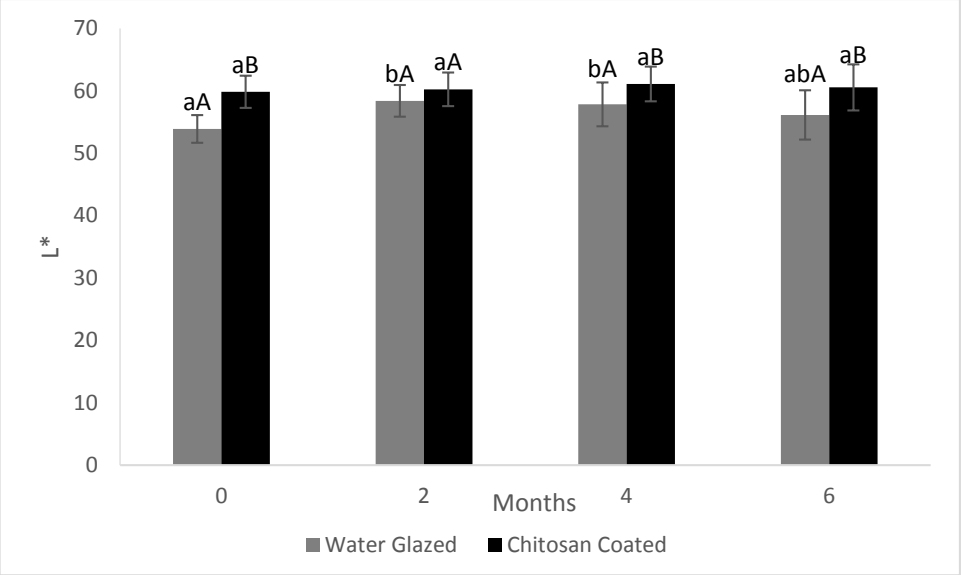

Figure A. 7 Values of the color parameter L* for thawed water glazed and chitosan coated salmon samples during six months of storage at -20 ºC; standard deviation corresponds to eighteen replications; different small letters in the same sample type, and different capital letters in the same time moment indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).

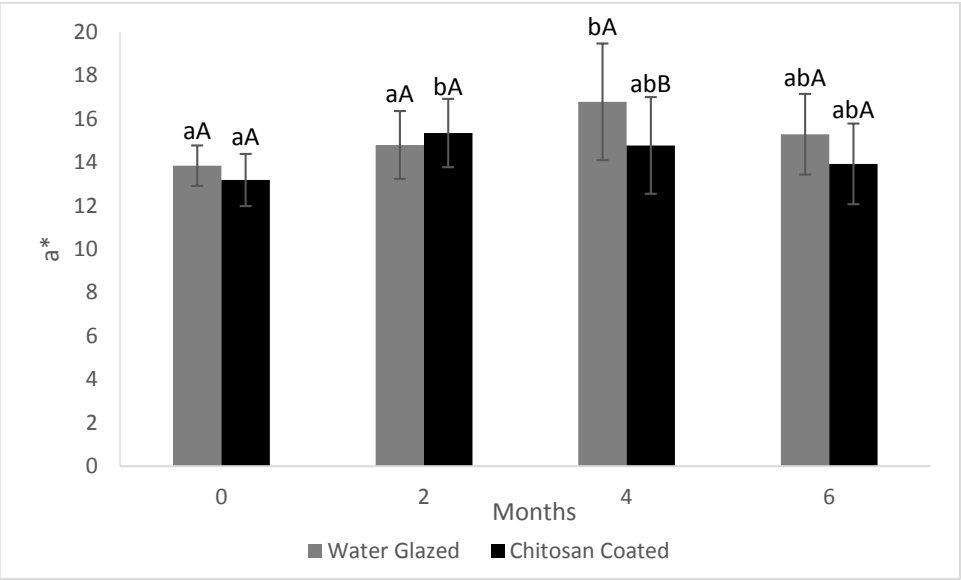

Figure A. 8 Values of the color parameter a* for thawed water glazed and chitosan coated salmon samples during six months of storage at -20 ºC; standard deviation corresponds to eighteen replications; different small letters in the same sample type, and different capital letters in the same time moment indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).

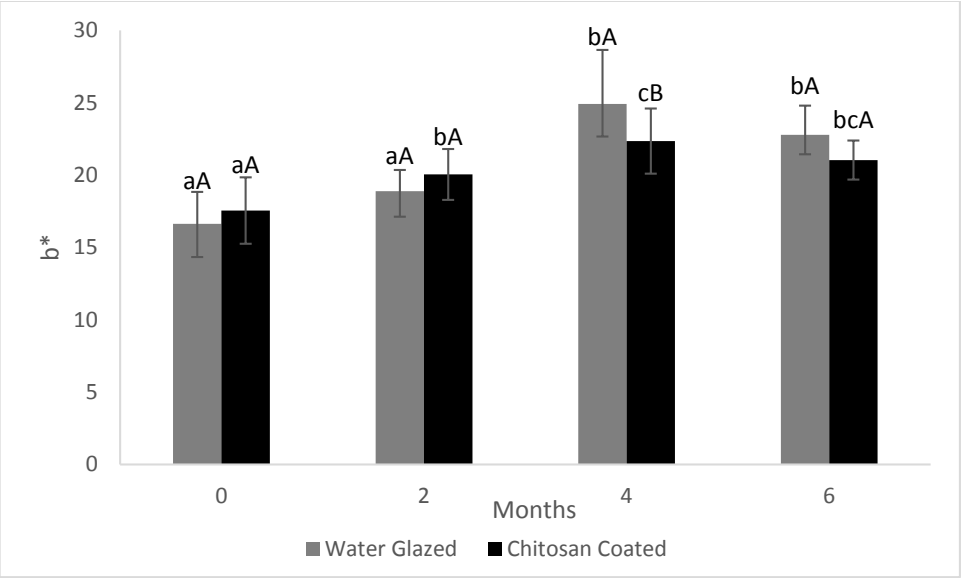

Figure A. 9 Values of the color parameter b* for thawed water glazed and chitosan coated salmon samples during six months of storage at -20 ºC; standard deviation corresponds to eighteen replications; different small letters in the same sample type, and different capital letters in the same time moment indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).

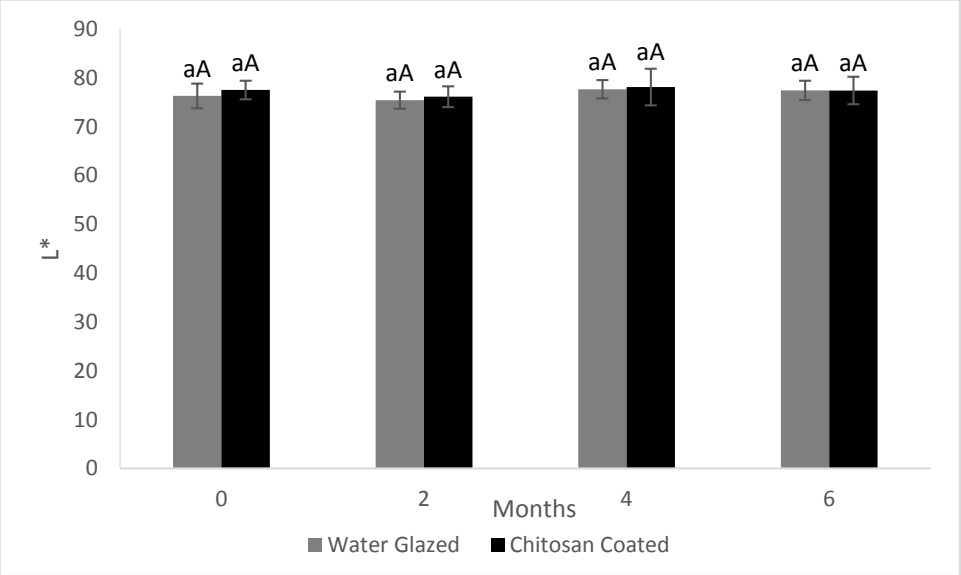

Figure A. 10 Values of the color parameter L* for cooked water glazed and chitosan coated salmon samples during six months of storage at -20 ºC; standard deviation corresponds to eighteen replications; different small letters in the same sample type, and different capital letters in the same time moment indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).

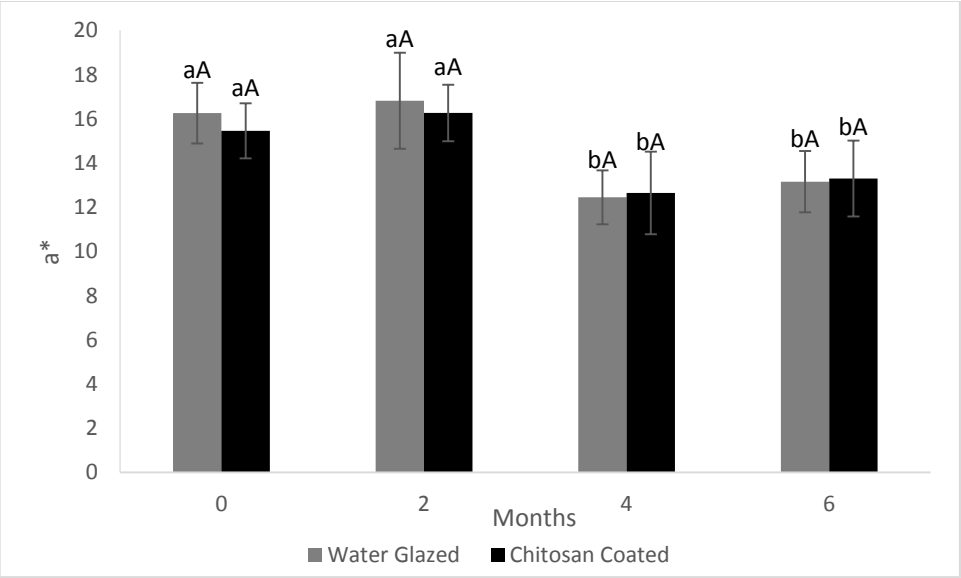

Figure A. 11 Values of the color parameter a* for cooked water glazed and chitosan coated salmon samples during six months of storage at -20 ºC; standard deviation corresponds to eighteen replications; different small letters in the same sample type, and different capital letters in the same time moment indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).

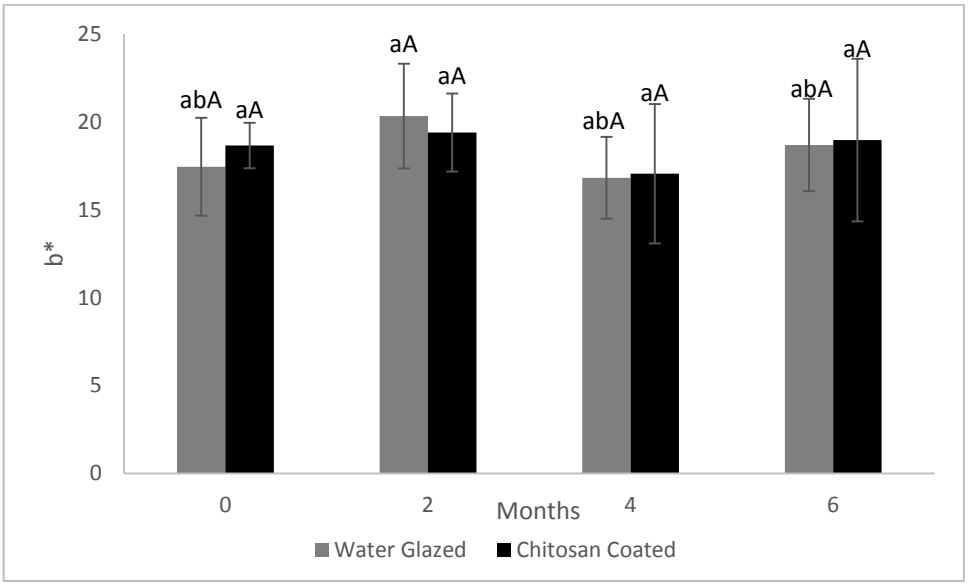

Figure A. 12 Values of the color parameter b* for cooked water glazed and chitosan coated salmon samples during six months of storage at -20 ºC; standard deviation corresponds to eighteen replications; different small letters in the same sample type, and different capital letters in the same time moment indicate a statistically significant difference (Tukey HSD test, $p < 0.05$).