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A thesis presented to the Faculty of Engineering of the University of Porto, in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemical and Biological Engineering

Thesis prepared under the supervision of Dr. Alberto M. Sereno, Associate Professor at the Department of Chemical Engineering of the University of Porto

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“Imagination is more important than knowledge”

Albert Einstein

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ABSTRACT

In recent years, increasing interest in biodegradable materials has developed mainly due to concern over the disposal of conventional synthetic plastic materials derived from petroleum. Degradation of plastics requires a long time and most of them end up over-burdening the environment. In terms of end-use applications, packaging remains one of the major uses of plastics in the world. Food packaging has become a central focus of waste reduction efforts, because it is in most cases based on a variety of petroleum-derived plastic materials, due to their availability in large quantities at low cost and favourable functional characteristics.

Edible and biodegradable natural polymer films offer alternative packaging with lower environmental costs. The main renewable and natural biopolymer films are obtained from polysaccharides, lipids and proteins. An intense search for new renewable sources to produce edible and biodegradable materials is observed, targeting in particular underexploited organisms and biological residues. Of great interest are unique carbohydrates found in marine organisms and crops, largely underexploited, that may become valuable source of new biomaterials.

The objective of this work was the production, characterization and optimization of biodegradable films and coatings for food obtained from biopolymers extracted from the macroalgae *Mastocarpus stellatus* and from a non-conventional starch source, the acorn from cork oak (*Quercus suber* L.).

The biopolymers carrageenan and starch extracted from *Mastocarpus stellatus* and cork oak's acorn (*Quercus suber* L.) were characterized for its subsequent utilization in new formulations for the production of films and coatings. Carrageenan biopolymer extracted from *Mastocarpus stellatus* seaweeds was shown to be a κ/ι-hybrid carrageenan with gel properties comparable to commercial k-carrageenan gel formers. This gelling ability of

carrageenan imparts excellent film forming properties. Cork oak's acorn, containing about 80% of carbohydrates, presents an important potential to be used as source of starch for films and edible coatings. The starch obtained in the laboratory was similar to other acorn varieties in terms of nutritional composition and gelatinization temperature range reported in the literature.

For the film production, two methods were investigated: casting and knife coating. Casting is a simple method to produce films, but has the disadvantage of being a batch procedure only used for the production of films in a very small-scale. To overcome such limitation, a semi-continuous knife-coating process was developed. This method allows an effective control of the thickness and application speed over a suitable support that can be chosen for each biopolymer solution used. Films produced by both methods presented very similar properties. The important advantage of films produced by knife coating results from their improved uniformity due to a strict thickness control, leading to a better property reproducibility, mainly in the tensile tests. Another advantage of the films produced by knife coating is the important reduction of drying time from 24 – 48 hours for casting to just 2 hours. Based on the results obtained, their good reproducibility and the ready possibility to turn it into a continuous industrial procedure, knife coating positions itself as a good alternative to produce films in a large scale.

Films produced with κ/ι-hybrid carrageenan extracted from *Mastocarpus stellatus* showed excellent properties in general. The films presented good optical properties in terms of transparency and UV-light barrier, in spite of its yellowness appearance. Water vapour permeability in carrageenan based films has an intermediate value among other protein and polysaccharide based films as reported in literature. Tensile strength of these films is excellent, reaching values around 60 MPa for pure carrageenan films. However, they showed poor elongation properties and to be very brittle. These drawbacks could be overcome with the addition of a plasticizer.

Hydrophilic and hydrophobic plasticizers were studied. The best results were obtained with glycerol, improving almost all properties. Films with glycerol became more deformable and easier to work; in spite of an increase of their hygroscopicity. On the other hand, films with hydrophobic plasticizers looked very poor due to moisture exudation. Only the barrier to UV-light improved with respect to those plasticized with glycerol.

Films produced from starch extracted from cork oak acorns (*Quercus suber* L.) were very brittle and difficult to remove from the support without breaking. To overcome such drawback, they were mixed with carrageenan. The results obtained were good in terms of mechanical properties, comparable with the mixture of rice starch and carrageenan. The acorn starch-carrageenan films showed a yellowness appearance and opacity, due to the use of non-purified starch, pale yellow in colour. In general, acorn starch showed to be an interesting alternative to produce films with good properties, but further studies are necessary to improve the extraction procedure and to include its purification.

A study of practical application for the carrageenan extracted from *Mastocarpus stellatus* for fruit coating was conducted. Cherries were coated with an aqueous solution of carrageenan, with and without the addition of a plasticizer (glycerol) and stored at 5ºC. The results indicate that with the coating application, the fruits lost about 30% less weight than the uncoated fruits. The coating application was also effective in retaining the firmness of the refrigerated cherries. External color change was not evident in this study, but the uncoated fruits lost their gloss whereas the coated fruits kept the brightness until the day 20 of storage. Coating based in κ/ι-hybrid carrageenan solution showed to be an interesting alternative to protect and extend shelf life of these fruits.

As a final conclusion, both biopolymers extracted from non-conventional low value renewable sources considered in this study proved to be promising materials for the production of edible coatings and biodegradable films,

with good properties. The new semi-continuous technique to produce films was able to produce homogeneous and uniform films, with an effective control over their thickness and opening sound perspectives for its scale up to an industrial scale.

RESUMO

Nos últimos anos tem havido um interesse crescente no desenvolvimento de materiais biodegradáveis devido à preocupação sobre a eliminação dos resíduos de materiais plásticos sintéticos convencionais derivados do petróleo. A degradação natural dos plásticos requer muito tempo e a maioria deles acaba causando uma sobrecarga do ambiente. As embalagens constituem uma das principais utilizações dos plásticos consumidos no mundo. As embalagens para alimentos tornaram-se pois um foco no esforço para a redução do lixo, pois são também produzidos a partir de materiais plásticos derivados do petróleo, devido à sua fácil disponibilidade, a baixo custo e com características funcionais favoráveis.

Os filmes comestíveis e biodegradáveis são uma alternativa de embalagem com menores custos ambientais. Os principais biopolímeros naturais e renováveis utilizados para a obtenção de filmes são os polissacáridos, os lípidos e as proteínas. Observa-se uma intensificação da procura de novas fontes renováveis de materiais biodegradáveis, nomedamente de organismos marinhos, produtos vegetais e resíduos biológicos naturais subaproveitados susceptíveis de se tornarem fonte de novos biomateriais.

O objectivo deste trabalho foi a produção, caracterização e optimização de filmes e revestimentos biodegradáveis para alimentos obtidos a partir de biopolímeros extraídos da macroalga *Mastocarpus stellatus* e a partir de uma fonte não convencional de amido, a bolota de sobreiro (*Quercus suber* L.).

Os biopolímeros extraídos das fontes indicadas, o carragenato e o amido respectivamente foram caracterizados para a sua subsequente utilização na formulação e produção de filmes e revestimentos. O carragenato extraído das algas de *Mastocarpus stellatus* foi um híbrido κ/ι-carragenato que apresenta propriedades gelificantes comparáveis às do κ-carragenato natural. Constata-se que as características gelificantes do carragenato são

excelentes para a formação de filmes. A bolota de sobreiro contém aproximadamente 80% de carbohidratos e por isso importante potencial como fonte de amido para filmes ou revestimentos comestíveis. O amido de bolota de sobreiro obtido em laboratório mostrou-se semelhante ao obtido a partir de outras variedades de bolota, em termos de composição nutricional e faixa de temperatura de gelatinização, de acordo com a informação reportada na literatura.

Foram investigados dois métodos para a produção de filmes: moldagem "*casting*" e aplicação sobre suporte com uma lámina "*knife coating*". A técnica de "*casting*" é um método simples de produção de filmes, mas tem a desvantagem de ser descontínuo e somente permitir o fabrico em muito pequena escala. Para contornar esta limitação, foi desenvolvido um processo semi-contínuo que utiliza a técnica de "*knife coating*". Este método permite um controle efectivo da espessura e da velocidade de aplicação sobre um suporte adequado a cada solução de biopolímero utilizada. Os filmes produzidos pelos dois métodos têm propriedades semelhantes. A principal vantagem dos filmes produzidos pela técnica de "*knife coating*" reside na maior uniformidade resultante de um controlo estrito da sua espessura, que conduz a uma maior reprodutibilidade das propriedades principalmente nos testes de tracção. Outra grande vantagem da técnica de "*knife coating*" reside na redução do tempo de secagem que passou de 24 a 48 horas nos filmes obtidos por *"casting"* para cerca de 2 horas. Com base nos resultados obtidos, na sua boa reprodutibilidade e na possibilidade de ser facilmente transformado num processo contínuo, a técnica de "*knife coating*" posiciona-se como uma boa alternativa para a produção de filmes em maior escala.

Os filmes produzidos com o híbrido κ/ι-carragenato extraído de *Mastocarpus stellatus* apresentaram em geral excelentes propriedades. Os filmes mostraram ter boas propriedades ópticas em termos de transparência e de barreira à luz ultra-violeta, apesar de sua aparência amarelada. A permeabilidade ao vapor de água dos filmes de carragenato situou-se num

valor intermediário, compararável à de outros filmes descritos na literatura, obtidos de proteínas e de polissacáridos. As suas propriedades mecânicas mostraram ser excelentes em termos de resistência à tracção, com valores próximos a 60 MPa para os filmes de carragenato puro. Apresentaram, contudo, um fraco comportamento à deformação, sendo muito quebradiços. Estas deficiências foram ultrapassadas pela adição de plastificantes. Foram estudados plastificantes hidrofílicos e hidrofóbicos. Os melhores resultados foram obtidos com o glicerol, que melhorou quase todas as propriedades estudadas. Os filmes plastificados com glicerol tornaram-se mais deformáveis e mais fáceis de se trabalhar, apesar da sua higroscopicidade ter aumentado. Os filmes produzidos com plastificantes hidrofóbicos apresentaram um aspecto bastante pobre devido à exudação de humidade. Somente as propriedades de barreira à luz ultravioleta foram melhores do que as dos filmes plastificados com glicerol.

Os filmes produzidos a partir do amido extraído da bolota de sobreiro (*Quercus suber* L.) mostraram-se muito quebradiços, sendo difícil destacá-los do suporte sem quebrar. A alternativa estudada foi misturar carragenato ao amido de bolota. Os resultados obtidos foram claramente melhores em termos de propriedades mecânicas, sendo semelhantes aos dos filmes produzidos a partir da mistura de amido de arroz com carragenato. Os filmes da mistura de amido de bolota e carragenato apresentaram maior opacidade e uma coloração amarelada, devido ao ao facto do amido não ter sido purificado e apresentar ele próprio uma coloração amarelada. Em termos gerais, o amido de bolota mostrou ser uma alternativa interessante para a produção de filmes com boas propriedades. São necessários todavia estudos complementares no sentido de optimizar a técnica de extracção e de introduzir um passo de purificação.

Como exemplo prático de aplicação do carragenato extraído de *Mastocarpus stellatus* foi estudado a aplicação de um revestimento na protecção de frutas frescas. Foram revestidas cerejas frescas com uma solução aquosa de carragenato, com e sem a adição de glicerol, que foram a seguir

armazenadas a uma temperatura de 5ºC. Os resultados indicaram que com a aplicação do revestimento, a perda de peso das frutas foi reduzida em cerca de 30% relativamente às frutas não revestidas. A aplicação do revestimento também foi eficaz na manutenção da textura das cerejas. Não foi observada mudança na coloração externa das cerejas. Numa apreciação visual observou-se que as frutas não revestidas perderam o brilho enquanto que as frutas revestidas mantiveram o brilho até o vigésimo dia de armazenamento. Este revestimento obtido a partir de soluções aquosas do híbrido κ/ι-carragenato mostrou pois ser uma boa alternativa para a proteção e aumento da vida útil destes frutos.

Como conclusão final pode dizer-se que os novos biopolímeros estudados mostraram ser materiais promissores para a produção de filmes biodegradáveis e de revestimentos comestíveis, com boas propriedades em geral. A nova técnica desenvolvida para a produção de filmes de aplicação com uma lámina sobre um suporte de maneira semi-contínua mostrou ser uma boa alternativa para a produção de filmes homogéneos e uniformes, que permite um eficaz controle da espessura e abre perspectivas para a produção contínua de filmes numa escala industrial.

RÉSUMÉ

Un intérêt grandissant pour les matériaux biodégradables a émergé ces dernières années, principalement à cause du problème associé à la destruction des matières plastiques synthétiques conventionnelles qui sont des dérivés du pétrole. La dégradation des plastiques requiert une longue durée et en grande majorité, ils deviennent un fardeau pour l'environnement. En termes d'utilisation, l'emballage est le secteur d'applications qui emploie le plus de plastique dans le monde. L'emballage alimentaire est devenu un point central des efforts de réduction des déchets, parce que dans la plupart des cas, il est constitué d'une variété de matières plastiques dérivées du pétrole. Ceci est du à la disponibilité en grande quantité et à bas prix des plastiques, ainsi qu'à leurs propriétés fonctionnelles favorables.

Les films de polymère naturel et biodégradable proposent une alternative pour des emballages ayant un moindre coût environnemental. Les films faits de polymères naturels et renouvelables sont obtenus essentiellement à partir de polysaccharides, de protéines et de lipides. On assiste aujourd'hui à une recherche accrue de nouvelles sources renouvelables permettant la production de matériaux biodégradables et comestibles. Les résidus biologiques et les sources sous exploitées sont en particulier les cibles de cette recherche. Les carbohydrates qui sont spécifiques aux organismes marins et aux cultures marines, sont sous exploités mais d'un grand intérêt car ils peuvent devenir une source précieuse pour de nouveaux biomatériaux.

L'objectif de ce travail est la production, la caractérisation et l'optimisation de films et de revêtements biodégradables alimentaires, obtenus à partir de biopolymères extraits d'algues de type *Mastocarpus stellatus* et à partir d'une source non conventionnelle d'amidon, le gland du chêne liège (*Quercus suber L.*).

La carraghénane et l'amidon extraits des algues de Mastocarpus stellatus et les glands du chêne liège (*Quercus suber L.*) ont été caractérisés afin de pouvoir les utiliser ultérieurement dans de nouvelles formulations visant à produire des films et des revêtements. La carraghénane extraite de l'algue *Mastocarpus stellatus* présente une structure chimique hybride de type κ/ι avec des propriétés gélifiantes comparables à celles de la carraghénane de type κ. Cette capacité à gélifier contribue pour donner à la carraghénane d'excellentes caractéristiques pour former des films. Le gland du chêne liège contient environ 80% de carbohydrates et présente un grand potentiel comme source d'amidon pour les films et les revêtements comestibles. L'amidon obtenu en laboratoire est similaire à ceux isolés à partir d'autres variétés de glands, dans la mesure où la composition nutritionnelle et la gamme de température de gélatinisation se révèlent être comparables à celles reportées dans la littérature.

Pour la production de film, deux méthodes ont été testées : le dépôt et le recouvrement au couteau. Le dépôt est une méthode qui permet de produire des films de manière simple, mais elle présente le désavantage d'être une technique de type batch, et par conséquent elle ne peut être utilisée que pour une production à petite échelle. Afin de surpasser cette limitation, un procédé semi continu de revêtement au couteau a été développé. Cette méthode permet un contrôle efficace de l'épaisseur et de la vitesse d'application de la solution qui formera le film sur un support qui peut être modifié en fonction de la formulation de bio polymère utilisée pour la solution. Les films produits avec les deux méthodes présentent des caractéristiques très similaires. Le principal avantage de la technique de revêtement au couteau réside dans l'aspect uniforme des films obtenu grâce à un control strict de l'épaisseur. Une conséquence de cet avantage est le fait de pouvoir atteindre une grande reproductibilité dans les tests mécaniques de tension. Un autre avantage de ce procédé est la réduction significative du temps de séchage. Celui-ci oscille entre 24 et 48 heures lorsque la technique de dépôt est employée, alors qu'il n'est que de deux heures pour la technique semi continue. A la vue des résultats obtenus, de

leur bonne reproductibilité, et du fait que cette technique peut facilement être adaptée à un procédé industriel continu, la méthode de recouvrement au couteau se révèle être une bonne alternative pour la production à grande échelle de films.

Les films produits à partir de la carraghénane hybride de type κ/ι extraite de l'algue *Mastocarpus stellatus* présentent d'une manière générale d'excellentes propriétés. Ces films ont de bonnes caractéristiques optiques en ce qui concerne la transparence et la barrière aux UV, en dépit de leur apparence légèrement jaunâtre. La perméabilité à la vapeur d'eau présente une valeur intermédiaire entre celles reportées dans la littérature pour des films formulés avec des protéines et d'autres polysaccharides. La résistance à la tension est excellente, puisque des valeurs avoisinant les 60 MPa sont mesurées pour les films obtenus uniquement à partir de la carraghénane. Cependant, ces films sont très cassants et présentent une mauvaise capacité d'élongation. Ces inconvénients ont néanmoins pu être surpassés en utilisant des plastifiants. Des plastifiants hydrophobes et hydrophiles ont été testés. Les meilleurs résultats furent obtenus avec le glycérol, qui améliore pratiquement toutes les propriétés. Les films contenant du glycérol sont plus facile à manipuler puisque mieux déformables. En revanche, les films contenant des plastifiants hydrophobes n'ont pas un aspect satisfaisant en raison de problèmes de d'exsudation d'humidité. Seules les propriétés de barrière à la lumière UV ont été améliorées par rapport à celles obtenues pour les films plastifiés avec du glycérol.

Les films produits à partir de l'amidon extrait des glands du chêne liège (*Quercus suber L.*) sont très cassants, et il est difficile de les décoller de leur support sans les endommager. Afin de résoudre ces impondérables, l'amidon a été mélangé avec de la carraghénane. De bonnes propriétés mécaniques ont ainsi été obtenues, puisque celles-ci sont comparables à celles des films produits à partir de mélanges de carraghénane et d'amidon de riz. Les films de carraghénane mélangée avec l'amidon du gland sont opaques et d'apparence jaunâtre, à cause de l'aspect même de l'amidon du

gland qui est jaune pâle puisqu'il n'a pas été purifié ni blanchi. D'une manière générale, cet amidon paraît être une alternative intéressante pour produire des films possédant de bonnes propriétés, mais des études supplémentaires sont nécessaires afin d'améliorer le procédé d'extraction qui doit inclure une purification.

L'étude de l'application pratique de la carraghénane extraite de l'algue *Mastocarpus stellatus* au revêtement de fruits de saison a été entreprise. Des cerises ont été revêtues avec une solution aqueuse de carraghénane contenant ou non un plastifiant (glycérol), et stockées à 5 ºC. Les résultats indiquent que le revêtement permet une réduction de la perte de poids des cerises d'environ 30 % par rapport aux cerises non traitées. L'application d'un revêtement s'avère également bénéfique pour la préservation de la fermeté des cerises. La variation de la couleur des fruits n'est pas significative au long de cette étude, cependant les fruits non recouverts perdent leur aspect brillant et attrayant rapidement, tandis que les fruits traités avec un revêtement de carraghénane maintiennent un aspect brillant jusqu'au vingtième jours de stockage. La solution de carraghénane hybride de type κ/ι se révèle être un moyen intéressant de protéger ces fruits et d'allonger leur état de fraîcheur et date limite de consommation.

En conclusion, les deux bio polymères extraits de sources renouvelables non conventionnelles et de bas coût qui ont été utilisés dans cette étude se sont avérés être des matériaux prometteurs pour la production de revêtements comestibles et de films biodégradables. La technique semi continue utilisée a permis de produire des films homogènes et uniformes, avec un contrôle efficace de leur épaisseur, et ouvre une perspective intéressante pour une production industrielle à grande échelle.

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Chapter 1
Introduction

1.1. General introduction

Petrochemical based plastics such as polyolefins, polyesters, polyamides, etc. have been increasingly used as packaging materials, because of their availability in large quantities at low cost and favourable functionality, characteristics such as good tensile and tear strength, good barrier properties to O_2 and aroma compounds and heat sealability. On the contrary they have a very low water vapour transmission rate and, most importantly, they are non-biodegradable, and therefore lead to environmental pollution, which pose serious ecological problems. Hence, their use in any form or shape has to be restricted and may be even gradually abandoned to avoid problems concerning waste disposal.

Edible coatings from polysaccharides, proteins, and lipids can extend the shelf-life of foods by functioning as solute, gas, and vapour barriers. Although the use of edible coatings and films is not a new concept, research in this field at academic, government and private industry laboratories has

intensified recently. Factors contributing to a renewed interest in development of edible coatings include consumer demand for high quality foods, environmental concerns over disposal of non-renewable food packaging materials and opportunities for creating new market outlets for film-forming ingredients derived from under-utilized agricultural resources.

The search for new renewable resources for the production of edible and biodegradable materials has steadily increased in recent years. In particular, non-conventional sources of carbohydrates have been extensively studied. There are various unique carbohydrates that are found in marine organisms that represent a largely unexplored source of valuable materials. These non-conventional and underexploited renewable materials can be used as an interesting alternative to produce edible films and coatings.

The biopolymers studied in this work to produce edible films and coatings were κ/ι-hybrid carrageenan extracted from *Mastocarpus stellatus*, an underexploited red algae present in the Portuguese marine coast and starch extracted from the acorn of cork oak (*Quercus suber* L.), which is usually used as feed source for free-ranging wild animals.

1.2. Objectives of the work

The general objective is to obtain safer and more stable foods using edible coatings and films as barriers to contamination and weight loss. This will involve the extraction of biopolymers from natural renewable sources, their formulation with food grade additives and development of improved technologies to produce edible coatings to be used to protect e.g. fresh or processed fruits and vegetables, and biodegradable films for food packaging.

The main goals are:

- to develop improved technologies to produce edible coatings and biodegradable films from low value renewable raw materials present in Portugal;

- to add value to existing renewable raw materials, the macroalgae *Mastocarpus stellatus* from the Portuguese coast and a non-conventional starch source, the acorn from cork oak (*Quercus suber* L.);

- to optimize formulations and develop new continuous film casting techniques leading to the right properties and the right functionality required.

This work is divided in several chapters that are briefly described below. Figure 1.1 shows a diagram of the thesis structure.

Chapter 2, *"Literature review"*, presents a review of the environmental impact of packaging made from synthetic polymers and a promising proposal to decrease this environmental impact: biodegradable films. A review of the main biopolymers used to produce biodegradable films, and the methods to produce and characterize the main properties of the films are also presented.

In Chapter 3, *"Selection of renewable sources of biopolymers"*, general aspects for the choice of the biopolymers studied in this work, as sources, properties, chemical structures and their use in the production of biodegradable films are presented.

Chapter 4, *"Biopolymer extraction and characterization"*, describes the procedures used for the extraction of the biopolymers from the natural sources chosen in Chapter 3, and their physicochemical characterization.

Chapter 5, *"Production and characterization of films"*, presents the methods used for film production and the procedures for the tests performed to characterize them.

In the Chapter 6, *"Film formulation and optimization"*, the main properties of different film formulations studied are presented. Based on the results obtained, such formulations were optimized and the corresponding film properties were evaluated.

Chapter 7, *"Application of a coating to fresh cherries"*, describes the procedures and results of the application of coatings based in the biopolymer extracted in this work (carrageenan), to protect and extend the shelf life of cherries.

Finally, in Chapter 8, *"Conclusions"*, major results of this work are identified and some perspectives for future work proposed.

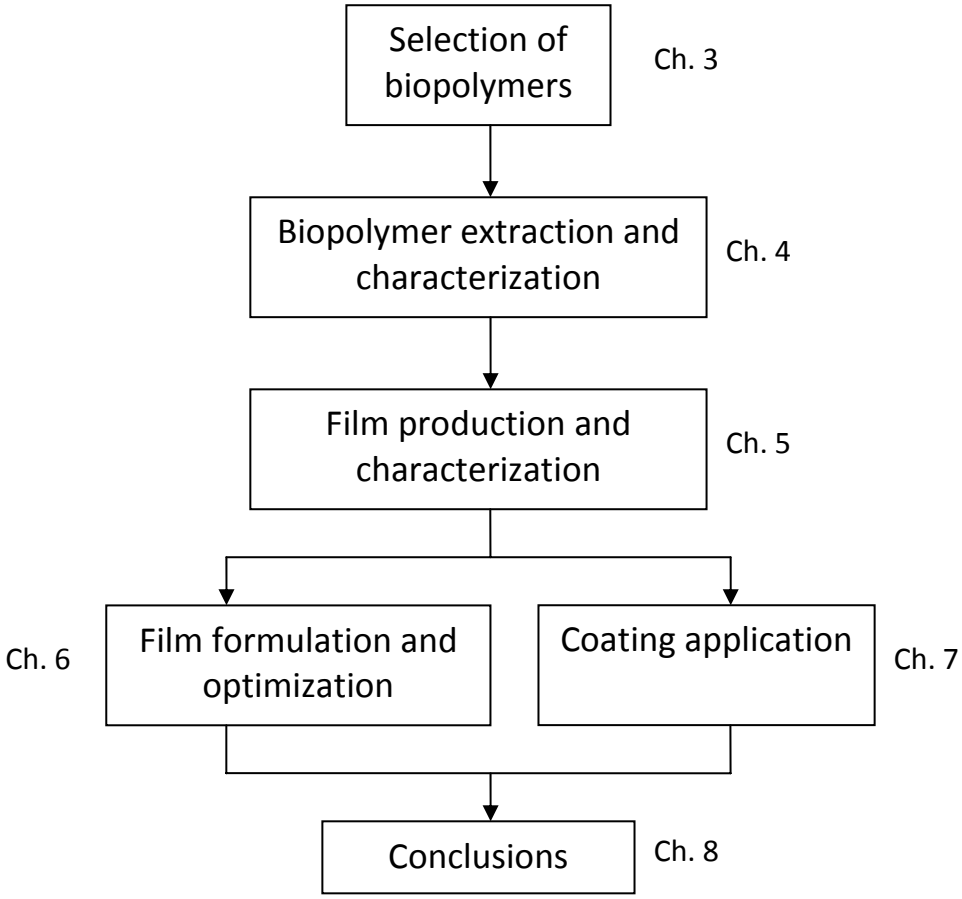


Figure 1.1 – Thesis structure.

Chapter 2

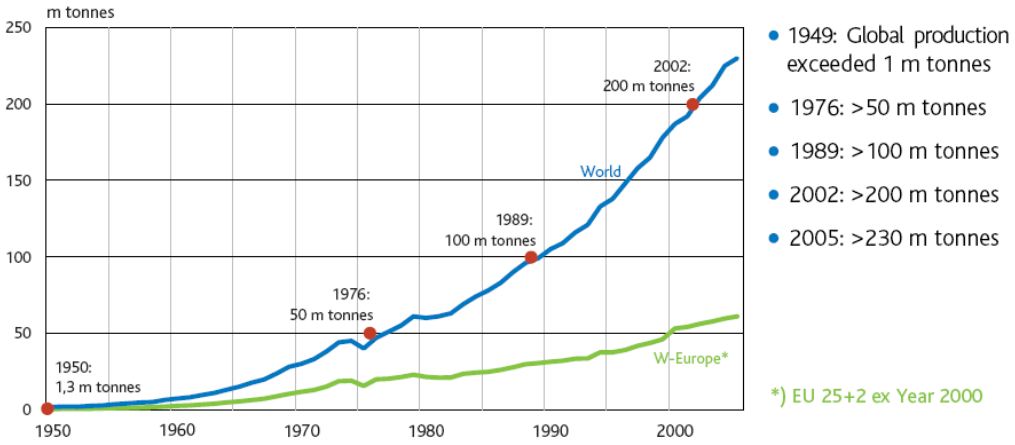
Literature review

In this chapter a literature review of the environmental problems caused by synthetic polymers, and a brief history of the use of biopolymers as edible films and coatings, including the technology for its production and their physical properties is presented.

2.1. The problem of plastic packaging waste

Polymers and plastics are typical materials of the last century and the continuous innovation around them helps to explain that since 1950, plastics production has increased by an average of almost 10% every year on a global basis. From around 1.3 million tons in 1950 the global production of plastics has grown to 230 million tons in 2005 (Figure 2.1). Western Europe accounts for 25% of that amount, a similar level to that of North America at 24% (Figure 2.2). An analysis of plastics consumption on a per capita basis shows that this has now grown to over 100kg/year in North America and Western Europe, with the potential to grow to up to 130kg/year per capita by 2010. Now, the highest potential for growth can be found in some rapidly

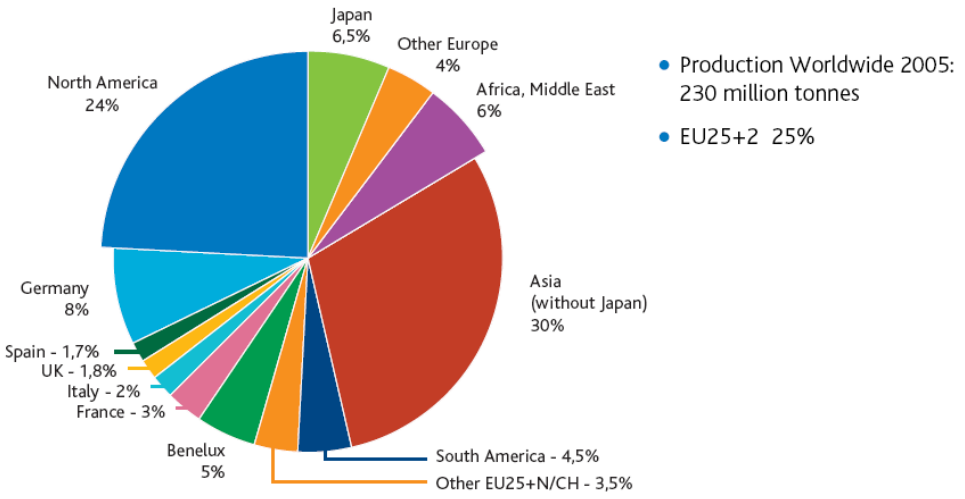
developing regions of Asia, where currently the per capita consumption is only around 20 kg/year (Figure 2.3). In the European context, it is the new Member States which are expected to see the biggest increase as their economies develop. Their current per capita consumption of 55 kg/year is currently little over half of the older Member States (Plastics*Europe*, 2007).



Note: Based on preliminary estimates by European Market Research & Statistics Working Group. Includes thermoplastics, thermosets, adhesives, coatings and dispersions. Fibers are not included.

Source: Plastics*Europe*, WG Market Research & Statistics

Figure 2.1 – World plastics production 1950-2005.



Source: Plastics*Europe*, WG Market Research & Statistics

Figure 2.2 – World plastics production per country/region.

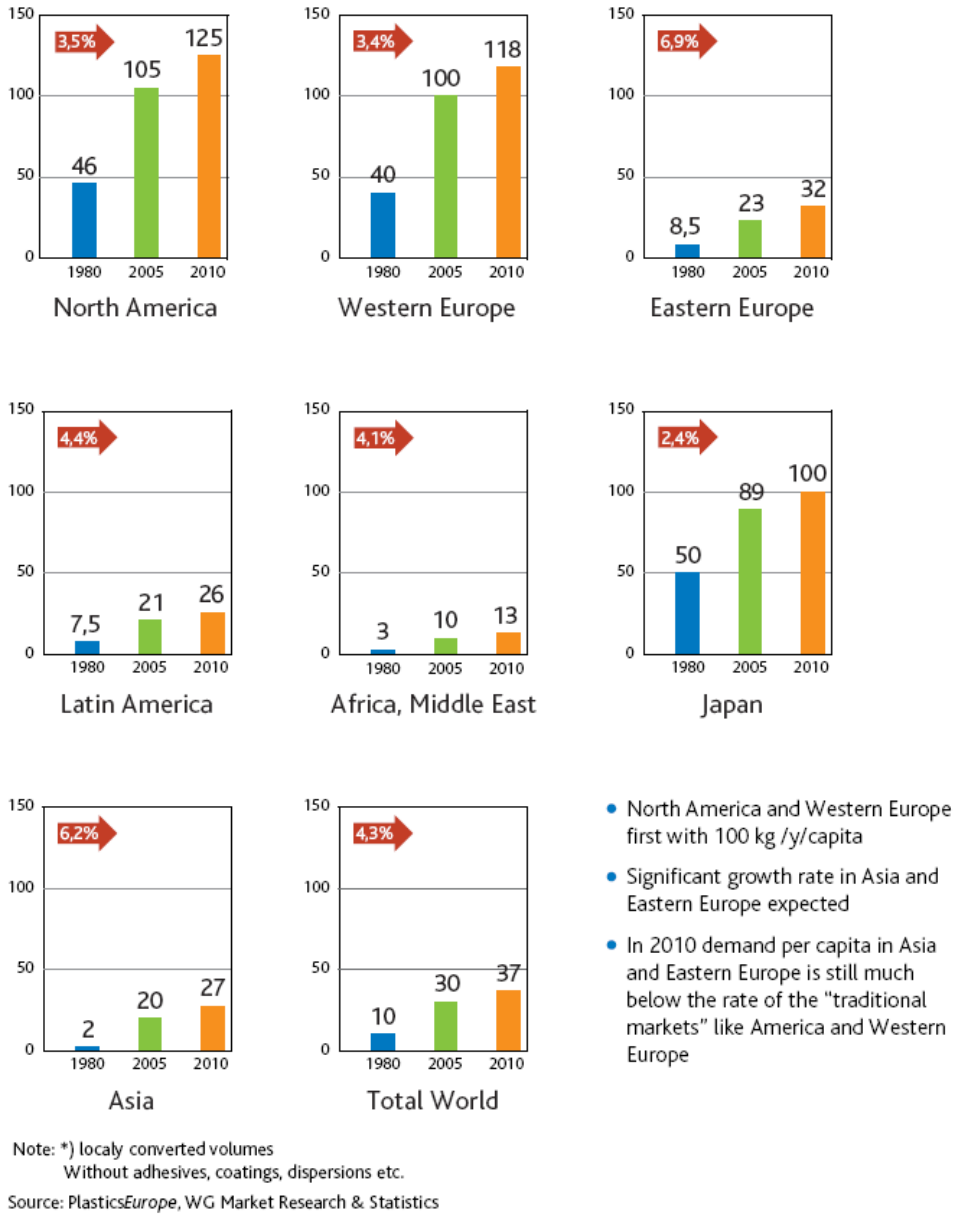


Figure 2.3 – Per capita demand/year of plastic materials* (in kg/year per capita 1980 – 2010 and growth p.a.).

In terms of end-use applications, packaging remains at 37% of total plastics demand in Western Europe (Figure 2.4). Total consumption of flexible packaging grew by 2.9% per year during 1992-1997, with the strongest growth in processed food and above average growth in chilled foods, fresh foods, detergents and pet foods. In this field plastics allow packaging to perform many necessary tasks, providing important properties such as

strength and stiffness, barrier to oxygen and moisture, resistance to food component attack and flexibility (Avella et al., 2001). Plastics used in food packaging must have good processability and be related to the melt flow behaviour and the thermal properties. Furthermore, these plastics should have excellent optical properties in being highly transparent (very important for the consumer) and possess good sealability and printing properties. In addition, it is required to meet legislation and consumer's demand for essential information about the content of the product (Vlieger, 2003).

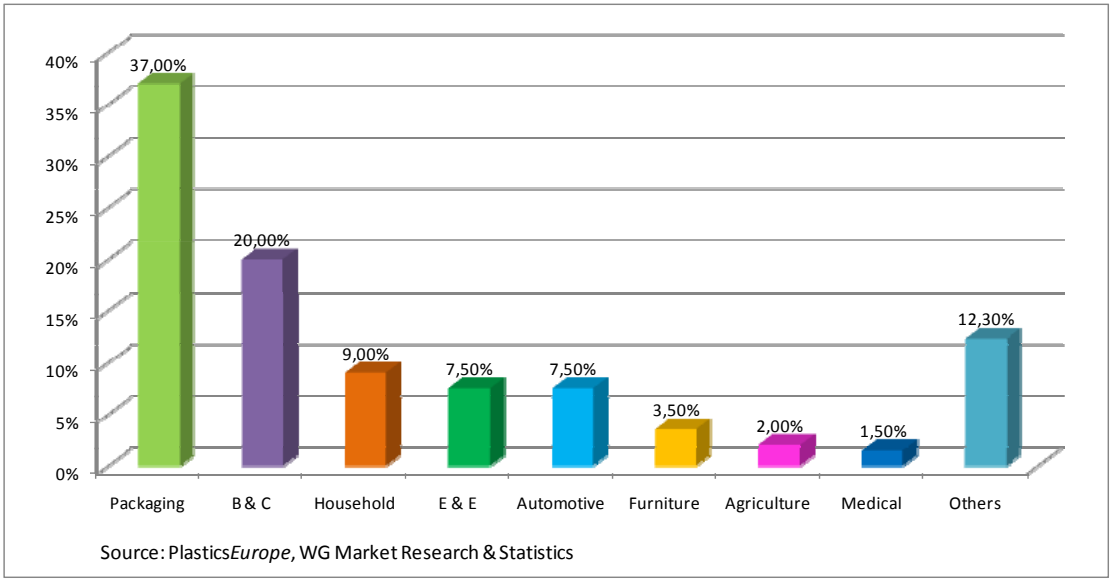


Figure 2.4 – Plastic demand: by end-use application sector (W. Europe).

Compared to the total amount of waste generated in the European Union, packaging accounts for only a small percentage of about 3%. Nevertheless, the total amount of packaging waste in Europe was about 76 million tons in 2004, and this amount has a big impact in the waste streams produced by households. Typically, the fraction of plastics in the total packaging waste generated is about 17%. In Portugal, plastics reach about 25% of the total amount of packaging waste generated (DG Environment, 2004).

Food packaging has become a central focus of waste reduction efforts. Packaging protects food from its environment. Quality and shelf life are reduced when food, through interaction with its environment, gains or loses moisture or aroma, takes up oxygen (leading to oxidative rancidity) or

becomes contaminated with microorganisms. In multicomponent foods, quality and shelf life are also reduced when moisture, aromas or lipid migrate from one food component to another (Krochta and De Mulder-Johnston, 1997). The polymers and materials used for food-packaging today consist of a variety of petroleum-derived plastic materials, metals, glass, paper and board, or combinations hereof, because of their availability in large quantities at low cost and favourable functionality characteristics. With the exception of paper and board, all major packaging materials are based on non-renewable materials, implying that at some point, more alternative packaging materials based on renewable resources have to be found to avoid problems concerning waste disposal (Weber et al., 2002).

Environmental concerns enhance and stimulate the use of renewable resources producing economically convenient applications to maintain or even improve life quality. Low cost agricultural and seafood commodities, commonly found in developing countries, produce high amounts of surplus and wastes that are under-utilized sources of biopolymers. Research on alternative uses and reprocessing of these biopolymers will add commercial value and reactivate depleted economies, as well as reduce pollution problems (Garcia et al., 2004).

Edible and biodegradable polymer films offer alternative packaging without the environmental costs. Although edible films are not meant to totally replace synthetic packaging films, they do have the potential to reduce packaging and to limit moisture, aroma, and lipid migration between food components where traditional packaging cannot function. Biodegradable packaging, on the other hand, has been viewed by many as having the potential to totally replace synthetic, non-biodegradable packaging in some applications (Krochta and De Mulder-Johnston, 1997). The functional characteristics required for the edible coatings and films depend on the product matrix (low to high moisture content) and the deterioration processes the product is subject to (Guilbert et al., 1996; Debeaufort et al., 1998). An edible film coating, acting as an efficient moisture, oxygen, or

aroma barrier, can reduce the amount of packaging. Edible films can be used to incorporate various food additives, such as antimicrobial agents and antioxidants, decreasing the overall concentration of the additive in the food (Krochta and De Mulder-Johnston, 1997).

2.2. Edible Films and Coatings History

The concept of employing edible films as protective coatings for foods to prolong storage life is not new. Coating of fresh oranges and lemons with wax to retard desiccation was practiced in China in the 12th and 13th centuries. Although the Chinese did not realize that the full function of edible coatings was to slow down respiratory gas exchange, they found that wax coated fruits could be stored longer than non-waxed fruits. In the 19th century, sucrose was initially applied as an edible protective coating on nuts, almonds, and hazelnuts to prevent oxidation and rancidness during storage. In the 1930s, hot-melt paraffin waxes became commercially available as edible coatings for fresh fruits as apples and pears, and in the 1950s carnauba wax oil-in-water emulsions were developed for coating fresh fruits and vegetables, to improve their appearance, such as their shininess, colour, softening, carriage of fungicides, and to better control their ripening and to retard the water loss (Kester and Fennema, 1986; Debeaufort et al., 1998; Park, 2003). Patents on edible films to extend the shelf-life of foods date back to the 1950s, comprising films for frozen meat, poultry and sea-food using alginates, fats, gums and starches (Bauer et al., 1968; Earle, 1968; Shaw et al., 1980; Macquarrie et al., 2004).

2.3. Edible polymer films

Generally, an edible film is defined as thin layer of edible material formed on a food as a coating or placed (pre-formed) on or between food components. Its purpose is to inhibit migration of moisture, oxygen, carbon

dioxide, aromas, and lipids, etc.; carry food ingredients (e.g., antioxidants, antimicrobials, and flavour); and/or improve mechanical integrity or handling characteristics of the food. In some cases, stand-alone edible films with good mechanical properties can replace synthetic packaging films. An edible film coating, acting as an efficient moisture, oxygen, or aroma barrier, can reduce the amount of packaging. Edible film coating may also help maintaining the quality of foods after packaging is opened by protecting against moisture change, oxygen uptake, and aroma loss. Edible films with adequate mechanical properties could conceivably also serve as edible packaging for select foods (e.g., pouches for dried soup which would become part of the prepared soup) (Krochta and De Mulder-Johnston, 1997).

The major benefit of the edible coatings is that they can be consumed along with the food, can provide additional nutrients, may enhance sensory characteristics and may include quality-enhancing antimicrobials. Because they may be consumed, the composition of edible films or coatings must conform to the regulations that apply to the food product concerned (Guilbert et al., 1996).

Edible films and coatings can be formed by the following mechanisms (Kester and Fennema, 1986; Gontard et al., 1992; Debeaufort et al., 1998):

- *Simple coacervation:* where a hydrocolloid dispersed in water is precipitated or undergoes a phase change after solvent evaporation (drying), after the addition of a hydrosoluble non-electrolyte in which the hydrocolloid is insoluble (e.g. ethanol), after pH adjustment of the addition of an electrolyte which induced salting out or cross-linking.

- *Complex coacervation:* where two hydrocolloid solutions with opposite electron charges are mixed, thus causing interaction and precipitation of the polymer complex.

- *Gelation or thermal coagulation:* where heating of the macromolecule, which leads to its denaturation, is followed by gelation (e.g. proteins such as egg albumin) or precipitation, or even cooling of a hydrocolloid dispersion causing gelation (e.g. gelatine or agar).

Films, that is, independent structures, have been obtained in laboratories after laying or spreading a film-forming solution on support, drying it, and then detaching it. These stand-alone films are also used as testing structures for determination of barrier, mechanical, solubility and other properties provided by a certain film material. For industrial scale production, techniques used for making flexible plastic films are in general applicable to produce edible and biodegradable films. These techniques can be extrusion or coextrusion for multilayer films, lamination, moulding and mainly roll-drying for the solvent removal of the polymer solution (Guilbert et al., 1996; Debeaufort et al., 1998).

Some researchers developed some alternatives to produce biodegradable films in a larger scale than the laboratorial. Lafargue et al. (2007) used the method of dip-moulding to produce films based in a modified starch/carrageenan mixture. In this method the gelled state is usually preferred to set hot solutions on a surface upon cooling. Kozempel and Tomasula (2004) developed a continuous process for making calcium caseinate films. In this method, the film forming solution was spread onto a moving belt and passed through a dryer. In this study was tried to develop a semi-continuous method to produce films based on the knife coating technique, and will be discussed on Chapter 5.

Edible packagings are mainly used as coatings in industrial processes, and the techniques used are traditional coating methods, such as spray fluidization, falling and pan coatings, spraying, dipping, or brushing. These processes are usually followed by drying steps for aqueous products, or by cooling for lipid-based coatings (Debeaufort et al., 1998).

Edible and biodegradable films must meet a number of specific functional requirements (moisture barrier, solute and/or gas barrier, water or lipid solubility, colour and appearance, mechanical and rheological characteristics, non-toxicity, etc.). These properties are dependent on the type of material used, its formation and application. Plasticizers, cross-linking agents, antimicrobials, antioxidants, texture agents, etc. can be added to enhance the functional properties of the film. In any polymeric packaging film or coating, two sets of forces are involved: between the film-forming polymer molecules for all polymeric films or coatings (cohesion), and between the film and the substrate for coatings only (adhesion). The degree of cohesion affects film properties such as resistance, flexibility, permeability, etc. Strong cohesion reduces flexibility, gas and solute barrier properties and increases porosity (Gontard et al., 1993). Cohesion depends on the biopolymer structure and chemistry, the fabrication procedure and parameters (temperature, pressure, solvent type and dilution, application technique, solvent evaporation technique, etc.), the presence of plasticizers and cross-linking additives and on the final thickness of the film. Film cohesion is favoured by high chain order polymers. Excessive solvent evaporation or cooling, which is generally required for industrial reasons, may sometimes produce non-cohesive films due to premature immobilization of the polymer molecule (Guilbert et al., 1996).

2.4. Role of Plasticizer

A plasticizer is a very important component affecting the physicochemical properties of the films. In general, plasticizers are added to overcome brittleness in the film caused by extensive intermolecular forces and to allow the film to be easily removed from its forming support. Addition of plasticizer results in decrease in intermolecular forces along the polymer chains which consequently improves flexibility, extensibility, toughness and tear resistance of the film. On the other hand, for good barrier properties of

the film, the polymer should have a high crosslink density. Since plasticizer lessens intermolecular forces, effectively dilutes and softens film structure and increases the chain mobility and intermolecular spacing. Therefore, the addition of plasticizer could lead to a marked increase in diffusion coefficients for gas or water vapour and a decrease in cohesion, tensile strength and glass transition temperature (T_g) of the film (Guilbert, 1986; McHugh et al., 1994).

The plasticizer must be compatible (miscible) with the polymer and if possible readily soluble in the solvent (to avoid premature separation during the film drying). In other words, effective plasticizers should resemble most closely the structure of the polymers they plasticize. The most commonly used plasticizers for biodegradable films are polyols (glycerol, sorbitol and polyethylene glycol 400, etc.), mono-, di- or oligosaccharides, lipids and their derivatives (Guilbert, 1986)

2.5. Biopolymers

Edible films are made of various materials, are formed by various processes, and have various properties. The main renewable and natural biopolymer films are obtained from polysaccharides, lipids and proteins. Polysaccharides may include cellulose derivatives, starches and their derivatives, seaweed extracts such as carrageenan and alginates, pectin, and chitosan. Protein film formers include collagen, gelatine, whey protein, corn zein, soy protein, and wheat gluten. Polysaccharide and protein film materials are characterized by high moisture permeability, low oxygen and lipid permeability at low relative humidity and compromised barrier and mechanical properties at high relative humidity (Brody, 2005). With regard to the polysaccharides, because of their wide variety of structures, various film-forming behaviours may occur during the manufacture. Consequently, films made from different types of polysaccharides will present a wide range of properties (Nisperos-Carriedo, 1994).

The diverse nature, chemical and physical properties of bio-based materials expand the possibilities of tailor made films used in packaging for the food industry. Biobased polymers used in the production of films and coatings are shown in Figure 2.5.

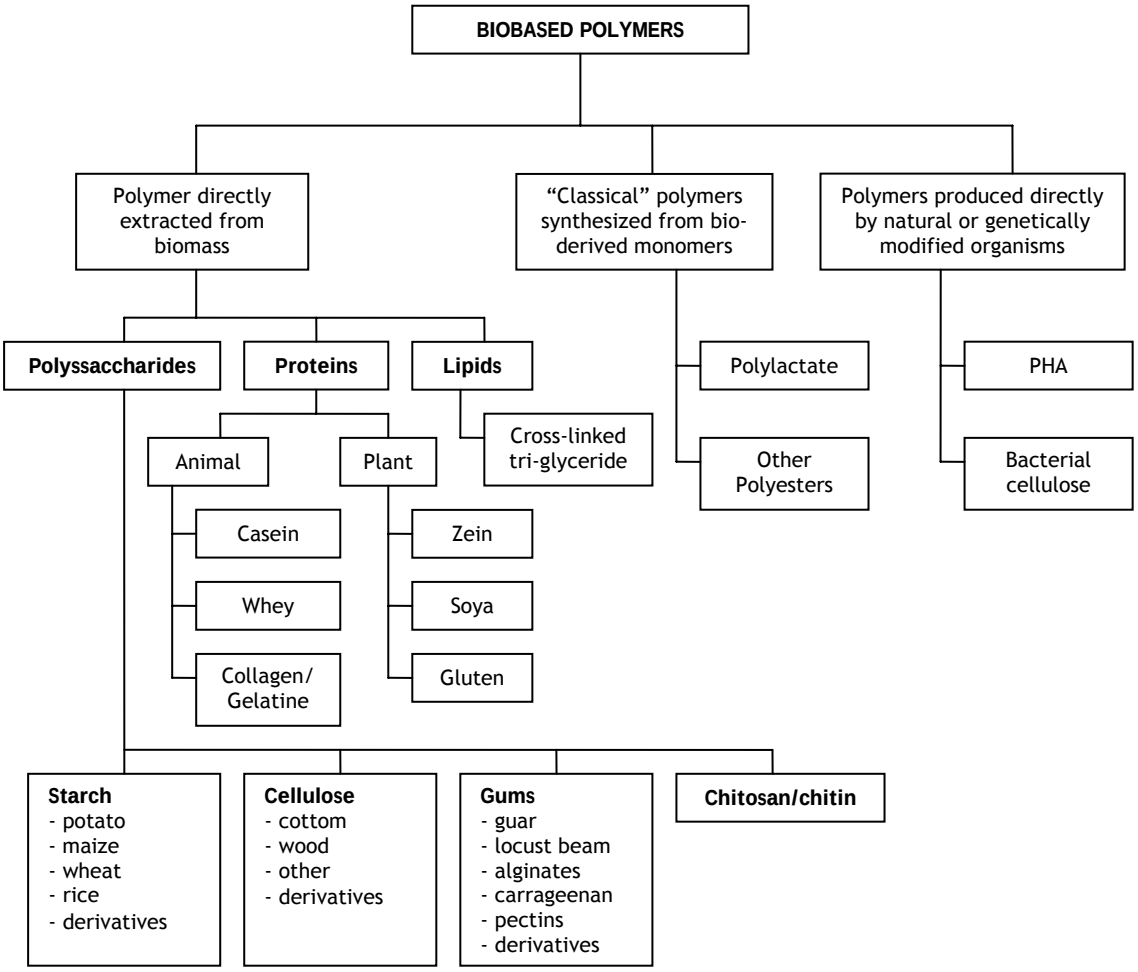


Figure 2.5 – Schematic presentation of biobased polymers based on their origin and method of production (van Tuil et al., 2000).

The most commonly available natural polymers are extracted from marine and agricultural sources (animals and plants). Examples are polysaccharides, such as cellulose, starch and chitin, and proteins such as casein, whey, collagen and soy. All these polymers are, by nature, hydrophilic and somewhat crystalline, and these characteristics causing processing and

performance problems, especially in relation to packaging of moist products. On the other hand, these polymers lead to materials with excellent gas barrier properties (van Tuil et al., 2000).

2.5.1. Polysaccharide-based films

Polysaccharides were the earliest and most extensively studied materials for biopackaging. A variety of polysaccharides and their derivatives have been tested for potential use as biodegradable/edible films, including alginate, pectin, carrageenan, konjac, chitosan, pullulan, cellulose and starch along with their derivatives.

Alginate, pectin, carrageenan and konjac: Sodium alginate is the salt of alginic acid extracted from brown seaweed. Pectin is a complex group of structural polysaccharides found in the middle lamella of plant cells. Carrageenan is an extract from red seaweed. Konjac is a polysaccharide obtained from a plant tuber. Alginate and pectin films can be formed by dipping the supporting plate into aqueous alginate, while low-methoxyl pectin solutions produce films followed by ionic crosslinking with a calcium salt and drying. Carrageenan and konjac films are simply formed by cooling their hot neutral or alkaline aqueous solutions to form a gel, followed by drying (5, 10). These polysaccharide films are water soluble and their mechanical strength is generally weaker than that of other polysaccharide films. Due to the hydrophilic characteristic of these films, only minimal moisture barrier properties are expected. However, they are good oxygen and lipid barriers that can retard lipid oxidation in homogeneous foods and lipid migration in heterogeneous foods. The primary applications of these polysaccharides as films for foods have been limited to edible, protective coatings for fruits, vegetables, cheese and meat products, aiming to retard water loss by acting as sacrificing agents and to carry antimicrobial and antioxidant agents (Kester and Fennema, 1986).

Chitosan and pullulan: chitosan is the deacetylated derivative of chitin, the second most abundant and naturally available biopolymer after cellulose, is found in the exoskeleton of crustaceans, in fungal cell walls and other biological materials (Srinivasa et al., 2002). Pullulan is a water soluble microbial polysaccharide extracellularly produced by a fungus (Kristo and Biliaderis, 2006). Films made with these two polysaccharides are potentially useful as food packaging films due to their good mechanical and oxygen barrier properties (Srinivasa et al., 2002; Kristo and Biliaderis, 2006). Pullulan has been commercialized in Japan where it is used primarily as food coating. Chitosan has been found to possess an antimicrobial function which may inhibit microbial growth on food surfaces if used as a food coating. Water stability of these films can be improved by chemically modifying the polymer structure. However, their biodegradability could be significantly slowed down due to such structural changes (El Ghaouth et al., 1991; Chen and Lin, 1994).

Starch, cellulose and their derivatives: Starch, the storage polysaccharide of cereals, legumes and tubers, is a renewable and widely available raw material suitable for a variety of industrial uses. As a packaging material, starch alone does not form films with adequate mechanical properties (high percent elongation, tensile and flexural strength) unless it is first treated by either plasticization, blending with other materials, genetic or chemical modification or combinations of the above approaches. Corn is the primary source of starch, although considerable amounts of starch are produced from potato, wheat and rice, in Europe, the Orient and the United States. Starch is economically competitive with petroleum and has been used in several methods for preparing compostable plastics. However, a challenge to the development of starch materials is the brittle nature of blends with high starch concentration. When starch is treated in an extruder by application of both thermal and mechanical energy, it is converted to a thermoplastic material. In the production of thermoplastic starches, plasticizers are expected to reduce the intermolecular hydrogen bonds effectively and to provide stability to product properties. Because of the

hydrophilicity of the starch, the performance of materials extruded with starch changes during and after processing, as water content changes. To overcome this challenge, many different starch derivatives have been synthesized; recently, site-selective modifications have been reported. Blending with more hydrophobic polymers produce formulations that are suitable for injection moulding and film blowing. Compatibility is an issue when these types of blends and laminates are used, and compatibilizers and other additives are used as processing aids. Starch-based thermoplastic materials have been commercialized during the last few years and are to day dominating the market of biobased, compostable materials (van Tuil et al., 2000).

Cellulose is the most abundant natural polymer on earth. Cellulose is a cheap raw material, but difficult to use because of its hydrophilic nature, insolubility in water and crystalline structure. To produce cellulose or cellophane film, cellulose is dissolved in an aggressive, toxic mixture of sodium hydroxide and carbon disulphide (”Xanthation”) and then recast into sulphuric acid. The cellophane produced is very hydrophilic and, therefore, moisture sensitive, but it has good mechanical properties. It is, however, not thermoplastic owing to the fact that its theoretical melting temperature is above its degradation temperature, and therefore cannot be heat-sealed. Cellophane is often coated with nitrocellulose wax or PVdC (Poly Vinylidene Chloride) to improve barrier properties and in such form it is used for packaging of baked goods, processed meat, cheese and candies. However, there is considerable potential for the development of an improved cellulose film product or an improved production method as the existing product is problematic in both respects. A number of cellulose derivatives are produced commercially, most commonly carboxy-methyl cellulose (CMC), methyl cellulose (MC), ethyl cellulose (EC), hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC) and cellulose acetate (CA). Of these derivatives only cellulose acetate is widely used in food packaging (baked goods and fresh produce). CA possesses relatively low gas and moisture barrier properties and has to be plasticized for film production. Many

cellulose derivatives possess excellent film-forming properties, but they are simply too expensive for bulk use. This is a direct consequence of the crystalline structure of cellulose making the initial steps of derivatization difficult and costly. Research is required to develop efficient processing technologies for the production of cellulose derivatives if this situation is to change (Kester and Fennema, 1986; van Tuil et al., 2000).

2.5.2. Protein-based films

The use of proteins as biodegradable/edible films has not been studied as extensively as polysaccharides. However, collagen and gelatine have been used for a long time as casings or wraps for sausages and other meat products. These films are transparent but highly permeable to moisture. Recently, many other proteins have been investigated for potential use as biodegradable/edible films. These proteins include wheat gluten, corn zein, whey protein, casein and soy protein.

Wheat gluten and corn zein: Wheat gluten, a major component of wheat flour proteins, is a mixture of gliadin and glutenins. Corn zein is an ethanol-soluble protein extracted from corn proteins. Wheat gluten and corn zein films can be produced by casting by drying their ethanolic solutions. The properties of wheat gluten and corn zein films, such as water solubility, opacity, mechanical and barrier properties, are strongly dependent on the film-formation conditions including the protein concentration, ethanol concentration and pH (Gennadios and Weller, 1990; Gontard et al., 1992). The aroma permeability of gluten films was found to be only one-tenth that of LDPE (Krochta and De Mulder-Johnston, 1997). Wheat gluten plastics exhibit high gloss (polypropylene like) and show good resistance to water under certain conditions. They do not dissolve in water, but they do adsorb water during immersion. Due to its abundance and low price, research on the use of gluten in edible films, adhesives, or for thermoplastic applications is currently being carried out (van Tuil et al., 2000). Zein films are well-suited for food service applications where short service life is

expected. However, longer or controlled time serviceability, for example, food packaging, horticultural covers, and consumer products, require further development (Wang and Padua, 2005).

Caseinate and whey protein: Casein are the major protein in milk, representing 80% of the total milk proteins, whereas whey proteins account for the other 20%. Casein is a milk-derived protein. It is easily processable due to its random coil structure. Upon processing with suitable plasticizers at temperatures of 80-100ºC, materials can be produced with mechanical performance varying from stiff and brittle to flexible and tough performance. Casein melts are highly stretchable making them suitable for film blowing. In general, casein films have an opaque appearance. Casein materials do not dissolve directly in water, but they show approx. 50% weight gain after 24 hours of immersion. The main drawback of casein is its relatively high price. Casein was used as a thermoset plastic for buttons in the 1940's and 50's. It is still used today for bottle labeling because of its excellent adhesive properties. Whey is a by-products from the cheese production and is particularly rich in ß-lactoglobulin. Whey proteins have a relatively high nutritional value, are available in large amounts world-wide and have been extensively investigated as edible coatings and films. This would seem to form the basis for a logical utilization strategy for this protein in packaging. Whey proteins are readily processable and have some potential as exterior films, if, as with gelatine, suitable modification strategies can be developed to reduce moisture sensitivity (van Tuil et al., 2000).

Soy protein: Soy proteins are commercially available as soy flour, soy concentrate and soy isolate, all differing in protein content. Soy protein consists of two major protein fractions referred to as the 7S (conglycinin, 35%) and 11S (glycinin, 52%) fraction. Both 7S and 11S fractions contain cysteine residues leading to disulphide bridge formation and processing is, therefore, similar to gluten with similar mechanical properties (van Tuil et al., 2000). The film can be produced either by heating aqueous soy protein

isolate dispersion to form a surface film or by casting and drying a prepared soy protein-based, film-forming solution. Two inherent problems with soy protein based plastics that have limited their usage are their low mechanical properties and high moisture sensitivity. Pure soy protein films also tend to be brittle. Various physical, enzymatic, chemical and physicochemical attempts have been made to modify soy protein films so as to improve their mechanical properties and increase their moisture resistance (Su et al., 2007). Some patents from the beginning of the 1900 describe the use of soy protein as adhesives or plastics. Even the ancient Chinese used soy protein for non-food applications such as oil for lubrication. The most successful applications of soy proteins were the use in adhesives, inks and paper coatings (van Tuil et al., 2000).

2.5.3. Lipid-based films

Among naturally occurring biopolymers, lipids have received the least study, probably due their poor self-supporting film-forming ability and the fairly weak mechanical strength of the formed films. The applications of lipid-based films to foods have been limited to edible coatings for fruits and meat products. The most common lipids used for edible film include acetoglycerides, beeswax and surfactants (Kester and Fennema, 1986).

Materials such as fatty acids, lipids (triglycerides), and waxes are commonly used in edible coatings to reduce surface water vapour permeability because these materials are non-polar or hydrophobic and, thus, are good barriers against moisture migration. Waxes and solid lipids in particular are good moisture barriers. They can also provide gloss, enhancing the visual appeal of products. Used alone, however, they require solvent or high-temperature casting and exhibit poor mechanical properties. Composite emulsion films, containing proteins or polysaccharides for structural integrity and waxes or lipids for hydrophobic character, exploit the advantages of each component to develop superior barrier and mechanical properties (Krochta, 2002). Incorporating lipid or wax in protein-based or

polysaccharide-based films may interfere with polymer chain-to-chain interactions and/or provide flexible domains within the film. The result can be a plasticizing effect, increasing film flexibility with a reduction of film strength, as seen in whey protein (Shellhammer and Krochta, 1997).

2.5.4. Polyester-based films

Poly(hidroxyalkanoate)s (PHAs) constitute a family consisting of renewable, biologically degradable, biocompatible, optically active polyesters. They are produced by many bacterial species in the form of intracellular particles, functioning as an energy and carbon reserve material. One of the determining factors for the properties of the final PHA-material, the specific monomer composition, is variable and can be controlled by choosing different bacteria strains and substrates for the synthesis of the biopolyesters (Weber et al., 2002). By manipulation of the growth medium, a random copolymer containing both hydroxyvalerate (HV) and hydroxybutyrate (HB) is obtained. The resulting copolymer poly(3-hydroxybutyrate)-co-(3-hydroxyvalerate) (PHB/V) is thermoplastic and fully biodegradable. By changing the ratio of HV to HB, the resulting copolymer can be made to resemble polypropylene (PP) or polyethylene (PE), with regard to flexibility, tensile strength and melting point. PHB/V has good moisture and aroma barrier properties due to its hydrophobic nature. While its oxygen barrier property is not as good as most polysaccharide or protein films al low relative humidity, it is being considered for use in beverage bottles, coated paperboard milk cartons and films. However, its high cost may limit its broad applications for food packaging (Krochta and De Mulder-Johnston, 1997).

2.5.5. Hydrocolloid/Lipid Composite films

Polysaccharides and proteins have good gas, aroma, and lipid barriers and can also adhere to fruits or vegetable cut surfaces, but they are inefficient against water transfer because of its hydrophilic characteristics. Lipids, on

the other hand, offer high water barrier properties; however, they form brittle films causing anaerobic conditions at higher storage temperatures and not sticking to hydrophilic cut surfaces (Kester and Fennema, 1986; Péroval et al., 2002). Composite films and coatings can be formulated to combine the advantages of both lipid and hydrocolloid components (Krochta and De Mulder-Johnston, 1997; Baldwin et al., 1997). The lipid component in the coating formulation can serve as a good barrier to water vapour while the hydrocolloid component can provide a selective barrier to oxygen and carbon dioxide and the necessary supporting matrix (Kester and Fennema, 1986; Baldwin et al., 1997). A composite film made of a protein or polysaccharide (or from a mixture of both) and lipids can be divided into laminates (lipid as a distinct layer within or atop the biopolymeric films) and emulsions (lipid uniformly dispersed throughout the film structure) (Anker et al., 2002).

2.5.6. Synthetic Polymer/Biopolymer Mixed films

The biodegradability of synthetic polymers is accelerated by incorporation of biopolymers which can be consumed by microorganisms. Presently, the main products of this type are starch-based. Other types of biopolymers such as cellulose, lipids and proteins have been investigated recently (e.g., cellulose/polyurethane mixtures, gluten/synthetic resin mixtures, vegetable protein/vinylic compound mixtures, lipid/synthetic polymer mixtures) but are not widely used. The idea of using starch inside a synthetic polymer matrix emerged in the 1970s. The first studies were based on introducing starch into the synthetic matrix at amounts lower than 10% while keeping the granular starch structure intact. In this case starch is only a filler susceptible to enzymatic degradation but unable to affect the mechanical properties of the final material. A second approach was to use polymer blends. To obtain this morphology, the granular structure of starch had to be modified (generally by extrusion) to allow thorough mixing of α-glucan and synthetic polymer. The notion of 'starch destructuration' refers to the loss of native granular structure and is achieved by melting under shearing

and low hydration. The main difficulties encountered in the addition of starch to synthetic polymer are due to the chemical incompatibility of these two types of polymers. The mechanical properties of these materials, which are dependent on the quantity of starch, diminish rapidly when starch level increases. Despite their poor mechanical properties, these materials have been recommended because of their biodegradability, and several commercial applications have been proposed (e.g. pharmaceutical bottles, container closures, cutlery and cotton swabs) (Lourdin et al., 1995). The physical properties of starch filled plastics depend on the starch type, granule size and its relative amount. However, full biodegradability and safety of these films are still highly controversial (Krochta and De Mulder-Johnston, 1997).

2.6. Film properties

The suitable use of edible packagings strongly depends on their functional properties. Biobased packaging materials must meet the criteria that apply to conventional food packaging materials. These relate to barrier properties (water vapour, gases, light, and aroma), optical properties (e.g. transparency), and mechanical properties (Debeaufort et al., 1998; Haugaard et al., 2001; Weber et al., 2002). Therefore, the importance of accurate methodologies for determining film performances has been developed (Debeaufort et al., 1998).

The methods used to measure film properties are derived from the classical methods applied to synthetic materials. However, these methods were adapted to biopolymer film characteristics, mainly due to the great influence of relative humidity and temperature in the film properties (Krochta and De Mulder-Johnston, 1997).

2.6.1. Mechanical Properties

Edible films should have adequate mechanical strength and extensibility to maintain integrity and withstand the external stress that occurs during processing, handling and storage (Yang and Paulson, 2000).

The mechanical properties of edible films and coatings depend on the type of film-forming material and especially on its structural cohesion. Cohesion is the result of a polymer's ability to form strong and/or numerous molecular bonds between polymeric chains, thus hindering their separation. This ability depends on the structure of the polymer and specially its molecular strength, geometry, molecular weight distribution and the type and position of its lateral functional groups. The mechanical properties are also linked with the film-forming conditions, e.g. type of process and solvent, cooling or evaporation rate, etc., and the coating technique (spraying, spreading, etc.) (Guilbert et al., 1996).

Classical methods used for evaluation of mechanical properties of synthetic materials can also be used for edible films. The mechanical properties of edible films and coatings are usually evaluated by puncture and tensile tests (Cuq et al., 1996), but tensile tests are more often presented in the literature. The properties evaluated in the tensile tests are tensile strength, elongation and elastic modulus. Tensile strength is defined as the maximum tensile stress which a material can sustain and is taken to be the maximum load exerted on the test specimen during the test. Elongation is the maximum change in the length of the test specimen before breaking and is expressed as the percent change of the original length of the material between the grip of the test machine. Elastic modulus (Young's modulus) is defined as the ratio of stress to strain in the initial linear part of the stress-strain curve. It is a fundamental measurement of inherent stiffness of a single film since it is nearly independent of dimensions and stress from small strains (McHugh and Krochta, 1994). A typical tensile test curve is shown in Figure 2.6. The materials can be classified in four types on the basis of

stress-strain curves, as shown in Figure 2.7. A hard brittle material such as an amorphous polymer far below its glass transition temperature (T_g) usually has an initial slope indicative of very high modulus, moderate strength, a low elongation at break, and a low area under the stress-strain curve. Hard and strong polymers have high modulus of elasticity, high strength, and elongation at break of approximately 5 per cent. The shape of the curve often suggests that the material breaks where a yield point might be expected. Hard and tough behaviour is shown by polymers that have high yield points and high modulus, high strengths and large elongations. Polymeric materials that are soft and tough show low modulus and yield values, moderate strength at break, and very high elongation ranging from 20 to 1000 per cent. Since the mechanical properties of edible films are strongly influenced by their environmental RH, film samples should be conditioned in a specified RH prior to the experiments.

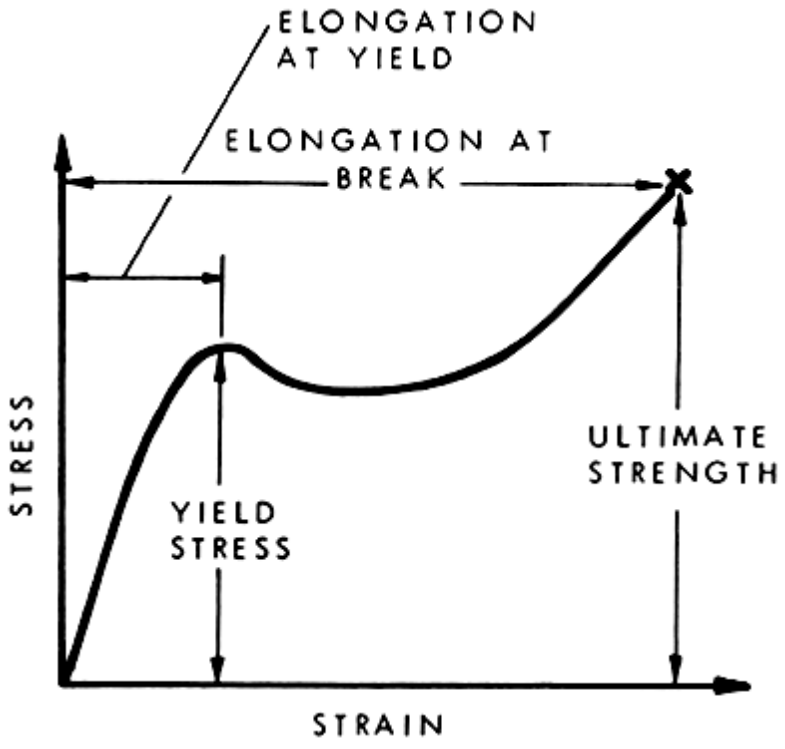


Figure 2.6 – Typical tensile stress-strain curve for polymeric materials (Blaga, 1973).

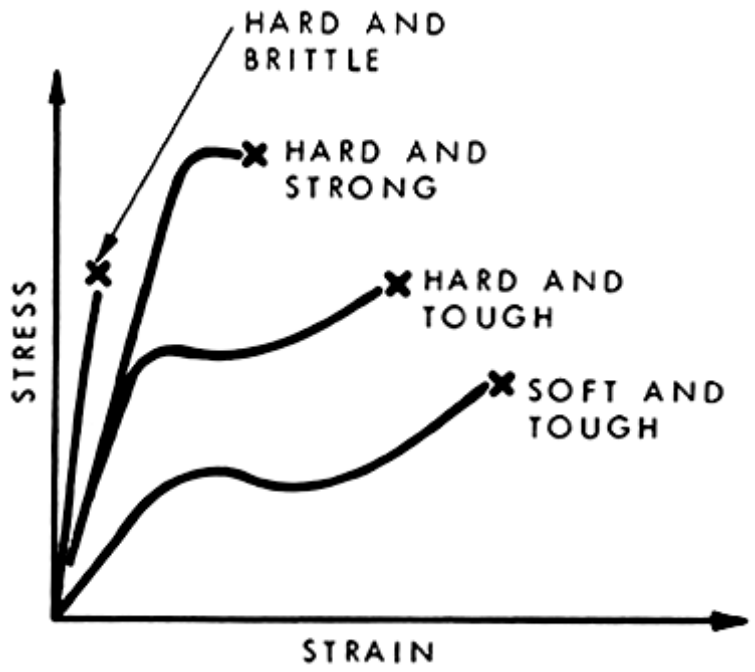


Figure 2.7 – Classification of materials on the basis of stress-strain curves (Blaga, 1973).

2.6.2. Barrier Properties

Water activity (a_w) is one of the critical factors affecting the sensory quality and shelf life of food products. During storage of foods, many deteriorative chemical and enzymatic reactions (lipid oxidation, Maillard browning and enzymatic browning), as well as microbial growth proceed at rates governed by water activity and water content of the foods. In addition, the textural properties of certain foods also largely dependent on their a_w. Dry and crispy cereal foods may become soggy and lose their crispness upon water adsorption, and soft-textured foods may become hard upon loss of water. Thus, water vapour permeability is an important property of film, indicating its ability to control water vapour transport between a food system and its surroundings. Control of moisture exchange of the food with its surrounding environment has been achieved in most instances through synthetic packaging films with good moisture barrier properties (Yang and Paulson, 2000).

Moisture Sorption

The moisture content of hydrocolloid films can affect significantly their physical and barrier properties; due to their inherent hydrophilic nature, these films tend to absorb large quantities of water at high relative humidity (RH) conditions (Cho and Rhee, 2002).

The moisture sorption isotherm is a means to characterize the water sorption property of the film, which in turn is transmitted to the product inside. Knowledge of sorption isotherm is also important for predicting stability and quality changes during packaging and storage for food product (Srinivasa et al., 2007). Moisture sorption isotherm equations are useful for predicting moisture sorption properties of hydrophilic films; they provide, however, little insight into the interaction of water and film components. There are several mathematical models proposed to describe moisture sorption isotherms of food system materials, but none gives accurate results throughout the whole range of water activities or for all types of food systems (Al-Muhtaseb et al., 2004). The GAB (Guggenheim, Anderson and de Boer) model is the most popular model in the area of food technology. It fits sorption data extremely well for many food materials over a wide range of water activity (a_w) (Srinivasa et al., 2007). The GAB model (van der Berg, 1984) used to represent the experimental sorption data is presented in Equation 2.1:

$$X = \frac{CkX_0 a_w}{\left[(1 - ka_w)(1 - ka_w + Cka_w)\right]} \qquad (2.1)$$

where X is the equilibrium moisture content at the water activity a_w, X_0 is the monolayer moisture content and represents the water content corresponding to saturation of all primary adsorption sites by one water molecule, C is the Guggenheim constant and represents the energy difference between the water molecules attached to primary sorption sites and those absorbed to successive sorption layers, and k is the corrective

constant taking into account properties of multilayer molecules with respect to the bulk liquid.

Water Vapour Permeability

Water vapour permeability can be useful to understand solute polymer interactions in edible films and possible mass transfer mechanisms. According to the thermodynamics of irreversible process, water chemical potential difference is the driving force for water transfer through a film. When the process occurs at constant temperature and pressure, the water chemical potential difference results proportional to water vapour concentration difference between the two faces of the film (Morillon et al., 2000).

Permeability is defined as the rate of vapour or gas transmission through a unit area of flat material of unit thickness induced by unit vapour or gas pressure difference between two specific surfaces, under specified temperature and humidity conditions (Yang and Paulson, 2000). The primary mechanism for gas or vapour transmission through a film is by activated diffusion (Figure 2.8). The penetrant dissolves in the matrix at the high concentration side, diffuses through the film driven by a concentration gradient, and evaporates from the other surface (Kester and Fennema, 1986).

The permeation can be described mathematically by Fick's first law. The flux (J) which is proportional to the concentration gradient can be defined in one direction as shown in Equation 2.2:

$$J = -D(\partial C / \partial X) \qquad (2.2)$$

where J is the flux, the net amount of solute that diffuses through unit area per unit time ($g/m^2.s$ or $ml/m^2.s$), D is the diffusivity constant (m^2/s), C is the concentration gradient of the diffusing substance and X (g/m^3 or ml/m^3)

is the thickness of the film (m) (Chang, 1981; Crank, 1975; Jost, 1960; Landrock and Proctor, 1952).

With two assumptions, (*i*) the diffusion is in steady state and (*ii*) there is a linear gradient through the film, the flux (J) is given by Equation 2.3:

$$J = D(C_2 - C_1)/X = Q/(A \cdot t) \tag{2.3}$$

where, Q is the amount of gas diffusing through the film (g or ml), A is the area of the film (m^2) and t is the time (s). After application of Henry's law, the driving force is expressed in terms of partial pressure differential of gas and a rearrangement of terms yields the Equation 2.4, in terms of permeability:

$$Q/(A \cdot t) = D \cdot S \cdot (p_2 - p_1)/X = P \cdot \Delta p/X \tag{2.4}$$

where, S is the Henry's law solubility coefficient (mole/atm), Δp is partial pressure difference of the gas across the film (Pa) and P is the permeability ((ml or g).m/m^2.s.Pa).

Then, the permeabilities of O_2, CO_2 and H_2O vapour can be calculated from the Equation 2.5 (Mannheim and Passy, 1985; Pascat, 1986):

$$P = Q \cdot X/(A \cdot t \cdot \Delta p) \tag{2.5}$$

The use of the above equation to calculate permeability as a universal property of a film is based on the assumption that there is no significant interaction between the film and water vapour. In the case of hydrophilic film, water molecules strongly interact with the film, and D and S are dependent on the water vapour partial pressure. The solubility coefficient increases with increased water vapour pressure because of the shape of the sorption isotherm, and the diffusion constant also increases with increased vapour pressure due to plasticization of the film by adsorbed water

molecules. Thus, the WVP strongly increases with increasing vapour pressure (Kester and Fennema, 1986).

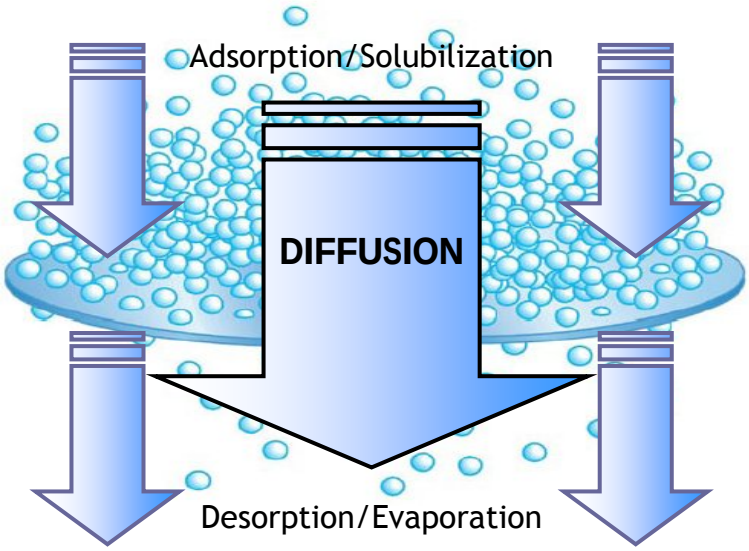


Figure 2.8 – Scheme of gas and vapour permeation through polymeric materials.

The most commonly used method for determining WVP is ASTM E96-95 (ASTM, 1996), known as the "cup method". This gravimetric method involves sealing a test film in a cup partially filled with either distilled water or desiccant, leaving an air gap under the film. The assembly (Figure 2.9) is placed in an air-tight desiccator under controlled RH and temperature, and weighed over time. Weight gain or loss is plotted over time. When steady state is reached, the plot is a straight line. WVP of the film is calculated by Equation 2.6:

$$WVP = \frac{W \cdot L}{A \cdot \Delta p} = (g) \cdot (m) / (m^2) \cdot (s) \cdot (Pa) \qquad (2.6)$$

where W is the weight gain or loss of the cup per hour (g/h) (slope of the straight line); L is the film thickness (m); A is the area of exposed film (m^2), and Δp is the vapour pressure differential across the test film (Pa).

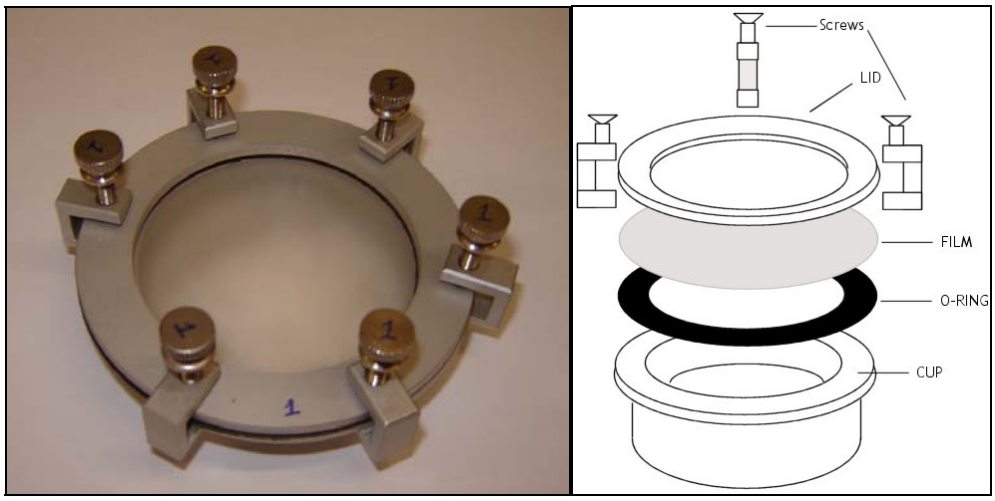


Figure 2.9 – Cup assembly to measure water vapour permeability (WVP) of films.

Oxygen and Carbon Dioxide Permeability

Oxygen is involved in many degradation reactions in foods, such as fat and oil rancidity, microorganism growth, enzymatic browning and vitamin loss. Thus, many packaging strategies seek to exclude oxygen to protect the food product (Gontard et al., 1996). On the other hand, the permeability to oxygen and carbon dioxide is essential for respiration of living tissues such as fresh fruits and vegetables. So, moderate barrier films and coatings are more appropriate. If a film or coating with the appropriate permeability is chosen, a controlled respiratory exchange can be established and thus the preservation of fresh fruits and vegetables can be prolonged (Ayranci and Tunc, 2003).

The measurement of oxygen and carbon dioxide permeabilities requires instruments which may not be easily available. Measurements are mostly based on the standard method described in ASTM D3985 (1988) for oxygen gas transmission through films. These methods involve flowing an oxygen gas stream on one side of the film and a nitrogen stream, to carry the transmitted oxygen gas to the analyzer, on the other side (Figure 2.10). A

coloumetric sensor, an infrared sensor, a gas chromatograph or a dedicated oxygen analyzer may be used for monitoring (Ayranci and Tunc, 2003).

The permeabilities of oxygen and carbon dioxide can be calculated from the Equation 2.6 presented above.

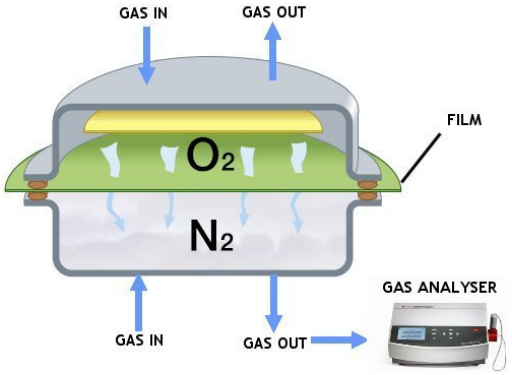


Figure 2.10 – Scheme of measurement of gas permeability to oxygen.

2.6.3. Optical Properties

Colour and transparency (opacity) of edible films are important properties when the films are used as food packaging materials. Colour may be considered an important parameter in the film characterization, because it is related to the raw material used in its production. The film colour can affect the consumer acceptance of coated produce (Kunte et al., 1997). The most commonly used systems for measurement of colour are Hunter Lab, CIE L*a*b*, CIE LCH, CIE XYZ, and CIE Yxy, which are based on the fact that human eye has three types of colour sensors which are sensitive to the colours red, green and blue and that all colours are seen as a mixture of these tree basic colours (Abbot, 1999). Measurements are conducted by using a colorimeter, in the case of the CIE L*a*b* scale, L* indicates lightness and a* and b* are the chromaticity coordinates (rectangular coordinates), +a* is the red direction, -a* is the green direction, +b* is the yellow direction, and –b* is the blue direction. The center of these coordinates is

achromatic and as a* and b* values increase, the saturation of the colour increases. In the L*a*b* colour space (Figure 2.11), colour difference can be expressed as a single numeric value, ΔE*, which indicates the size of the colour difference but not in what way the colours are different. ΔE* is defined by the Equation 2.7 (Konica Minolta, 1998).

$$\Delta E^* = \sqrt{(L^* - L_s^*)^2 + (a^* - a_s^*)^2 + (b^* - b_s^*)^2} \quad (2.7)$$

where L^*_s, a^*_s and b^*_s are the CIE L*a*b* standards for the white standard.

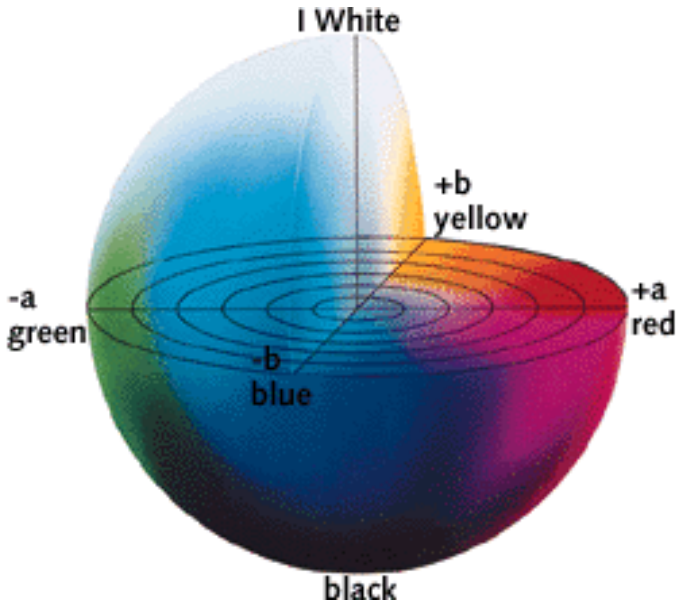


Figure 2.11 - Representation of colour solid CIE L*a*b* colour space.

2.6.4. Dielectric Properties

Dielectric analysis has been widely used in studying the macroscopic electrical properties of polymeric materials. The measurement of dielectric properties is useful in understanding the material's composition, mobility, and structure of the constituent molecules. Typically, dielectric analysis is performed by placing the sample between two parallel electrodes and exposing the sample to an external alternating electric field, which

interacts with free and bound charges in the sample, causing molecular polarization and dipole orientation. Dielectric analysis measures the two fundamental electrical characteristics of a material as a function of time, temperature and frequency: *(a) capacitance*, which indicates its ability to store electric charge; and *(b) conductance*, which indicates its ability to transfer electric charge. Four major properties are reported during dielectric analysis:

ε' – permittivity (dielectric constant)
ε'' – loss factor
tan δ - dissipation factor ($\varepsilon''/\varepsilon'$)
σ - ionic conductivity (derived from measurement of ε'')

Permittivity (ε') measures the alignment of dipoles and is proportional to capacitance, whereas ε'' represents the energy required to align dipoles and move ions, and is proportional to conductance.

A polar molecule has a permanent dipole moment in the absence of an applied electric field, whereas a non-polar molecule has an induced dipole moment in the presence of an electric field. When a sinusoidal electric field is applied, the orientation direction of the charged side-chains changes. As the molecules rotate, energy is stored from the external field. Polar molecules store more energy than non-polar molecules, thus they possess a higher dielectric constant than non-polar material.

The dielectric constant refers to the polarity of the film. Since the barrier properties tend to be dependent on the polarity of the film, it is expected that WVP would be related to the dielectric constant of the film. In a relevant study on the dielectric properties of biofilms, Kohyama et al. (1992) examined the dielectric constant of Konjac glucomannan films as a function of temperature at a fixed frequency of 10 Hz for various moisture levels. Their results showed that dielectric constant of the film were highly related to water content of the film.

2.7. Potential food applications

The choice of an edible packaging mainly depends on the specific characteristics of the food product that requires protection and on storage conditions. Edible films and coatings have been applied on meat, poultry, seafood, fruits, vegetables, grains, candies, heterogeneous and complex foods, or fresh, cured, freezed, and processed foods (Debeaufort et al., 1998).

The markets of biobased food-packaging materials are expected to start up as niche markets, where the unique properties of the biobased materials apply an advantage to the packaging concept (Weber et al., 2002). The applications cited in Table 2.1 only give examples of all potentialities of edible films and coatings in function of the nature of the food product and of the polymer-film-forming agents, but certainly more possibilities will open up for these materials as the developments seem to go very fast.

Potential applications of biobased materials for specific food products have been identified, using the product as a starting point. However, to succeed, biobased packaging of foods must comply with the quality and safety requirements of the food product and meet legal standards. Additionally, they should enhance the value of the product to justify any extra material cost. In this context, shelf life testing is vital, along with testing of durability and migration, and verification of consumer acceptance (Haugaard et al., 2001).

Table 2.1 – Potential food applications using edible film/coatings (adapted from Haugaard et al., 2000)

Product example	Critical functions of packaging	Value added function	Examples of materials
FRESH MEAT PRODUCTS			
Fresh meat products in general		reduction of oxidation (warmed over flavor), antioxidant release and moisture barrier	different edible coatings combined with antioxidants
READY MEALS			
Pizza base/sauce	high moisture barrier and heat resistance	reduction of water migration and softening of the base	edible film/coating of e.g. alginate or pectin between base and sauce
	high gas barrier (oxygen and carbon dioxide) and heat resistance	reduction of oxidation (warmed over flavor), antioxidant release and moisture barrier	different edible coatings combined with antioxidants
DAIRY PRODUCTS			
Dairy products in general		moisture barrier	medium chain length polyhydroxyalkanoate-latex
FRUITS AND VEGETABLES			
Fruits and vegetables in general		moisture barrier prevention of microbial growth and oxidation	wheat gluten, pectin, beeswax
FROZEN PRODUCTS			
Frozen products in general		prevention moisture loss	various edible coatings

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Chapter 3
Selection of renewable sources of biopolymers

In this chapter, a justification for the choice of the two biopolymers studied in this work, carrageenan from marine algae and starch from cork oak acorn, is given. Some general aspects of these biopolymers sources, properties and chemical structure are also revised.

3.1. Introduction

The search for new renewable resources for the production of edible and biodegradable materials is increasing in the last years. Non-conventional sources of carbohydrates are an interesting alternative and have been studied by many researchers. Biodegradable films have been produced from different sources of starch like yam (Mali et al., 2002), 'cará' (Wilhelm et al., 2003), amaranth (Tapia-Blácido et al., 2005; Colla et al., 2006), pea (Han et al., 2006), mango and banana (Romero-Bastida et al., 2005). Fruit and vegetable purees such as peach, strawberry, apple, and cranberry have

been used also to form edible films (McHugh et al., 1996; McHugh and Senesi, 2000; Park and Zhao, 2006).

There are various unique carbohydrate residues found in marine organisms that represent a largely unexplored source of valuable materials (De Ruiter and Rudolph, 1997). The three commercially exploited carbohydrate polymers from marine organisms are: alginates from brown seaweeds, agar and carrageenan from red seaweeds. These biopolymers from marine sources have also been used to produce films. Alginate and carrageenan extracted from seaweeds possess good film forming properties (Park et al., 2001; Xiao et al., 2001; Cha et al., 2002; Briones et al., 2004) and they are good carriers for antimicrobial agents (Kester and Fennema, 1986; Krochta and De Mulder-Johnston, 1997; Karbowiak et al., 2006).

Portugal possesses some of these underexploited renewable sources of biopolymers. A non-conventional source of starch founded in Portugal is the acorn from cork oak (*Quercus suber* L.). Other polysaccharide, from marine sources, carrageenan, can be extracted from the underexploited seaweed *Mastocarpus stellatus*, present in the Portuguese coast. These polymers can be used to produce edible films and coatings.

3.2. Cork oak (*Quercus suber* L.)

One of the most characteristic tree species of the Mediterranean ecosystem is the cork oak, *Quercus suber* L., which in addition to being of great ecological value produces cork, a natural renewable product of relevant economic interest. This species is being actively planted within the framework of European Union funded programs of reforestation of abandoned former agricultural lands (Álvarez, 2004).

Portugal has the highest surface of cork oak (*Quercus suber L.*) plantations in the world. In fact, the Portuguese territory includes around one-third of

the world's cork oak surface and is the origin of more than half of the world's cork production (Silva and Catry, 2006).

Cork oak (*Quercus suber* L.) is an evergreen oak (Figure 3.1), from the family of *Fagaceae*, native to the maritime countries of Europe and North Africa that surround the western Mediterranean basin. During the Discoveries Period, in the 16th and early 17th centuries, cork oak wood was extensively used and very appreciated for boat construction due to its high mechanical resistance and natural durability. Nowadays cork oaks are exclusively oriented towards the production of cork and therefore, research has been mainly focused on issues related to cork characterization and production (Leal et al., 2007).

Figure 3.1 – Cork oak (*Quercus suber* L.).

Cork oak acorns (Figure 3.2) are economically less important, because cork alone is enough to justify trees exploitation (Pereira and Fonseca, 2003). Acorns from *Quercus* spp. are usually used as feed source for free-ranging wild animals. In south-western continental Europe, acorns mainly come from *Q. ilex*, *Q. rotundifolia* and *Q. suber.* and are frequently used by domestic animals under free-range conditions, especially the Iberian pig, an autochthonous breed from the Iberian Peninsula (Cantos et al., 2003).

Figure 3.2 – Cork oak (*Quercus suber* L.) acorn.

Starch is a major reserve polysaccharide of green plants. Starch has been obtained from many plant sources with extensive research focusing on cereal, legume, root and tuber crops. While studies have investigated starch content of presently uncommercialized plants, little research has been done on the structural and functional properties of these starches. These lesser studied starches may possess unique structural and functional properties that potentially might have niche industrial applications. Acorn, the fruit from oak trees, is one such source of starch that has had very little research into its structural and functional characteristics (Stevenson et al., 2006).

Despite being overlooked by many societies today, acorns were used extensively in Europe, Asia, North Africa, Middle East and North America over the last 6000 years. Even now, acorns, particularly its starch, are still consumed especially in Korea, but also in China and Japan (Stevenson et al., 2006). These acorns have been consumed in the form of bread, cakes and confectionary in Europe and are commonly used for making 'mook' in Korea (Aee et al., 1998). Naturally, acorns show a high content of tannins, which must be removed in the process of producing starch for food applications (Stevenson et al., 2006).

The use of acorn starch to produce films is an interesting alternative to add value and new applications to this underexploited non-conventional source of starch raw material.

3.2.1. Starch

In addition to being a major nutrient, starch is widely used in technical applications and has recently gained interest as a renewable and biodegradable plastic. Films can be made from starch or its components, amylose and amylopectin, by various techniques such as thermoplastic processing and solution casting (Rindlav-Westling et al., 2002).

Native starch is the main form of stored energy in plants and occurs as easily isolatable granules. The granules occur in a variety of shape and dimension, and are insoluble in cold water and in most organic solvents. Starch is a complex homopolymer of α-D-glucose units and is found mainly in two structurally and functionally distinct forms – amylose and amylopectin. Amylose is a linear or sparsely branched polymer with a molecular mass up to 10^6Da (Figure 3.3.a), whilst amylopectin is highly branched, with a molecular mass of about 10^8Da (Figure 3.3.b). Native starches differ in both water content and amylose:amylopectin ratio (van Soest and Vliegenthart, 1997). In most common types of cereal endosperm starches, the mass percent of amylopectin ranges between 72% and 82% while amylose ranges from 18% to 33% (Buléon et al., 1998). Acorn starch is composed of a mixture of amylose and amylopectin and minor impurities, such as crude fat, proteins, fibers, minerals and tannin (Aee et al., 1998).

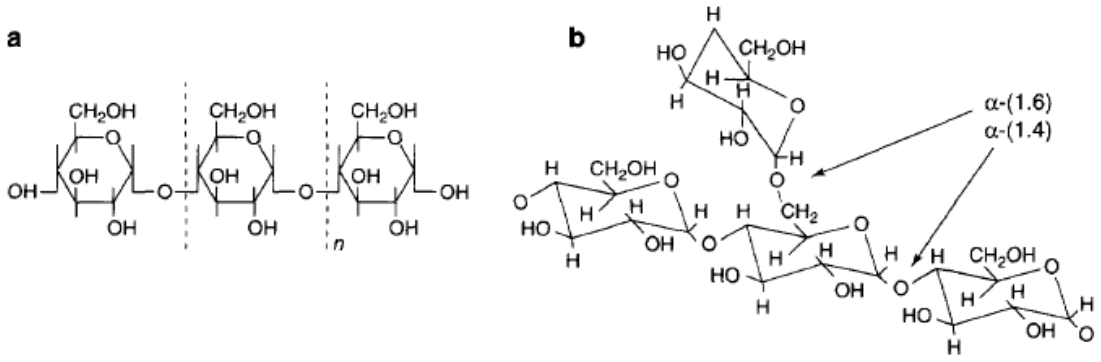

Figure 3.3 – Structure of starch main constituents: (a) amylose; (b) amylopectin (van Soest and Vliegenthart, 1997).

Starch films and coatings have been used for various food and pharmaceutical applications. Films prepared from starch are isotropic, odorless, tasteless, colourless, non-toxic and biodegradable. Edible films and coatings can be prepared from native and modified starch (Pareta and Edirisinghe, 2006). Starch may be used as barrier for food protection and preservation or as carrier of various components used in food processing. When acting as barrier the film formation property of starches can be utilized, and their mechanical properties can be controlled by addition of plasticizers. One of the advantages of using edible hydrophilic films like starches for food protection is their ability to act as oxygen barriers (Forsell et al., 2002). Starch films are excellent oxygen barriers (Krochta and De Mulder-Johnston, 1997; Rindlav-Westling et al., 1998), due to their tightly packed, ordered hydrogen-bonded network structure and low solubility (McHugh & Krochta, 1994). However, two inherent disadvantages of neat starch-based film have limited such utilization: relatively low tensile strength and very high water absorbency. The low tensile strength limits its mechanical potential (handling is more difficult), whereas, more importantly, a high water uptake limits its viability, since it will succumb to decomposition much more readily (Ban et al., 2007).

Starch can be modified chemically, physically or enzimatically to suit various needs. Starch films are usually improved by the addition of

plasticizers. Polyols (glycerol, sorbitol and polyethylene glycol) are the most common plasticizers (Gontard et al., 1993). These additives decrease the intermolecular attraction between adjacent polymeric chains, resulting in a higher film flexibility in spite of a decrease of its strength (Guilbert, 1986; McHugh et al., 1994; Laohakunjit and Noomhorm, 2004).

Many studies have reported on starch based films cast from solutions or gels since 1950, as were described by several authors (Lourdin et al., 1997; Parra et al., 2004; Famá et al., 2005; Liu and Han, 2005; Zhang and Han, 2006).

To obtain a homogeneous, essentially amorphous polymeric matrix, granular starch should be submitted to thermal and mechanical treatments (Souza and Andrade, 2001) to destruct its semi-crystalline granular structure and make it more processable. When heated in the presence of water, starch undergoes a number of irreversible changes commonly referred to as gelatinization. The granules are observed to swell, absorb water, lose crystallinity and birefringence and leach amylose (Biliaderis et al., 1980; Jenkins and Donald, 1998; Wootton and Bamunuarachchi, 1979). The structural changes that take place during gelatinization include a simultaneous crystallite melting and double-helix unwinding, absorption of water in the amorphous growth ring, changes in shape and size of granules, dispersion of blocklet-like structures and leaching of amylose from the granules. In this way, aqueous suspensions of discrete semicrystalline granules are transformed into a continuous amorphous gel phase (Vermeylen et al., 2006).

In order to gelatinize starch, a plasticizer is necessary since the glass transition temperature (T_g) and the melting temperature of pure dry starch are higher than its decomposition temperature (Shogren, 1993). Therefore, the presence of a plasticizer ensures that starch undergoes gelatinization rather than degradation in the presence of heat. Water is the most commonly used plasticizer in starch processing. The minimum moisture content for wheat starch to gelatinize is generally reported to be around

33% (Wootton and Bamunuarachchi, 1979; Eliasson, 1980). Gelatinization at lower moisture contents requires significantly higher processing temperatures, which may not be desirable in some applications (Nashed et al., 2003). It is generally accepted that during gelatinization water first penetrates the amorphous region, initiating swelling and resulting in a decrease in birefringence. Additionally, water strips starch chains from the surface of crystallites as the temperature increases. With increasing temperature, the thermal motion of the molecules and the solvation by the swelling forces lead to an increasing disorder and the disruption of the crystalline regions with uncoiling of the double helices until the granular structure is nearly completely disrupted and a sol is obtained, as shown in Figure 3.4 (Lai and Kokini, 1991).

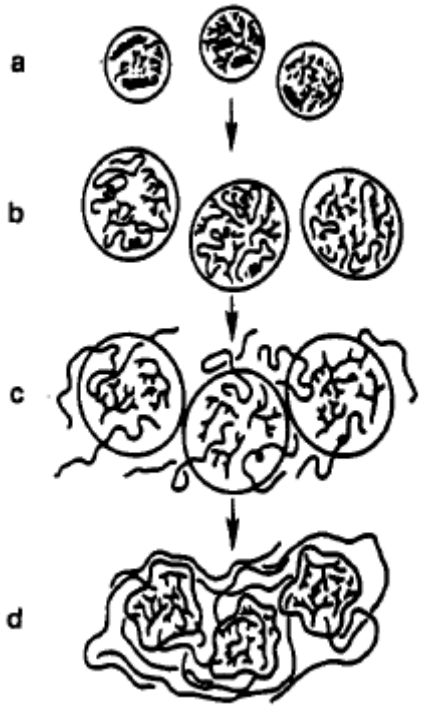

Figure 3.4 – Mechanism of starch gelatinization (Adapted from Lai and Kokini, 1991).

Figure 3.4 show the mechanism of starch gelatinization, where (a) raw starch granules made up of amylose (helix) and amylopectin (branched); (b) addition of water breaks up amylose crystallinity and disrupts helices,

granules swell; (c) addition of heat and more water causes more swelling, amylose begins to diffuse out of granule; (d) granules, now containing mostly amylopectin, have collapsed and are held in a matrix of amylose forming a gel (Lai and Kokini, 1991).

3.3. Seaweeds

Marine algae are among the most primitive members of the plant kingdom. They occur in an incredible variety of life-forms, from uni-cellular species to giant kelp which may extend in length up to 40 m. Algae are generally classified into four main groups, largely upon the basis of pigmentation: green algae (the *Chlorophyceae*), blue-green algae (*Cyanophyceae*), red algae (*Rhodophyceae*) and brown algae (*Phaeophyceae*). Green and blue-green algae, while present in salt water, are more commonly associated with freshwater. Red and brown algae on the other hand, are found almost exclusively in marine environments. Brown algae are the most familiar, conspicuous, largest and most abundant of the seaweeds, but in number and diversity are exceeded by the group of red algae of which there are some 4000 different species. The former are particularly abundant in cold northern waters and few species are found in tropical regions; red algae are present at all latitudes (Naylor, 1976).

The cell walls of seaweeds contain long chain polysaccharides, which give flexibility to the algae and allow then to adapt to the variety of water movements in which they grow. For example, some brown seaweeds grow attached to rocks in very turbulent waters, requiring maximum flexibility to survive, and these contain a higher amount of these polysaccharides than brown seaweeds growing in calm waters. These polysaccharides are referred to as hydrocolloids because they disperse in water to give a solution with colloidal properties. Polysaccharides from other sources such as land plants behave in a similar way so sometimes the term “phycocolloid” is used to designate the hydrocolloids derived from seaweeds (from the term,

phycology, the study of algae including seaweeds). When hydrocolloids are dispersed in water they increase its viscosity and so find many applications as thickening agents. Under some conditions they will also form gels and this property is useful for other applications. Their colloidal properties can lead to still other applications where their mode of action is less easily defined; for example, alginate, the hydrocolloid from brown seaweeds is often added to ice cream where it inhibits the formation of ice crystals if the ice cream partly melts and is refrozen (on the way from the supermarket to home).

As mentioned before, the hydrocolloids of commercial importance extracted from seaweeds, are alginate, agar and carrageenan. The polysaccharide in brown seaweed is a carboxylic acid, alginic acid, present as its sodium, potassium, magnesium and calcium salts. Red seaweeds contain a variety of polysaccharides but those of commercial importance are agar and carrageenan; they are called sulfated polysaccharides because they contain negatively charged sulfate groups and in the seaweed are combined with a positively charged ion such as those found with alginic acid (McHugh, 2002).

Alginate, agar and carrageenan form the main components extracted from seaweeds and the basis for their industrial use. Seaweeds as a source of these hydrocolloids dates back to 1658 when the gelling properties of agar, extracted with hot water from red seaweed, were first discovered in Japan. Extracts of Irish Moss, another red seaweed, contain carrageenan and were popular as thickening agents in the nineteenth century but it was not until the 1930s that extracts of brown seaweeds, containing alginate, were produced commercially and sold as thickening and gelling agents. Industrial use of seaweed extracts expanded rapidly after World War II but was sometimes limited by the availability of raw materials. Today, approximately 1 million tons of wet seaweed are harvested and extracted to produce the above three hydrocolloids per year, leading to produce 55,000 tons of hydrocolloids with a total value of US$ 585 millions. All alginate production (US$ 213 million), by extraction from brown seaweeds, is obtained frow seaweeds harvested from the wild; cultivation of brown

seaweeds is too expensive to provide raw material for industrial uses. Agar production (US$ 132 million) is obtained mainly from two types of red seaweed, one of which has been cultivated since the 1960-70s, but on a much larger scale since 1990, allowing the expansion of the agar industry. Carrageenan production (US$ 240 million) was originally dependent on wild seaweeds, especially Irish Moss, a small alga growing in cold waters with a limited resource base. However since the early 1970s the industry has expanded rapidly because of the availability of other carrageenan-containing seaweeds that have been successfully cultivated in warm-water countries with low labour costs. Today most of the seaweed used for carrageenan production comes from cultivation although there is still a small demand for Irish Moss and some other wild species from South America (McHugh, 2002).

3.3.1. Seaweeds in Portugal

On the Portuguese coast, macroalgal communities from rock pools are dominated by the algae group Rhodophyta (Araújo et al., 2006). Red seaweeds, from the algae group Rhodophyta, contain a variety of polysaccharides but the ones of commercial importance are agar and carrageenan (McHugh, 2002).

The carrageenophytes pertaining to the Gigartinaceae, Petrocelidaceae, Phyllophoraceae and Cystocloniaceae families (Gigartinales, Rhodophyta) are widely distributed in the central and northern Atlantic coast of Portugal. However, *Mastocarpus stellatus* (Petrocelidaceae) and *Chondrus crispus* (Gigartinaceae) are the only species currently harvested for industrial aims, mainly in the north coast of Portugal (Pereira and Mesquita, 2003).

Mastocarpus stellatus (Stackhouse) Guiry (Figure 3.5) is a common intertidal red alga in the north Atlantic (Zucarello et al., 2005). It is cartilaginous, purplish brown fronds, often in dense tufts, arising from a discoid holdfast, to 0.20 m high. Narrow compressed stipe expands into strap-like blade,

usually inrolled to form a channel, with thickened margins (Guiry and Guiry, 2007).

Figure 3.5 – *Matocarpus stellatus* (Guiry and Guiry, 2007).

3.3.2. Carrageenan

Different species of *Rhodophycae* contain naturally occurring polysaccharides which fill the voids within the cellulose structure of the plant. This family of polysaccharides includes carrageenan, furcellaran and agar. These polymers have a backbone of galactose but differ in the proportion and location of ester sulfate groups and the proportion of 3,6-anhydrogalactose. The differences in composition and conformation produce a wide range of rheological properties which are useful for a large number of foods (Imeson, 2000).

The use of carrageenan in Europe started more than 600 years ago in Ireland. In the village of Carraghen on the south Irish coast, flans were made by coking the so-called Irish moss (red seaweed species *Chondrus crispus*) in milk. The name carragenin, the old name for carrageenan, was first used in 1862 for the extract from *C. crispus* and was dedicated to this village. Since then, Irish moss also has been used for industrial beer clarification and textile sizing. The commercial production began in the 1930s in the U.S.

During that time, the trading shifted from dried seaweed meal to refined carrageenan. After the Second World War, a general increase in the standard of living forced an increase in carrageenan production. Fractionation of crude carrageenan extracts started in the early 1950s, resulting in the characterization of the different carrageenan types. A Greek prefix was introduced to identify the different carrageenans. In the same period, the molecular structure of carrageenans was determined. The structure of 3,6-anhydro-D-galactose in κ-carrageenan, as well as the type of linkages, between galactose and anhydrogalactose rings, was determined. Today, the industrial manufacture of carrageenan is no longer limited to extraction from Irish moss, and numerous red seaweed species are used. Traditionally, these seaweeds have been harvested from naturally occurring populations. Seaweed farming to increase the production started almost 200 years ago in Japan. Scientific information about the seaweed life cycles allowed artificial seeding in the 1950s. Today, nearly a dozen seaweed taxa (group of organisms) are commercially cultivated, lowering the pressure on naturally occurring populations. During the past few years, the total carrageenan market has shown a growth rate of 3% per year, reaching estimated worldwide sales of 310 million US$ in 2000. At the end of the 20th century, a few large corporations that account 80% of the supply dominate the carrageenan market, including (van de Velde and de Ruiter, 2002):

- FMC Corportation (USA),
- CP Kelco (USA),
- Degussa (Germany),
- Danisco (Denmark),
- Ceamsa (Spain), and
- Quest International (The Netherlands).

Different carrageenans cover a wide spectrum of rheological behaviour going from a viscous thickener to thermally reversible gels which range in texture from soft and elastic to firm and brittle. Kappa carrageenan is able to interact synergistically with other gums, such as locust bean gum and

konjac mannan, to modify further the gel texture. A specific interaction between kappa carrageenan and kappa casein is widely used to stabilise dairy products (Imeson, 2000).

Carrageenan is a water soluble linear biopolymer, increasingly used as natural thickener, formulation stabilizer, or gelling agent in applications ranging from food industry (mainly dairy products) to pharmaceutics (van de Velde et al., 2002). The dairy sector accounts for a large part of the carrageenan applications in food products, such as frozen desserts, chocolate milk, cottage cheese and whipped cream. In addition to this, carrageenans are used in various non-dairy food products, such as instant products, jellies, pet foods, sauces, and non-food products, such as pharmaceutical formulations, cosmetics and oil well drilling fluid (Imeson, 2000; van de Velde and De Ruiter, 2002). Except starch, carrageenan is, with pectin, the main natural gelling polysaccharide extracted from plants or seaweeds and used as high-value functional ingredient in foods, cosmetics and pharmaceuticals (De Ruiter and Rudolph, 1997).

Carrageenan is usually classified in three industrially relevant types: λ-carrageenan, which is a highly sulfated galactan with viscosity enhancer properties; ι-carrageenan, which forms thermoreversible soft gels; and κ-carrageenan, which gives strong and brittle gels with water syneresis. But in reality, carrageenan biopolymers possess a complex hybrid chemical structure comprising λ-, ι-, or κ-carrageenan monomers together with nongelling biological precursors monomers such as ν- or μ-carrageenan monomers (Lahaye, 2001).

The chemical structure of the monomers corresponding to λ-, ι-, and κ-carrageenans are presented in Figure 3.6, together with the chemical structures of μ- or ν-monomers which are the biological precursors of κ- and ι-monomers, respectively.

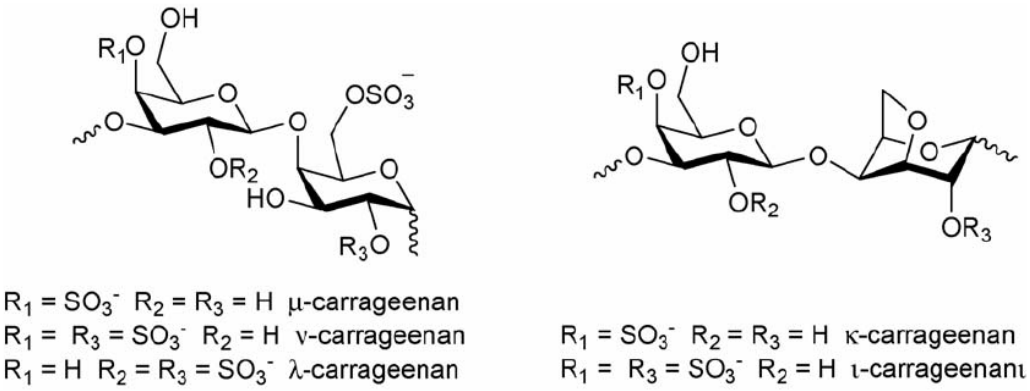

Figure 3.6 – Molecular structure of carrageenan monomers (Hilliou et al., 2006).

κ-Carrageenan has only one negative charge per disaccharide with a tendency to form a strong and rigid gel. ι-Carrageenan has two and λ-carrageenan bears on the average 2.7 charges per disaccharide unit. The gelling power of κ- and ι-carrageenans imparts excellent film forming properties (Park et al., 2001). κ-Carrageenan exhibits the highest tensile strength when compared with that of λ- and ι-carrageenan films (Cha et al., 2002).

The use of carrageenan as edible films and coatings already covers various fields of the food industry such as application on fresh and frozen meat, poultry and fish to prevent superficial dehydration (Shaw et. al, 1980), ham or sausage-casings (Macquarrie et al., 2004), granulation-coated powders, dry solid foods, oily foods (Ninomiya et al., 1997), etc., but also manufacturing soft capsules (Tanner et al., 2002; Bartkowiak and Hunkeler, 2001), and especially non-gelatin capsules (Fonkwe et al., 2006). Indeed, this protective barrier can also be used in food domain in order to prevent the transfer of moisture, gases, flavours, or lipids and thus to maintain or improve food quality and to increase food product shelf life (Krochta and De Mulder-Johnston, 1997). Another promising emerging technology that has been applied to various biopolymers, including, carrageenan-based coating, is their use as antimicrobial agent carriers in active packaging systems (Hong et al., 2005; Choi et al., 2005).

3.4. Starch/carrageenan mixtures

Industrial application of starch films for non-food uses is limited due to their brittleness and their hydrophilic nature. As indicated before, plasticizers such as glycerol are often used to overcome these limitations by improving the processing and the flexibility of starch films (Lourdin et al. 2007; Krogars et al., 2003; Mehyar and Han, 2004). Another way to reduce these drawbacks is the incorporation of another biopolymer (Lafargue et al., 2007).

Composite films from mixed polysaccharides can be produced to combine the intrinsic properties of each component to obtain an enhancement of the properties of the association. Other polysaccharides have been shown to reinforce the mechanical properties of the starch films like cellulose and its derivatives (Psomiadou et al., 1996), pullulan (Biliaderis et al., 1999) and chitosan (Rueda et al., 1999). Although a great interest has been focused on polysaccharides films, film-forming properties of starch/carrageenan mixtures have not been investigated before, despite the fact that κ-carrageenan itself is known for its good film forming ability (Park et al., 2001; Briones et al., 2004; Choi et al., 2005).

The addition of carrageenan to starch-based systems results in various physical effects which are now well documented. Carrageenan can equally act as a starch solution thickener, a gel-accelerating or gel-retarding agent, a gel-strengthening or gel-weakening agent, depending on the polysaccharide type (Lai et al, 1999). However, little is known about the effect of carrageenan on the ultimate properties of film from carrageenan-starch mixtures.

3.5. Final justification for the materials selected

As was mentioned before, the search for new renewable resources for the production biodegradable materials has steadly increased in recent years. The use of non-conventional sources of carbohydrates is an interesting alternative to produce biodegradable materials and have been investigated by many researchers.

Some underexploited renewable sources of biopolymers are found in Portugal, in abundance and at low cost. One of them is the acorn from cork oak (*Quercus suber* L.), a non-conventional source of starch. Carrageenan is other polysaccharide obtained from marine sources and with interesting film forming properties, can be extracted from the underexploited seaweed *Mastocarpus stellatus*, present in the Portuguese coast.

The purpose of this study was create a new markets, add value and new applications to underexploited renewable sources of Portugal, acorn starch from cork oak (*Quercus suber* L.) and carrageenan from *Mastocarpus stellatus*, developing biodegradable films and edible coatings based on these biopolymers, for food and non-food applications, and combining the intrinsic properties of these two polysaccharides in order to improve the properties of the material obtained.

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Chapter 4
Biopolymer extraction and characterization

In this chapter, the procedures used for the extraction and characterization of the biopolymers studied, starch from cork oak's acorn (*Quercus suber* L.), and carrageenan from the *Mastocarpus stellatus* seaweed are presented.

4.1. Starch

4.1.1. Materials and methods

4.1.1.1. Acorn sampling

Acorns from cork oak (*Quercus suber* L.) were collected and supplied by the Association of Forest Producers of Vale do Sado (ANSUB), Alcácer do Sal, Portugal, during November 2006. The acorns were inspected in order to separate fruits with defects or disease, and stored at room temperature. Acorns of uniform size were selected for the extraction of starch.

4.1.1.2. Extraction

The selected acorns were peeled and cut into small pieces. Representative samples of 1 kg were ground in a blender with 2 L of water containing a small amount of potassium disulfite (5 g/L H_2O), to avoid acorn browning. The slurry was filtered through sieves and then separated in residue and filtrate. The residue was ground again with the same proportion of water (1:2) and filtered. The second filtrate was added to the first filtrate, and let to settle over 24 h. Settled solids were separated from the supernatant and washed with distilled water several times with the aid of a vacuum system, until a clear wash was obtained and the starch was free of colour; the starch was then dried in a ventilated oven at 40ºC. Figure 4.1 shows the flow diagram used to for the acorn starch extraction.

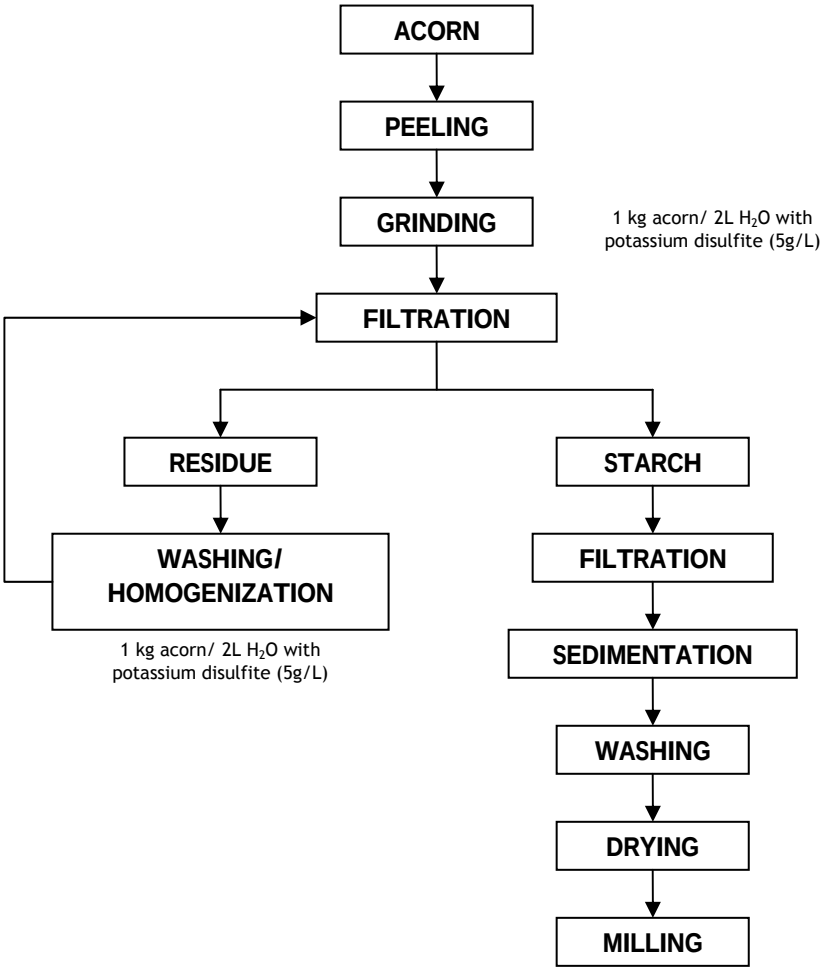


Figure 4.1 – Flow diagram for the starch extraction (adapted from Leonel et al., 2003; Leonel et al., 2002; Demiate et al., 2001; Tulyathan et al., 2005).

4.1.1.3. Chemical analysis

A nutritional analysis was performed to determine the chemical composition of the starch extracted from acorn. The same analysis was carried out for the acorn, which was dry milled and sieved, and in this study was called as acorn flour.

The methodologies used for the nutritional analysis were:

- Moisture: oven desiccation at 100±2ºC (AOAC 24.003);
- Ash: dry incineration method (Netto, 1959);
- Lipid: Soxhlet extraction method (AOAC 24.005) using petroleum ether as extractor solvent (b.p. 40-60ºC) and a minimum extraction time of 36 hours;
- Protein: Kjeldahl method using as conversion factor of nitrogen in protein the value 6.25 (AOAC 24.038);
- Carbohydrate: determined by the difference of the others components.

The chemical analysis of acorn flour and starch were performed by the Bromatology Service of the Faculty of Pharmacy of University of Porto.

4.1.1.4. Rheological analysis

Experiments were carried out to determine the gelatinization temperature range for acorn starch, using a suspension of 50 g/l acorn starch in water using a TA AR2000 controlled stress rheometer (TA Instruments, USA) fitted with cone-plate geometry (4º cone angle, 4.0 cm diameter). The analysis was made in the temperature range of 25º to 90ºC, with a heating rate of 5ºC/min, at frequency of 1 Hz and deformation of 5%.

4.1.2. Results and discussion

4.1.2.1. Starch extraction

Oak acorns are a rich source of carbohydrates, amino acids, proteins, lipids and various sterols. The main part of carbohydrates is composed of starch (Saricicek and Kilic, 2004; Rakić et al., 2007). Later studies of several *Quercus* sp. found acorns containing 48-85% of carbohydrates (dry weight with most varieties over 72%) (Stevenson et al., 2006), and starch content of 59% (dry weight) (Saffarzadeh et al., 1999), with starch described as beige to yellow-brown in colour with onset gelatinization temperature of 61-68ºC. Acorns contained a considerable amount of starch especially because their moisture content was lower than that of most fruit (Stevenson et al., 2006).

Acorn extraction had a yield of 21.2% (based on 1 kg of fresh unpeeled fruits), and it was found that carbohydrate was the major component, comprising 80% of acorn in this study. Starch extracted from cork oak's acorn presented a pale yellow coloration (Figure 4.2) with moisture content of 10%. The methodology of extraction was based in other studies that extracted starch from non-conventional sources. Starch extraction from water chestnut (*Trapa bispinosa* Roxb.) had a yield of 37.4% and the extract was composed by 84.8% of carbohydrates (Tulyathan et al., 2005). Chestnut (*Castanea sativa* Mill.) studied by Demiate et al. (2001) was composed by 78.8% of starch. Starches were extracted by Leonel et al. (2002) from *Canna edulis,* obtaining a yield of 11.4% and 56.2% of starch, and Leonel et al. (2003) extracted starch from *Pachyrhizus ahipa* with a yield of 4.3 % and 46.2% of starch.

Figure 4.2 – Starch extracted from acorn cork oak (*Quercus suber* L.).

4.1.2.2. Chemical analysis

Chemical composition of acorn flour and acorn starch extracted in this study is shown in Table 4.1. The chemical composition for the acorn from cork oak (*Quercus suber* L.) of the present study agrees with the values found in the literature. Bainbridge (1986) present the acorn composition from 18 different species, and the values ranged from 8.7 to 44.6% of moisture, 2.3 to 8.6% of protein, 1.1 to 31.3% of fat and 32.7 to 89.7% of carbohydrates. González et al. (2006) found for *Quercus suber* acorn values of 5.9% for fat, 6% of protein and 84.1% of carbohydrate. Table 4.2 shows the nutritional compositions of several varieties of acorns.

Table 4.1 – Chemical composition of acorn flour and acorn starch.

Composition	Acorn flour*	Acorn starch*
Moisture	6.90±0.04	10.00±0.04
Ash	2.30±0.01	0.40±0.06
Crude fat	4.70±0.01	5.60±0.05
Crude protein	6.10±0.04	5.90±0.01
Carbohydrate**	80	78

* Values are expressed in g per 100 g of product.

** Values were determined by difference

Table 4.2 – Nutritional compositions of acorns (all values are expressed as percent of dry weight).

source	*Ref.*	*MC*	*Ash*	*CF*	*CP*	*C*	*S*
Q. suber	this study	6.9	2.3	4.7	6.1	80.0	-
Q. suber	González et al. (2006)	-	1.9	5.9	6.0	84.1	-
Q. rotundifolia	González et al. (2006)	-	1.8	7.6	4.1	83.0	-
Q. rotundifolia	Rey et al. (2006)	-	1.8	11.0	4.8	81.4	-
Q. palustris	Stevenson et al. (2006)	37.9	-	4.2	6.7	-	17.3
Q. agrifolia	Bainbridge (1986)	9.0	-	16.8	6.3	54.6	-
Q. chrysolepis	Bainbridge (1986)	9.0	-	8.7	4.1	63.5	-
Q. douglasii	Bainbridge (1986)	9.0	-	8.1	5.5	65.5	-
Q. dumosa	Bainbridge (1986)	44.6	-	3.4	2.3	40.7	-
Q. kellogii	Bainbridge (1986)	9.0	-	18.0	4.6	55.5	-
Q. branti	Saffarzadeh et al. (1999)	-	1.5	7.7	3.9	75.2	58.8
Q. variabilis	Xiao et al. (2007)	-	-	3.9	5.9	-	54.2
Q. serrata	Xiao et al. (2007)	-	-	3.0	6.1	-	54.0
Q. serrata	Shimada (2001)	-	2.8	2.5	4.5	88.3	-
Q. mongolica	Shimada (2001)	-	1.5	1.7	4.4	90.3	-
Q. acutissima	Shimada and Saitoh (2006)	-	1.8	4.6	5.2	-	-
Q. aliena	Shimada and Saitoh (2006)	-	1.5	2.8	5.3	-	-
Q. robur	Shimada and Saitoh (2006)	-	1.6	4.9	6.2	-	-
Q. petraeae	Shimada and Saitoh (2006)	-	2.1	4.9	5.9	-	-
Q. aegilops	Al Jassim et al. (1998)	-	2.4	23.6	3.9	-	-
Q. coccifera	Al Jassim et al. (1998)	-	2.8	25.3	2.9	-	-
Q. pubescens	Saricicek and Kilic (2004)	-	1.5	3.8	3.6	45.0	-

Ref. – reference; MC – moisture content; CF – crude fat; CP – crude protein; Carb – carbohydrates; S – starch.

The presence of small amounts of protein and fat (Table 4.1) indicates that starch extracted in this study was not purified. Some authors (Rayas and Hernández, 1997; Mariniello et al., 2003; Tapia-Blácido et al., 2005) reported that the presence of native lipids and proteins can be an interesting alternative to produce films and improve their properties combining the properties of each component (starch, protein and lipid).

In general, starch films present good mechanical properties and good oxygen barrier properties, but these films are sensitivity to humidity (Forsell et al., 2002). In order to improve the properties of these materials, some authors

have elaborated films based on starch and protein mixtures (Jagannath et al., 2003; Coughlan et al., 2004), and, with the particular objective of improving water vapour permeability, some lipids have also been added to the film formulations (Garcia et al., 2000; Shaw et al., 2002; Bravin et al., 2004; Colla et al., 2006).

Various studies have attempted to improve the functional properties of these films, by using mixtures of protein and starch as the raw material for the formation of edible films. To produce such films, researchers have commonly used extracted and purified biopolymers (such as commercial starches and proteins) and lipids, that are the mixed during film processing. Parris et al. (1997) verified that the addition of 1% of corn starch to Zein films decreased the water vapour permeability. Whereas blends of gelatine and potato starch showed greater elongation and tensile stress at break in comparison with pure starch films (Arvanitoyannis et al., 1997). However, the addition of casein was reported to decrease the mechanical resistance of corn starch films (Arvanitoyannis et al., 1996). When dealing with cereals, it is not necessary to extract the starch or the proteins for film production. Rayas and Hernández (1997) prepared edible films from three types of wheat flours, and more recently, Mariniello et al. (2003) used whole soy flour and apple pectin as the raw materials for producing edible films. Tapia-Blácido et al. (2005) produced amaranth flour films with interesting mechanical and water vapour barrier characteristics. According to these authors, these characteristics were a result of the natural interactions occurring between starch, protein and lipids during drying of the filmogenic solution.

4.1.2.3. Rheological analysis

The determination of the gelatinization temperature range is an important parameter for the starch based film production. Heating the film forming solution above the gelatinization temperature will ensure a total

solubilization of starch in the film forming solution, destructing its semi-crystalline granular structure, and improving its film forming capacity.

Figure 4.3 shows the gelatinization temperature range of acorn starch as determined by oscillatory rheometry. Figure 4.3A shows variations of the storage modulus G' and loss modulus G" between 35 and 85ºC; its ratio G"/G' is known as *tan* δ *and is* illustrated in Figure 4.3B as function of the temperature. At temperatures below 60ºC, G">G' (*tan* δ > *1*), which indicates a liquid-like behaviour of the sample. Both moduli sharply increased at around 60ºC, and when *tan* δ becomes lower than 1, the starch gelatinization begins resulting in an increase in the storage modulus (G'), reflecting a solid-like behaviour (formation of the gel). The gelatinization temperature range was determined in the interval where *tan* δ became lower than 1 (G" < G'), and ranging from 63.7 to 67.4ºC. Similar onset gelatinization temperatures (60-65ºC) were reported for starch isolated from acorns of *Q. alba*, *Q. prinus*, *Q. rubra* and *Q. palustris* (Stevenson et al., 2006). Aee et al. (1998) reported an interval of gelatinization temperature between 55.0ºC to 74.3ºC for commercial acorn starch. The interval of gelatinization temperature range of acorn starch is similar to starches from other sources (Table 4.3).

Table 4.3 - Gelatinization temperature range of some starches.

Starch source	Gelatinization temperature range (ºC)
acorn*	63.7 – 67.4
potato	56.0 - 66.0
cassava	58.0 - 70.0
corn	62.0 - 72.0
sorghum	68.0 - 75.0
wheat	52.0 - 63.0
rice	61.0 - 77.0

Adapted from Bobbio and Bobbio (1995); * this study.

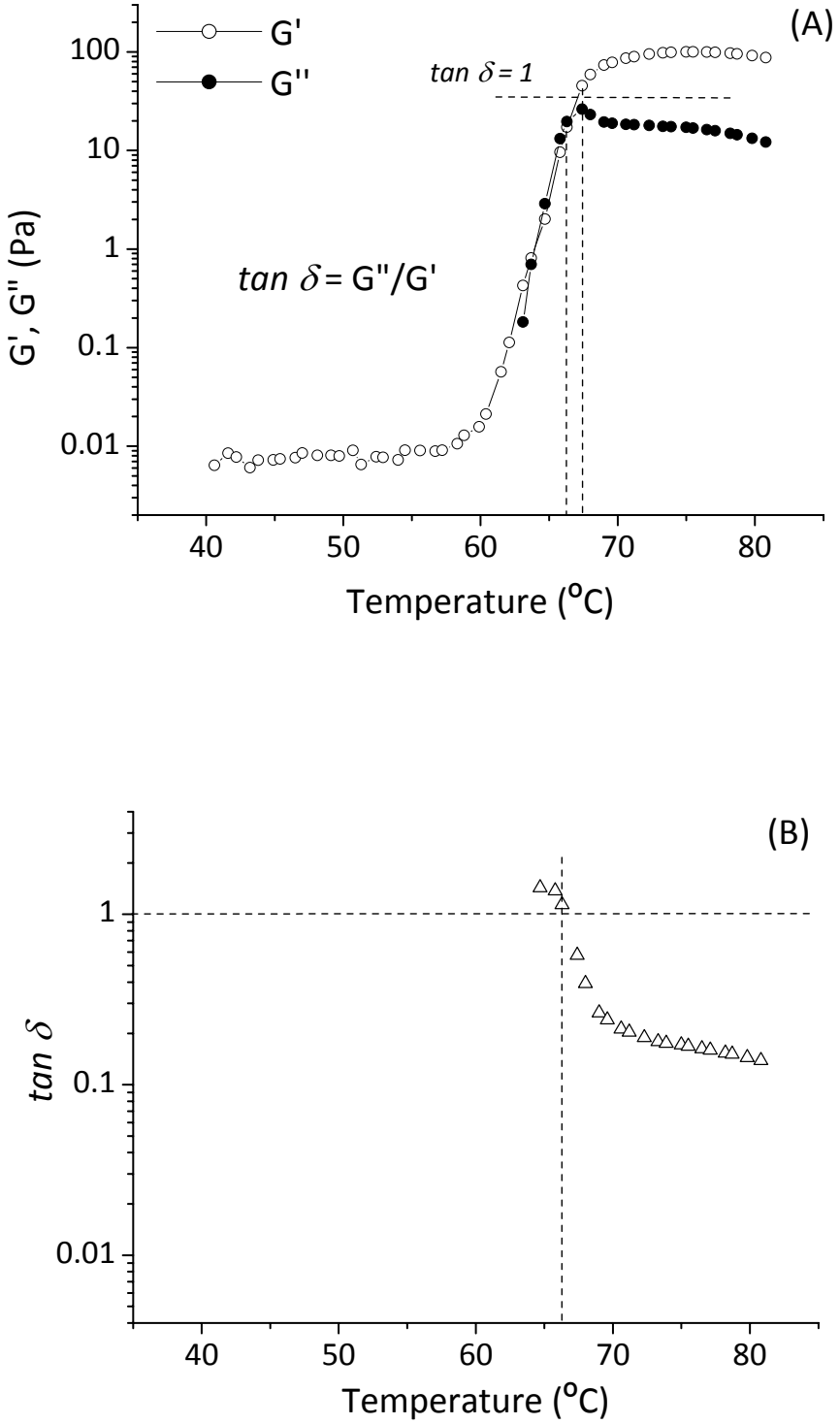


Figure 4.3 – Rheological analysis of acorn starch suspension at a heating rate of 5ºC/min and 50 g/l, at a frequency of 1 Hz and deformation of 5%: (A) G' anf G" as function of the temperature; (B) *tan* δ as function of the temperature.

4.1.3. Conclusions

Cork oak's acorn containing about 80% of carbohydrates presents an important potential to be used as source of carbohydrates. At present, most of these acorns are used as feed source for free-ranging pigs in Portugal. As starch source this cork oak's acorn can be used to produce a renewable material, in the form of films or edible coatings. The extract obtained in the laboratory was similar to other acorn varieties in terms of nutritional composition and gelatinization temperature range reported in the literature. While presently it does not seem feasible to commercially utilize acorn starch, enormous quantities of acorn starch are produced globally each year by oak trees and left to rot. With trends to convert biomass from an increasingly diverse range of crops into biofuels and biobased plastics, acorns in the future could be a potential source of carbohydrates for industrial products. In terms of biodegradable films production, the extract from cork oak's acorn showed good results in the film formation as will be discussed in Chapter 6, constituting an opportunity to add value to this natural resource underexploited in Portugal.

4.2. Carrageenan

4.2.1. Materials and methods

4.2.1.1. Seaweeds sampling

Mastocarpus stellatus seaweeds were hand collected at low tides during November 2004, on the intertidal rocks located in Vila Praia de Ancora (Northern coast of Portugal). Right after sampling, seaweeds were washed several times with tap water in order to remove sand and other non algal materials. No attempt was made to separate gametophyte thalli from carposporophyte thalli (specimen with different life phases), as this process

is difficult to implement on a plant-scale carrageenan production unit. Clean seaweeds were then dried at 60 ºC for 48 hours in a ventilated oven, and stored at room temperature.

4.2.1.2. Alkaline treatment

Dried seaweeds were submitted to an alkaline treatment (40g dried algae poured into a 0.1M $NaHCO_3$ tap water solution at room temperature) during a time *PT* prior to the extraction procedure. Alkali is used because it causes a chemical change that leads to increased gel strength in the final product. In chemical terms, it removes some of the sulfate groups from the molecules and increases the formation of 3,6-anhydrogalactose: the more of the latter, the better the gel strength (McHugh, 2003).

4.2.1.3. Extraction

Alkali treated seaweeds were then washed several times with tap water in order to get rid of the excess of $NaHCO_3$ salt, as demonstrated by the decrease in the algae pH from 9 to 7. Extraction (40g dried algae in 4 liters tap water) was performed at a temperature *T* during a time *t* and at a defined pH controlled by addition of 0.01M HCl or $NaHCO_3$ dry powder. Parameters *PT*, *T*, *t* and pH were varied from 0 to 70 h, from 80 to 110ºC, from 30 min to 6 h, and from 7 to 9, respectively. The extraction parameters are presented in Table 4.4. The extracts were then filtered with metallic sieves. In a subsequent step, a filtration employing a cotton cloth was conducted, prior to concentration by water evaporation of the filtrate, performed at 60 ºC. One volume of the resulting concentrated extract was then precipitated in two volumes of commercial ethanol (95%). The precipitate was separated from the water-ethanol mixture by filtration, and the filter cake containing the recovered polysaccharide was washed with further ethanol. This product was dried at 60 ºC under vacuum until constant weight was reached, and milled. The resulting powder was then purified by mixing 1 g of isolated product with 49 ml in hot distilled water

during 1 hour and subsequent centrifugation performed at 25000 rpm and 40 ºC during 40 minutes. The supernatant was finally recovered, dried at 60 ºC under vacuum and weighted to calculate the extraction yield with respect to the dried algae weight initially used for alkali treatment. Selected extractions were performed in triplicate in order to determine the experimental error associated with the yield and to check the good qualitative reproducibility in the chemical structure of the extracted carrageenan as well as in the corresponding gelling properties. Figure 4.4 shows the flow diagram used to for the carrageenan extraction.

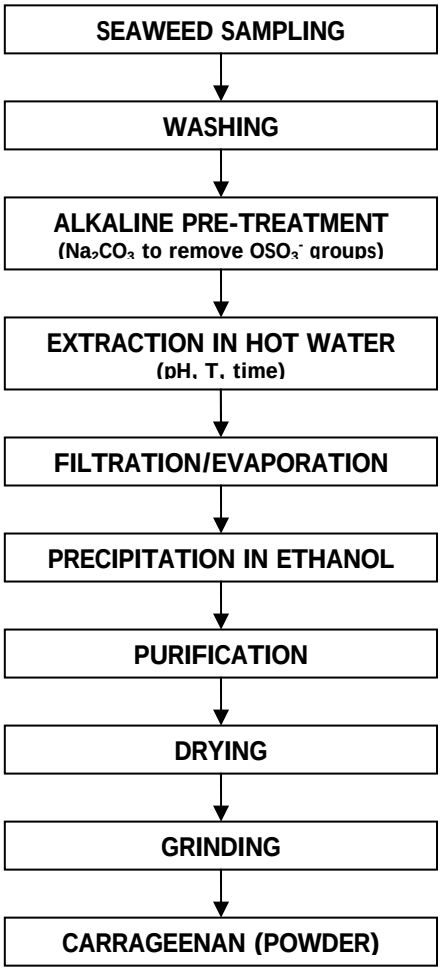


Figure 4.4 – Flow diagram for the carrageenan extraction (adapted from Bixler, 1996).

Table 4.4 – Extraction parameters (alkaline pre-treatment duration *PT*, duration *t*, temperature *T* and *pH*) and yield of extraction.

Sample label	Extraction parameters				Yield (%) *
	PT (hours)	*t (hours)*	*T (ºC)*	*pH*	
M8	0	2	95	7	26
M9	42	2	95	8	17
M11	72	2	95	8	14
M15	0	2	95	8	35
M16	20	2	95	8	27
M19	0	2	95	9	38
M21	0	2	80	8	24
M22	0	2	110	8	32
M23	48	0.5	95	8	13
M24	48	1	95	8	18
M25	48	2	95	8	20
M26	48	4	95	8	25
M27	48	6	95	8	24

* dried carageenan weight over dried seaweeds weight

4.2.1.4. Chemical structure

Assessment of the chemical structure of extracted phycocolloids was performed by combining Fourier transform infrared spectroscopy (FTIR) and ^{1}H NMR spectroscopy. FTIR spectra, obtained with a Perkin-Elmer 157G infrared spectrometer using a HeNe laser with continuous wave radiation at 633 nm, are the average of four scans (with a 2 cm^{-1} resolution) performed on carrageenan films cast from dilute water solutions. The ^{1}H NMR spectra were recorded at 80ºC, on a Bruker ARX 400 NMR spectrometer (400 MHz), using D_2O as solvent and TMSPSA as internal standard. Typical phycocolloid concentration in D_2O was 10 mg/ml. κ-Carrageenan (lot 083K125), ι-carrageenan (lot 121K1200) and λ-carrageenan (lot 122K1444) were purchased from Sigma Chemical Co. as reference materials, and used as received. The ^{1}H NMR spectrum of λ-carrageenan sample showed a peak assigned to Floridean starch and a less intense one assigned to ι-monomers, together with traces of ν-, κ- and λ-monomers. As such this product appears to be a non-gelling carrageenan, rather than a polymer basically containing λ-monomers. FTIR and ^{1}H NMR spectroscopies were performed at the Departament of Chemistry of Universidade Nova de Lisboa.

4.2.1.5. Determination of molecular weight distribution

Number and weight average molecular weights, M_n and M_w, respectively, as well as the polydispersity index (M_n/M_w) were obtained by size exclusion chromatography (SEC) in a low temperature Waters Co. apparatus equipped with a Waters Ultrahydrogel Linear column and a differential refractive index detector (Waters 2410). A 0.1M NaCl solution at 40ºC was used as eluent, and the carrageenan concentration was less than 0.1wt.%, thus ensuring the pumping of essentially non-aggregated polysaccharides in coil conformation by a Waters 510 Solvent Delivery System. The values of M_n and M_w were calculated using a calibration curve generated with eight monodisperse pullulan standards (Shodex, Showa Denko, Japan, in the range 0.59 x 10^4 to 78.8 x 10^4 g/mol). Molecular weight distribution determination was performed at Departament of Chemistry of Universidade Nova de Lisboa.

4.2.1.6. Characterization of gel mechanical properties

Gel strength was determined by means of penetration tests performed on equilibrated gels with a texture analyzer (TA-XT2 from Stable Microsystems Ltd.) equipped with a cylindrical probe (diameter 1 cm). Hot solutions of 1.5 wt.% extracted carrageenans in 0.05M KCl were prepared by vigorous stirring (for at least 30 min) of phycocolloid powders added to an adequate volume of 0.05M KCl solution at 70ºC. These hot solutions were poured into plastic cups (diameter 3 cm, depth 1 cm) and left to cool down to 25ºC. During cooling, carrageenan gels were formed; a 24 h aging period was allowed for the gels to reach structural equilibrium. Penetration tests were performed with the lower penetration velocity allowed by the equipment (0.1 mm s^{-1}) in order to ensure quasi-static conditions for the record of the force response *F*. Penetration depth was typically 1 mm. This distance roughly corresponds to a tenth of the sample height and allows neglecting wall effects. No correction for buoyancy force actuating on the penetrating probe was made (Gregson et al., 1999), and gel Young's moduli *E* were

directly estimated by computing the ratio of measured stress (force *F* (N) by the probe surface (m^2)) over imposed strain. *E* values reported in Table 4.5 are the average of three penetration tests performed on three identical gel samples.

Gel's melting temperatures were measured by differential scanning calorimetry (DSC). Tests were performed by a DSC50 – Shimadzu apparatus on gel samples (1.5 wt.% biopolymer in 0.05M KCl) previously equilibrated during 24 h in the DSC pans. The heating rate was 2.5ºC/min and the reference pan was filled with 0.05M KCl solution.

4.2.1.7. Characterization of gel thermal properties

Figure 4.5 illustrates the experimental protocol used to characterize the gel setting temperature (T_{gel}), the gel melting temperature (T_{melt}), and the gel viscoelastic properties of all extracted κ/ι-hybrid carrageenans (data for sample M11 are reported in Figure 4.5). Hot carrageenan solutions (1.5% w/w biopolymer in 0.05 mol/dm^3 KCl) were loaded at 90ºC in the preheated plate-plate geometry of a stress-controlled rheometer (AR2000, TA Instruments). Water loss was avoided by covering the geometry with paraffin oil. Hot solutions were first cooled (5ºC/min) down to 20ºC while small amplitude oscillatory shear strain with 0.1% amplitude was applied at 1 Hz in order to probe the temperature evolution of linear viscoelastic properties such as *tan* δ, the tangent of the phase shift angle δ between imposed sinusoidal strain and measured sinusoidal stress (Figure 4.5A). The point for which *tan* δ = 1 was defined as the gel-setting temperature. It should be noted that the Winter-Chambon criterion (Winter and Mours, 1997) for liquid-solid transition determination could not be used, as the time evolution of dynamic moduli was too fast at temperatures close to the liquid-solid transition and did not allow proper measurement of mechanical spectra on a sufficiently wide frequency range. The time evolution of the storage modulus *G'* and the loss modulus *G"* was followed at 20ºC (0.1%

strain at 1 Hz), allowing the determination of gel equilibrium conditions (Figure 4.5B). Mechanical spectra of equilibrated gels were then obtained in the linear regime by performing frequency sweeps with a 0.1% strain (Figure 4.5C). From this test, one can extract the value of the gel storage modulus *G'* at 1 Hz (G_0), which essentially mirrors the gel elasticity at equilibrium and 20ºC. Finally, the gels were heated (5ºC/min) up to 90ºC and *G'* and *G"* were simultaneously measured at a 0.1% strain and a frequency of 1 Hz, enabling the characterization of the gel melting point (chart D) again defined as the temperature for which $tan\ \delta = 1$.

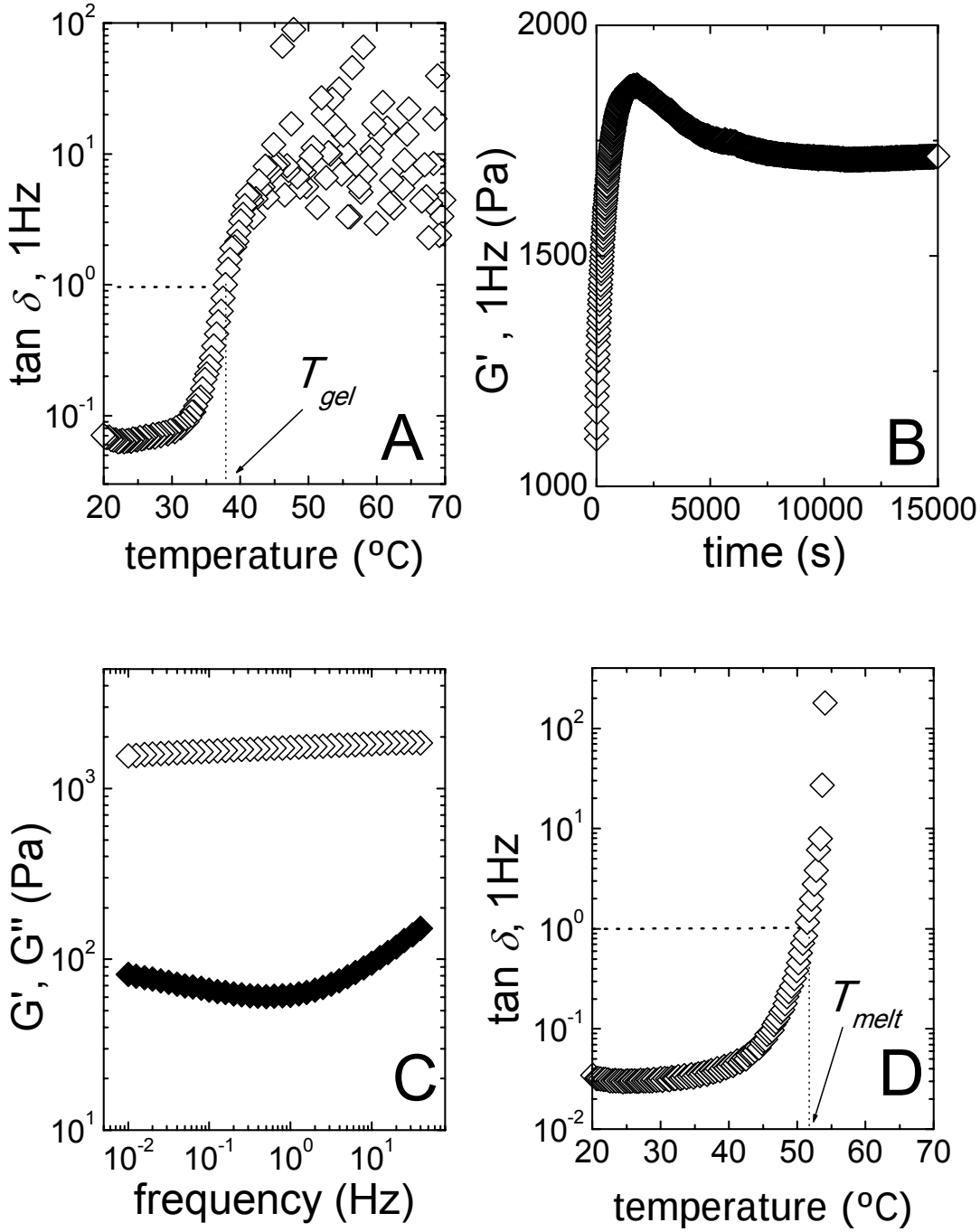


Figure 4.5 - Experimental protocol used for the rheological characterization of κ/ι-hybrid carrageenan gels. Dotted lines in A indicate the gel setting point T_{gel}. Dotted lines in D indicate the gel melting temperature T_{melt}.

4.2.2. Results

4.2.2.1. Identification of the carrageenan biopolymers extracted from Mastocarpus stellatus

Figure 4.6 shows the ^{1}H NMR spectrum of a representative phycocolloid extracted from *Mastocarpus stellatus* with the following parameters: *PT* = 70 h, *t* = 2 h, pH 8 and *T* = 95ºC, and hereafter labeled as M11. The peaks at 5.32 and 5.11 ppm reveal the presence of ι- and κ-monomers, respectively (van de Velde et al., 2002a, b) and are indicated by vertical arrows in Figure 4.6, a small peak located at 5.52 ppm can be resolved, together with a shoulder at 5.26 ppm. These two signals are indicative of the presence of ν- and μ-monomers, respectively. The ^{1}H NMR spectrum demonstrates that the biopolymers extracted from *Mastocarpus stellatus* are essentially made of κ- and ι-monomers and contains traces (above 5% in relative content, if one considers the detection limit of NMR spectroscopy) of μ- and ν-monomers. Therefore, these biopolymers can be seen as κ/ι-hybrid carrageenans (blocks of κ- and ι-monomers distributed on the same macromolecule) or equally as mixtures of κ- and ι-carrageenan biopolymers. Carrageenan fractionation in a KCl solution, with adequate salt and biopolymer concentrations, provides a way to differentiate between the two types of macromolecular structure (van de Velde et al. 2001). Based on the phase diagrams established earlier by Michel et al. (1997) at 20ºC with model carrageenans, two solutions were prepared. A first solution corresponding to the sol phase of ι-carrageenan and the turbid gel phase (with water syneresis) of κ-carrageenan was prepared by mixing 1.5 wt.% of the sample M11 in 0.1M KCl at 70ºC under strong stirring during 1 h. A second solution containing 0.2 wt.% of M11 in 0.03M KCl was prepared in a similar way. Under the latter polymer concentration and salt conditions, ι-carrageenan is still in the liquid phase whereas κ-carrageenan forms a much weaker gel with water syneresis. Both solutions were left to cool down to room temperature for 2 days. At that point, no phase separation took place, although the more concentrated solution showed to be a gel with no water

syneresis. Both solution and gel were then centrifugated at 10ºC and 10 000 x g for 2 h. Again, no phase separation into a gel state and a solution state was observed. These results are therefore in favour of a κ/ι-hybrid carrageenan, possessing a block copolymer structure allowing gel formation (van de Velde et al., 2001).

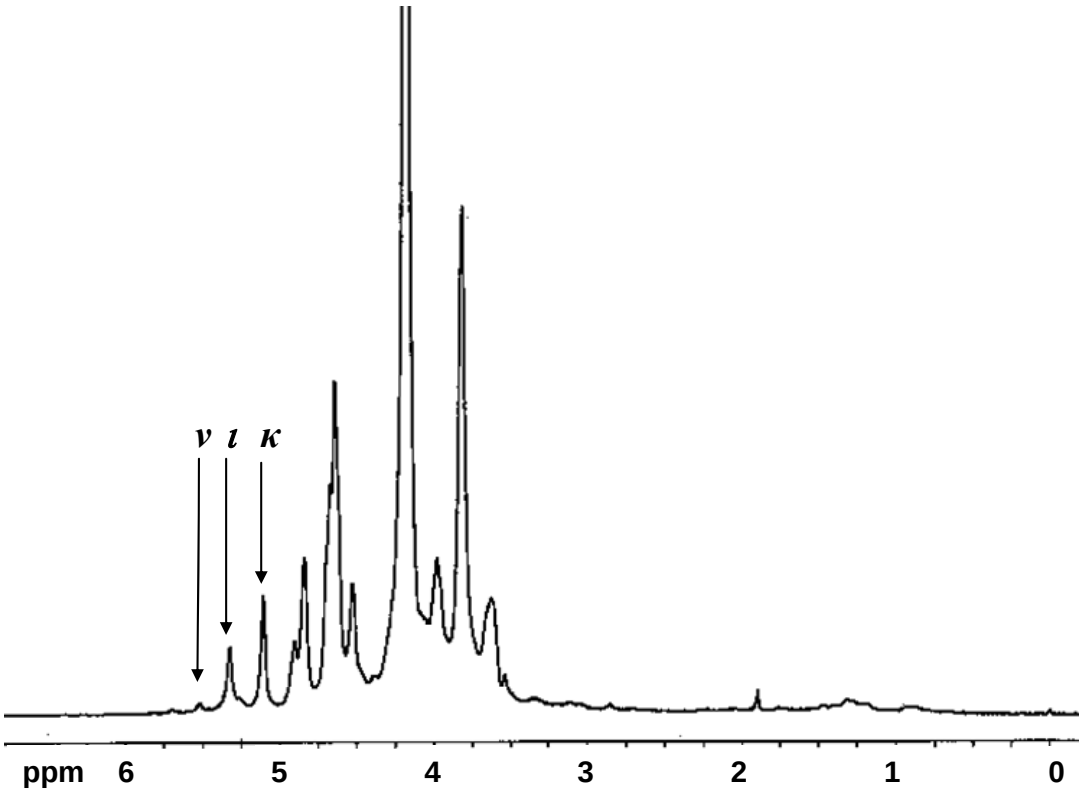


Figure 4.6 - ^{1}H NMR spectrum of a phycocolloid extracted from *Mastocarpus stellatus*.

The FTIR spectrum in Figure 4.7 confirms the complex structure of the extracted biopolymer, whose main spectral peaks are indicated by three vertical dotted lines: an absorption band at 805 cm^{-1} reminiscent of ι-carrageenan monomers (Pereira and Mesquita, 2003); a stronger band at 930 cm^{-1}, which is a common feature of both κ- and ι-monomers (Volery et al., 2004); and a band at 845 cm^{-1}, which is reported to be specific to κ-, ι-, ν-, and μ-monomers (Chopin et al., 1999) as it relates to the COS group located on the fourth carbon of the galactose (Figure 4.8). These results are in

harmony with a recent report on κ/ι-hybrid carrageenans extracted from gametophyte samples of similar seaweeds collected on the northern coast of Portugal (Pereira and Mesquita, 2003). Furthermore, KCl fractionation carried out in demonstrates that κ-carrageenan biopolymers cannot be separated from ι-carrageenan biopolymers. Instead, either a homogeneous viscous solution, a transparent gel, or a grainy, turbid gel (when KCl concentrations in excess of 0.1 mol/dm^3 and biopolymer concentrations above 1.5% w/w are used) is obtained after centrifugation. This is indicative of the copolymer nature (van de Velde et al., 2001) of the κ/ι-hybrid carrageenan extracted from *Mastocarpus stellatus*, which is to be seen as a macromolecular chain containing long sequences of κ- and ι-carrageenan monomers (with sufficient length for allowing the formation of aggregating helices) separated by shorter sequences and/or units of ν- and μ-carrageenan monomers, rather than a mixture of essentially ideal κ-carrageenan and ι-carrageenan biopolymers.

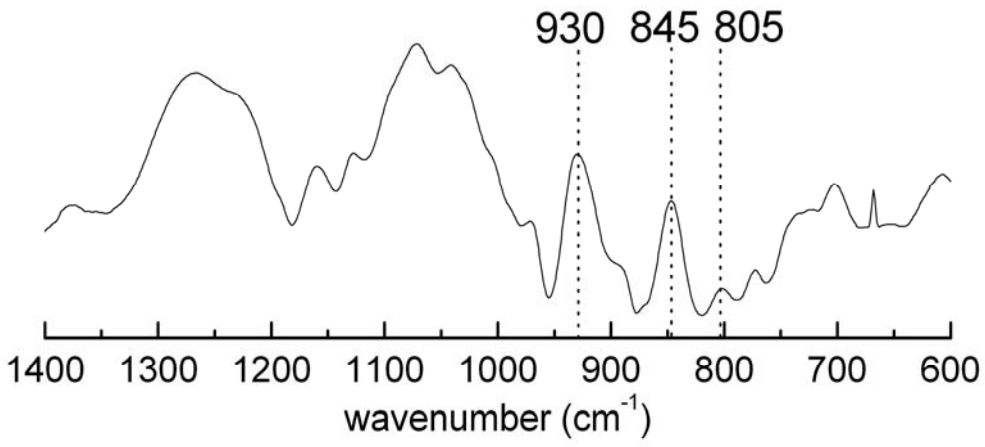
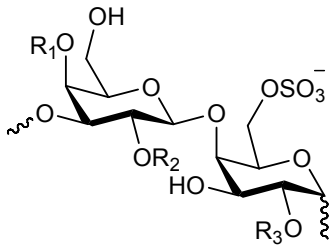
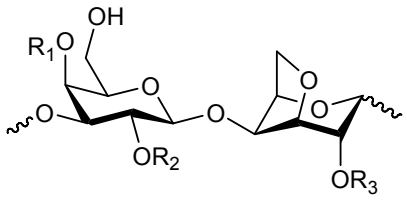


Figure 4.7 – FTIR spectrum of phycocolloid extracted from *Mastocarpus stellatus* (sample obtained after 4h of extraction at pH 8 and *T* = 95ºC performed on 48 h alkali-treated seaweeds).



$R_1 = SO_3^-$ $R_2 = R_3 = H$ μ-carrageenan
$R_1 = R_3 = SO_3^-$ $R_2 = H$ ν-carrageenan
$R_1 = H$ $R_2 = R_3 = SO_3^-$ λ-carrageenan



$R_1 = SO_3^-$ $R_2 = R_3 = H$ κ-carrageenan
$R_1 = R_3 = SO_3^-$ $R_2 = H$ ι-carrageenanι

Figure 4.8 – Molecular structure of carrageenan monomers.

4.2.2.2. Effect of extraction time t on chemical structure and gel properties of the κ/ι-hybrid carrageenans

Phycocolloids obtained from *Mastocarpus stellatus* after different extraction time *t*, while all other extraction parameters were kept constant (*PT* = 48 h, *T* = 95ºC and pH 8), showed qualitatively unchanged ^{1}H NMR spectra. This result suggests that κ/ι-hybrid carrageenans are extracted from *Mastocarpus stellatus*, whatever the extraction time. On the other hand, the relative peak intensities are changed with parameter *t*. This is illustrated in Figure 4.9 where peak intensity ratios giving the relative amount (peak integrated area relative to the total peaks integrated area) of corresponding chemical structures are plotted as a function of parameter *t*. This analysis shows that the κ/ι-hybrid carrageenan chemical structure, described in terms of relative amount in ι- or ν-monomers, is basically unchanged after 1 hour of extraction. However, the relative amount in μ-monomer shows a minimum at 2 hours of extraction, which corresponds to a maximum in the κ-monomer relative content.

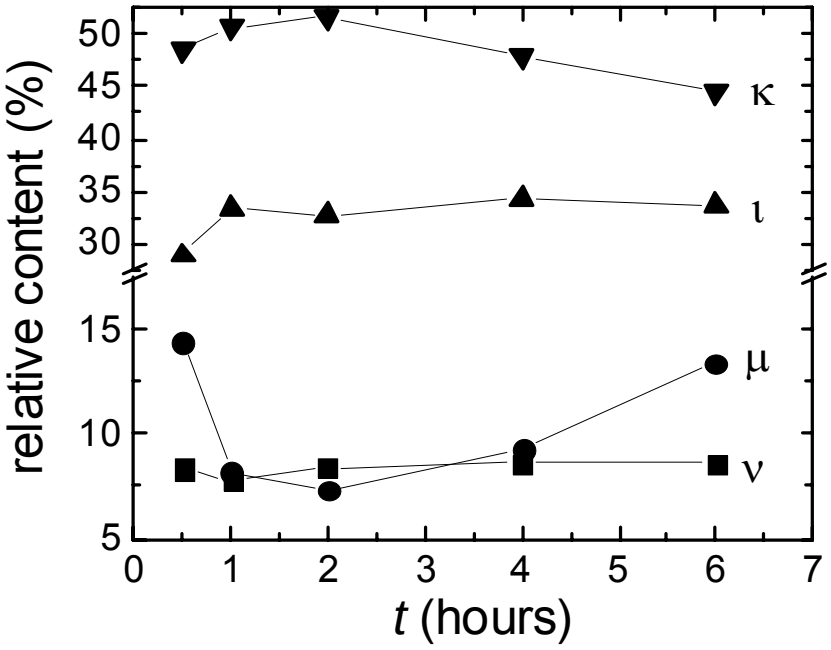


Figure 4.9 **-** Effect of parameter *t* on ^{1}H-NMR relative peak intensities assigned to *ν*-monomer (■), *μ*-monomer (●), *ι*-monomer (▲) and *κ*-monomer (▼).

Figure 4.10 presents the DSC scans obtained with gels made from the phycocolloid samples whose chemical structures have been examined in Figure 4.9. The curves have been vertically shifted to allow a comparison of the broad gel melting process, which is similar to a second order transition such as the glass transition in synthetic polymers. This transition has been analyzed with the definition of three temperatures: the melting temperature T_m, which is the midpoint between the onset temperature T_{on} and the end set temperature T_{end}. The latter is reported in Table 4.5 and no peculiar variation with parameter t is evident. This result also holds for temperatures T_m and T_{on} (Figure 4.10), thus indicating that gel thermal properties do not seem to be affected by the extraction time. In contrast, the extraction yield, reported in Table 4.4 for each extraction time, is an increasing function of parameter *t*, reaching a maximum yield at 4 hours of extraction. The Young's modulus *E*, extracted from penetration tests (displayed in Figure 4.11) and both M_n and M_w, reported in Table 4.5 appear

not be affected by the extraction time, not showing significant variations after the first 30 minutes of extraction.

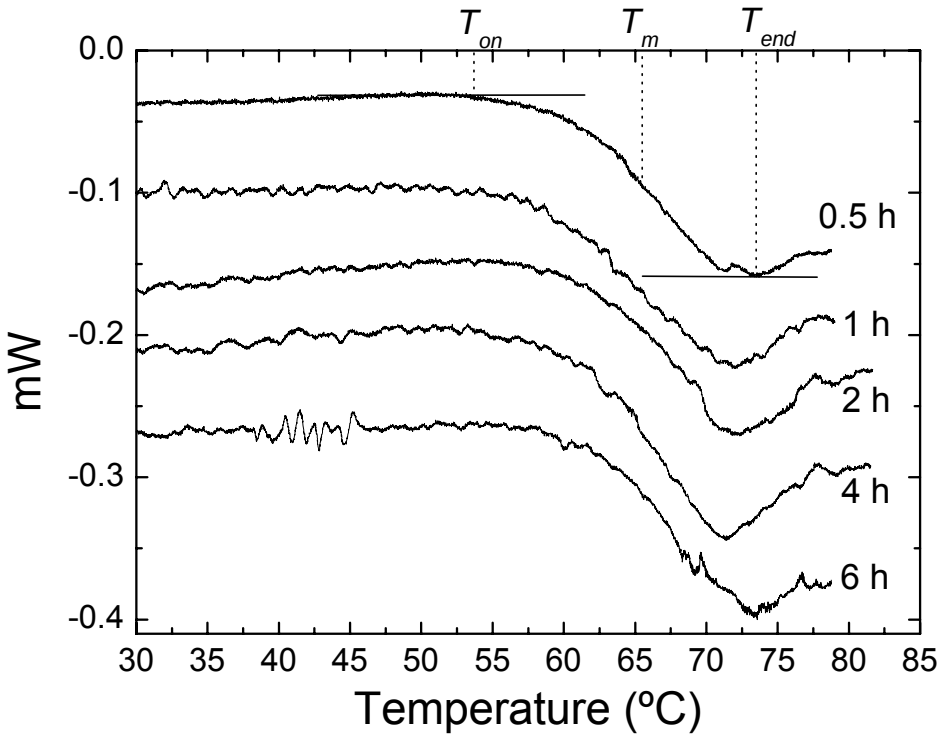


Figure 4.10 - DSC curves of equilibrated gels containing κ/ι-hybrid carrageenans (1.5%wt in 0.05 M KCl) obtained with different extraction times (from top to bottom): 0.5 hour, 1 hour, 2 hours, 4 hours and 6 hours (vertical dotted lines and horizontal solid lines indicate the graphical determination of temperatures T_{on}, T_m and T_{end}).

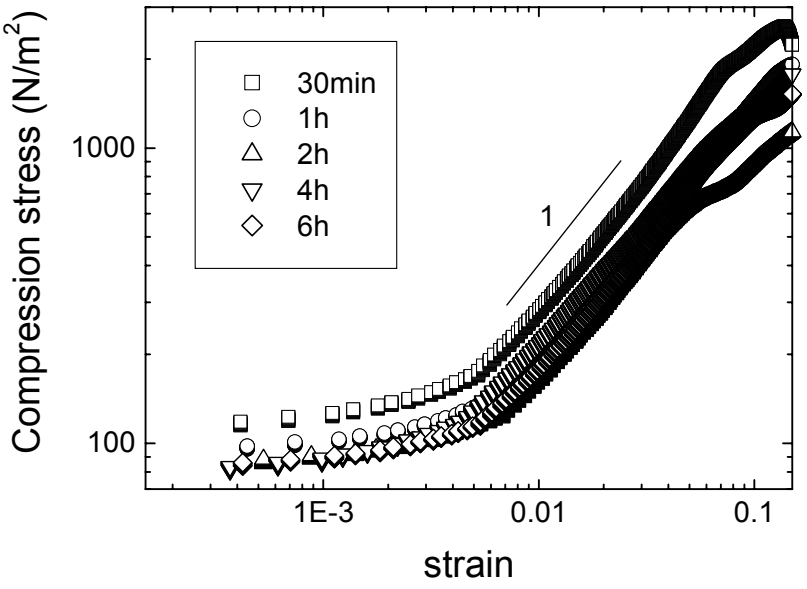


Figure 4.11 - Stress-strain curves recorded during penetration tests performed on equilibrated gels containing κ/ι-hybrid carrageenans (1.5%wt in 0.05M KCl) obtained with different extraction times: 0.5 hour (□), 1 hour (○), 2 hours(△), 4 hours(▽) and 6 hours(◇). The solid line indicates the linear regime where the Young modulus is calculated, whereas the (non linear) regime below 1% strain corresponds to the probe accommodation to the gel surface.

Table 4.5 – Molecular weight distribution, chemical structure and gel properties of carrageenan extracted from *M. stellatus* varying the extraction parameters (alkaline pre-treatment duration *PT*, duration *t*, temperature *T* and *pH*).

	Molecular weight distribution			Chemical structure [a]		Gel properties [b]	
Sample label	M_n (10^6 g/mol)	M_w (10^6 g/mol)	I [c]	κ (%)	*Precursors* (%)	T_{end} (ºC)	*E* (kPa)
M8	0.50	1.20	2.4	51.2	21.3	71.0 ± 0.5	1.2 ± 0.2
M9	0.50	1.90	3.8	54.1	15.0	72.0 ± 1.0	16.6 ± 0.2
M11	0.47	1.10	2.4	44.7	10.1	72.7 ± 0.5	16.6 ± 0.1
M15	0.70	2.20	3.2	56.8	16.5	70.0 ± 0.2	2.6 ± 0.1
M16	0.55	2.20	4.0	51.3	16.3	72.7 ± 1.5	5.1 ± 3.3
M19	0.40	1.20	2.7	50.0	17.2	72.0 ± 1.0	1.3 ± 0.1
M21	0.27	0.70	2.5	48.4	23.7	73.1 ± 0.5	1.3 ± 0.1
M22	0.15	0.40	2.7	48.6	19.3	72.1 ± 0.1	7.2 ± 0.7
M23	0.30	1.00	3.5	48.5	22.7	73.7 ± 1.0	24.5 ± 0.8
M24	0.40	1.14	3.0	50.6	16.0	72.4 ± 0.2	14.0 ± 1.0
M25	0.25	0.80	3.2	51.7	15.7	73.0 ± 1.0	14.0 ± 1.5
M26	0.26	0.65	2.5	47.9	17.9	71.1 ± 0.1	15.8 ± 5.2
M27	0.25	0.79	3.2	44.5	22.0	73.4 ± 0.5	13.2 ± 4.5

[a] integrated area relative to the total integrated area

[b] 1.5%wt carrageenan in 0.05M KCl

[c] polydispersity index I = M_w/M_n

4.2.2.3. Effect of alkaline pre-treatment duration PT on chemical structure and gel properties

Increasing the alkaline pre-treatment duration *PT* and keeping all other extraction parameters unchanged (*t* = 2 h, *T* = 95ºC and pH 8) did not modify qualitatively the FTIR or ^{1}H-NMR spectra of extracted biopolymers, which all remained of the κ/ι-hybrid carrageenan type. Rather, a fine alteration in the chemical structure is inferred from a quantitative analysis of FTIR and ^{1}H-NMR spectra. Figure 4.12A shows the band intensity ratio computed from FTIR spectra as a function of parameter *PT*. The ratio 1240/930, which can be related to the sulfate content in the extracted biopolymer (as it is calculated from the band intensity associated with the sulfate groups at 1240 cm^{-1} over the band intensity of C-O-C groups at 930 cm^{-1}), decreases with increasing pre-treatment time. The ratio 845/930, calculated from the ratio of band intensities measured at wavelengths 845 and 930 cm^{-1}, also decreases with increasing *PT;* the meaning of this band intensity ratio will be discussed below (section 4.2.3). The ratio of band intensity at 805 cm^{-1} to band intensity at 845 cm^{-1}, usually associated in the literature with the relative amount of ι-carrageenan monomers with respect to κ-carrageenan monomers (Pereira and Mesquita, 2003), does not exhibit a meaningful variation with parameter *PT*: a minimum is observed for 20 h pre-treatment duration, but values corresponding to non-pre-treated seaweeds are reached at longer times. Relative monomer contents calculated from ^{1}H-NMR peaks are displayed in Figure 4.12B. A clear drop in μ-monomers, the sulfated biological precursors of κ-monomers, is evidenced as the pre-treatment duration is extended, which agrees with results on sulfate content obtained from FTIR spectra analysis. However, the ι-monomer content seems to level off after 20 h, whereas biopolymers appear to contain relatively more ν-monomers as the pre-treatment duration is increased.

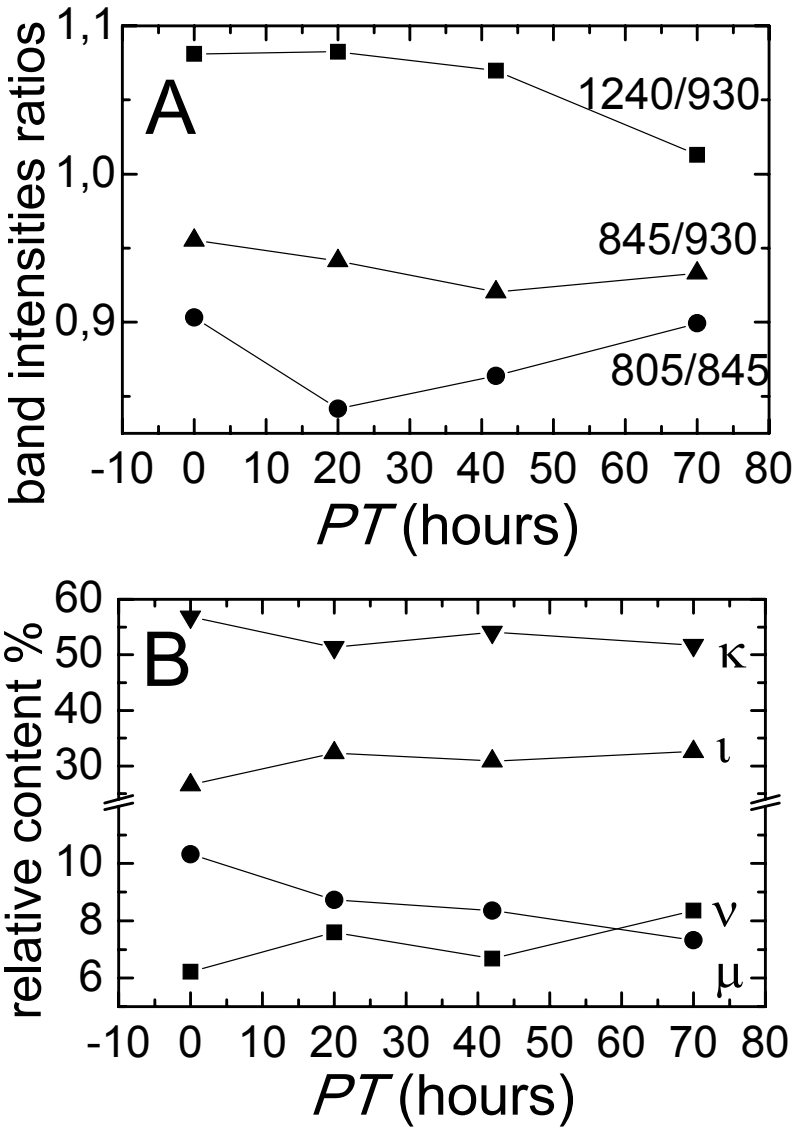


Figure 4.12 - Effect of parameter *PT* on FTIR band intensity ratios (A) and ^{1}H-NMR relative peak intensities (B) assigned to ν-monomers (■), μ-monomers (●), ι-monomers (▲) and κ-monomers (▼).

The corresponding gel mechanical and thermal properties are summarized in Table 4.5 for each pre-treatment duration *PT*, together with a characterization of the molecular weight distribution and the extraction yield. Both *E* and T_{end} are increasing with *PT*, which can be directly related to the drop in biopolymer sulfate content and μ-monomers relative content (Figure 4.12). However, after 42 hours, longer alkaline pre-treatment do not lead to gels with improved mechanical or thermal properties. The cost to pay for biopolymers with better gel elasticity is a loss in extraction yield and

in molecular weight. Therefore, a compromise between pre-treatment duration and gel strength is to be found, if one wants to recover gelling biopolymers in an acceptable amount.

4.2.2.4. Effect of extraction pH and temperature T on chemical structure and gel properties

The impact of *pH* on the gel properties of phycocolloids was screened by performing extractions during t = 2 h with raw seaweeds (PT = 0) at T = 95ºC. Mechanical and thermal characterization of the resulting κ/ι-hybrid carrageenans are compiled in Table 4.5, along with some molecular weight characterization. Alkaline conditions favour the yield of extracted carrageenans, but the corresponding gel properties are poor when compared to the ones obtained with alkali treated seaweeds at room temperature, prior to the hot extraction process. Best gels (with respect to elastic properties) were obtained for a pH 8. ^{1}H-NMR analyses reveal that under this pH condition, a biopolymer with comparatively lower ι-monomers is obtained, whereas the sulfate content as determined by FTIR spectroscopy does not depend on the pH used during extraction of raw seaweeds.

The role of the extraction temperature T on the gel and thermal properties of recovered biopolymers is summarized in the two last rows of Table 4.5. Again, raw algae were used (PT = 0) and other parameters were kept constant (pH 8 and t = 2 h). The Young's modulus is an increasing function of parameter T, whereas other physical properties do not present any monotonic variation with this parameter. In particular, the extraction performed at T = 80 ºC yields κ/ι-hybrid carrageenans with a better melting temperature T_{end} than the polysaccharide obtained at 110 ºC or 95 ºC. The highest gel melting temperature reached in the present study is 73.7 ºC for the sample M23 (PT = 48 h, T = 95ºC and pH 8).

4.2.2.5. Mechanical properties of κ/ι-hybrid carrageenan gels

Gels obtained by cooling κ/ι-hybrid carrageenan hot solutions down to 20ºC need more than 3 h to reach equilibrium at this temperature, as illustrated by the time evolution of the storage shear modulus *G'* measured at 1 Hz and presented in chart B of Figure 4.5. Shortly after reaching 20ºC, the gel elasticity rapidly increases up to a maximum before slowly decaying down to its equilibrium value. Similar gel kinetics has been measured with mixtures of ideal κ-carrageenan and ι-carrageenan homopolymers (Parker et al., 1993) and with ideal ι-carrageenan homopolymers (Hermansson, 1989). Microstructural investigations (Hermansson, 1989; Hermansson et al., 1991) carried out at different times during equilibration evidenced a structural rearrangement of the three-dimensional network responsible for the water syneresis that accompanies the gel kinetics. Visual inspection of κ/ι-hybrid carrageenan gels equilibrated at 20ºC during 24 h in sealed plastic cups shows that the gels do not release any water during equilibration. To further test the hypothesis that shearing plates can stress the building gel and force trapped water to migrate toward the gel-plate interfaces, the experiment depicted in Figure 4.5 was repeated with serrated plates. A comparison of the gel kinetics measured with the two shearing plates on sample M11 is provided in Figure 4.13. The good qualitative reproducibility of both gel kinetics (Figure 4.13A) and gel equilibrium mechanical spectra (Figure 4.13B) rules out the syneresis of water during gel equilibration; the structural rearrangement of the gel network is probably the explanation for the observed gel kinetics. A data analysis inspired from Fourier transform rheology concepts (Wilhelm, 2002) is proposed in Figure 4.14 in order to check whether the structural evolution is induced by the 0.1% strain used during the mechanical testing or is an intrinsic gel building up. Any strain-induced structure or damage developing in the κ/ι-hybrid carrageenan gels is reflected by some non-sinusoidal components (nonlinearity) showing up in the measured stress response to the imposed sinusoidal strain. Signal nonlinearity can be, to a first approximation, estimated by fitting the experimental data to the following periodic function:

$$\sigma(t) = A\sin(wt) + AI_{3w}\sin(3wt + \phi_{3w}) \quad (4.1)$$

where *A* is the amplitude of the signal measured at a frequency ω = 1 Hz (the excitation frequency), $I_{3\omega}$ is the scaled amplitude of the nonlinearity showing up at the third harmonic with phase shift ϕ_{3w}. In the insets of Figure 4.14, where the time evolution of the gel storage modulus *G'* measured with sample M24 is presented, the stress data are displayed at different times during the gel kinetics, together with the corresponding fits obtained with Equation 4.1 using the reported I_{3w} values. This satisfactory data analysis (standard errors of 10% were typically observed for all the I_{3w} values obtained with the fitting procedure, which is acceptable, given the neglect of higher harmonics needed to fully describe a non-sinusoidal waveform) demonstrates that the stress third harmonic scaled amplitude keeps values below 0.2%. The latter are smaller than the I_{3w} parameters calculated for all the corresponding strain signals throughout the duration of the experiment. In other words, the rather small torque nonlinearity originates from the nonlinear deformation imposed by the rheometer (possibly caused by the use of pseudo-strain controlled experiments) and not from any strain-induced damaging of the gels. On the contrary, the time evolution of *G'* evidenced in Figures 4.13 and 4.14 may rather originate from intrinsic structural rearrangements taking place during the equilibration of κ/ι-hybrid carrageenan gels at 20ºC (as discussed below).

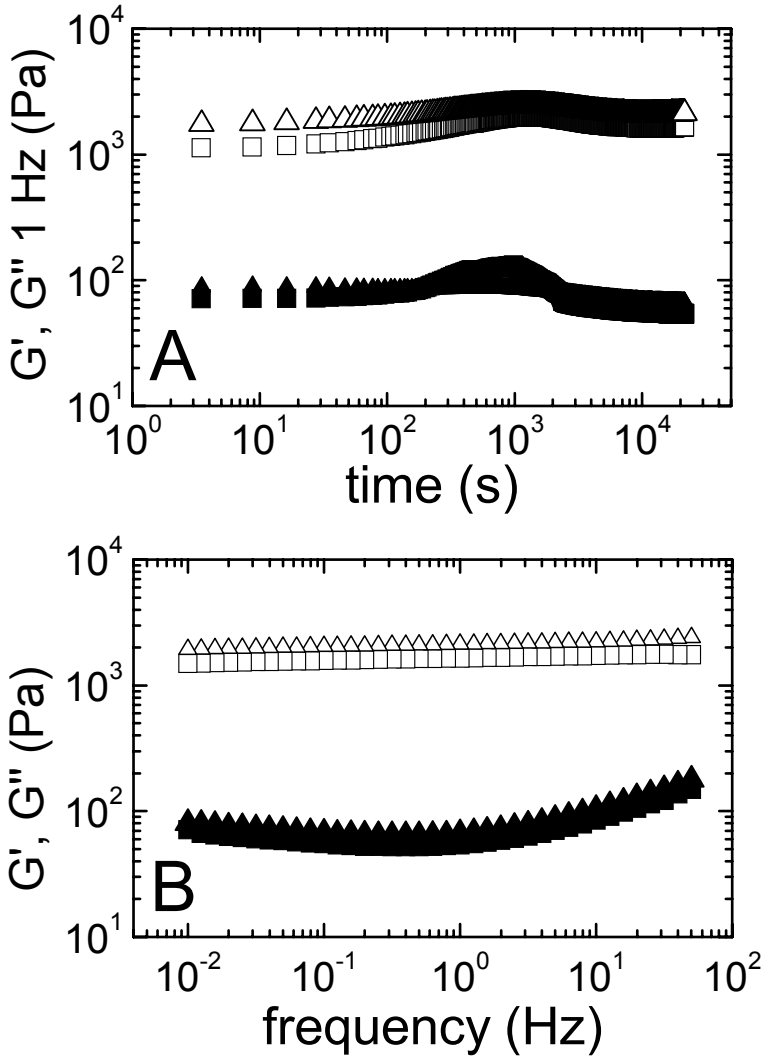


Figure 4.13 - Effect of shearing plates surfaces (□: serrated plates; △: smooth plates) on the reproducibility of experimental data collected for sample M11 at 20ºC. A: time dependence of the storage modulus (open symbols) and loss modulus (solid symbols) measured at 1 Hz. B: frequency dependence of storage and loss moduli (same symbols as in A) measured on the equilibrated gels at 20ºC.

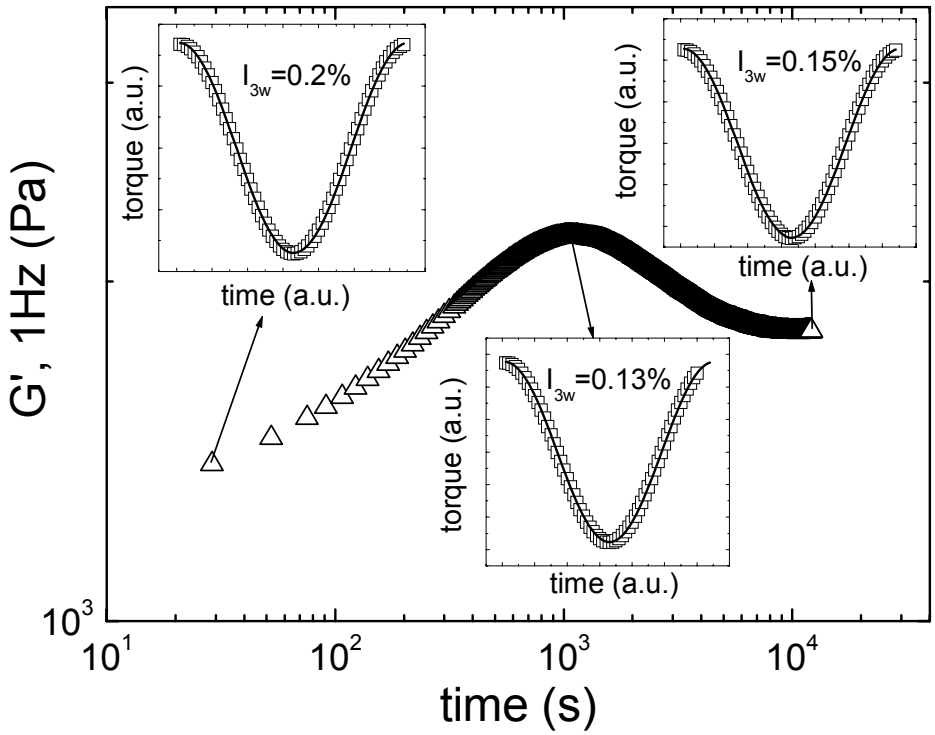


Figure 4.14 - Analysis of the linearity of the stress response measured during equilibration at 20ºC of a κ/ι-hybrid carrageenan gel (sample M24). In the insets, lines are the fits of equation (4.1) to the stress data (□) recorded at selected times (indicated by arrows) during the gel kinetics.

Examples of mechanical spectra measured on equilibrated gels are presented in Figure 4.15. Qualitatively similar viscoelastic behaviours were observed with the remaining κ/ι-hybrid carrageenans listed in Table 4.5. For the three gels depicted in Figure 4.15, the frequency dependence of the tangent of the phase shift angle δ (*tan* δ = *G"*/*G'*) shows a minimum, which suggests the existence of two relaxation times located at frequencies higher and smaller than the experimentally accessible frequencies. Comparison of storage moduli *G'* plotted in the inset of Figure 4.15 with *tan* δ data reveals that increased gel strength (higher *G'*) correlates with increased elastic properties (smaller *tan* δ). This increase in gel elasticity is accompanied by a shift in the location of the *tan* δ minimum toward higher frequencies and a

flattening of the shift angle frequency dependence. The observed flattening is indicative of the widening of the time distribution of the relaxation processes. In a recent study on gels made with ideal κ-carrageenans in 0.1 mol/dm^3 NaCl, Meunier et al. (1999) measured mechanical spectra very similar to the ones shown in Figure 4.15: this suggests that the gels made from κ/ι-hybrid carrageenans structurally resemble the gels made with pure κ-carrageenans. In contrast to these features, weak gels obtained from non-aggregating κ-carrageenan in NaI salt (Ikeda and Nishinari, 2001) as well as gels made with ideal ι-carrageenans (Hossain et al., 2001) both show *G'* and *G"* moduli with very weak frequency dependences.

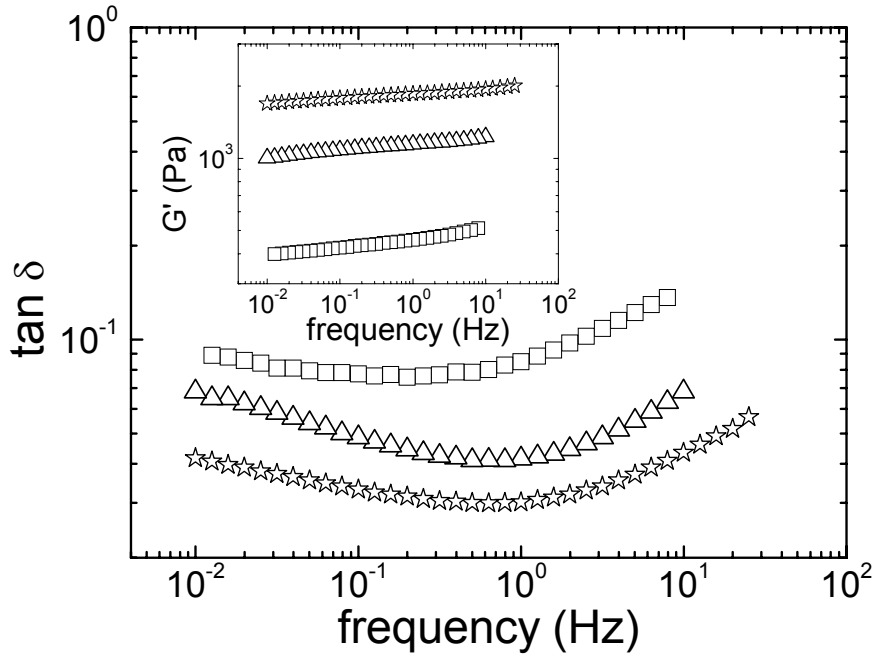


Figure 4.15 - Frequency dependence of the tangent of mechanical phase shift angle δ for equilibrated gels formed at 20ºC with samples M16 (□), M9 (△) and M26 (☆). Inset: frequency dependence of corresponding gels storage moduli *G'*.

4.2.2.6. Interplay between gels thermal properties and gels elasticity

The gel-setting temperatures (T_{gel}) and the gel thermal hysteresis (ΔT, calculated as $\Delta T = T_{melt} - T_{gel}$) are plotted in Figure 4.16 as a function of

their corresponding gel equilibrium storage modulus G_0 measured at 20ºC for all κ/ι-hybrid carrageenan samples listed in Table 4.5. T_{gel} and ΔT are increasing functions of G_0. However, both thermal properties seem to level off for G_0 values higher than 1500 Pa. The data representation proposed in Figure 4.16 suggests that gel thermal properties are closely related to gel elastic properties: stronger gels are easier to form (their gel-setting temperatures are higher) and melt at higher temperatures. Thermal hysteresis is usually reported as a κ-carrageenan gel fingerprint (Piculell, 1995; Takemasa et al., 2001) and is associated with the existence of big aggregates of helical conformers (Ikeda and Kumagai, 1998) exhibiting melting temperatures T_{melt} higher than the temperature T_{gel} at which smaller aggregates start to percolate to form a three-dimensional elastic network. The widely accepted gel mechanism (Piculell, 1995; van de Velde et al., 2002a,b; Lahaye, 2001; Hermansson, 1989; MacArtain et al., 2003; Ikeda et al., 2001; Meunier et al., 1999; Ikeda and Kumagai, 1998; Takemasa et al., 2001; Ikeda and Nishinari, 2001) for κ-carrageenans provides a good explanation for the interplay between thermal and mechanical behaviours: as more helices are bundled to form a mechanically effective cluster, the latter becomes more elastic, since the density of cross-links inside the aggregate is raised, and higher temperatures are needed to disrupt the bundles of helices. T_{gel} and T_{melt} values measured in the present study for the whole set of extracted κ/ι-hybrid carrageenans are in fairly good agreement with values recently reported (Chanvrier et al., 2004) for a commercial κ/ι-hybrid carrageenan containing 50% κ-carrageenan monomers. A final comment on the thermal properties of the κ/ι-hybrid carrageenan gels extracted from *Mastocarpus stellatus* deals here with the monotonic decrease or increase of *tan* δ observed during the cooling (Figure 4.5A) or the heating (Figure 4.5D), respectively, of all samples listed in Table 4.5. Such smooth temperature dependences contrast with the thermal behaviour measured some time ago by Parker et al. (1993) for a mixture of κ-carrageenan and ι-carrageenan under similar salt conditions. The authors described *G'* temperature dependence as a "two-step gelation", attributed to the build-up of two phase-separated networks formed by ι-carrageenan at

higher temperatures and by κ-carrageenan at lower temperatures. The discrepancy between this non-monotonic thermal behaviour and the temperature sweeps depicted in Figure 4.5 (the thermal dependence of *G'* was checked to be equally monotonic) is in favour of a statistical distribution of blocks of ι-carrageenan and κ-carrageenan monomers making up the present κ/ι-hybrid carrageenan copolymer. Actually, such macromolecular architecture prevents the microphase separation of κ- and ι-carrageenan monomers, and the formation of two phase-separated or interpenetrated networks is consequently forbidden. Alternatively, under the present salt and polymer concentration conditions, the network associated with ι-carrageenan monomers is elastically far weaker than the network associated with κ-carrageenan monomers and cannot be resolved during the temperature sweeps. Indeed, for all concentration tested so far and ranging from 0.2% to 2.5% w/w (Hilliou and Gonçalves, 2006), temperature sweeps with a single step in *G'* and *G"* have been recorded. However, experiments carried out with 0.1 mol/dm^3 NaCl salt and with biopolymer concentrations above 2% w/w (Hilliou and Gonçalves, 2006) revealed that the ι-carrageenan network shows up at high temperatures (above 50ºC) as a kink in the otherwise monotonic temperature dependence of *G'*, prior to the development of the elastically stronger κ-carrageenan network.

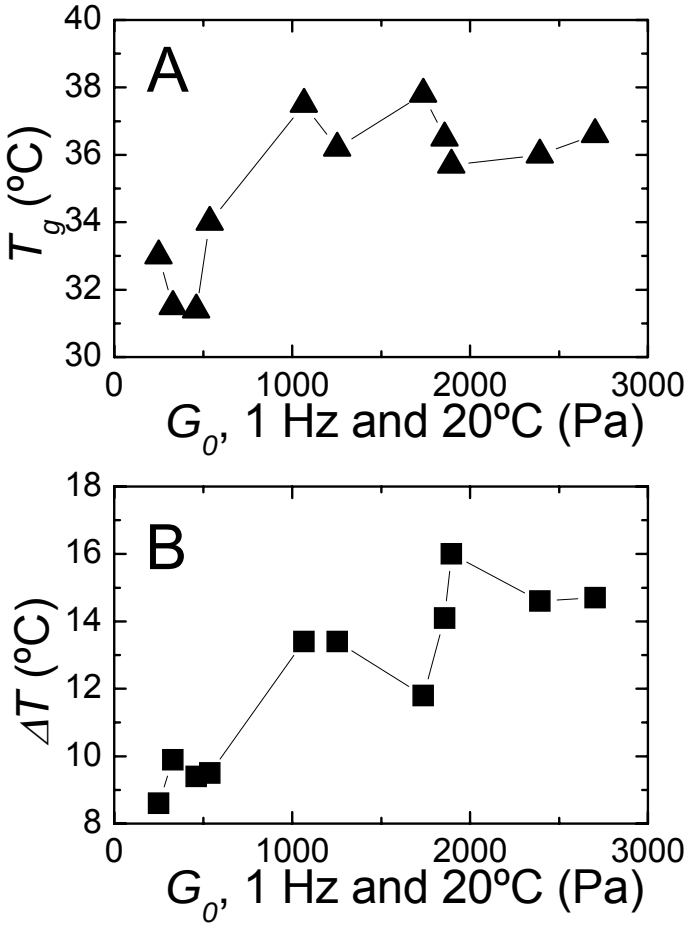


Figure 4.16 - Relationship between gels thermal properties and gel elastic properties at 20ºC. A: gel-setting temperature T_g as a function of the corresponding gel elastic modulus G_0. B: thermal hysteresis ΔT as a function of the corresponding gel elastic modulus G_0.

4.2.2.7. Correlation between gel elasticity and molecular weight distribution

A comparison between the molecular weight distributions (characterized by M_w and M_n) of the κ/ι-hybrid carrageenan biopolymers and the elastic modulus G_0 of the corresponding gels is proposed in Figure 4.17. This representation clearly indicates that both M_w and M_n are decreasing functions of G_0. This result is rather surprising if one notes here that all M_w values are above the κ-carrageenan ideal homopolymer critical molecular

weight beyond which gel elasticity shows no molecular weight dependence (Rochas et al., 1990). This unexpected behaviour can be understood by bearing in mind the copolymer nature of the κ/ι-hybrid carrageenans. Indeed, a prerequisite for gel formation is the existence of blocks of pure κ-carrageenan or pure ι-carrageenan monomers with lengths bigger than the critical one for helix formation. In a recent study (van de Velde et al., 2005), minimum lengths for helix formation have been calculated: these are eight or nine κ-carrageenan monomers and two or three ι-carrageenan monomers. However, the presence of biological precursors may lead to a dramatic increase in this minimum length. The monomers in the κ/ι-hybrid carrageenan macromolecular chain that does not participate in helix formation and subsequent aggregation will essentially act as free dandling chains, thereby causing a plasticizing effect to the gel network. As such, bigger κ/ι-hybrid carrageenan molecular weights bring more free dandling chains to the gel structure, which exhibits inherent softer elasticity. One may conclude at that point (this result is discussed below, based on the statistical analysis of the data) that the gel elasticity of κ/ι-hybrid carrageenans extracted from *Mastocarpus stellatus* can be tuned by controlling the molecular weight of the polysaccharides. As was showed in section *4.2.2.2*, this control could be achieved by monitoring the extraction time: the longer the extraction duration, the shorter the resulting biopolymers, with no modification of the polysaccharide chemical structure.

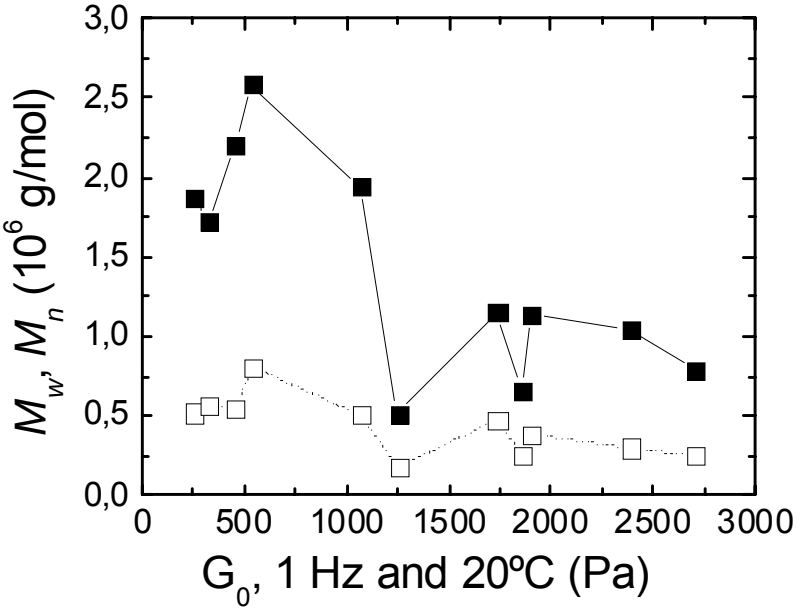


Figure 4.17 – κ/ι-hybrid carrageenans molecular weights M_w (■) and M_n (□) as a function of the corresponding equilibrium gels elastic modulus G_0.

4.2.2.8. Relationships between chemical structure and gel mechanical properties

Systematic plots of mechanically relevant chemical properties as a function of gels elasticity G_0 were prepared in an attempt to correlate the κ/ι-hybrid carrageenan chemical structure to product functional properties such as gel elasticity. In Figure 4.18, the chemical characteristics determined with FTIR spectroscopy are compared to G_0. The degree of κ-ι hybridization can be calculated by computing a FTIR band intensity ratio (Pereira and Mesquita, 2003; McCandless et al., 1983): the 805 cm^{-1} absorption band intensity over the 845 cm^{-1} absorption band intensity. This ratio, hereafter denoted as ι-content, can be seen as an indication of the relative amount of ι-carrageenan monomers with respect to all other gelling and non-gelling carrageenan monomers in the polysaccharide. As such, and under the assumption of (*i*) a blockwise distribution of ι-carrageenan monomers along the copolymer chain, (*ii*) the absence of correlations between the ι-content and other elastically relevant parameters (as discussed below), and (*iii*) a

gel elasticity solely arising from ι-carrageenan monomers, the ι-content is likely to be an increasing function of G_0. The ι-content is plotted as a function of the gels elasticity G_0 in Figure 4.18A. Despite some scattering in the data at low G_0 values (softer gels), results suggest that a higher content in ι-carrageenan monomers results in improved gel elastic properties. In a similar way, the degree of sulfate groups in the biopolymer (DS) is usually estimated from FTIR data (Rochas et al., 1986) by computing the ratio of the band intensity at 1250 cm^{-1} over the band intensity at 2920 cm^{-1} (the latter corresponding to CH groups). Comparison of DS with gel elastic properties is relevant, since DS is a direct measure of the amount of nongelling carrageenan units in the polysaccharide. The increase in gel elasticity with decreasing values of DS is widely documented in the literature (Piculell, 1995) for ideal κ- or ι-carrageenan homopolymers and is explained by the fact that biological sulfated precursors locally stop the homopolymer stereochemical regularity (the blockwise distribution of gelling monomers is interrupted by nongelling monomers), thus restricting the polysaccharide conformational transition from coil to aggregating helices (van de Velde et al, 2002a). However, due to spectral width limitations in FTIR data (absorptions were recorded between 600 and 1400 cm^{-1}), it was prompted to propose the ratio between the band intensity at 1250 cm^{-1} and the band intensity measured at 930 cm^{-1}, as an alternative to the estimation of DS. The use of the band showing up at 930 cm^{-1} is motivated by the fact that the latter is specific to less sulfated κ- and ι-carrageenan monomers (Chopin et al., 1999; Pereira and Mesquita, 2003; Volery et al., 2004). The new ratio DS is plotted in Figure 4.18B, again as a function of G_0. As expected, data indicate that more elastic gels are obtained for κ/ι-hybrid carrageenans bearing less sulfated groups and thus validate the use of the proposed DS calculation.

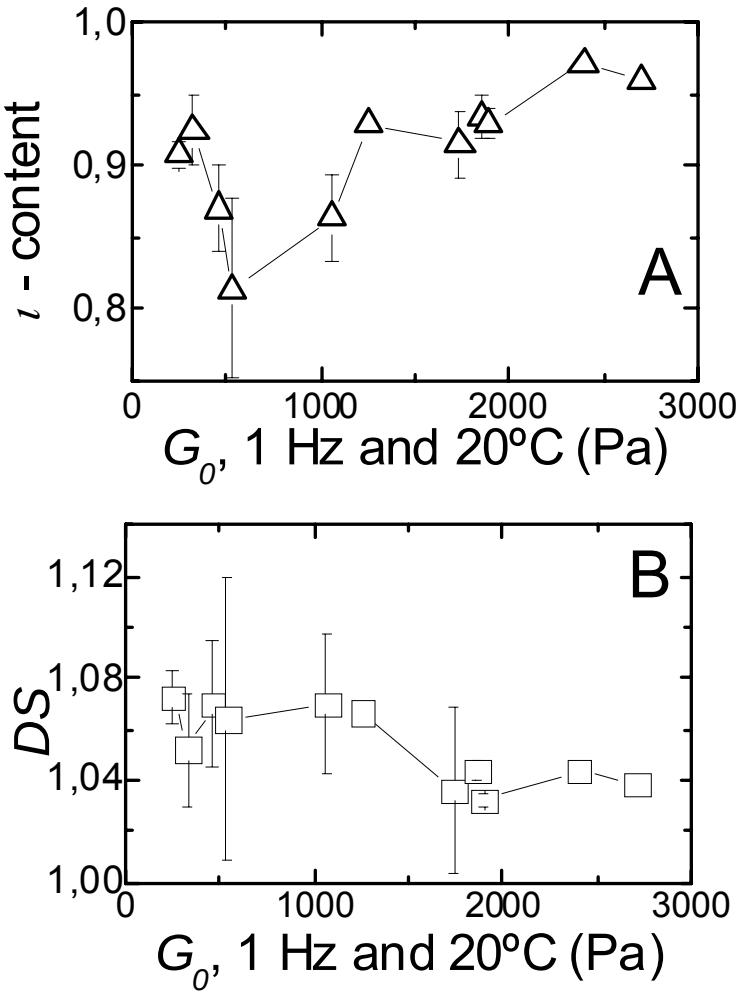


Figure 4.18 - Relationship between κ/ι-hybrid carrageenan chemical structure as measured by FTIR spectroscopy, and equilibrium gel elastic modulus G_0. A: ι-content as a function of G_0. B: degree of sulfate *DS* as a function of G_0.

A comparison between ^{1}H NMR quantitative analysis and G_0 is summarized in Figure 4.19. The ^{1}H NMR analysis relies on the computation of ratios of peak intensities (integrated areas) assigned to a specific carrageenan monomer over the sum of all peak intensities considered in the analysis. For instance, the relative content in κ-carrageenan monomers is obtained from the ratio of the peak intensity measured at 5.11 ppm over the sum of peak intensities recorded at 5.11, 5.32 (for ι-carrageenan monomers), 5.52 (for ν-carrageenan monomers) and 5.26 ppm (for μ-carrageenan monomers). Data gathered in Figure 4.19 show that the ^{1}H NMR peak intensity ratio giving the

relative content in κ-carrageenan monomers is roughly an increasing function of G_0, if data scattering at low G_0 is neglected: relative content ranges from 43% (softer gels) to 52% (stronger gels) for a corresponding 10-fold increase in gel elasticity (G_0 increases from 250 to 2700 Pa). A recent study (van de Velde et al., 2005) on a series of κ/ι-hybrid carrageenans extracted from different seaweeds and containing from 60% to 100% κ-carrageenan monomers with less than 2% nongelling monomers showed a very similar relationship between the gels elasticity and the relative content in κ-carrageenan monomers. In contrast to that, peak intensity ratios computed for both ι-carrageenan and biological carrageenan precursor monomers ν and μ (the latter ratio is obtained by summing the ratios corresponding to ν-carrageenan and μ-carrageenan monomers) and displayed in Figure 4.19 are virtually not depending on G_0. Poor NMR detection sensitivity (peak relative intensity ranging from 5 to 10% for ν-carrageenan monomers) is apparently responsible for the appreciable lack of correlation between G_0 and the relative content in biological carrageenan precursor monomers. Note here that, in the light of the discussion developed above for DS, depressed gel elasticity should correlate with polysaccharides containing more biological precursors. The non-dependence of ι-carrageenan monomers relative content on gel elastic properties is more questionable, as this result is hard to reconcile with the ι-content measured with FTIR spectroscopy (Figure 4.18). Actually, both chemical analyses should render the same experimental output, as both spectroscopic methods assess the same chemical property. A plausible reason for the absence of consistent results might again lie in the poor ^{1}H NMR quantitative sensitivity to small amounts of biological precursors, which hampers the interpretation of computed peak intensity ratios. In particular, μ-carrageenan monomers show up as a shoulder to the ι-carrageenan peak in the ^{1}H NMR line shape (Figure 4.6), thus posing serious limitations to a correct quantitative measure of respective signals intensities. In the light of the results presented above, it seems that for the peculiar case of *Mastocarpus stellatus* seaweeds, a quantitative analysis of the biopolymers chemical structure is better achieved by means of FTIR spectroscopy, since the latter

technique produces chemical parameters that reliably correlate with the elastic properties of κ/ι-hybrid carrageenan gels.

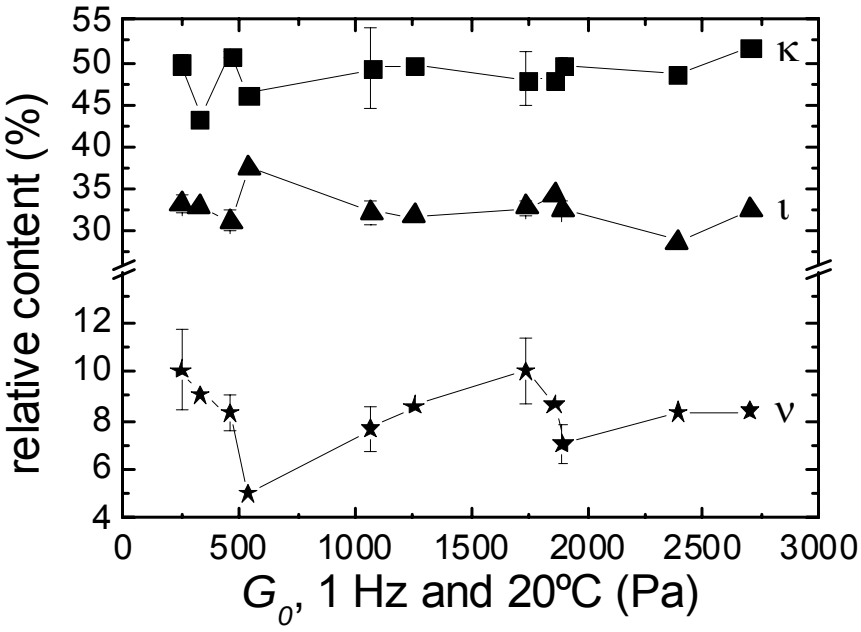


Figure 4.19 - Relationship between κ/ι-hybrid carrageenan chemical structure as measured by ^{1}H-NMR spectroscopy and gel elastic modulus G_0: relative content of κ-carrageenan monomers (■), ι-carrageenan monomers (▲), and biological precursor monomers (★).

4.2.3. Discussion

The phycocolloids with best gelling properties obtained throughout this study exhibit Young's modulus ranging from 14 to 24.5 kPa (data obtained with *PT* = 48 h in Table 4.5). This is roughly two orders of magnitude higher than values reported in the literature for ι-carrageenan gels under equivalent salt, polymer concentration and test temperature conditions (Michel et al., 1997). On the other hand, these gels show only slightly weaker elasticity than their κ-carrageenan counterparts (Rochas et al. 1990), but with no water syneresis. This intermediate gel elasticity is an intrinsic property of κ/ι-hybrid carrageenan biopolymers (Chanvrier et al., 2004), which are now recognized as statistical block copolymers (van de

Velde et al., 2001, 2005; Bixler et al., 2001; Falshaw et al., 2003; Villanueva et al., 2004) produced by all carrageenophytes (Lahaye, 2001; Chopin et al., 1999) in a wide variety of block composition.

Extraction parameters are seen to affect the κ/ι-hybrid carrageenan composition. For instance, the content in μ-monomers, which can be seen as a non gelling monomer and therefore a defect in the gel structure, can be varied from 10 to 7% by increasing the pre-treatment duration (Figure 4.12B). The alkali induced μ-monomer transformation into less sulfated κ-monomers is also mirrored by the slow decrease in the 1240/930 ratio (Figure 4.12A) and by the noticeable decrease in the 845/930 ratio. Indeed, this ratio decreases when the band intensity related to C-O-C groups in the 3,6-anhydrogalactose increases as a result of the substitution of the sulfate groups. Note that all monomers except the λ-carrageenan ones show a band at 845 cm^{-1} (Chopin et al., 1999; Prado-Fernández et al., 2003), and therefore this band intensity should not vary with any chemical treatment, since no λ-carrageenan monomers are detected in the present phycocolloids. Therefore, the alkali pre-treatment, performed prior to extraction, leads to essentially less sulfated κ/ι-hybrid carrageenans with correspondingly better gelling properties. This result comes as no surprise, since alkali pre-treatment of a wide variety of carrageenophyte seaweeds is performed in extracting industries, or alternatively, hot extractions in alkaline conditions are performed (Piculell, 1995; van de Velde et al., 2002a,b; Lahaye, 2001). It was simply note here that, in the specific case of *Mastocarpus stellatus* seaweeds, the latter process will not give κ/ι-hybrid carrageenans with improved gelling properties when compared to seaweeds submitted to a cold and long pre-treatment, as suggested from the results reported for pH 9 in Table 4.5. Extraction time is not the parameter of choice for tuning the end-product chemical structure, as far as ι-monomers and ν-monomers are concerned (Figure 4.9). After 1 hour extraction, the κ/ι-hybrid carrageenan chemical composition does not change, and mechanical or thermal properties of the resulting gels are accordingly unaffected (Table 4.5). Actually, longer extraction times will be only

preferred if one wants to increase the yield in extracted biopolymers with minor changes in the ratio between κ-monomers and ι-monomers, as suggested from the ^{1}H-NMR data in Figure 4.9. Extraction temperature, or equally pH, is a more efficient parameter in modifying the biopolymer chemistry. These features are illustrated in Figure 4.20 where the gel elasticity of all products extracted during this study, is plotted as a function of their corresponding chemical composition (relative content in μ- and κ-monomers). This figure indicates that the content in κ-monomers can be varied from 57% to 45% and the content in non gelling μ-monomers can be varied from 6% to 14% of the total amount in monomers, by choosing specific sets of extraction parameters.

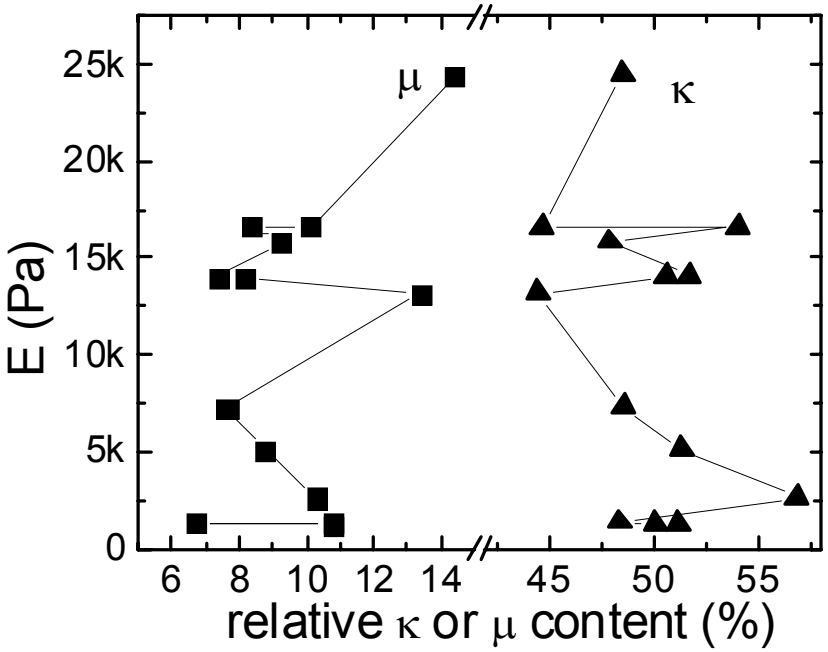


Figure 4.20 – Young's modulus *E* of equilibrated gels as a function of the κ/ι-hybrid carrageenans chemical structures characterized by their relative content in μ-monomers and κ-monomers.

Consequently, gel elasticity can be tuned from 1.2 up to 24.5 kPa, but no correlation with the biopolymers chemical structure can be inferred from Figure 4.20. Actually, one could expect that more κ-monomers in the biopolymer contribute to build a more elastic gel, as recently reported in a

study (van de Velde et al., 2005), where phycocolloids containing an increasing fraction of κ-monomers (with a minimum amount of 60 mol%) showed increasing gel elasticity. In the present case, the low amount in κ-monomers, together with the low sensitivity of the texture analyzer (which was evident from the scattering in data at low Young's modulus values in Figure 4.20), may explain the absence of correlation between gel mechanical properties and biopolymer chemical structure. In addition, the fraction in κ-monomers is perhaps not the relevant parameter to relate to gel elasticity. In fact, the fraction of blocks of κ-monomers or ι-monomers with sufficient lengths for gel formation (M_w greater than 40 000 g/mol for κ-carrageenans; Rochas et al., 1990) are the physically relevant entities to be quantified in κ/ι-hybrid carrageenans, but this characterization is beyond the scope of the present study.

An attempt to relate gel melting properties to the κ/ι-hybrid carrageenan chemical structure is proposed in Figure 4.21. The gel melting process is usually described as the heat induced disruption of primarily small bundles of few helices or even isolated helices at lower temperatures, followed by the destruction of bigger helix aggregates at higher temperatures (Ridou et al., 1996). As such, the gel melting characterization offers the possibility to investigate the aggregate size distribution in the gel. T_{end} can therefore be associated with the size of the largest clusters, whereas the broadness of the melting process (roughly quantified by $\Delta T = T_{end} - T_{on}$) reflects the width of the aggregates size distribution. Data gathered in Figure 4.21 suggest that temperature T_{end} does not truly correlate with the relative content in μ-monomers. This lack of correlation can be explained by the rather weak NMR signal (corresponding to the shoulder at 5.26 ppm in Figure 4.6) originating from the small amount of μ-monomers in the biopolymers, which complicates the exact determination of the associated relative content. To overcome this lack of resolution, ^{13}C-NMR spectroscopy should be preferred to ^{1}H-NMR spectroscopy (van de Velde et al., 2002a,b). On the other hand, Figure 4.21 shows that T_{end} is higher when less κ-monomers are relatively present in the biopolymer. The rather high gel melting temperatures

measured here (roughly ranging from 70 to 74ºC, Table 4.5), suggest that T_{end} is raised as a result of the increase in the fraction of ι-carrageenan gelling blocks (actually, T_{end} is also seen to increase with the FTIR band intensity ratio 805/845 corresponding to the relative content in ι-monomers), which are known to form gelling structures with higher melting points than the ones formed by κ-carrageenan blocks (Ridou et al., 1996), given the salt and biopolymer concentrations used here.

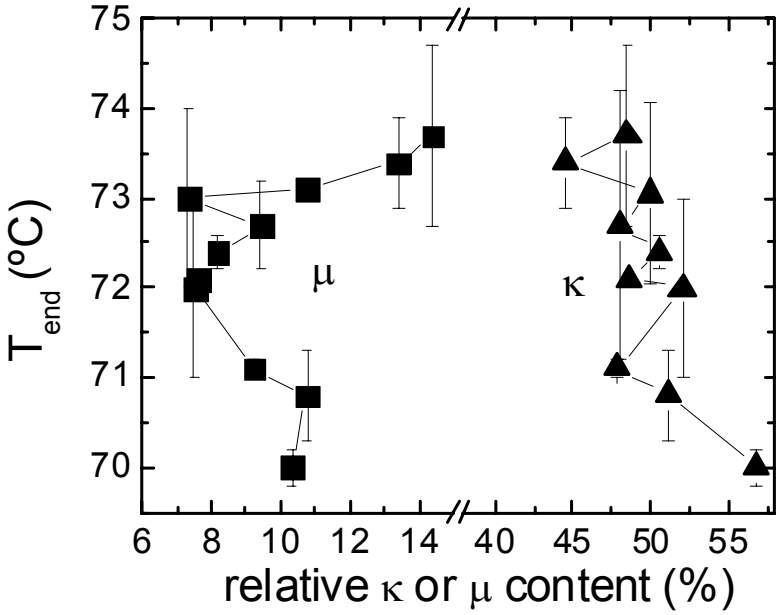


Figure 4.21 – Gel melting temperature T_{end} of equilibrated gels as a function of the κ/ι-hybrid carrageenans chemical structures characterized by their relative content in μ-monomers and κ-monomers.

Figures 4.20 and 4.21 show that the elastic and thermal properties of the gels obtained from the κ/ι-hybrid carrageenan isolated from *Mastocarpus stellatus* under various extraction conditions cannot be simply correlated to the polysaccharides chemical structure. This is partly explained by the complex interplay between gel properties and elastically relevant chemical or physical parameters such as the total amount in biological precursors (van de Velde et al., 2002a,b), the ratio between κ-monomers and ι-monomers (van de Velde et al., 2005), or the polysaccharide molecular weight (Rochas et al., 1990). A statistical treatment of data reported in Table 4.5 has been

carried out in an attempt to separate the effects of such parameters (the content in biological precursors monomers in Table 4.5 has been computed from the addition of intensities of peaks assigned to both μ-monomers and ν-monomers and labelled as precursors), on the measured E, T_{end} and ΔT. No conclusive correlations could emerge from this analysis, as the too small set of data showed rather bad statistics, especially for the Young's modulus (big scattering in the data associated with weaker gels) and parameter ΔT (low sensitivity in DSC signals impeding a reliable determination of T_{on}).

The time evolution of the storage modulus *G'* shows a maximum as the gel progresses toward its mechanical equilibrium. Before providing an interpretation for this maximum, a check for possible experimental artefacts first needs to be carried out. It is worth noting here that gel kinetics similar to the ones displayed in Figures 4.5, 4.13, and 4.14 have been systematically observed for all biopolymers listed in Table 4.5 and for all sample thicknesses (ranging from 0.8 to 1 mm) used throughout this study. In addition, a maximum in *G'* was also resolved in tests performed with a cone and plate geometry: both maximum amplitude and time location showed good reproducibility with tests performed with smooth plates. The use of excessive strain as a possible source for experimental error has been suggested above, and Fourier transform analysis of both torque and strain signals has been performed to establish the absence of strain-induced structural rearrangements within the gels. As an ultimate check, experiments depicted in Figure 4.5A, B have been reproduced with the strain set to 0. The mechanical spectrum of the gel obtained after this sample treatment showed a satisfactory reproducibility (*G'* and *G"* values differing by less than 15%) when compared to data displayed in Figure 4.5C: this result confirms that strain-induced structural rearrangement is not related with the observed gel kinetics. The latter is rather the signature of a structural property of carrageenan gels (Hermansson, 1989) and should therefore depend on parameters such as the biopolymer concentration. Preliminary studies are presented in Figure 4.22, where the time dependence of *G'* is plotted for five different concentrations of biopolymers

solutions obtained with sample M25 (the latter showed the best gelling properties of the κ/ι-hybrid carrageenans studied here). For concentrations below 0.5% w/w, *G'* exhibits a monotonic increase before reaching an equilibrium plateau. This gel kinetics is reminiscent from the one obtained some time ago for κ-carrageenan solutions with similar concentrations (Meunier et al., 1999). When the concentration is raised above 0.5% w/w, the *G'* maximum is resolved. The latter appears at earlier times in the gel kinetics and with increased amplitude as the concentration is increased. The maximum time location and amplitude also depend on the cooling rate used to set the gel: a bigger maximum occurs at earlier times if the cooling rate is slower. These features suggest that aggregation phenomena are involved in the intrinsic structural rearrangement of the gel. As the temperature is lowered, helices are formed and aggregate in bundles of helices (Piculell, 1995; Hermansson, 1989, MacArtain et al., 2003; Ikeda et al., 2001). These bundles act as cross-links between the remaining parts of macromolecules that did not adopt a helix conformation, as biological precursor monomers limit the extent of conformational transition (van de Velde et al., 2002b, van de Velde et al., 2005). As time proceeds, more bundles are formed, thereby increasing the cross-link density in the forming gel. Consequently, the gel elasticity rises and the time evolution of the storage modulus *G'* witnesses this structural build-up. At longer times, the bundles are merging as helix aggregation proceeds, resulting in a decrease in cross-link density, which is mirrored by the decrease in *G'*. As such, a maximum in the storage modulus is expected to show up during the gel kinetics. A simple way to validate this structural picture is to look for correlations between the maximum amplitude or time location and parameters such as T_m or ΔT, which are known to be related to aggregation (Ikeda and Kumagai, 1998; Takemasa et al., 2001). Such a statistical analysis is provided in Table 4.6, where linear correlation coefficients computed with the Statistica software, version 6.1 (Statsoft, USA), are reproduced. The rather low values of the coefficients in Table 4.6 indicate that the kinetics of gel rigidity does not correlate with the gel melting behaviour or gel thermal hysteresis, suggesting that bundles of helices are not the only structural units that

contribute to gel elasticity. Clearly, additional work including in situ structural characterization is needed if one wants to provide a better understanding of *G'* maximum, as no further correlations between the maximum amplitude or time location and κ/ι-hybrid carrageenans chemical composition, molecular weight distribution, and equilibrium elastic modulus G_0 could be evidenced.

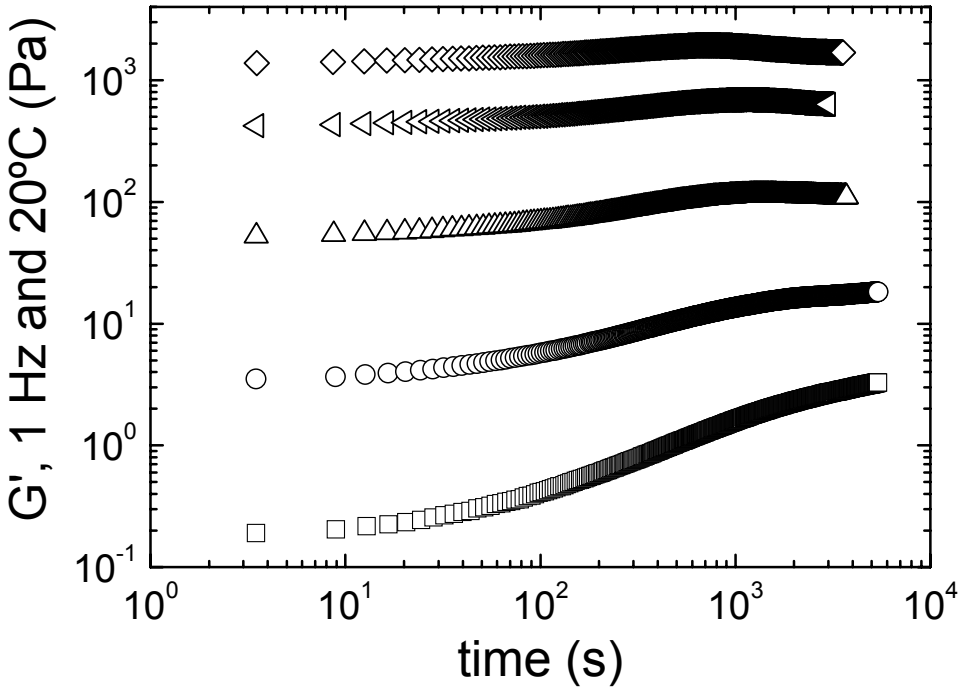


Figure 4.22 – Time dependence of the storage modulus *G'* measured at 1 Hz and 20ºC during the equilibration of the gels obtained for different concentrations of sample M25 in 0.05 mol/dm^3 KCl: (□) 0.2% w/w; (○) 0.3% w/w; (△) 0.5% w/w; (◁) 0.9% w/w; (◇) 1.3% w/w.

Table 4.6 – Correlation coefficients between *G'* maximum amplitude (MAX) and time location (TIME) and gel thermal parameters (T_m, T_g, ΔT) measured for all samples listed in Table 4.2.

	MAX	TIME	T_m	T_g	ΔT
MAX	-	-0.2	-0.4	-0.38	-0.37
TIME		-	0.23	0.36	0.08
T_m			-	0.92	0.94
T_g				-	0.73
ΔT					-

Gels obtained from κ/ι-hybrid carrageenans exhibit an equilibrium elastic modulus G_0 that is related to gel thermal properties (Figure 4.16). Moreover, G_0 depends on the molecular weight distribution (Figure 4.17) and on the biopolymer chemical composition as determined by FTIR experiments (Figure 4.18). A tentative interpretation has been given for each of these results, namely, helix aggregation and tridimensional networking, free dandling chains acting as gel plasticizers, and biological precursor monomers that limit the extent of helix formation and subsequent aggregation. These interpretations are partly motivated by earlier reports on virtual model carrageenan systems (Piculell, 1995; van de Velde et al., 2002a,b; Lahaye, 2001; Hermansson, 1989; MacArtain et al., 2003; Ikeda et al., 2001; Meunier et al., 1999; Ikeda and Kumagai, 1998; Takemasa et al., 2001; Ikeda and Nishinari, 2001; van de Velde et al., 2001; Falshaw et al., 2001; van de Velde et al., 2005) and are based on the hypothesis that G_0 solely depends on the parameter under study, whereas all the others are either kept constant or are simply not correlated with the studied parameter or physical property. The κ/ι-hybrid carrageenans extraction process results in a set of samples that vary by more than one parameter. Therefore, statistical analysis of the correlations between all elastically relevant parameters (T_g, molecular weight distribution and chemical composition) is required in order to discern their respective impact on G_0 and validate the above-mentioned interpretation or alternatively underpin their complex interplay with G_0.

The results of the statistical analysis (performed as mentioned above) of data presented in Figures 4.16, 4.17, 4.18 and 4.19 are displayed in Table 4.7, where linear correlation coefficients between all studied parameters are reported. The gel-setting temperature T_g is not correlated with any chemical parameters nor with the molecular weight distribution, since corresponding coefficients are kept between -0.59 and 0.36. Thus, the dependence of G_0 on chemical parameters and on M_w or M_n will not be blurred by its dependence on T_g. The molecular weight distribution is strongly correlated with the content in ι-carrageenan monomers as determined by FTIR (coefficients of -0.84 and -0.83 for parameters M_w and M_n, respectively). This suggests that longer κ/ι-hybrid carrageenan macromolecules possess proportionally less ι-carrageenan monomers. Unfortunately, chemical parameters, which were obtained from ^{1}H NMR spectra (with inherently big data scattering and experimental errors as evidenced in Figure 4.19) are not correlated with either M_w or M_n. This analysis thus cannot indicate if absent ι-carrageenan monomers in longer biopolymers chains are substituted by κ-carrageenan monomers or rather non-gelling units, which would favour the occurrence of free dandling chains in the gels. A way to overcome this lack of information is to focus now on the thermal hysteresis ΔT, which reflects the extent of aggregation of helices in the gels. ΔT becomes smaller as the molecular weight is bigger (correlation coefficients are truly negative for both M_w and M_n parameters), which suggests that long κ/ι-hybrid carrageenan chains form less aggregates because they contain relatively less helical conformers, whereas shorter biopolymers contain relatively more helices. A final comment deals with the increased gel elasticity G_0 with the increasing relative content in ι-carrageenan monomers, as depicted in Figure 4.18. This positive correlation (the corresponding coefficient reads 0.69 in Table 4.7) is difficult to reconcile with the κ/ι-hybrid carrageenan gel elasticity, which seems rather dominated by a κ-carrageenan-like behaviour as mentioned earlier: gel kinetics is slow and presents a maximum in G', mechanical spectra qualitatively resemble that of pure κ-carrageenan gels, and both ΔT and G_0 values are seemingly bigger than those found in pure ι-carrageenan gels

under similar salt and concentration conditions (Hilliou and Gonçalves, 2006). As mentioned earlier, the ι-content is not an independent parameter, as it strongly correlates with the molecular weight distribution. Therefore, it does not allow the simple rationalization provided in the Results section and based on too strong or unverified assumptions.

Table 4.7 – Correlation coefficients between gel physical parameters (T_m, T_g, ΔT, G_0) and biopolymers chemical parameters (M_w, M_n, DS, ι-content, ι, κ, PREC) measured for all samples listed in Table 4.5.

	T_g	ΔT	G_0	M_w	M_n	DS	ι-content	ι[a]	κ[a]	PREC[a]
T_m	0.92	0.94	0.87	-0.72	-0.69	-0.60	0.49	-0.26	0.35	-0.11
T_g	-	0.73	0.74	-0.59	-0.52	-0.45	0.30	-0.10	0.31	-0.19
ΔT		-	0.87	-0.74	-0.74	-0.65	0.60	-0.36	0.35	-0.01
G_0			-	-0.75	-0.71	-0.77	0.69	-0.36	0.40	-0.06
M_w				-	0.95	0.58	-0.84	0.40	-0.27	-0.09
M_n					-	0.40	-0.83	0.56	-0.47	-0.04
DS						-	-0.62	0.13	0.04	-0.15
ι-content							-	-0.63	0.19	0.36
ι								-	-0.35	-0.52
κ									-	-0.62

[a] ι, κ, and PREC stand for the relative contents in ι-monomers, κ-monomers, and biological precursor monomers, respectively, as determined by ^{1}H NMR and plotted in Figure 4.17.

4.2.4. Conclusions

Carrageenan biopolymers have been extracted from *Mastocarpus stellatus* seaweeds harvested on the northern Portuguese coast. FTIR and ^{1}H NMR spectra revealed that this seaweed mainly contains κ- and ι-monomers with minor amounts (up to a maximum of 15% in relative monomer content) of ν- and μ-monomers. The biopolymer chemical structure can be controlled by monitoring the extraction conditions. Results confirm the benefits of an already industrially utilized process: a long and cold alkaline pre-treatment favours the subsequent extraction of biopolymers containing a reduced quantity of biological carrageenan precursors (ν- and μ-monomers) with no

modification in the relative amount of κ- and ι-monomers, but with an improvement in the resulting gel elasticity. Extraction duration is not seen to change the biopolymers chemical structure that much, whereas both extraction pH and extraction temperature can have a dramatic impact on the biopolymer chemical properties and corresponding gel properties. The main output of this systematic screening of the effect of extraction parameters on the physico-chemical properties of κ/ι-hybrid carrageenans is the delivery of hydrocolloids exhibiting a wide range of gel elastic properties in 0.05 M KCl, with no algal fastidious sorting. In particular, a set of optimized parameters (*PT* = 48 h, *T* = 95ºC, *t* = 4 h and *pH* 8) with respect to the end product gel properties and extraction yield has been determined. The corresponding κ/ι-hybrid carrageenan, obtained with an extraction yield of 24 wt.% on a dry basis, has a molecular weight M_w = 6.5 x 10^5 g/mol with a polydispersity index M_w/M_n = 2.5. The product presents gel properties comparable to k-carrageenan gel formers when mixed at a concentration of 1.5 wt.% in 0.05 M KCl, but with no water syneresis: after an equilibration time of 24 h, the gel showed a Young's modulus *E* = 15.8 kPa at T = 25ºC, and a gel melting temperature of 65.5ºC. An improvement of gel properties and extraction yield is foreseen (preliminary results will be presented elsewhere), based on the fact that seasonal variation in the chemical structure of biopolymers extracted from *Mastocarpus stellatus* has been suggested elsewhere (Pereira and Mesquita, 2003). Gel thermal properties were seen to be correlated with the κ/ι-hybrid carrageenan chemical structure, as less sulfate groups and a higher content in ι-carrageenan monomers result in gel with higher melting temperatures. However, experimental limitations prevent us from obtaining a clear correlation between the biopolymer macromolecular structure properties and the mechanical properties of the corresponding gels. Rheological study performed allowed a better assessment of the thermal and elastic properties of the κ/ι-hybrid carrageenan gels.

The gelling power of carrageenan imparts excellent film forming properties, and due to these properties was decided to study the production of

biodegradable films and coatings from κ/ι-hybrid carrageenan extracted from *Mastocarpus stellatus*, and give a new application to this underexploited macroalgae founded in the Portuguese coast.

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Chapter 5
Production and characterization of films

In this chapter the methods used to produce and to measure the properties of biodegradable films from the biopolymers studied are presented.

5.1. Film production

Various techniques have been proposed to produce films. The simplest process is casting, which is used for free-standing films. The liquid solution is poured onto a smooth, flat surface to a controlled thickness. Films can be also prepared with a spreader. The spreader draws solution from a product reservoir through an adjustable gap to control its thickness. The spreader is drawn over a substrate to deposit the solution at the desired thickness. These are batch processes not conducive to continuous processing and are normally too costly (Kozempel and Tomazula, 2004). The solution can sometimes be extruded into a continuous film (Gennadios, 2002). For example, some films are produced by extruding a viscous aqueous

suspension into a neutralizing coagulation bath, washing, plasticizing, and drying. This usually applies to highly concentrated, viscous solutions or melts at elevated temperatures. Sometimes, the best method is to dip rollers into the solution to pick up the solution (Shepherd, 1995). The film forms on the rolls and is the doctored off. For a dilute aqueous solution, such as studied in this research, the alternatives tested were casting and knife coating methods.

5.1.1. Casting

Films as stand-alone structures have been obtained in laboratories by laying or spreading a film-forming solution on a support, drying it and then detaching it. These stand-alone films are also used as testing structures for determination of barrier, mechanical, solubility and other properties.

Aqueous solutions of biopolymers or their mixtures were heated to 90°C under stirring during 30 minutes for their total solubilization (Figure 5.1A). The film-forming solution was then spread over a Teflon® plate (Figure 5.1B) and let to dry at room temperature for 24h to form the film (Figure 5.1C). The film formulations are described in Chapter 6.

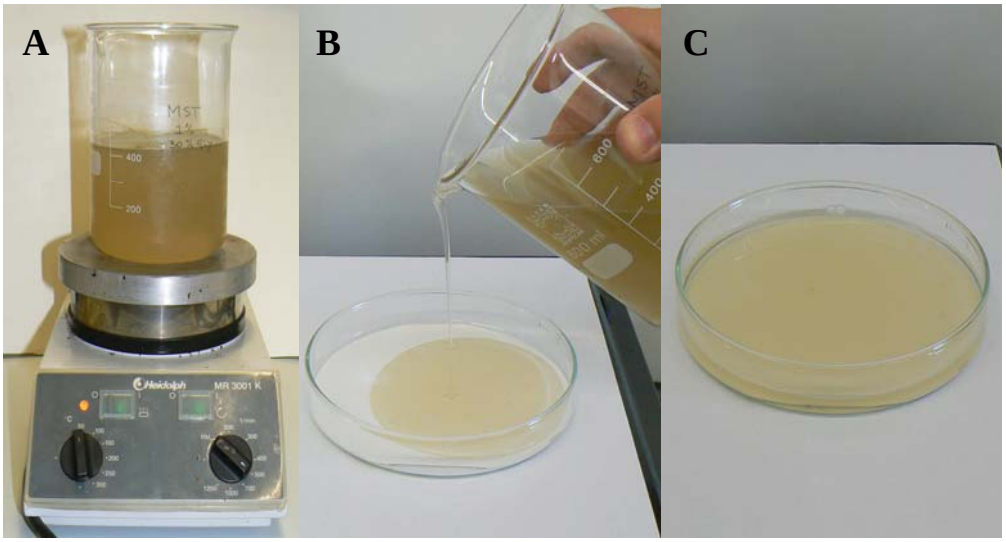

Figure 5.1 – Film production by the casting method: (A) preparation of film forming solution; (B) spreading over a support; (C) drying at room temperature.

5.1.2. Knife coating

It is relatively easy to make films in the laboratory by pouring a solution of biopolymers and water into a dish and allowing it to dry undisturbed to properly form a thin film (casting method). While this method is the most commonly used at laboratory scale by most researchers, it can not be turned into a large-scale film making technique.

To overcome such limitation, a semi-continuous knife-coating process was developed to produce films with the biopolymers under study. An Automatic Film Applicator model 1132N (Sheen, UK), with a constant speed guide driving a knife maintained at an adjustable gap from the film support (Figure 5.2), was used to spread the biopolymer solutions uniformly over an acrylic plate. These coatings of uniform thickness were let to dry at room temperature using a fan, forming the film that can be easily removed from the support (Figure 5.3). In this method, thickness, application speed and film length can be controlled and different suitable supports can be chosen for each biopolymer solution used. Furthermore, industrial equipments using this technique exist and are currently used to produce coatings and films from synthetic polymers.

With this knife-coating method, a higher concentrated biopolymer solution (~10% w/w) must be used in order to obtain a more uniform spread over the support and the films took only 2 to 3 hours to be dried at room temperature. The casting method, using more dilute solutions (2% w/w), take about 24 to 48 hours to produce the films at room temperature, which are typically less uniform in thickness and limited in size.

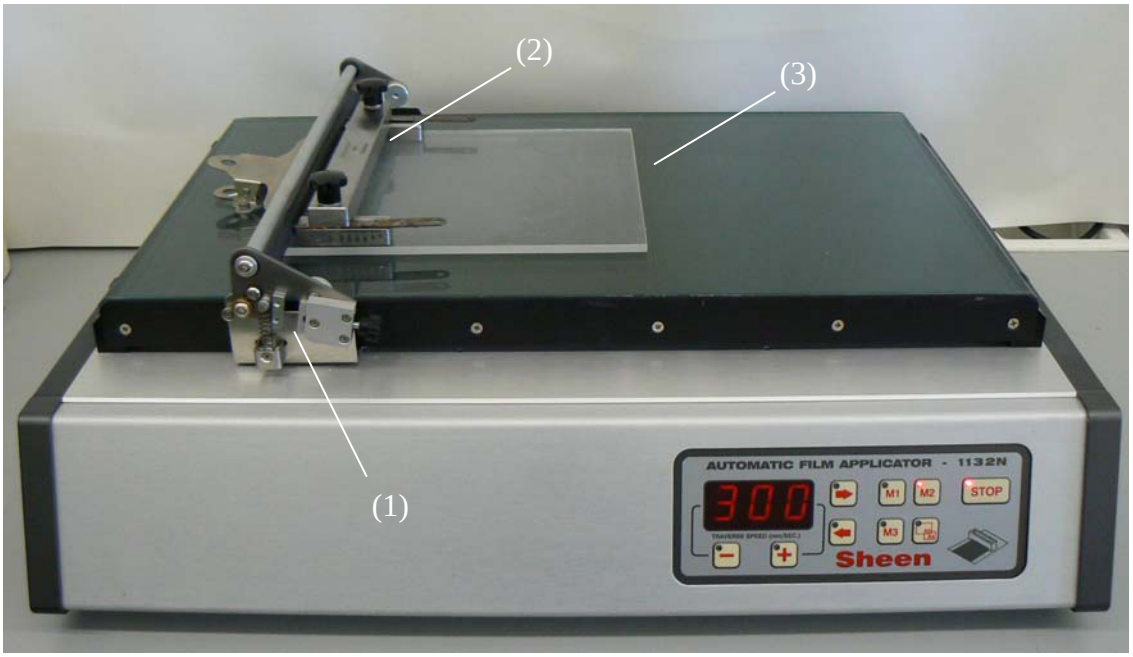

Figure 5.2 – Automatic film applicator: (1) constant speed driver; (2) adjustable gap knife to control thickness; (3) acrylic support.

Aqueous solutions of biopolymers or their mixtures were submitted to heating up to 90°C, under stirring, during 30 minutes for their total solubilization. The film-forming solution was then put over the acrylic support, spread over the support by the Automatic Film Applicator and let to dry at room temperature using a fan to form the film (Figure 5.3). The speed of application is constant, but may be adjusted between 50 and 500 m/s. The film formulations and process parameters used are described in Chapter 6.

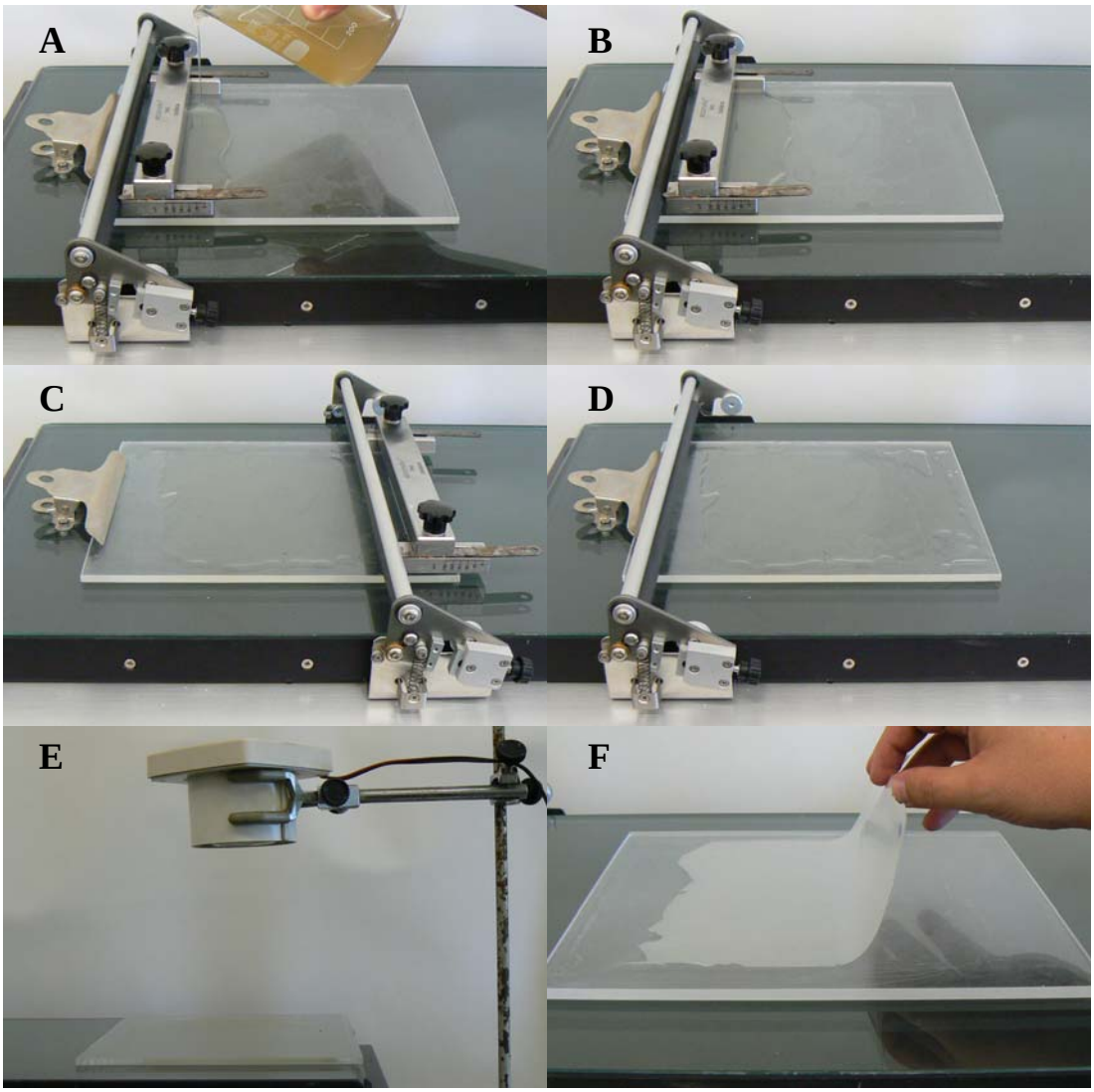

Figure 5.3 – Knife coating semi-continuous method: (A and B) preparing the application of film-forming solution (FFS) over the acrylic support; (C) spreading FFS at constant speed; (D) FFS after spreading; (E) drying at room temperature with help of a fan; (F) peeling off the film from the support.

5.2. Film characterization

5.2.1. Film thickness

The thickness of film samples was measured using a thickness comparator Absolute Digimatic Indicator model ID-F150 (Mitutoyo Co., Japan) with a resolution 1 μm (Figure 5.4). Reported thickness values are means of five measurements.

Figure 5.4 – Film thickness measurement apparatus.

5.2.2. Moisture sorption isotherms

Water sorption isotherms were determined by the gravimetric method. Samples with dimensions of 30 x 30 mm were previously dried at 50°C during 24 h in a vacuum oven. The samples were then placed in desiccators with different relative humidity, imposed by the use of saturated salt solutions (Table 5.1), for a_w ranging from 0.11 to 0.90. The experiment was carried out at 25°C. Values for the water activity of the salt solutions at 25ºC were obtained from the literature (Greenspan, 1977; Spiess and Wolf,

1983). The samples were weighed periodically, using a balance Sartorius BP211D (Sartorius AG, Germany) with a resolution of 0.01 mg, until they reached constant weight, after which the sample moistures were determined by the gravimetric method. The Guggenheim-Anderson-de Boer (GAB) model (Eq. 5.1) (van der Berg, 1984) was used to represent the experimental sorption data.

$$X = \frac{CkX_0 a_w}{\left[(1 - ka_w)(1 - ka_w + Cka_w)\right]} \quad (5.1)$$

where X is the equilibrium moisture content at the water activity a_w, X_0 is the monolayer moisture content and represents the water content corresponding to saturation of all primary adsorption sites by one water molecule, C is the Guggenheim constant and represents the energy difference between the water molecules attached to primary sorption sites and those absorbed to successive sorption layers, and k is the corrective constant taking into account properties of multilayer molecules with respect to the bulk liquid. GAB equation parameters were calculated with STATISTICA software (version 6.0).

Table 5.1 - Saturated salt solutions used to control water activity (Greenspan, 1977; Spiess and Wolf, 1983).

Salt	Chemical formula	Water activity of the saturated salt solution (at 25ºC)
Lithium chloride	LiCl	0.112
Magnesium chloride	$MgCl_2$	0.328
Potassium carbonate	K_2CO_3	0.432
Magnesium nitrate	$Mg(NO_3)_2$	0.529
Sodium bromide	NaBr	0.576
Strontium choride	$SrCl_2$	0.709
Sodium choride	NaCl	0.753
Barium choride	$BaCl_2$	0.902

5.2.3. Mechanical properties

The procedure used to prepare the films for testing mechanical properties was adapted from ASTM standard test method for tensile properties of thin plastic sheeting (ASTM, 1996a). Film samples were cut into 25 x 100 mm strips and film thickness was measured at four different points along the midsection of the film strip. Prior to testing, strips were stored 7 days at room temperature in a desiccator with a relative humidity of 53% (using a saturated salt solution of magnesium nitrate).

The tensile properties were determined using a TA-XT2 Stable Micro Systems texture analyzer (Surrey, England). Film samples were clamped between grips, leaving an initial distance between the grips of 60 mm. A strain rate of 0.2 mm/s was used. Force (N) and deformation (mm) were recorded during the test. The parameters determined were: tensile strength (MPa), elongation at break (%) and Young's modulus (MPa). At least five replicates of each film were tested.

5.2.4. Water vapour permeability (WVP)

In this study, WVP tests were conducted using ASTM method E96-95 (ASTM, 1996b) with some modifications. Film samples were cut into discs of 80 mm diameter (that were conditioned for 7 days at room temperature in a desiccator under a relative humidity of 53%), and sealed over a circular opening 0.003m^2 of the permeation cell, stored at 25ºC in a desiccator. The driving force applied corresponded to a relative humidity (RH) gradient across the film maintained by placing anhydrous calcium chloride (2% RH) inside the cell and distilled water (100% RH) in the desiccator. This driving force, expressed as water vapour partial pressure, was 3106.51Pa. To maintain this driving force a fan was operated within the chamber to avoid the stagnant air. The test cell was periodically weighted, using a balance Sartorius BP211D (Sartorius AG, Germany) with a resolution of 0.01 mg, after steady state conditions were reached. Typically, after about 2 hours, a

constant weight variation rate was observed. WVP (g m^{-1} s^{-1} Pa^{-1}) was calculated using the Eq. 5.2:

$$WVP = \frac{\Delta m \cdot x}{A \cdot \Delta t \cdot \Delta p} \quad (5.2)$$

where Δm is the weight gain (g) of the test cell, x is the film thickness (m), and A is exposed area (0.003 m^2) during Δt duration (s) under Δp partial water vapour pressure (Pa).

5.2.5. Oxygen permeability

Oxygen transmission rate tests were performed using a static method, simulating the real package situation. This method consisted of a static diffusion cell with a purging gas circulating through a compartment of volume V separated from the atmosphere by the film sample under test (Figure 5.5). The purging gas-compartment has an inlet and an outlet for gas flushing, purging the oxygen permeated through the film. A sampling port allows the collection of gas samples. Film samples were cut as 90 mm diameter discs, which were conditioned for 7 days at room temperature in a desiccator under 53% relative humidity. The discs were attached over and sealed a circular opening 0.0046 m^2 of the diffusion cell. For measuring the rate of oxygen diffusion, helium was allowed to flow through the cell for a period of time t, so that the cell is completely purged with until there is no detectable amount of oxygen in the cell. The valves were closed and the test started. At given time intervals, a sample was taken out of the sampling and its O_2 concentration measured using a CheckMate II gas analyzer (PBI Dansensor, Denmark), allowing the calculation of amount of O_2 crossing the film during that time interval. The oxygen transmission rate (OTR) (cm^3 s^{-1}) was determined by the slope of the curve of volume of oxygen crossing through the film during a determined time. Oxygen Permeability (ml m m^{-2} s^{-1} Pa^{-1}) was calculated using the Eq. 5.3:

$$OP = \frac{OTR \cdot x}{A \cdot \Delta p} \tag{5.3}$$

where x is the film thickness (m), A is exposed area (0.0046 m^2), and Δp is the O_2 partial pressure differential across the film (Pa), in this case is the difference of the partial pressure of oxygen in atmospheric air and the partial pressure of oxygen inside the diffusion cell (approx. 0% O_2).

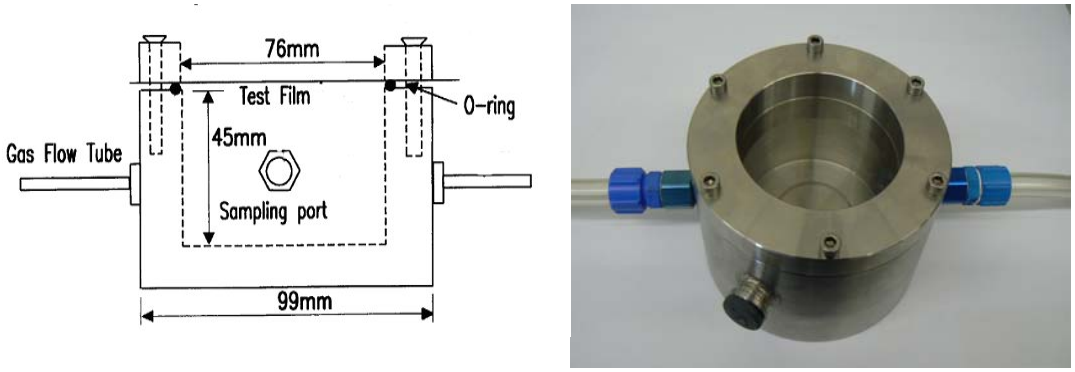

Figure 5.5 – Static diffusion cell used for the determination of oxygen permeability of the film samples.

The system was calibrated using aluminium sheets in order to verify possible leaks. The calibration was done in triplicate, and the Box-Lucas model was used to fit the experimental data. The Box-Lucas model is expressed by the following equation (Eq. 5.4):

$$y = a(1 - e^{-bx}) \tag{5.4}$$

where x is a variable, a and b are parameters with $0<b<1$.

Experimental data and the respective Box-Lucas fitting are presented in Figure 5.6. The equation obtained by fitting the Box-Lucas model (Eq. 5.5) was used to correct the data obtained for the films tested.

$$\%O_2 = 24.164 \cdot (1 - e^{-0.182 \cdot t}) \tag{5.5}$$

where $\%O_2$ is the volume percent of O_2 that enter into diffusion cell through the leaks at the time *t*.

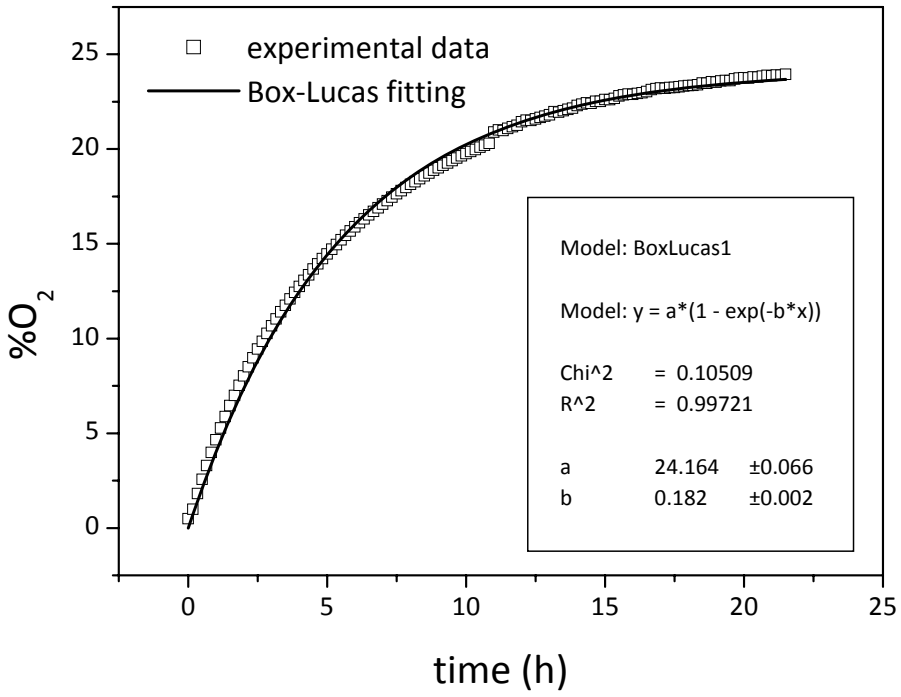

Figure 5.6 – Cell diffusion calibration with aluminium sheets: experimental data and fitting curve obtained by the Box-Lucas model.

Oxygen transmission rate (OTR) for all the films tested were corrected by the Equation 5.5, and used to calculate the oxygen permeability (OP) using the Equation 5.3.

5.2.6. Optical properties

Film colour was determined by a Minolta colorimeter CR300 series (Tokyo, Japan) using the CIELab colour parameters, lightness (L*) and chromaticity parameters a* (red – green) and b* (yellow – blue) were measured. The colour of the films was expressed as the difference of colour (ΔE*) (Eq. 5.6).

$$\Delta E^* = \sqrt{(L^* - L_s^*)^2 + (a^* - a_s^*)^2 + (b^* - b_s^*)^2} \quad (5.6)$$

where L_s^*, a_s^* and b_s^* are the CIELab standards for the white standard, used as the film background.

The ultraviolet (UV) and visible light barrier properties of the films were measured at selected wavelengths from 200 to 600 nm using a UV-visible Unicam spectrometer model Helios Alpha. Transmittance and absorbance were measured.

Transparency of the films was calculated by the equation of Han and Floros (1997) (Eq. 5.7):

$$Transparency = \frac{A_{600}}{Thickness} or -\frac{\log T_{600}}{Thickness} \quad (5.7)$$

where A_{600} and T_{600} are absorbance and transmittance at 600 nm, respectively.

5.2.7. Differential scanning calorimetry (DSC)

A Shimadzu DSC-50 differential scanning calorimeter was used. The instrument was calibrated for heat flow and temperature using Indium (m.p., 156.6ºC; ΔHm, 28.5 J g^{-1}). Helium at a flow rate of 50 ml min^{-1} was used as carrier gas. Film samples of approximately 25 µg, previously conditioned under a relative humidity of 90% during seven days, were directly weighed into Perkin-Elmer hermetically sealable 40 µl aluminium pans. After sealing, pans were left to equilibrate for 1 h. A pan containing an equal amount of distilled water was used as a reference. The temperature was raised from 20 to 90ºC at a heating rate of 2.5ºC/min.

5.2.8. Dielectric spectroscopy

Dielectric spectroscopy measurements were carried out at the Max Planck Institute for Polymer Research, Germany to determine the dielectric properties of the films samples, the dielectric relaxation and conductivity using a Novocontrol Dielectric Broadband Spectroscopy. The dielectric measurements were carried out in a temperature range of -150 to 100ºC, and a frequency range of 10^{-3} to 10^{7} Hz. The experiments were carried out with the samples equilibrated at room conditions (20ºC; 58%RH). The dielectric constant (ε') and loss factor (ε") were calculated as a function of frequency and temperature.

5.2.9. Rheology characterization of the film-forming solution

Mixtures of rice starch and commercial carrageenan were prepared for rheology characterization varying the amount of carrageenan (0, 16.7, 28.6 and 100% in dry basis) in a total polymer concentration of 5 % (in weight) in water. The mixtures were submitted to heating to 90°C under stirring during 30 minutes for total solubilization of starch and carrageenan. Mixtures of rice starch and carrageenan extracted from *Mastocarpus stellatus* were also prepared in a similar way, in order to perform a comparative study with respect to commercial carrageenan films and mixtures (section *6.1.4.8*). The following procedure was used: a TA AR2000 controlled stress rheometer (TA Instruments, New Castle, USA) fitted with cone-plate geometry (4º cone angle, 4.0 cm diameter) was used. Dynamic shear modules were recorded for imposed sinusoidal strain of fixed amplitude at 1.0%, ensuring satisfactory sinusoidal torque response for all experiments. The hot mixture was poured directly onto the plate of the rheometer, at 60ºC, and covered with paraffin oil to prevent water loss. A system temperature was reduced from 90 to 20ºC, at the rate of -5ºC/min and at a constant frequency of 1 Hz. Mixtures were then allowed to equilibrate at 20ºC in order to obtain non time dependent dynamic shear modules; a frequency sweep from 100 to 0.01 Hz was subsequently performed.

In order to evaluate the effect of plasticizer addition on the film-forming solution viscosity (section *6.6.4.6)*, rheological analysis was carried out for solutions used to produce films based in carrageenan extracted from *Mastocarpus stellatus*, with the addition and variation of plasticizer content (0, 10, 20 and 40%), keeping a total weight of material (carrageenan and plasticizer) in solution of 4%. For the rheological characterization of these film-forming solutions, the same TA AR2000 controlled stress rheometer (TA Instruments, USA) fitted with cone-plate geometry (4º cone angle, 4.0 cm diameter) was used. Hot pure carrageenan and mixtures (carrageenan+plasticizer) solutions were poured directly onto the plate of the rheometer, at 60ºC, and covered with paraffin oil to prevent water loss. The sample was conditioned at 80ºC for 5 minutes. A frequency sweep was performed from 100 to 0.01 Hz with a 20% strain. The system was then cooled from 80 to 25ºC, at the rate of -5ºC/min and at a constant frequency of 1 Hz. Another frequency sweep from 100 to 0.01 Hz was performed at 25ºC. A steady flow sweep test was carried out, with a shear rate ranging from 0.1 to 500 s^{-1}. Each data point corresponds to a time independent viscosity achieved by allowing enough time (typically less than 2 minutes are needed to reach a steady stress, as inferred from the time dependence of the recorded torque signal) to the measured stress to reach a steady state value (less than 2% variation). Upon flow cessation, G' and G'' were recorded by performing small amplitude oscillatory shear (5% strain, 1 Hz) during 10 minutes in order to probe the relaxation mechanism of the polymer solution. Finally another steady flow sweep test, with the shear rate ranging from 500 to 0.1 s^{-1} was subsequently performed, in order to evidence any possible thixotropic behaviour.

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Chapter 6
Film formulation and optimization

In this chapter the film's formulation and its optimization is described on the basis of its more relevant physical properties (hygroscopic, mechanical, optical, and barrier properties).

6.1. Preliminary studies

Preliminary studies were carried out with mixtures of rice starch and carrageenan, to evaluate the performance of carrageenan on the film formation and properties and to compare a commercial κ-carrageenan with the κ/ι-hybrid carrageenan extracted from *Mastocarpus stellatus*.

6.1.1. Materials

Rice starch, commercial κ-carrageenan (SKW Biosystems, France) and κ/ι-hybrid carrageenan extracted from *Mastocarpus stellatus* (Hilliou et al.,

2006) were used as the film forming component to provide a continuous matrix of edible film.

6.1.2. Preparation of starch-carrageenan based films

Rice starch and carrageenan were used to prepare starch-carrageenan mixtures, with different amount of carrageenan (0, 16.67, 33.33, 44.44 and 100% in dry basis). The mixtures with commercial κ-carrageenan were codified as SKW, and the mixtures with κ/ι-hybrid carrageenan were codified as MST. Films were prepared by the casting method, as described in Chapter 5. The thickness of film samples had a mean value of 50 ± 5μm. The result was translucent films, which can be easily removed from the support.

6.1.3. Film characterization

Film samples were characterized as described in Chapter 5, measuring the following film properties: moisture sorption isotherms, mechanical properties, water vapour permeability, optical properties (colour and light transmission), thermal properties (DSC) and dielectric spectroscopy. A rheological characterization of the film-forming solution was also performed.

6.1.4. Results and discussion

Homogeneous, thin, flexible and transparent films were obtained from rice starch-carrageenan mixtures by the casting method. All the films were easily removed from the Teflon® plate and showed smooth surfaces. The films were very sensible to water, being easily dissolved in distilled water. Rice starch and SKW films were colourless and MST ones had a slightly yellow appearance.

6.1.4.1. Moisture sorption isotherms

Moisture sorption isotherms at 25ºC for films containing 0, 16.7, 33.3, 44.4 and 100% of carrageenan (SKW and MST) (w/w rice starch) are presented in Figure 6.1, together with the GAB model (van der Berg, 1984) fitted for each sample. The GAB equation parameters and the correlation coefficients are presented in Table 6.1. The values of k (<1) and the correlation coefficient (r>0.98) show that GAB equation gives a good fit to experimental values.

Because of their hydrophilic nature, hydrocolloid film properties are highly dependent on environmental conditions such as relative humidity and temperature (Karbowiak et al, 2006). An important quantity of water can be held within their cross-linked polymer network. As presented in Figure 6.1, the water content of starch-carrageenan based films is more sensitive to relative humidity when the amount of carrageenan was increased. The isotherms curves presented a sigmoid shape and indicate that the equilibrium moisture content increases slowly with increasing environmental a_w up to 0.7, beyond which a steep rise in moisture content in film samples was observed.

The isotherm corresponding to the pure rice starch film (data denoted by ✯) exhibits a less hygroscopic behaviour, as the moisture content measured for all water activities is the lowest of all studied films. Rice starch film shows higher monolayer moisture content compared to the other film samples, which indicated that the monolayer moisture content and moisture sorption were attributable to the nature of the film-forming substance. Data associated to pure carrageenan film lie over all other data, which indicates that carrageenan is more hydrophilic than rice starch. The main output from moisture sorption isotherms is that addition of carrageenan in film formulation results in increased hydrophilic properties of the films, which is expected if one considers the higher hydrophobic character of starch, when compared to carrageenan. The effect of carrageenan content on the film

moisture content are presented in Figure 6.2, that suggests a linear dependence of the moisture content with the relative amount of carrageenan. It can be also noted that the dependence is steeper at bigger a_w.

Sorbed water acts as plasticizer by lowering the glass transition temperature, T_g, of the polymer (Beck et al., 1996). Plasticizers can also act as lubricants of the polymer chains improving flexibility of the film (Debeaufort and Volley, 1997).

Table 6.1 - GAB equation parameters for moisture sorption isotherms (r is the correlation coefficient) for the rice starch-carrageenan based films.

Sample	X_0	C	k	r
RST	1.772	29.639	0.005	0.969
SKW (16.7%)	0.088	29.978	0.706	0.994
SKW (33.3%)	0.084	113.626	0.778	0.997
SKW (44.4%)	0.095	11.339	0.786	0.998
SKW (100%)	0.114	23.320	0.854	0.999
MST (16.7%)	0.053	34.909	0.868	0.989
MST (33.3%)	0.052	24.864	0.893	0.993
MST (44.4%)	0.056	151.516	0.886	0.990
MST (100%)	0.048	261.226	0.965	0.997

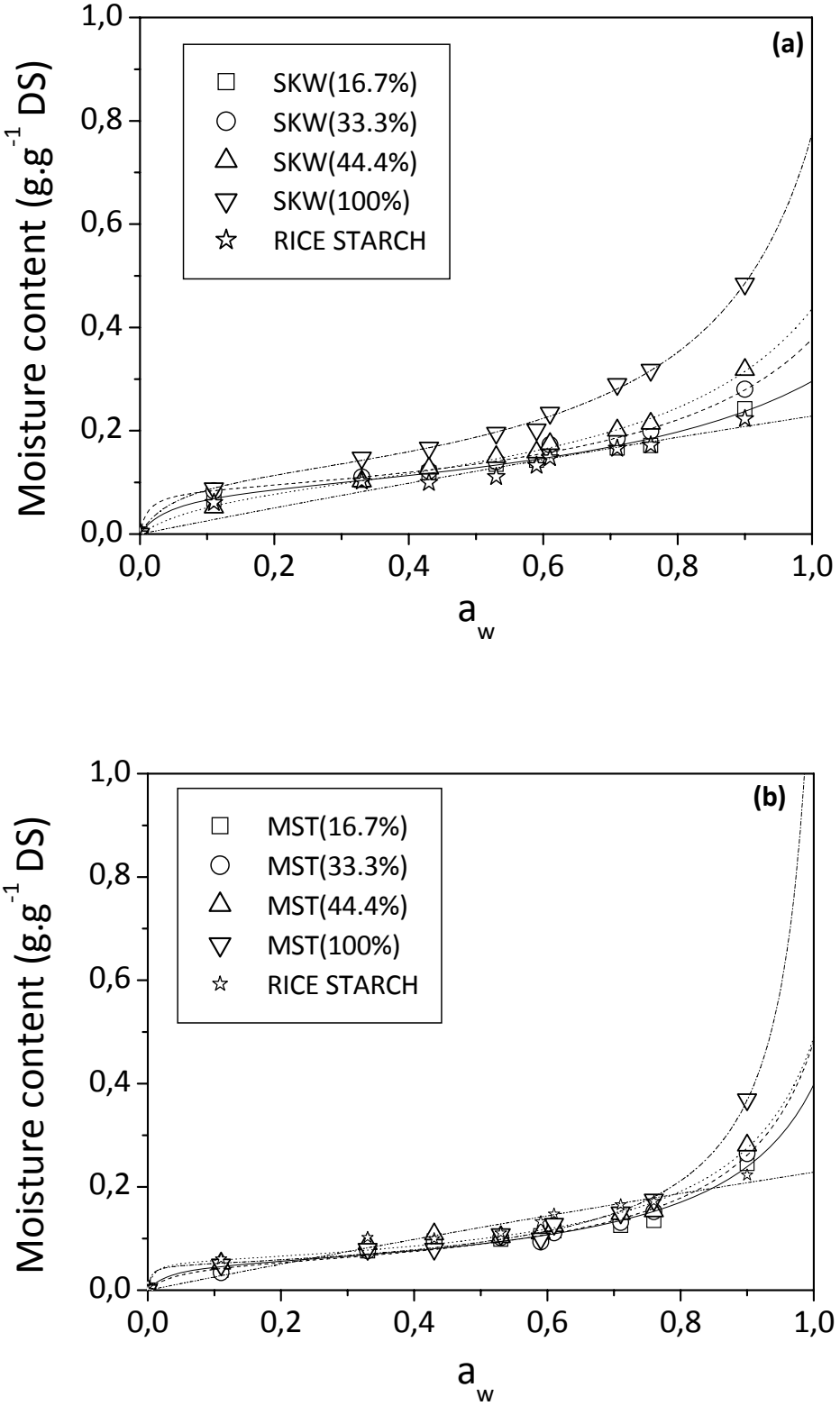

Figure 6.1 - Experimental data (□, ○, △,▽, ☆) for moisture sorption isotherms of the film obtained from mixtures of rice starch and commercial carrageenan, varying the amount of carrageenan (0, 16.7, 33.3, 44.4 and 100%), and the respective fitted GAB curves: (a)SKW; (b) MST.

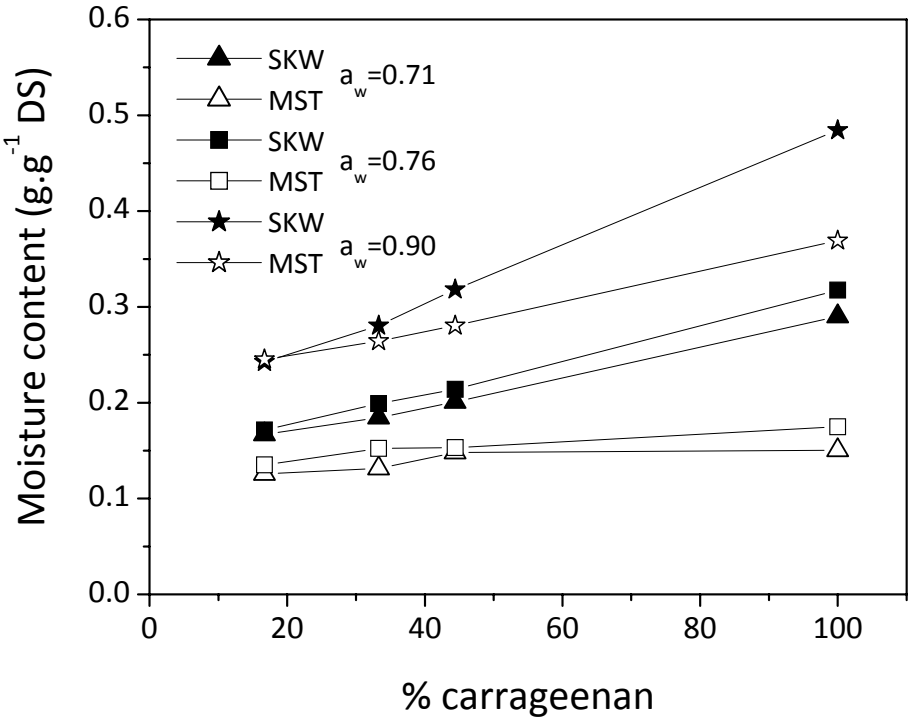

Figure 6.2 - Effect of carrageenan (SKW and MST) content on the film moisture content for three different a_w.

6.1.4.2. Mechanical properties

The tensile properties of the starch-carrageenan films are presented in Figures 6.3, 6.4 and 6.5. The behaviour of tensile strength (TS) and elongation at break (E) seems to vary according to the carrageenan content. The TS results (Figure 6.3) are not significantly different ($p>0.05$) for the films with 33.3, 44.4 and 100% of carrageenan, comparing SKW and MST. TS significantly increases ($p<0.05$) with the increase of carrageenan in the mixture, for both types of carrageenan used. The κ and ι types of carrageenan differ in their chemical structure and properties. The κ-carrageenan exhibited higher tensile strength than the ι-carrageenan. This shows that κ-carrageenan produces a stronger film than ι-carrageenan (Briones et al, 2004). In this study a κ/ι-hybrid carrageenan was used leading to slightly higher TS results than those for the commercial

carrageenan, which was composed mainly by κ-carrageenan. Elongation results (Figure 6.4) were significantly different for the SKW films, increasing with the increase of carrageenan in the mixture. For the MST films, elongation did not have significant variation. These results of elongation can be explained because carrageenan is more hygroscopic than starch; hence water uptake could explain the plasticization behaviour (increased elongation at break) which accompanies the increase in carrageenan content. The carrageenan content dependence of the Young's modulus (Figure 6.5) contrasts with the mechanical data discussed above, for the SKW films. The Young's modulus increases with the increasing of the amount of carrageenan in the mixture until 33.3%, and decreases for 44.4% and 100%. The bell shape is reminiscent from fracture stress and storage shear modulus data reported for various polysaccharide-galactomannan mixtures in the gel phase (Williams and Phillips, 1995), and has been assigned to some specific synergistic effects between the macromolecules. For MST films, Young's modulus presented the same tendency that for the tensile strength, increasing with the increase of carrageenan in the mixture.

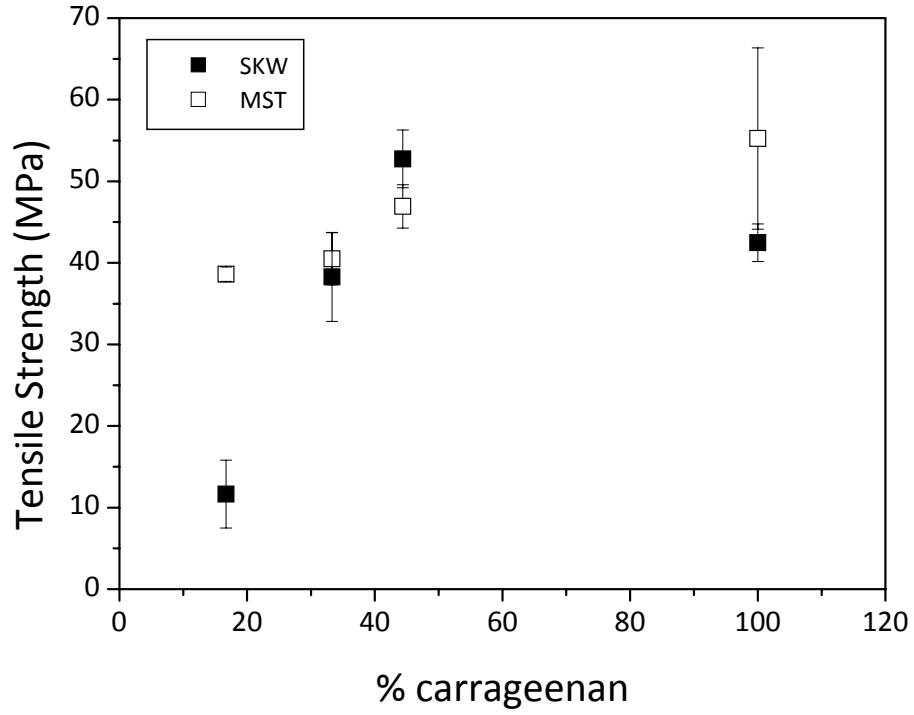

Figure 6.3 -Tensile strength for the starch-carrageenan films varying the amount (16.7, 33.3, 44.4 and 100%) and type (■ SKW, □ MST) of carrageenan.

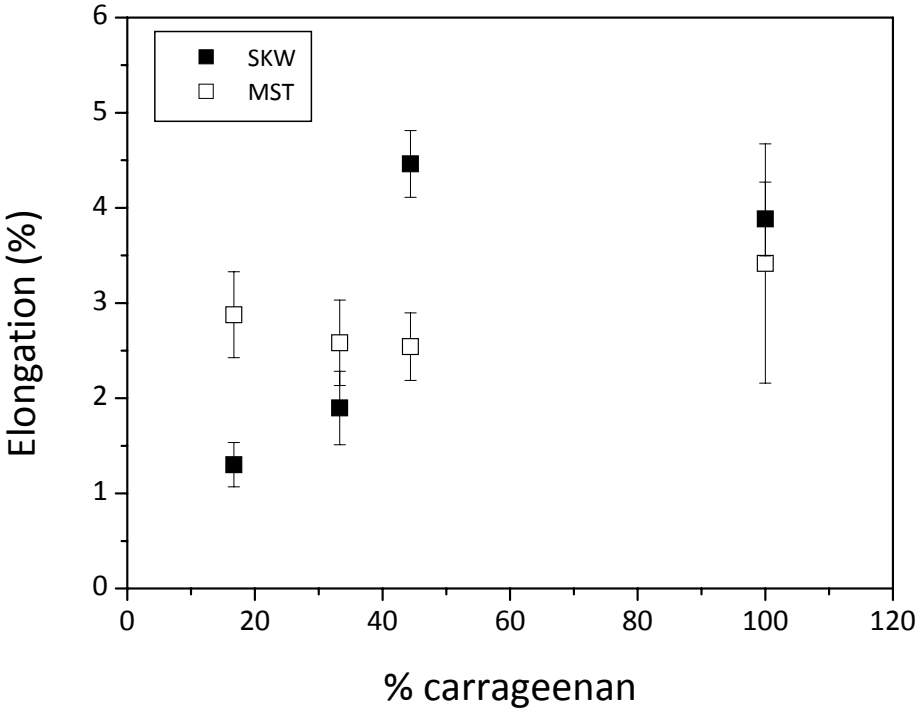

Figure 6.4 - Elongation for the starch-carrageenan films varying the amount (16.7, 33.3, 44.4 and 100%) and type (■ SKW, □ MST) of carrageenan.

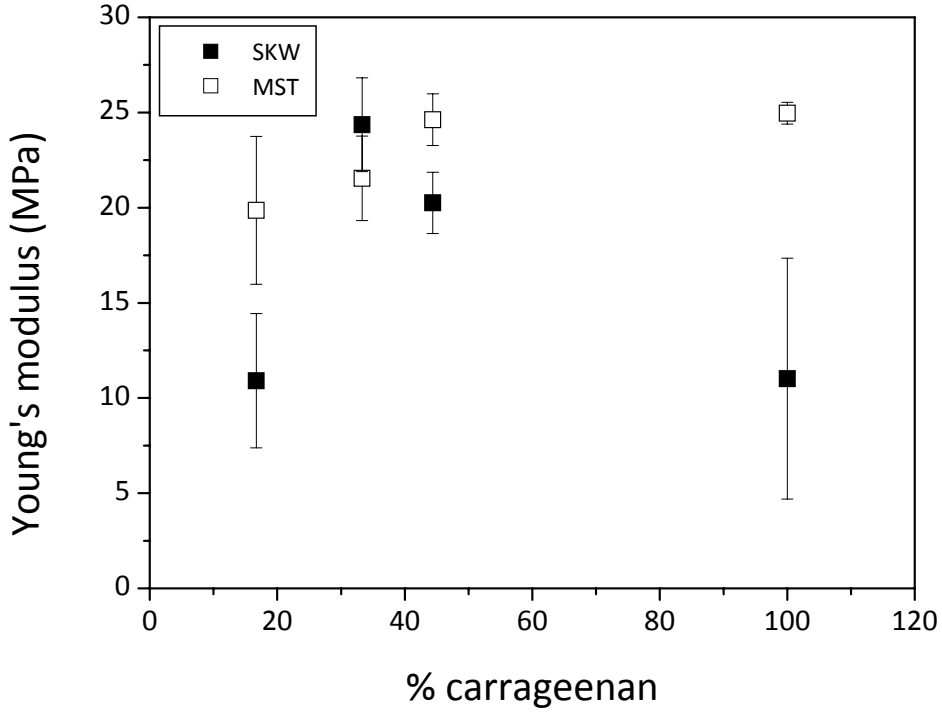

Figure 6.5 - Young's modulus for the starch-carrageenan films varying the amount (16.7, 33.3, 44.4 and 100%) and type (■ SKW, □ MST) of carrageenan.

6.1.4.3. Water vapour permeability (WVP)

Figure 6.6 shows the effect of carrageenan content (16.7, 33.3, 44.4 and 100%, w/w) on the WVP of the starch-carrageenan films. ANOVA test indicated that films formulated with SKW did not differ significantly ($p>0.05$), but the films with MST were significantly different decreasing WVP with the increase of carrageenan. WVP values ranged between 0.67 and 1.54 x 10^{-10} g m^{-1} s^{-1} Pa^{-1} for films formulated with MST and 0.75 and 0.91 x 10^{-10} g m^{-1} s^{-1} Pa^{-1} for films with SKW. Hydrocolloid films are characterized by their good gas barrier properties but poor water vapour permeability (Baldwin et al, 1997; Debeaufort and Voilley, 1997; Krochta and De Mulder-Johnston, 1997). Compared with other films based in proteins and polysaccharides reported in literature, films based in carrageenan (commercial and domestic) show similar WVP values, and in some cases lower with an order

of magnitude below (Table 6.2). With regard to synthetic polymers, carrageenan films have similar values to those of cellophane. However they are higher than low density polyethylene (LDPE), the most commonly polymer used in the food packaging industry (Table 6.2).

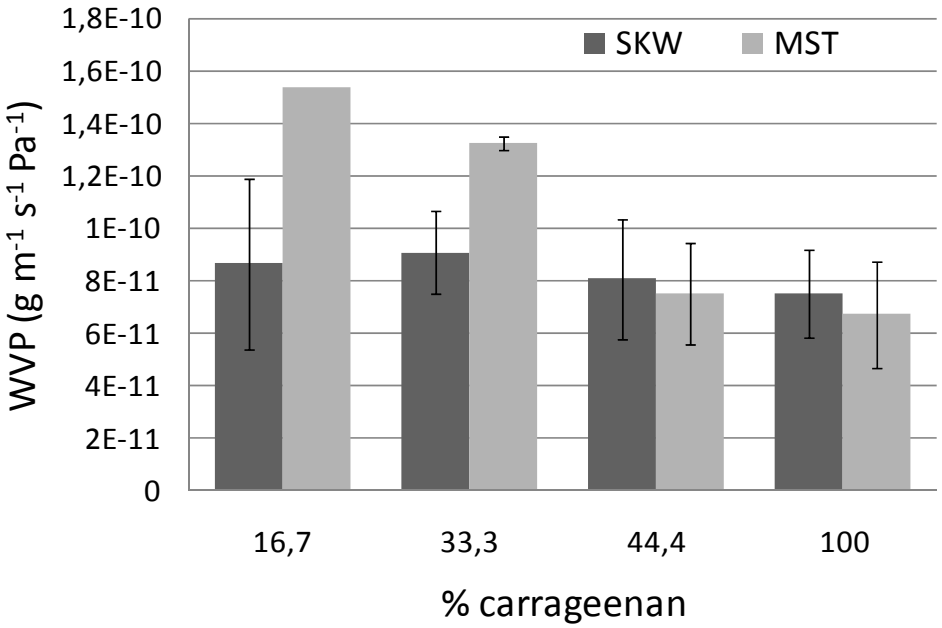

Figure 6.6 – Water vapour permeability (WVP) of starch-carrageenan films varying the amount (16.7, 33.3, 44.4 and 100%) and type (kappa and kappa/iota hybrid) carrageenan.

Table 6.2 – Comparison of WVP values of biodegradable and synthetic films.

Film formulation	WVP ($g\ m^{-1}\ s^{-1}\ Pa^{-1}$)	Reference
Whey protein	3.8×10^{-9}	Anker et al, 2002
Corn starch	1.8×10^{-10}	Garcia et al, 2006
Methylcellulose 1%	7.6×10^{-11}	Garcia et al, 2004
κ-carrageenan	7.5×10^{-11}	Present study
κ/ι-hybrid carrageenan	6.7×10^{-11}	Present study
Chitosan	4.5×10^{-11}	Garcia et al, 2006
Cellophane	8.4×10^{-11}	Shellhammer and Krochta, 1997
Low density polyethylene	9.1×10^{-13}	Smith, 1986
High density polyethylene	2.3×10^{-13}	Smith, 1986
Poly(vinylidene chloride)	4.4×10^{-13}	Parris et al, 1995

6.1.4.4. Oxygen permeability (OP)

Oxygen permeability (OP) is the next most commonly studied transport property of edible films after water vapour permeability (Miller and Krochta, 1997). Oxygen is involved in many degradation reactions in foods, such as fat and oil rancidity, micro-organism growth, enzymatic browning and vitamin loss. Thus, many packaging strategies seek to exclude oxygen to protect the food product (Gontard et al., 1996). On the other hand, the permeability to oxygen and carbon dioxide is essential for respiration of living tissues such as fresh fruits and vegetables. So, moderate barrier coatings are more appropriate. If a coating with the appropriate permeability is chosen, a controlled respiratory exchange can be established and thus the preservation of fresh fruits and vegetables can be prolonged (Ayranci and Tunc, 2003)

Most edible films are water sensible, but their hydrophilic nature makes them excellent barriers against the non-polar substances such as aromas and oxygen (Khwaldia et al., 2004). Dry hydrocolloid films have good oxygen barrier properties. In the presence of moisture, the macromolecule chains become more mobile, leading to a substantial increase in O_2 permeability (Kumins, 1965). As observed in section *6.1.4.1*, the addition of carrageenan to the film formulation results in increased hydrophilic properties of the films and more water adsorbed, which can act as a plasticizer. The plasticizing and/or swelling effect of water on hydrophilic polymers results in increased permeability values (Ashley, 1985). Figure 6.7 shows the effect of carrageenan content (16.7, 44.4 and 100% w/w) on the *OP* of the starch-carrageenan films. ANOVA test indicated that films formulated with SKW and MST were significantly different ($p<0.05$), with increasing OP with carrageenan content in both cases. OP values ranged between 0.94 and 5.21 x 10^{-12} ml m m^{-2} s^{-1} Pa^{-1} for films formulated with SKW and 0.81 and 3.33 x 10^{-12} ml m m^{-2} s^{-1} Pa^{-1} for films with MST. Compared with other films based in proteins and polysaccharides reported in literature, films based in carrageenan (commercial and domestic) show similar OP values, in some

cases an order of magnitude below (Table 6.3). With regard to synthetic polymers, carrageenan films have similar values to those of cellophane and high density polyethylene (HDPE). However they are lower than low density polyethylene (LDPE), the most commonly polymer used in the food packaging industry (Table 6.3).

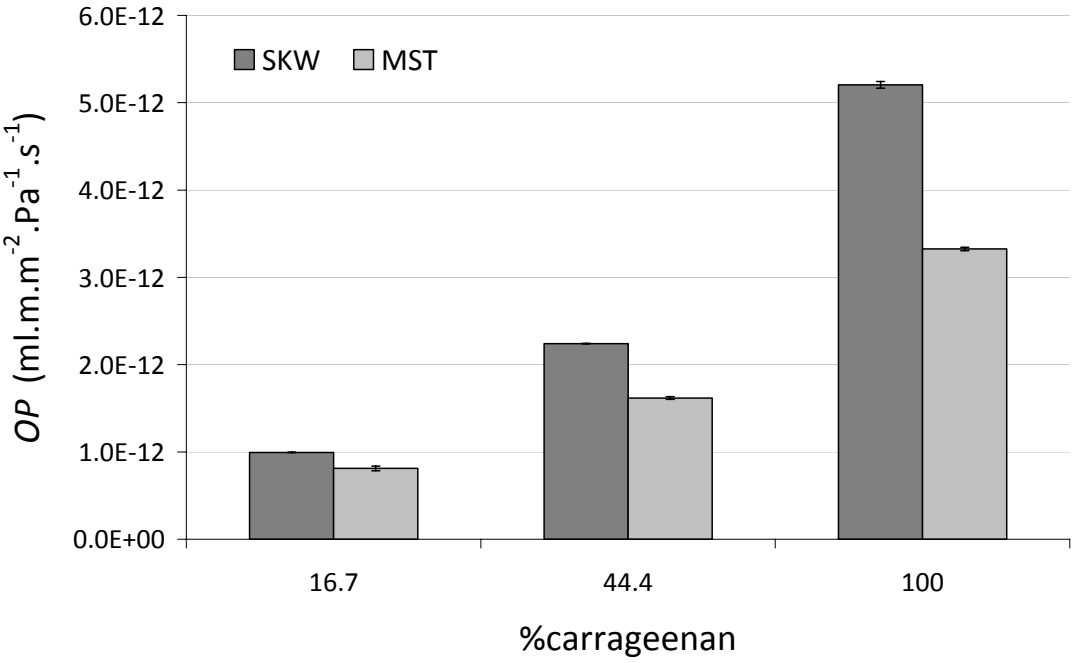

Figure 6.7 – Oxygen permeability (OP) of starch-carrageenan films varying the amount (16.7, 44.4 and 100%) and type (kappa (SKW) and kappa/iota hybrid (MST)) carrageenan.

Table 6.3 – Oxygen permeability of edible films.

Film	Test conditions	O_2 Permeability x10^{12}($cm^3.m/m^2.s.Pa$)
MST *(present study)*	23ºC;50 %RH	3.32
SKW *(present study)*	23ºC;50 %RH	5.21
Carrageenan[a]	-	4.19
Corn starch[b]	24ºC; -	0.15
Amylomaize[c]	25ºC; -	17.13
Pectin[d]	25ºC; 96 %RH	29.56
Chitosan[d]	25ºC; 93 %RH	10.44
Pullulane[d]	25ºC; 30 %RH	0.38
Wheat gluten[d]	25ºC; 91 %RH	21.70
Fish proteins[d]	25ºC; 86 %RH	6.397
Beeswax[e]	25ºC; 0 %RH	10.78
Carnauba wax[e]	25ºC; 0 %RH	1.82
Whey protein/Sorbitol[f]	23ºC;75 %RH	1.68
Peach puree[g]	23ºC; 58 %RH	0.81
LDPE[h]	23ºC; 50 %RH	21.64
HDPE[h]	23ºC; 50 %RH	4.94
Cellophane[i]	23ºC; 95 %RH	2.92

[a] Ribeiro et al. (2007); [b] Allen et al. (1963); [c] Mark et al. (1966); [d] Guilbert et al. (1996); [e] Greener (1992); [f] McHugh and Krochta (1994); [g] McHugh et al. (1996); [h] Salame (1986); [i] Taylor (1986); Abbreviations: LDPE: low density polyethylene; HDPE: high density polyethylene.

6.1.4.5. Optical properties

Colour coordinates of several films studied are presented in Table 6.4. SKW films were colourless while MST ones had a slightly yellow-green appearance. This yellow-green appearance for MST films, represented by an increase of the *b** parameter and a decrease of a* with the increase of carrageenan content, because κ/ι-hybrid carrageenan extracted in the laboratory did not suffer a bleaching process. This result agreed with visual observations. Since *b** was the parameter with the highest contribution to

colour difference, *ΔE** showed a similar trend for the MST films. All the colour parameters (*L**, *a** and *b**) differ significantly ($p<0.05$) for all film formulations.

Table 6.4 – Colour standards of the film samples.

Film samples	Lightness (*L**)	Chromaticity parameter *a**	Chromaticity parameter *b**	Colour difference (*ΔE**)
Starch	99.37 ± 0.08	0.57 ± 0.03	2.05 ± 0.02	0.05 ± 0.31
SKW (16%)	99.34 ± 0.07	0.59 ± 0.04	2.62 ± 0.16	0.59 ± 0.32
SKW (33%)	98.49 ± 0.11	0.44 ± 0.05	4.31 ± 0.35	2.44 ± 0.49
SKW (44%)	99.09 ± 0.13	0.50 ± 0.05	3.61 ± 0.21	1.60 ± 0.46
SKW (100%)	98.97 ± 0.12	0.57 ± 0.02	3.11 ± 0.20	1.14 ± 0.34
MST (16%)	97.91 ± 0.32	0.26 ± 0.04	5.31 ± 0.37	3.60 ± 0.50
MST (33%)	95.73 ± 0.33	0.00 ± 0.04	9.37 ± 0.12	8.20 ± 0.15
MST (44%)	93.87 ± 0.24	-0.35 ± 0.04	12.79 ± 0.41	12.11 ± 0.33
MST (100%)	93.77 ± 0.13	-0.40 ± 0.08	12.72 ± 0.35	12.10 ± 0.54

* CIELab standards for the white standard, used as the film background were: L_s^* (99.33), a_s^* (0.61) and b_s^* (2.03).

The protection provided to a product by the packaging material depends on its light transmission, which in turn depends on the chemical structure and on the molecular arrangement of the material. Table 6.5 shows the transmission and transparency of starch-carrageenan films at 600 nm, which is the commonly used wavelength to report film transparency (Han and Floros, 1997; Fang et al., 2002; Shiku et al., 2003).

The addition of carrageenan to the mixture decreases the light transmission of the films, improving the barrier to light transmission, at the selected wavelength of 600 nm (Table 6.5). The pure carrageenan films and 16.7 and 33.3% mixtures blocked most light in the wavelength of 600 nm because they were less transparent than the pure starch film.

Table 6.5 – Light transmission (%, T_{600}) and transparency (A_{600}/mm) of starch-carrageenan based films.

Light Transmission and Transparency	Starch	SKW 16.7%	SKW 33.3%	SKW 100%	MST 16.7%	MST 33.3%	MST 100%
%, T_{600}	64.7	34.7	34.2	50.8	31.8	28.2	50.1
A_{600}/mm	7.9	19.2	19.4	12.2	20.7	22.9	12.5

Figure 6.8 presents the absorbance of light in the range of 200 to 600 nm. It was observed that the addition of carrageenan improves the barrier properties to UV radiation in the range of 200 to 280 nm for both κ/ι-hybrid carrageenan (MST) and κ-carrageenan (SKW) films. These excellent barrier properties to UV light (in the 200-280 nm region) suggest that these films can prevent the lipid oxidation induced by UV light. The results observed for MST films were better than for the SKW films.

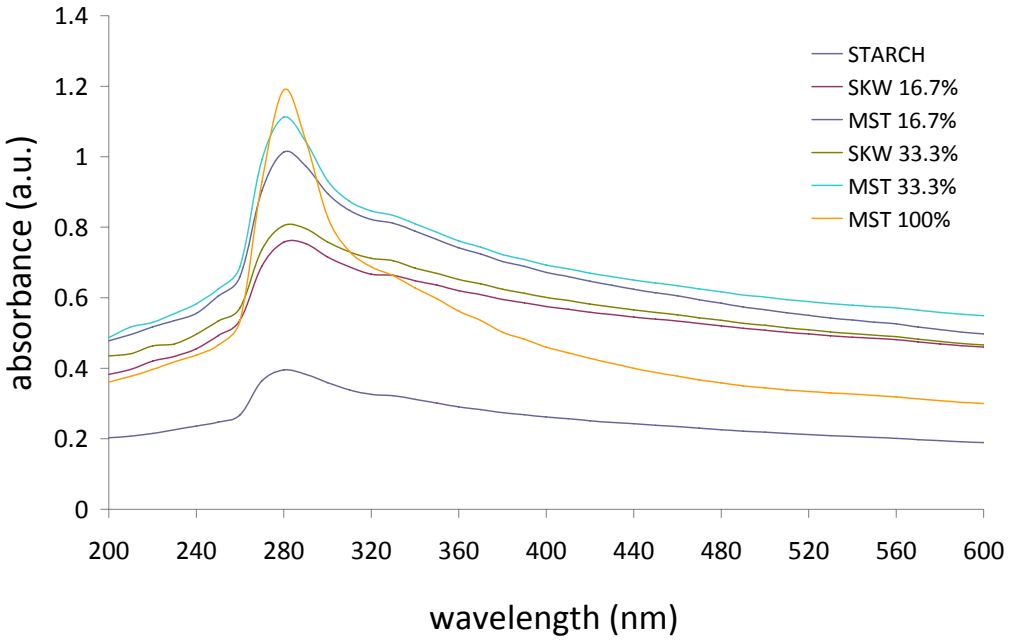

Figure 6.8 – Light transmission spectra (absorbance) of starch-carrageenan based films varying the content and type of carrageenan.

6.1.4.6. Differential Scanning Calorimetry (DSC)

DSC curves displayed in Figure 6.9 clearly show that increasing the amount of carrageenan in the films leads to a decrease in the glass transition temperature (T_g). This result may be explained by the increased hygroscopic properties of film containing carrageenan as observed in the moisture sorption isotherms (section *6.1.4.1*). The higher water content of the films favours the inherent plasticizing effect which leads to the decrease in T_g. Again, films formulated with domestic carrageenan (MST) show a glass transition temperature similar to its commercial counterpart (Figure 6.10). T_g of pure carrageenan based films was also strongly affected by a_w or water content. A linear relationship (r^2 = 0.99) was observed between T_g and a_w (Figure 6.11), where the increase of a_w causes a decrease in T_g, which confirms the plasticizing effect of the water content in the films.

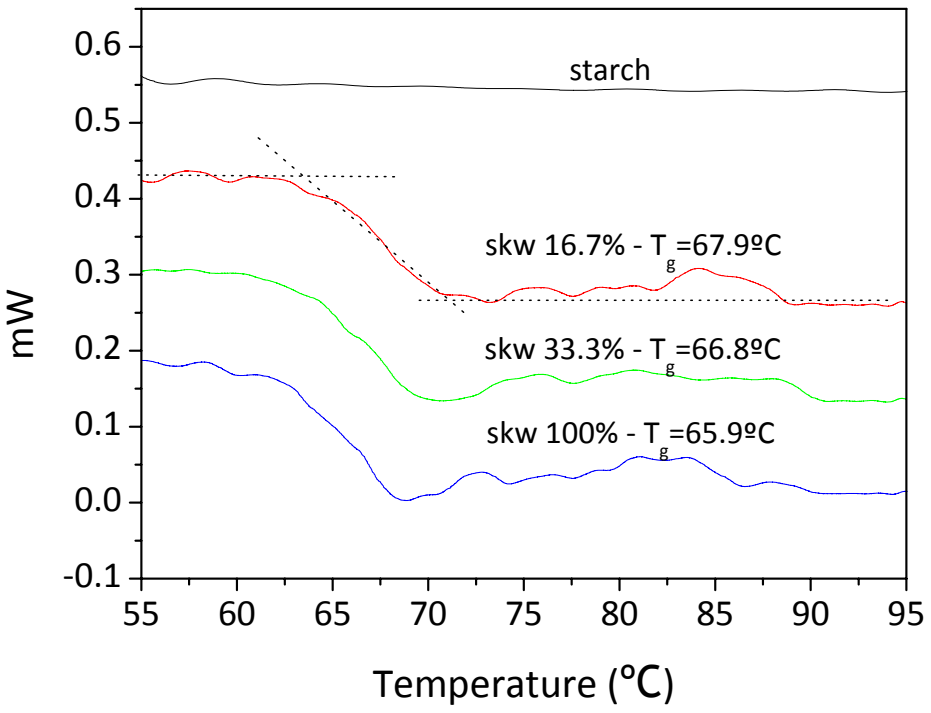

Figure 6.9 - DSC thermograms of film samples obtained from mixtures of rice starch and carrageenan, varying the amount of carrageenan (0, 16.7, 33.3 and 100%).

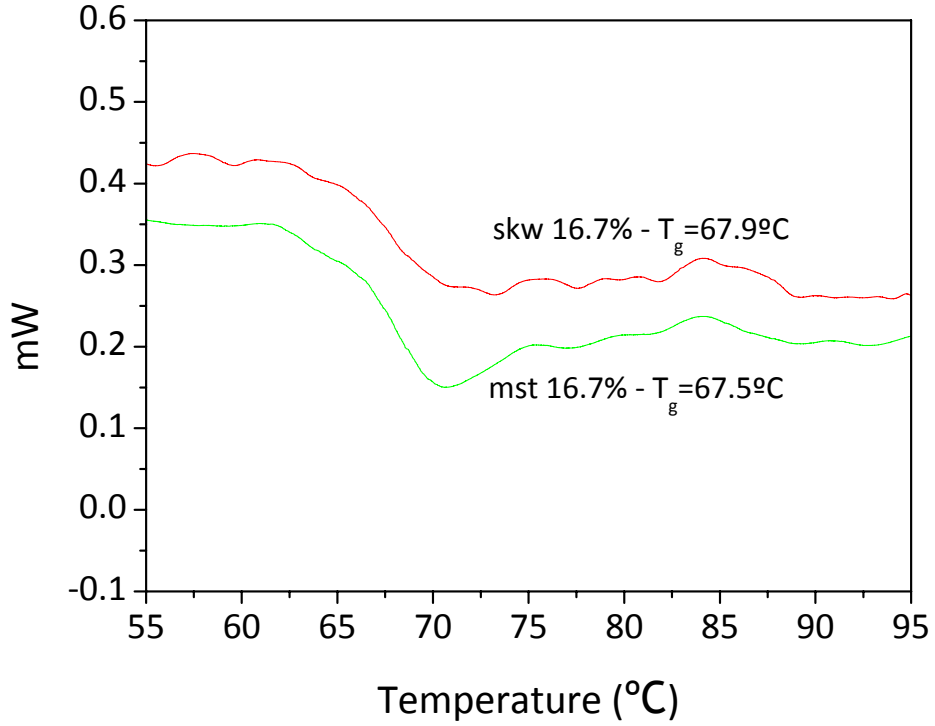

Figure 6.10 - Comparison of T_g of films formulated with SKW and MST.

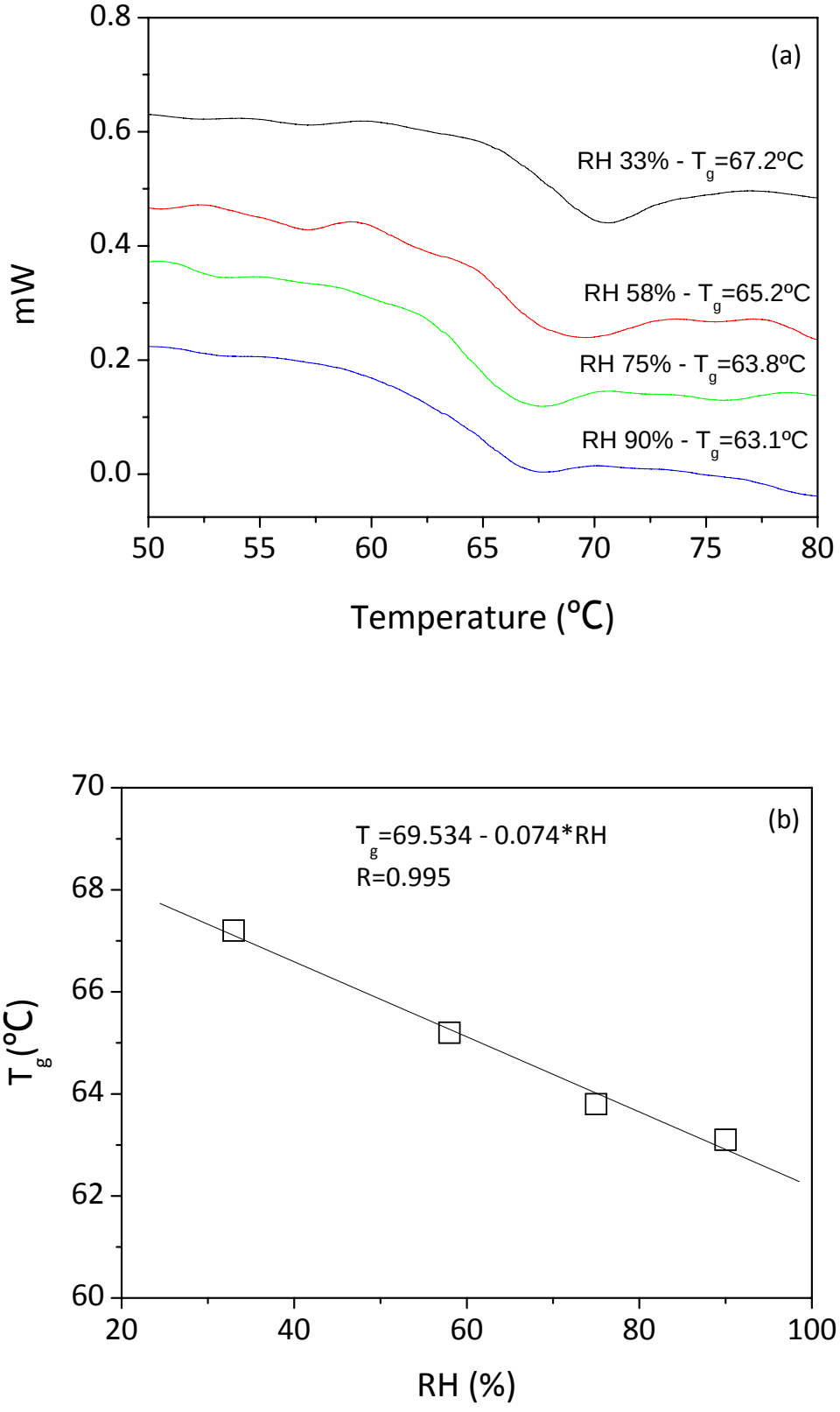

Figure 6.11 – (a) DSC thermograms of pure κ/ι-hybrid carrageenan films at different relative humidity; (b) effect of RH on T_g.

6.1.4.7. Dielectric spectroscopy

The dielectric constant (permittivity ε') of film is usually related to the film polarity. The dielectric properties of synthetic films have been extensively explored. However, very little information is available on the dielectric properties of biodegradable films. The dielectric constant (ε') of starch-carrageenan films was measured over a frequency range of 10^{-3} to 10^{7} Hz and a temperature range of -150 to 100ºC. The results showed that ε' of starch-carrageenan films was much lower than that of semi-solid or liquid biological samples due to the relatively lower water content and limited mobility of the polymer molecules in the films, but was similar to that of common synthetic films. As expected, the films with more carrageenan exhibited higher ε' values (Figure 6.12). Carrageenan is more hygroscopic than starch and adsorbs more water (Figure 6.1); water is polar and also acts as a plasticizer, so the addition of carrageenan increasing water content, facilitates the mobility of the polar side-chains and enhances dipole orientation of polar groups in the film. These results showed that dielectric constant of the films were highly related to their water content.

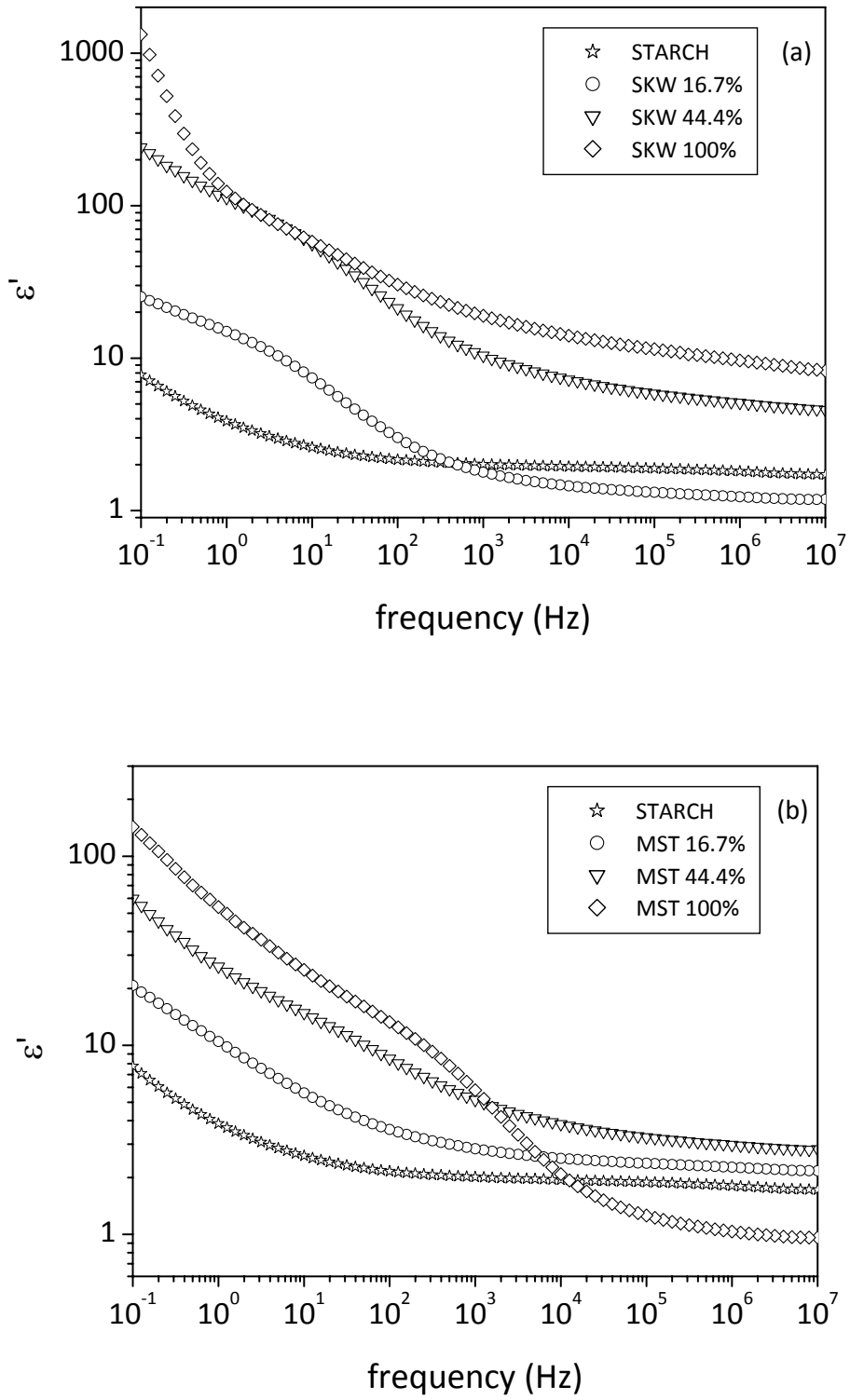

Figure 6.12 – Dielectric constant (ε' – permittivity) at 25ºC of starch-carrageenan based films as a function of frequency at different amount of carrageenan in the mixture (0, 16.7, 44.4 and 100%) – (a) commercial κ-carrageenan (SKW); (b) κ/ι-hybrid carrageenan (MST).

6.1.4.8. Rheology

Figure 6.13 shows the frequency dependence of the mechanical moduli for mixtures of rice starch and commercial κ-carrageenan at 20ºC and a 5% total polymer concentration in water. The sample with 100% of carrageenan exhibited typical gel-type mechanical spectra (G' > G") in the entire frequency range examined. A drastic change in rheological properties was observed for samples with 16.7 and 28.6% of carrageenan. Their viscoelastic behaviour (elastic at low frequencies and viscous at higher frequencies) is reminiscent of gels close to their gel point.

The decrease in the gel rigidity with increased content of carrageenan in the mixture is better seen in Figure 6.14, which displays the variation of the storage modulus (G') for various starch-carrageenan mixtures in water measured at 0.01 Hz and 20ºC, as a function of the carrageenan concentration. This behaviour is counterintuitive if one notes that the storage modulus of pure carrageenan gel is roughly 2 orders of magnitude higher than the storage modulus of pure rice starch gel. However, data definitely show the plasticizing effect associated with the addition of carrageenan to the starch solutions. This can be understood if one realizes that the critical concentration for carrageenan gel-setting is close to 4.5%, as indicated in Figure 6.14 by the star symbol, which denotes the first gel-like storage modulus measured in pure carrageenan solutions. Below this concentration, carrageenan molecules do not form a three dimensional network. Therefore, for all mixtures containing less than 90% carrageenan, the system is essentially a rice starch gel swollen by carrageenan biopolymers which possibly limit the full formation of the starch network, and thereby weaken the elastic modulus of the gels. Note that mixtures with SKW (16.7%) and SKW (28.6%) correspond to solutions with 0.8 and 1.4% of carrageenan in water, respectively.

The rheological behaviour exhibited by mixtures containing domestic carrageenan extracted from *Mastocarpus stellatus* collected in November

2004 (samples with open circles in Figure 6.14) seem in qualitative agreement with the one evidenced for the commercial samples SKW, as a decrease in G' with increasing content of MST is equally suggested by the few data presented here. However, additional rheological characterization is clearly needed to further assess the similarity in mixing properties of the two carrageenan types. Gel rigidity of the pure MST sample is 100 times lower than the gel rigidity corresponding to the pure SKW solution in water. This result comes as no surprise if one considers that MST is a κ/ι-hybrid carrageenan (Hilliou et al, 2005), and that ι-carrageenan is known to form much softer gels than κ-carrageenan.

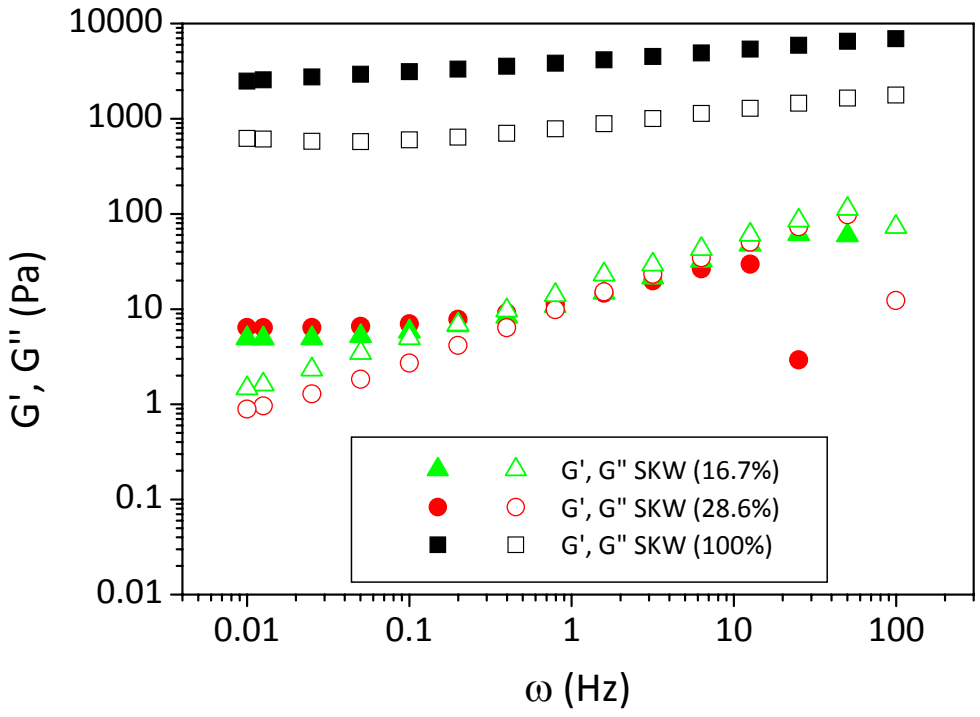

Figure 6.13 - Dynamic frequency sweeps for mixtures of rice starch and varying the amount of carrageenan (SKW) (16.7, 28.6 and 100%).

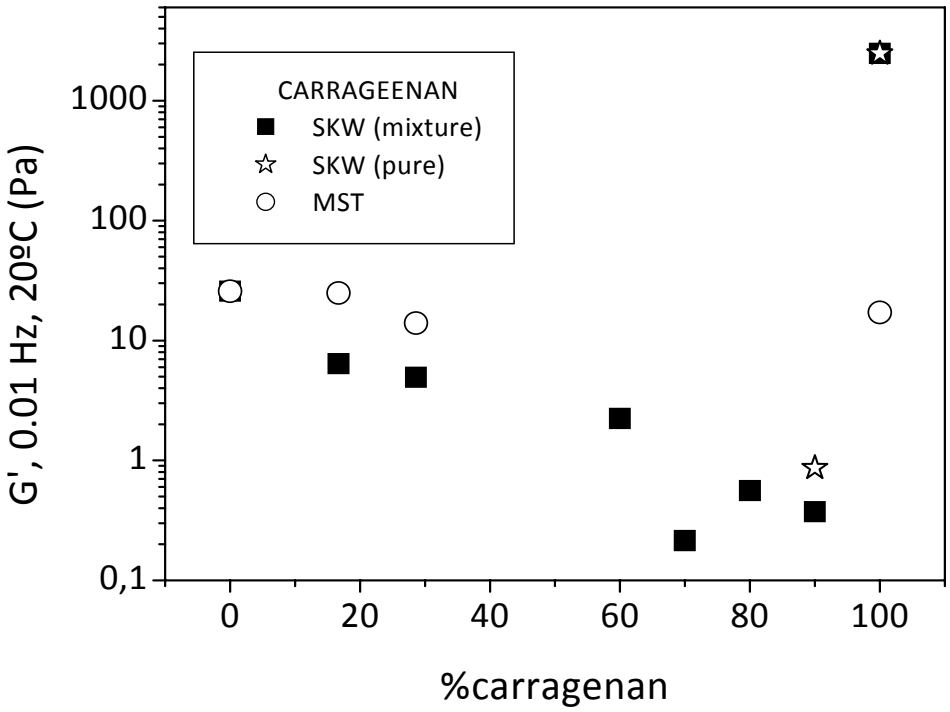

Figure 6.14 - Storage modulus variation with concentration of carrageenan (SKW and MST) at 0.01Hz and 20ºC.

6.1.5. Conclusions

Homogeneous, thin, flexible and translucent films were obtained from rice starch-carrageenan mixtures. Increasing carrageenan content in the film formulation resulted in an increasing film higroscopicity and a decreasing glass transition T_g of films. Accordingly, film's mechanical properties are seen to be improved, since both tensile strength and elongation at break increased. Water vapour permeability of SKW and MST films were similar to WVP of other polysaccharides (methylcellulose and chitosan), even to cellophane, a synthetic polymer. SKW films were colourless whereas MST films had a slightly yellow-green appearance. Rheological characterization of the mixtures at a 5% total polymer concentration suggests that carrageenan actually acts as a plasticizer, and that water plasticization alone does not account for the increased mechanical properties of the films. Additional rheological studies, together with structural characterization of

the mixtures and films are however needed to assess the origin of the carrageenan plasticizing effect. The use of κ/ι-hybrid carrageenan, extracted from *Mastocarpus stellatus*, as a substitute to commercial κ-carrageenan, seems to be validated by the results obtained so far, as films hygroscopic characteristics and mechanical properties are qualitatively similar.

The carrageenan used in the subsequent sections (6.2, 6.3, 6.4 and 6.5) in the films formulation was the commercial κ-carrageenan face the similar results obtained for both carrageenans (commercial and extracted from *Mastocarpus stellatus*) and due to its higher availability comparing with κ/ι-hybrid carrageenan.

6.2. Comparing casting with knife coating

Studies were carried out to compare the characteristics of films produced by casting and by knife coating, using mixtures of rice starch and commercial carrageenan.

6.2.1. Materials

Rice starch (S7260, Sigma-Aldrich, USA) and commercial κ-carrageenan (SKW Biosystems, France) were used as the film forming components to provide a continuous matrix of edible film.

6.2.2. Preparation of starch-carrageenan based films

Rice starch and carrageenan were used to prepare starch-carrageenan mixtures, with different amounts of carrageenan (16.67, 33.33, 44.44 and 100% dry basis). Films were prepared by the casting and by knife coating methods, as described in Chapter 5. The thickness of film samples had a

mean value of 32.5 ± 10 μm for the films produced by the knife coating method and 11.5 ± 2 μm for the films produced by the casting.

6.2.3. Film characterization

Film samples were characterized as was described in Chapter 5. Film mechanical properties and water vapour permeability were evaluated in order to assess differences due to each of the two methods to produce films (casting and knife coating).

6.2.4. Results and discussion

Homogeneous, thin, flexible and translucent films were obtained from rice starch-carrageenan mixtures by the methods used, casting and knife coating. All the films were easily removed from the supports, a Teflon® plate (casting) and an acrylic plate (knife coating), showing smooth surfaces. The films made by knife coating were thicker and more uniform than those made by casting. Because the film-forming solution for the knife coating process (>7% polymer in solution) is more concentrated than for the casting process (<3% polymer in solution), drying is faster for the former (about 2 hours for knife coating and 24 hours for casting).

6.2.4.1. Mechanical properties

The tensile properties (tensile strength, elongation and Young's modulus) of the starch-carrageenan based films are presented in Figures 6.15, 6.16 and 6.17. The behaviour of tensile strength (TS) and elongation at break (E) varying according to the carrageenan content. The TS results (Figure 6.15) are significantly different ($p<0.05$) for the films with 16.67, 33.33 and 44.44% of carrageenan made by casting and knife coating. TS significantly increases ($p<0.05$) with the increase of carrageenan content in the mixture, for both methods of film production. TS results were higher for the films made by knife coating and the results obtained for each carrageenan

content more reproducible. Elongation results (Figure 6.16) were significantly different ($p<0.05$) for the films with 16.67, 33.33 and 44.44% carrageenan content, comparing films made by casting and knife coating. Films produced by casting were significantly different, increasing with the increase of carrageenan content in the mixture. For the films made by knife coating, elongation did not have significant variation. The behaviour observed for elongation results was similar for both methods used to produce the films; this aspect is discussed in the section *6.1.4.2*. The Young's modulus results (Figure 6.17) were significantly different ($p<0.05$) for the films with 16.67 and 44.44% carrageenan content by the two production methods. This behaviour is also discussed in the section *6.1.4.2*. For the films produced by knife coating, Young's modulus presented the same tendency of the tensile strength, increasing with the increase of carrageenan content in the mixture but decreasing for a pure carrageenan film.

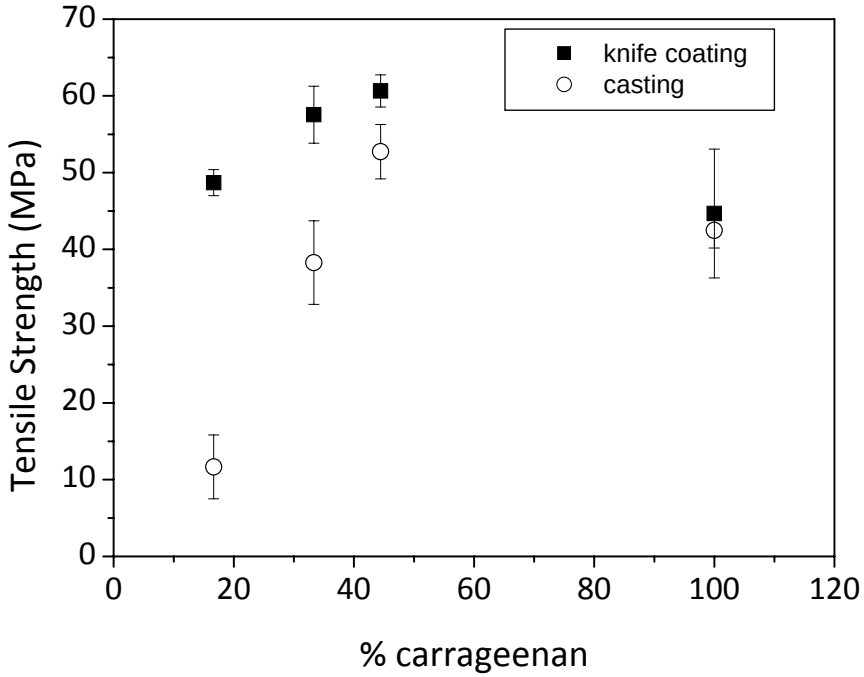

Figure 6.15 - Tensile strength for the starch-carrageenan films varying the amount of carrageenan (16.67, 33.33, 44.44 and 100%) and method of production (■ knife coating, ○ casting).

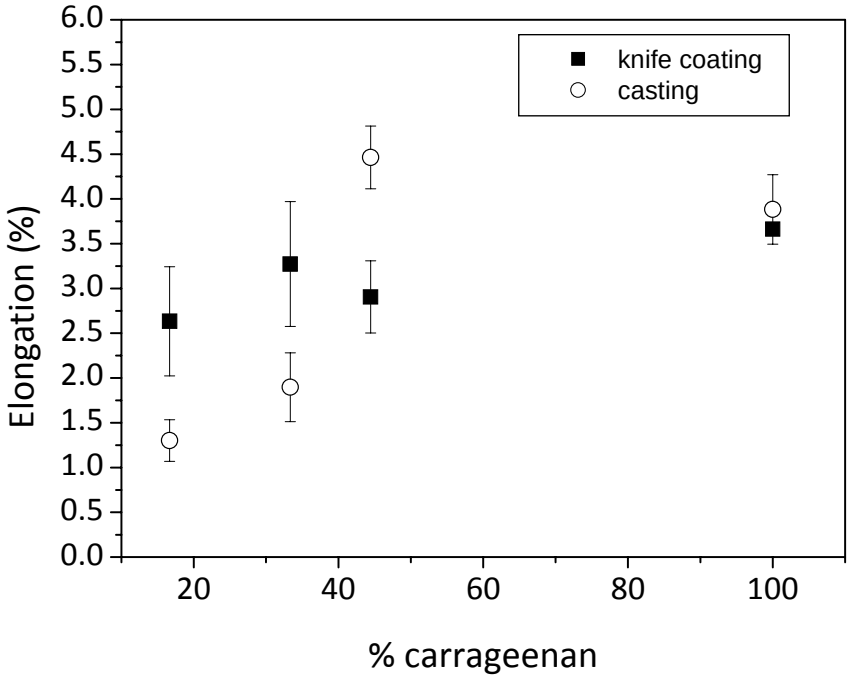

Figure 6.16 - Elongation for the starch-carrageenan films varying the amount of carrageenan (16.67, 33.33, 44.44 and 100%) and method of production (■ knife coating, ○ casting).

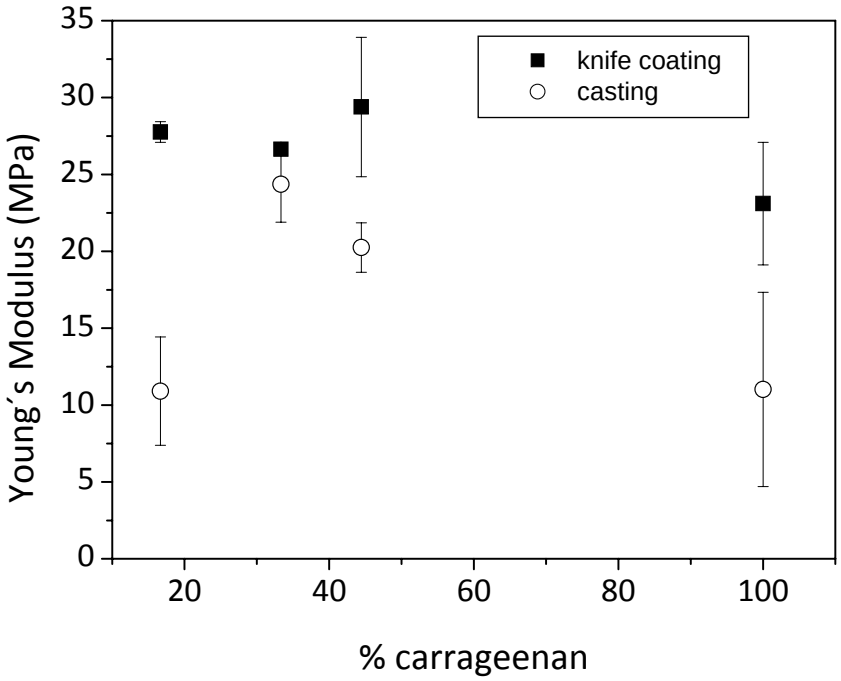

Figure 6.17 - Young's modulus for the starch-carrageenan films varying the amount of carrageenan (16.67, 33.33, 44.44 and 100%) and method of production (■ knife coating, ○ casting).

6.2.4.2. Water vapour permeability

Figure 6.18 shows the comparison of WVP of starch-carrageenan films made by knife coating and by casting. ANOVA test indicated that films produced by both methods did not differ significantly ($p>0.05$). WVP values ranged between 1.23 and 2.74 x 10^{-10} g m^{-1} s^{-1} Pa^{-1} for films produced by knife coating and 0.75 and 0.91 x 10^{-10} g m^{-1} s^{-1} Pa^{-1} for films made by casting. The films presented a similar trend with the variation of carrageenan content in the mixture. The WVP values were higher for the films produced by knife coating, because they were thicker than those produced by casting. The film thicknesses are presented in Table 6.6.

Table 6.6 – Thickness of starch-carrageenan based films produced by knife coating and casting methods for the WVP measurements.

Composition (carrageenan content)	Thickness (μm)	
	Knife coating	Casting
16.7%	36	12
33.3%	43	13
44.4%	32	12
100%	19	9

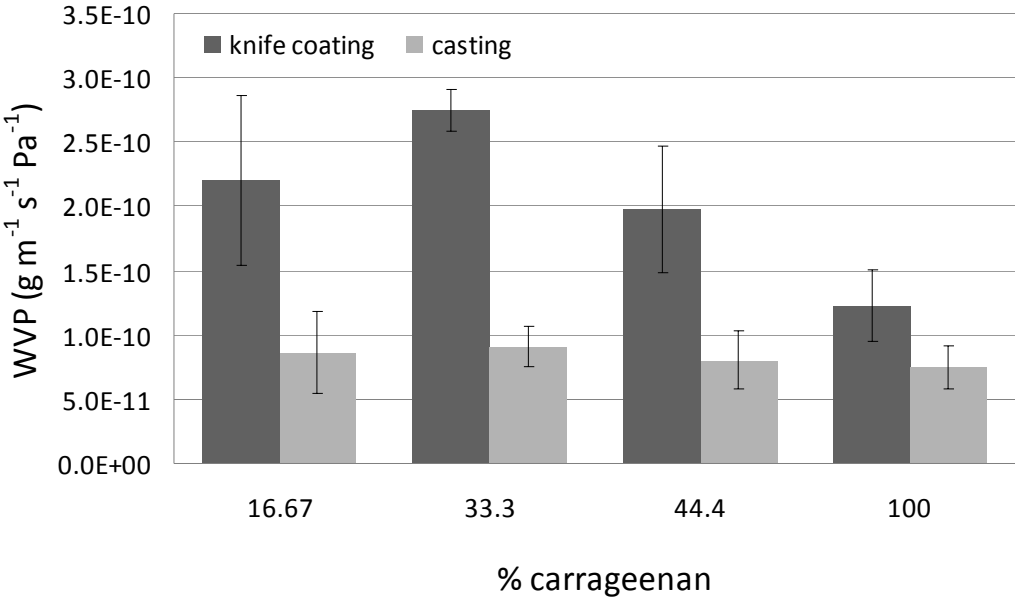

Figure 6.18 – Water vapour permeability (WVP) of starch-carrageenan films varying the amount of carrageenan (16.7, 33.3, 44.4 and 100%) and method of production (knife coating and casting).

In ideal polymeric structures gas and vapour permeability are independent of the film thickness (Schwartzberg, 1986). However, there is experimental evidence (McHugh et al., 1993; Park and Chinnan, 1995) of non ideal behaviour. In this study it was observed that WVP of starch-carrageenan based films increased linearly with film thickness, regardless the composition (Figure 6.19a). The linear fitting of the experimental data leads to a regression coefficient (R^2) of 0.99. Considering the formulation, the linear fittings obtained were the same for all compositions (Figure 6.19b).

This positive deviation from the ideal behaviour indicates that starch-carrageenan based films have an affinity for moisture that is not taken into account in Fick's and Henry's laws as can be seen in Figure 6.1a (non linear isotherms, for the SKW films made by casting). Several explanations for thickness effects on edible films have been reported. McHugh et al. (1993) observed that, as film thickness increased, the film provided and increased resistance to mass transfer across it; consequently, the equilibrium water vapour partial pressure at inner film surface increased. Other authors attributed thickness effect to film swelling as a result of attractive forces between polymer and water (Park et al., 1993). Film swelling could result in varying film structures.

From the permeability equation (Eq. 5.2) and experimental data it becomes quite clear that water vapour flux through the film (weight gain/time x area) (g s^{-1} m^{-2}) remains independent of the film thickness, which would imply that flux in this type of edible films does not depend on thickness in the range of film thickness studied (9 to 43 μm). Due to the strong hygroscopic nature of starch-carrageenan films, it could be possible that during permeation process the film matrix, exposed to the high relative humidity side, sorbed enough water that desorption rate becomes independent of thickness resistance. As a whole the film behaves as only a thin portion of it, at the low relative humidity side, which is responsible of the mass transfer resistance. Under these conditions the flux can become thickness independent and permeability will increase linearly with thickness (Bertuzzi et al., 2007).

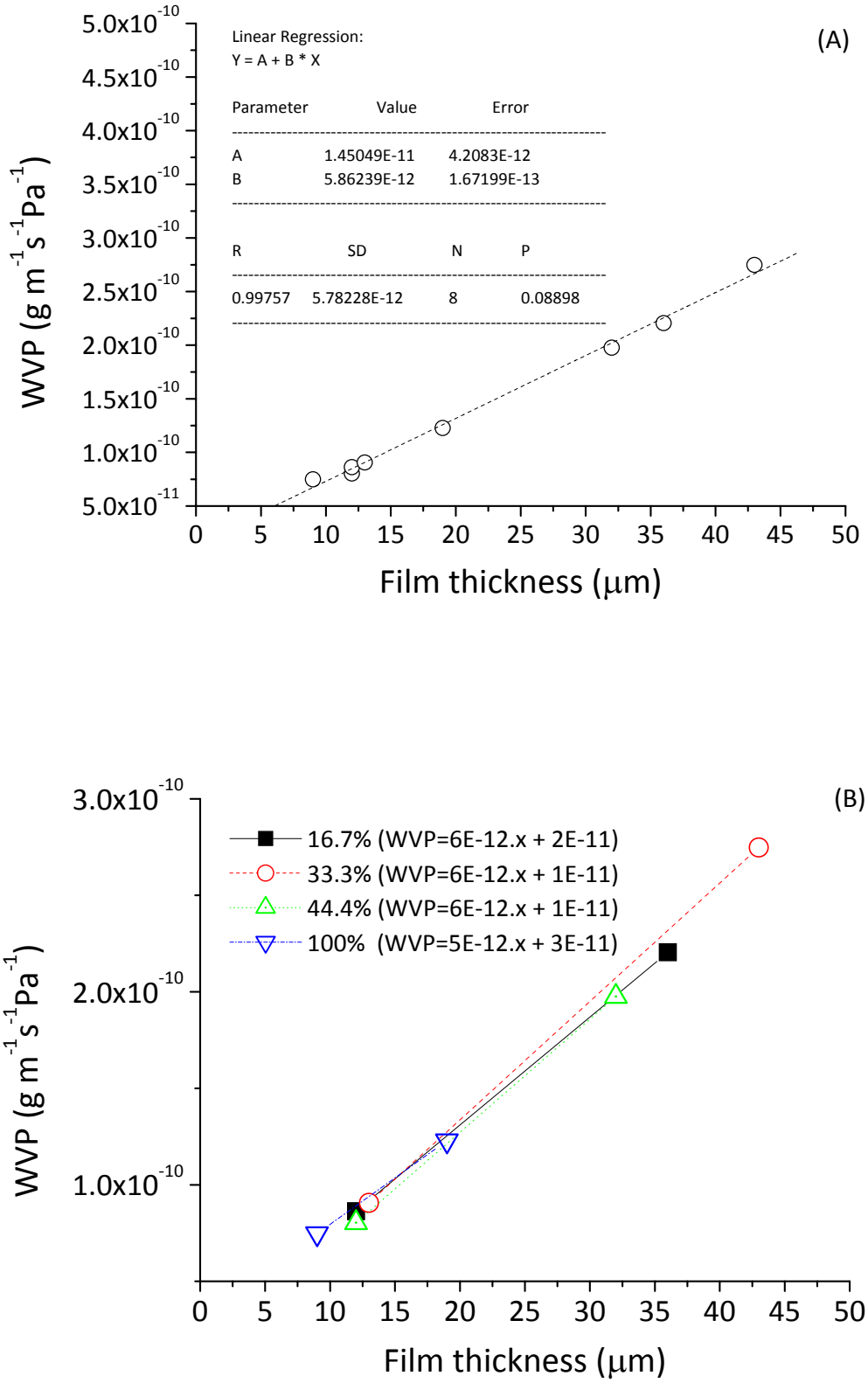

Figure 6.19 – Effect of film thickness on water vapour permeability for starch-carrageenan based films: (A) Linear fitting not considering the film formulation; (B) Linear fittings considering the film formulation.

6.2.5. Conclusions

The comparison of both methods of films production, knife coating and casting, did not show great differences on the properties studied. The main output in this study was that the films produced by knife coating method had more uniformity in their thickness over the film than the films produced by casting. This characteristic led to more uniform results for the film properties and a better reproducibility, mainly in the tensile tests. The mechanical properties showed a similar behaviour for all the properties studied (tensile strength, elongation and Young's modulus). The WVP results showed a linear increase with the film thickness and because of this the WVP values were higher for the films produced by knife coating. Based on the results obtained and their good reproducibility, the use of the knife coating method as a semi-continuous process showed to be a good alternative to produce films in a larger scale.

6.3. Assessment of the effect of possible molecular orientation by knife coating

Tensile tests were carried out to assess whether direction of application inherent to the knife coating method produced any molecular orientation and a consequent anisotropy of the resulting film.

6.3.1. Materials

Corn starch (S4126, Sigma-Aldrich, USA) and commercial κ-carrageenan (SKW Biosystems, France) were used as the film forming components to provide a continuous matrix of edible film.

6.3.2. Preparation of corn starch-carrageenan based films

Corn starch and carrageenan were utilized to prepare starch-carrageenan mixtures. The amount of carrageenan in the mixture was 30 g/100 g of corn starch. Films were prepared by the knife coating method, as described in Chapter 5. The average thickness of film samples was 50 ± 1 μm.

6.3.3. Film characterization

Tensile tests were performed, as was described in Chapter 5, to assess the effect of application direction on the mechanical properties of films. The film samples were cut with the two orientations, parallel and perpendicular to the application direction as explained in Figure 6.20.

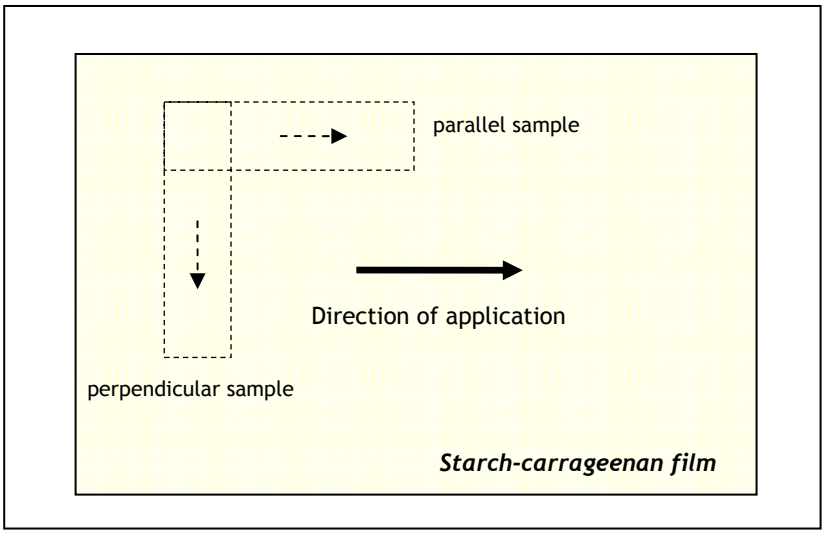

Figure 6.20 – Scheme of sample preparation to verify the effect of the direction of application on mechanical properties of the starch-carrageenan films.

6.3.4. Results and discussion

Homogeneous, thin, flexible and transparent films were obtained from corn starch-carrageenan mixtures by the knife coating method. All the films were easily removed from the acrylic support and showed smooth surfaces.

The mechanical properties results for the starch-carrageenan based films are presented in Figures 6.21, 6.22 and 6.23. The starch-carrageenan films were not significantly different ($p>0.05$) in terms of all mechanical properties studied. These results indicate that the film's mechanical properties were independent of the direction of application and exhibited an isotropic behaviour.

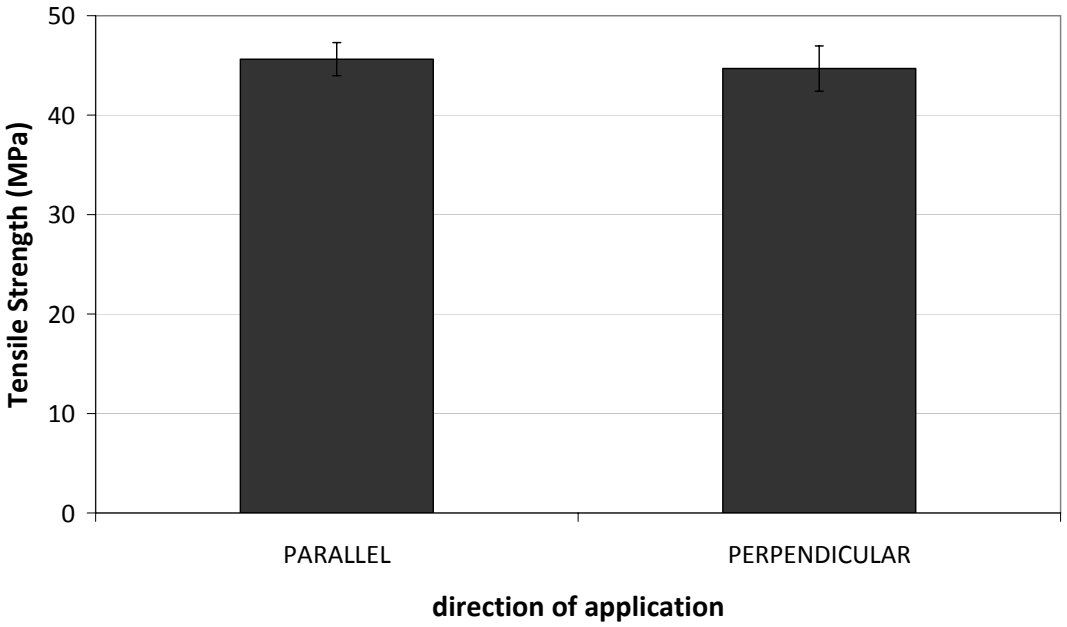

Figure 6.21 – Tensile strength of starch-carrageenan based films cut in the directions parallel and perpendicular to application.

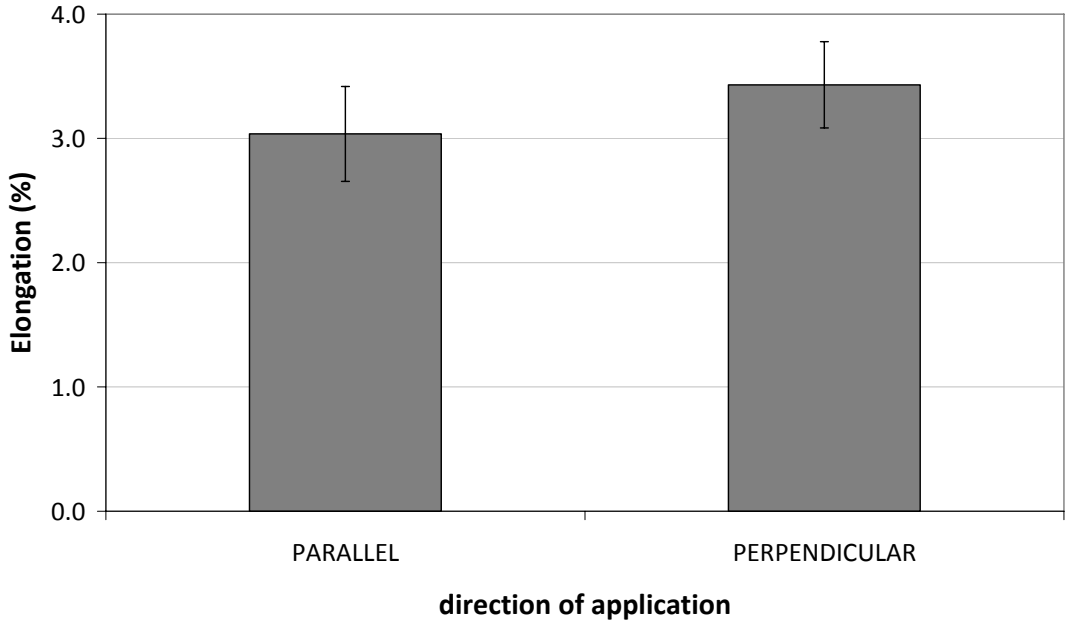

Figure 6.22 – Elongation of starch-carrageenan based films cut in the directions parallel and perpendicular to application.

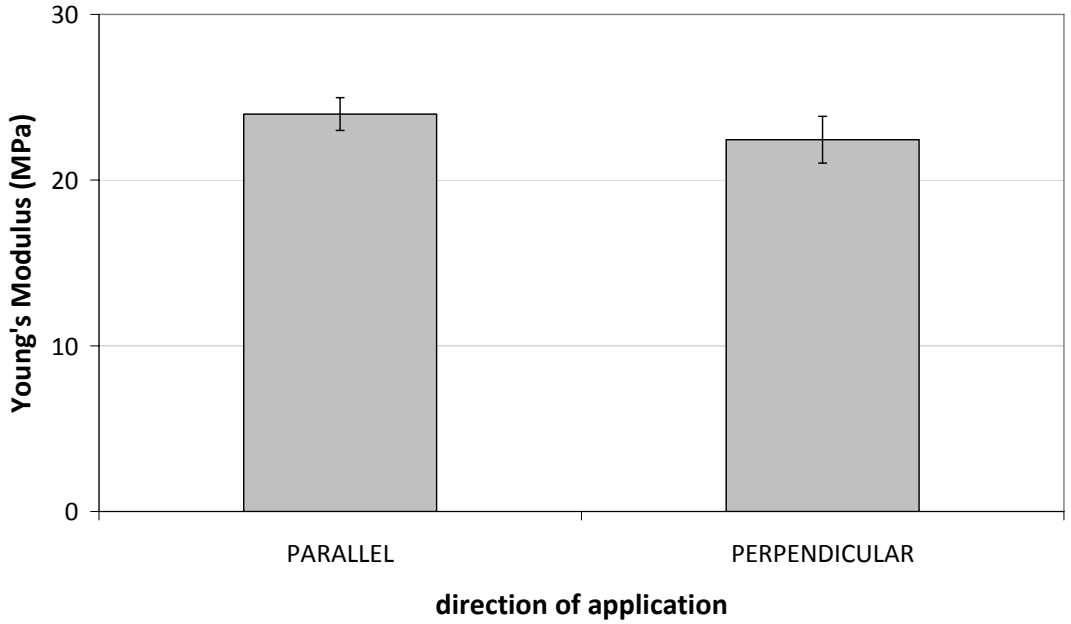

Figure 6.23 –Young's Modulus of starch-carrageenan based films cut in the directions parallel and perpendicular to application.

6.3.5. Conclusions

The starch-carrageenan films obtained by the knife coating method were homogeneous and very uniform, presenting a maximum variation of 1 μm in the thickness of all samples tested. The direction of application to produce films in this semi-continuous process did not show significantly differences in the mechanical properties studied, concluding that the starch-carrageenan based films obtained possess an isotropic behaviour, so the films can be analyzed independent of the direction of application.

6.4. Effect of different plasticizers in the film properties

Studies were carried out to evaluate the effect of different plasticizers (hydrophilic and hydrophobic) on the properties of starch-carrageenan based films.

6.4.1. Materials

Corn starch (S4126, Sigma-Aldrich, USA) and commercial κ-carrageenan (SKW Biosystems, France) were used as the film forming component to provide a continuous matrix of edible film. Hydrophilic and hydrophobic plasticizers were used in the film formulation. The plasticizers used were:

- Hydrophilic:
    - Glycerol (GLY) (Merck, Germany);
    - Polyethylene glycol 400 (PEG) (VWR – Prolabo, France);
- Hydrophobic:
    - Tributyl citrate (TBC) (Merck, Germany);
    - Tributylacetyl citrate (TBAC) (Merck, Germany);
    - Triethyl citrate (TEC) (Fluka, Germany);
    - Triethyl *O*-acetylcitrate (TEAC) (Sigma-Aldrich, Germany).

6.4.2. Preparation of corn starch-carrageenan based films

Corn starch and carrageenan were used to prepare starch-carrageenan mixtures. The amount of carrageenan in the mixture was 30 g/100 g of corn starch, and the plasticizer was added in the proportion of 10 g/100 g of corn starch, for each film formulation with the different plasticizers. Films were prepared by the knife coating method, as described in Chapter 5. The plasticizer was added to the mixture after the solubilization of starch and carrageenan. The average thickness of film samples was 40 ± 5 μm.

6.4.3. Film characterization

Film samples were characterized as described in Chapter 5. Film properties evaluated to compare the effect of different plasticizers in the film properties were: moisture sorption isotherms, mechanical and optical properties.

6.4.4. Results and discussion

Homogeneous, thin, flexible and transparent films were obtained from corn starch-carrageenan mixtures by the knife coating method, using different plasticizers. All films were easily removed from the acrylic supporting plate and showed smooth surfaces. All the films were colourless (Figure 6.24). The films with hydrophobic plasticizers presented moisture exudation after drying.

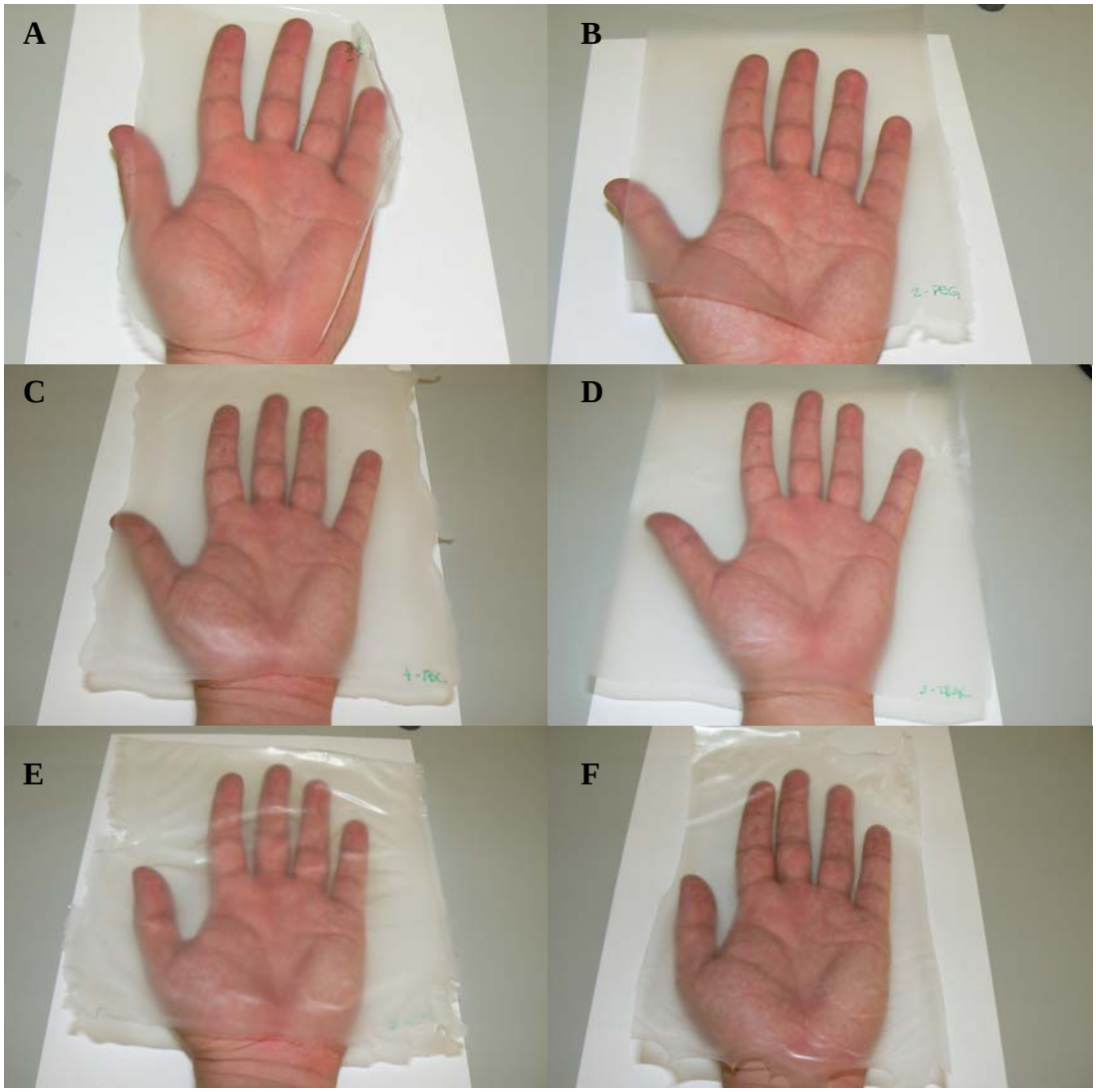

Figure 6.24 – Starch-carrageenan based films with different plasticizers: (A) GLY; (B) PEG; (C) TBC; (D) TBAC; (E) TEC; (F) TEAC.

6.4.4.1. Moisture sorption isotherms

Moisture sorption isotherms at 25ºC for films containing different plasticizers (glycerol (GLY), polyethylene glycol 400 (PEG), tributyl citrate (TBC), tributylacetyl citrate (TBAC), triethyl citrate (TEC), and triethyl *O*-acetylcitrate (TEAC)) are presented in Figure 6.25, together with the GAB model (van der Berg, 1984) fitted for each sample. The GAB equation parameters and the correlation coefficients are presented in Table 6.7. The

values of k (<1) and the correlation coefficient (r>0.98) show that GAB equation gives a good fit to experimental values.

As presented in Figures 6.25, 6.26 and 6.27, the isotherm curves presented a sigmoid shape and indicate that the equilibrium moisture content increases slowly with increasing environmental a_w up to 0.7, beyond which a steep rise in moisture content in film samples was observed. Due to their hydrophilic nature, films with hydrophilic plasticizers tend to absorb larger quantities of water at high relative humidity conditions. For higher water activities (a_w>0.8), the moisture content of starch-carrageenan based films was more sensitive to relative humidity when a hydrophilic plasticizer (GLY and PEG) was used. The isotherms corresponding to films with hydrophobic plasticizers (TBC, TBAC, TEC and TEAC) exhibited a less hygroscopic behaviour for high water activities, as expected, due to their hydrophobic nature. Although there are studies focused on sorption properties of edible films, little information is available on the effect of type of plasticizer on sorption characteristics of these films (Cho and Rhee, 2002). Films with GLY and PEG presented similar behaviour in terms of moisture sorption, and the same trend was observed between the films with hydrophobic plasticizers (TBC, TBAC, TEC and TEAC). The higher water uptake shown by films with hydrophobic plasticizers compared with the films with hydrophilic plasticizers at water activities below 0.7, can be explained by the water exudation presented in films with hydrophobic plasticizers, because the samples were weighed with the water eliminated present in the sample surface, which was not adsorbed by the film. The moisture sorption isotherm is a means to characterize the water absorption property of the film, which in turn is transmitted to the product inside (Srinivasa et al., 2007).

Table 6.7 - GAB equation parameters for moisture sorption isotherms (r is the correlation coefficient) for the rice starch-commercial carrageenan based films.

Sample	X_0	C	k	r
GLY	0.051	7.264	0.941	0.997
PEG	0.049	7.472	0.910	0.993
TBC	0.084	29.388	0.760	0.992
TBAC	0.084	21.136	0.768	0.997
TEC	0.087	25.413	0.749	0.993
TEAC	0.089	30.718	0.725	0.995

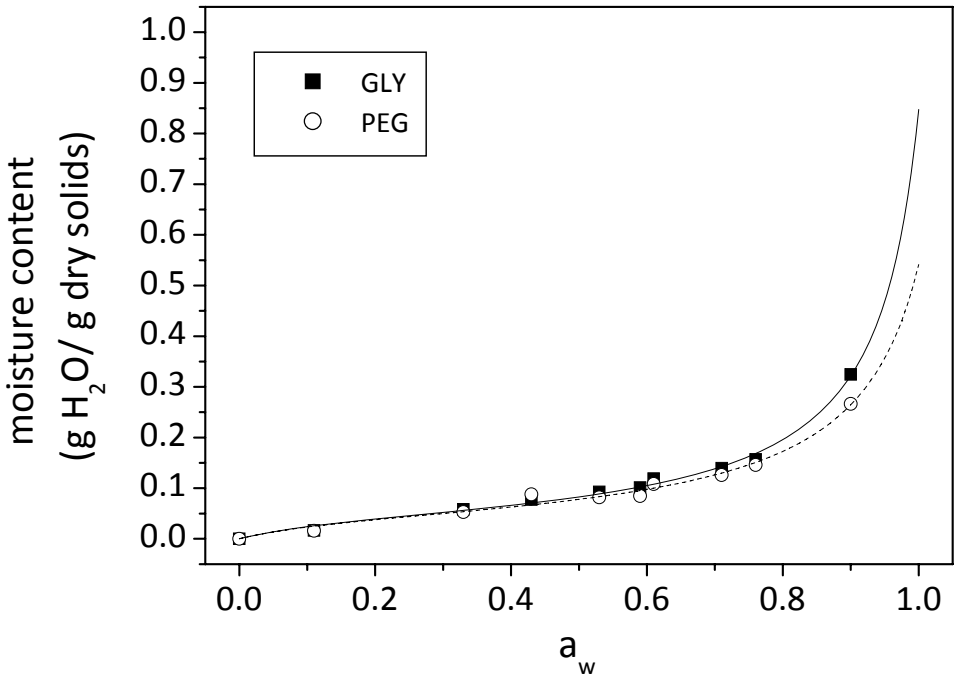

Figure 6.25 - Experimental data for moisture sorption isotherms of the starch-carrageenan based films varying the plasticizer, and the respective fitted GAB curves: comparison GLY-PEG.

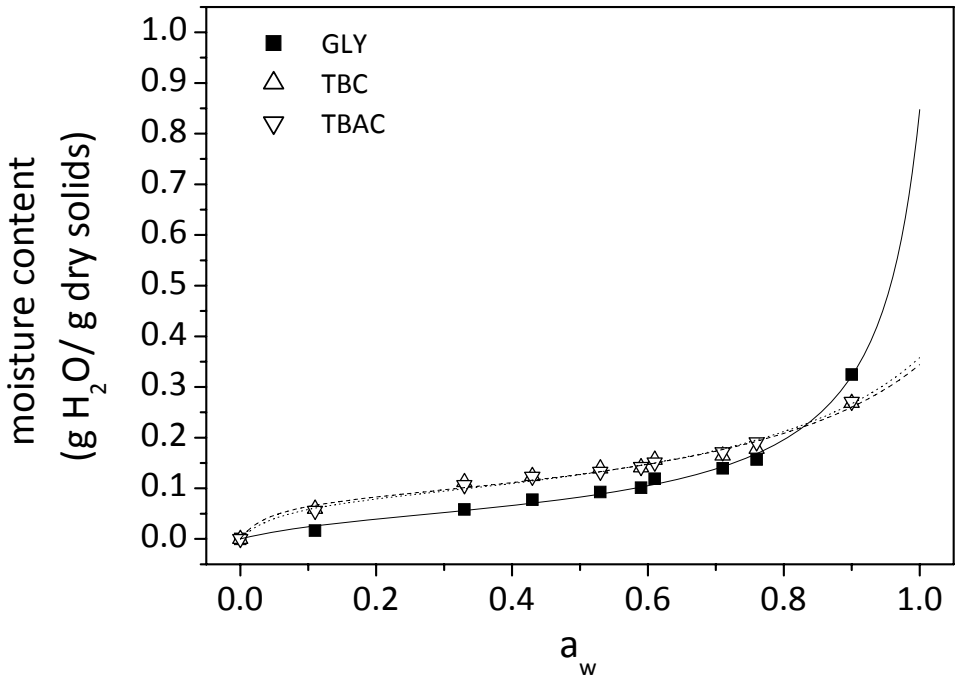

Figure 6.26 - Experimental data for moisture sorption isotherms of the starch-carrageenan based films varying the plasticizer, and the respective fitted GAB curves: comparison GLY-TBC-TBAC.

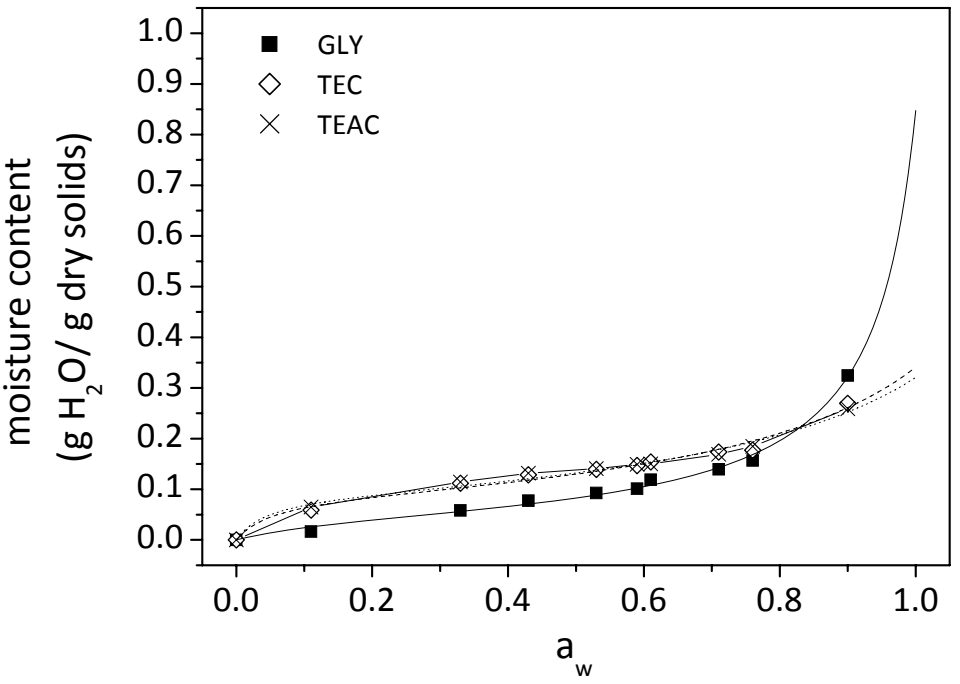

Figure 6.27 - Experimental data for moisture sorption isotherms of the starch-carrageenan based films varying the plasticizer, and the respective fitted GAB curves: comparison GLY-TEC-TEAC.

6.4.4.2. Mechanical properties

Mechanical strength of films decreases due to plasticizer addition and water sorption resulting in decreased Young's modulus and tensile strength, and increased elongation (Lourdin et al., 1997; Mathew and Dufresne, 2002). As expected, the addition of plasticizers produced films that were less stiff and rigid, and more extendible. Values of Young's modulus decreased with the addition of plasticizers with a concurrent increase in elongation of films at break, and decrease in tensile strength (Figure 6.28). All the films were different significantly ($p<0.05$) for the tensile properties studied (tensile strength, elongation and Young's modulus), and the best results were obtained for the films formulated with hydrophilic plasticizers. Good results were also reached for the plasticizers TBAC and TEC, comparing the films with hydrophobic plasticizers. Effects of plasticizers on elongation were largest for the hydrophilic plasticizers (GLY and PEG) and for the triethyl citrate (TEC). The decrease in the tensile strength and Young's modulus was not so evidenced for the films plasticized with glycerol comparing with the unplasticized film.

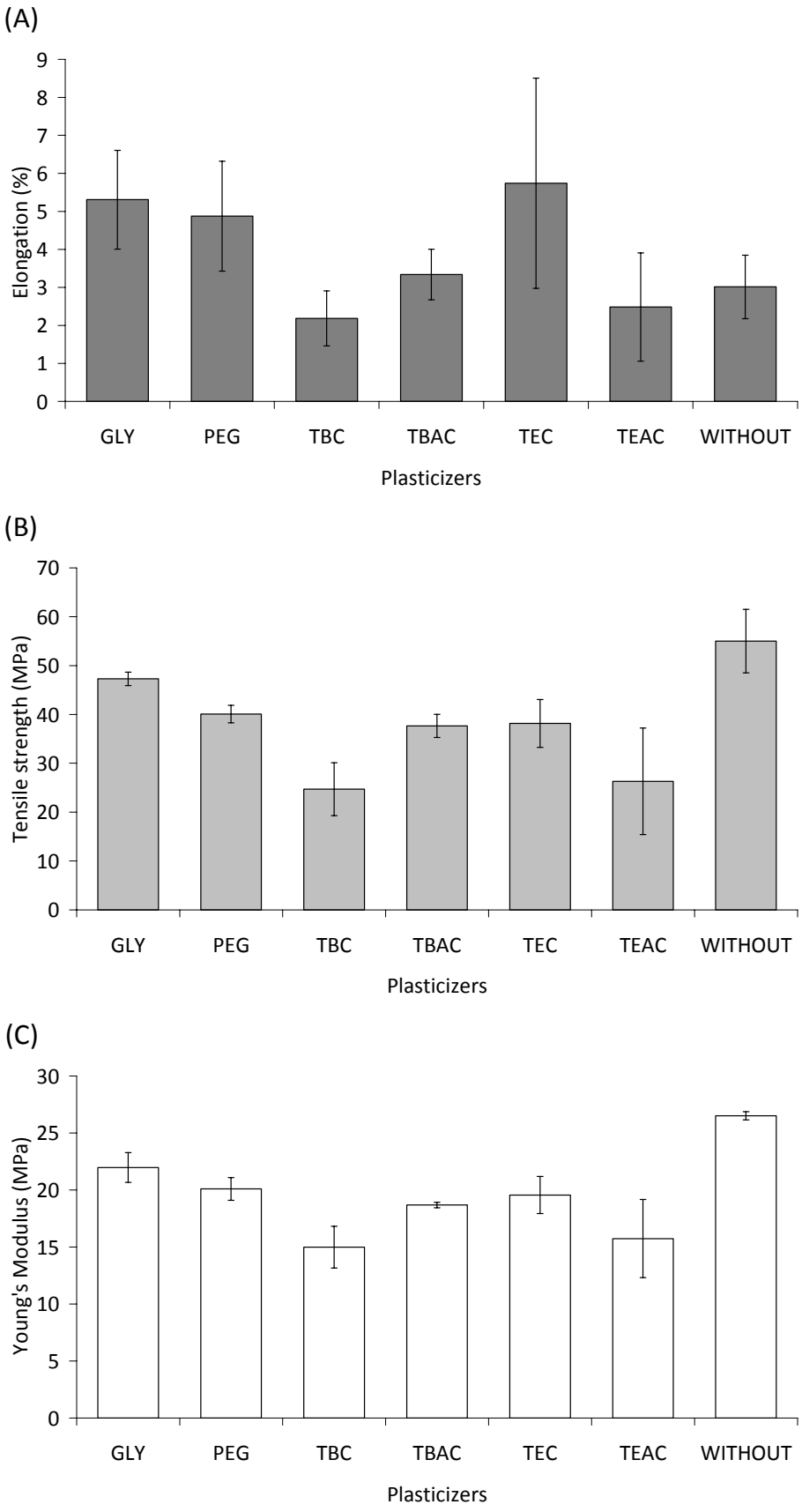

Figure 6.28 – Elongation (A), tensile strength (B) and Young's modulus (C) for starch-carrageenan based films with different plasticizers.

6.4.4.3. Optical properties

The results of colour standards of the starch-carrageenan based films with different plasticizers are presented in Table 6.8. All the films studied presented very similar results in terms of colour. Instrumental colour determination performed on films showed significant differences ($p<0.05$) in the *L** values for all assayed films. For the chromaticity parameters (*a** and *b**) did not have significant differences ($p>0.05$) between the studied films.

Table 6.8 – Colour standards of the starch-carrageenan film with different plasticizers.

Film samples	Lightness (*L**)	Chromaticity parameter *a**	Chromaticity parameter *b**	Colour differences (*ΔE**)
GLY	96.26 ± 0.42	0.02 ± 0.03	2.90 ± 0.09	1.34 ± 0.20
PEG	96.58 ± 0.08	0.05 ± 0.03	2.65 ± 0.11	0.95 ± 0.04
TBC	97.08 ± 0.02	-0.01 ± 0.01	2.95 ± 0.04	1.07 ± 0.03
TBAC	96.94 ± 0.02	0.02 ± 0.02	2.79 ± 0.06	0.93 ± 0.06
TEC	97.23 ± 0.19	-0.03 ± 0.02	2.98 ± 0.17	1.11 ± 0.19
TEAC	96.97 ± 0.32	0.04 ± 0.01	2.81 ± 0.03	0.94 ± 0.02

* CIELab standards for the white standard, used as the film background were: L_s^* (97.14), a_s^* (0.06) and b_s^* (1.88).

Table 6.9 shows the light transmission and transparency of starch-carrageenan films with different plasticizers at 600 nm that is commonly used wavelength for the film transparency (Han and Floros, 1997; Fang et al., 2002; Shiku et al., 2003).

The addition of hydrophobic plasticizers in the formulation decreases the light transmission of the films, improving the barrier to light transmission, at the selected wavelength of 600 nm (Table 6.9), when compared with the films with hydrophilic plasticizers. The films with hydrophobic plasticizers blocked most light in the wavelength range of 600 nm because they are less transparent than those with hydrophilic plasticizers.

Table 6.9 – Light transmission (%, T_{600}) and transparency (A_{600}/mm) of starch-carrageenan based films varying the plasticizer.

Light Transmission and Transparency	PLASTICIZERS					
	GLY	PEG	TBC	TBAC	TEC	TEAC
%, T_{600}	68.00	66.20	40.33	42.59	42.47	36.52
A_{600}/mm	4.02	4.48	9.86	10.02	10.53	10.50

Figure 6.29 presents the absorbance of light in the range of 200 to 600 nm. It can be observed that in the UV light range of 200 to 280 nm, the addition of hydrophobic plasticizers improved the barrier properties to UV light of the films, when compared to the films with hydrophilic plasticizers. Starch-carrageenan film plasticized with TEC was the one that had the higher absorbance of UV light (in the 200-280 nm region).

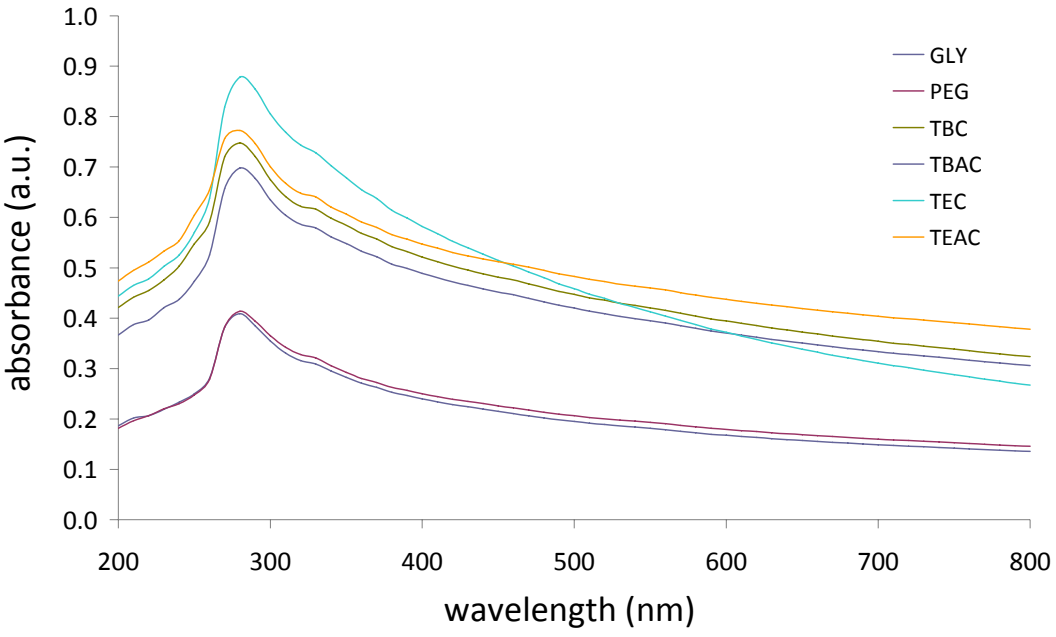

Figure 6.29 - Light transmission spectra (absorbance) of starch-carrageenan based films with different plasticizers.

6.4.5. Conclusions

In summary, the starch-carrageenan based films prepared using different plasticizers showed several interesting characteristics. Plasticizer type was found to affect physical and mechanical properties of the films. Water content was higher at lower water activities for films with hydrophobic plasticizers as compared to films with hydrophilic plasticizers. At higher water activities the opposite occurred, the films with hydrophilic plasticizers adsorbed more water than the films with hydrophobic plasticizers. In terms of mechanical properties, the tensile strength of the films decreased while elongation increased with the addition of plasticizer. Glycerol was found to be the most suitable plasticizer to incorporate into starch-carrageenan films when compared with other plasticizers studied. Glycerol films were more flexible than the other plasticized films. The films with hydrophobic plasticizers presented moisture exudation as a non-favourable characteristic. In terms of optical properties, all the films were very similar, and the films with hydrophobic plasticizers had better UV barrier properties than the films with hydrophilic plasticizers. The use of plasticizer produces films with good workability and properties. Further studies were done with the plasticizers with the best performance in each group of plasticizers, glycerol (GLY) and triethyl citrate (TEC); these will be presented in section 6.6 of this Chapter.

6.5. Effect of carrageenan and plasticizer contents and application speed on the mechanical properties of starch-carrageenan based films.

An experimental design was used to evaluate the influence of carrageenan and plasticizer contents and application speed on the mechanical properties of starch-carrageenan based films.

6.5.1. Materials

Corn starch (S4126, Sigma-Aldrich, USA) and commercial κ-carrageenan (SKW Biosystems, France) were used as the film forming component to provide a continuous matrix of edible film. Triethyl citrate (TEC) (Fluka, Germany) was used as plasticizer because it had the better results among the hydrophobic plasticizers in section 6.4.

6.5.2. Preparation of corn starch-carrageenan based films

Corn starch and carrageenan were used to prepare starch-carrageenan mixtures. Films were prepared by the knife coating method, as was described in Chapter 5. Film formulations are presented in Table 6.10.

6.5.3. Film characterization

Tensile tests were performed to evaluate the mechanical properties of the films, as was described in Chapter 5.

6.5.4. Experimental design

A full factorial design (2^3) with two replicates at the central point was performed. The selected variables were carrageenan content, TEC content and application speed of film forming solution in the knife coating process. The experimental design with the coded and actual values of the variables is shown in Table 6.10. All experiments were performed randomly and data were treated with the aid of STATISTICA 6.0 from Statsoft Inc.

Table 6.10 - Experimental design matrix with code and real values of the variables.

run	% carrageenan (x_1)*	% plasticizer (x_2)*	speed (mm/s)(x_3)*
1	30 (-1)	10 (-1)	100 (-1)
2	50 (+1)	10 (-1)	100 (-1)
3	30 (-1)	20 (+1)	100 (-1)
4	50 (+1)	20 (+1)	100 (-1)
5	30 (-1)	10 (-1)	300 (+1)
6	50 (+1)	10 (-1)	300 (+1)
7	30 (-1)	20 (+1)	300 (+1)
8	50 (+1)	20 (+1)	300 (+1)
9	40 (0)	15 (0)	200 (0)
10	40 (0)	15 (0)	200 (0)

* Independent variable values (the values in parentheses are the coded variable values).

6.5.5. Results and discussion

Under stress conditions that would occur during processing, such as handling and storage, the expected film integrity can be indicated by its mechanical properties. The resistance and flexibility of films can be described by its tensile strength (TS), elongation at break (E) and Young's modulus (Y) (Park et al., 2001).

The films obtained from the mixture of corn starch and carrageenan with plasticizer (TEC) have homogeneous and smooth surfaces with their thickness in the range of 50-70 μm. The results of film mechanical properties obtained for the 2^3 full factorial design are presented in Table 6.11.

Table 6.11 – Mechanical properties (tensile strength, elongation and Young's modulus) of the films of the experimental design matrix (Table 6.9).

run	Tensile Strength (MPa)	Elongation (%)	Young's Modulus (MPa)
1	16.32	1.37	13.96
2	27.83	1.68	18.31
3	21.67	1.53	15.51
4	20.89	1.51	15.95
5	29.35	1.90	18.77
6	26.52	1.64	18.59
7	12.35	0.82	15.17
8	11.97	0.78	14.05
9	14.85	1.03	15.24
10	17.67	0.91	20.22

The film corresponding to sample 5, with 30% carrageenan and 10% plasticizer, applied at 300 mm/s, showed the highest tensile strength (29.35 MPa) and elongation (1.90%). Samples 2 and 6, with 50% of carrageenan, showed also good results in terms of tensile strength and elongation. These results can be explained by the statistical analysis of the effects of the variables studied over these properties, given in Table 6.12. It can be observed that carrageenan concentration has a positive effect on tensile strength and Young's modulus, but slightly negative effect on elongation. This behaviour observed for carrageenan agrees with the results presented in sections 6.1 and 6.2 of this Chapter, where the increase of carrageenan in the mixture resulted in increased tensile strength and elongation. This increase in elongation is probably related to the hygroscopicity of carrageenan. The plasticizer presented a negative effect on the film properties that may be explained by its hydrophobic nature, reducing water adsorption and decreasing the plasticizing effect of carrageenan. The application speed showed a negative effect on tensile strength and elongation and a positive effect on the Young's modulus.

Table 6.12 – Main effects and interaction analysis for the response of mechanical properties of tensile strength, elongation and Young's modulus.

	Tensile Strength			***Elongation***			***Young's Modulus***		
factor	effect (MPa)	standard error	*p* value	effect (%)	standard error	*p* value	effect (MPa)	standard error	*p* value
carrageenan (x_1)	1.880	3.292	0.608	-0.002	0.240	0.992	0.872	1.675	0.638
plasticizer (x_2)	-8.285	3.292	0.086	-0.487	0.240	0.135	-2.237	1.675	0.274
application speed (x_3)	-1.630	3.292	0.654	-0.237	0.240	0.396	0.712	1.675	0.699
x_1x_2	-2.460	3.292	0.509	-0.027	0.240	0.916	-1.212	1.675	0.521
x_1x_3	-3.485	3.292	0.367	-0.147	0.240	0.582	-1.522	1.675	0.430
x_2x_3	-7.490	3.292	0.107	-0.482	0.240	0.138	-1.832	1.675	0.354

A regression analysis was performed to obtain first-order model equations (Equations 6.1-3) for the mechanical properties as a function of carrageenan and plasticizer concentration and application speed.

$$\textit{Tensile Strength} = -20.9305 + 0.8115x_1 + 1.6535x_2 + 0.1739x_3 - 0.0246x_1x_2 - 0.0017x_1x_3 - 0.0075x_2x_3 \quad (6.1)$$

$$\textit{Elongation} = 0.08825 + 0.01875x_1 + 0.05875x_2 + 0.0090x_3 - 0.000275x_1x_2 - 0.000074x_1x_3 - 0.000482x_2x_3 \quad (6.2)$$

$$\textit{Young's Modulus} = -1.38675 + 0.37775x_1 + 0.62775x_2 + 0.06150x_3 - 0.01213x_1x_2 - 0.00076x_1x_3 - 0.00183x_2x_3 \quad (6.3)$$

where x_1 is the carrageenan content, x_2 is the plasticizer (TEC) concentration and x_3 is the application speed.

Response surfaces generated from equations 6.1 to 6.3 are presented in Figures 6.30 to 6.32. These contain the results of the tensile tests (tensile strength, elongation and Young's Modulus). As expected, tensile strength decreased as a function of plasticizer content and increased as a function of carrageenan content (Figure 6.30). With the increase of application speed, it can be noted that the tensile strength increased at the maximum concentration of carrageenan (50%) and minimum plasticizer content (10%). At plasticizer concentration (30%) and carrageenan concentration (50%), the tensile strength decreased with the increase of application speed. Elongation showed the same trend for the tensile strength (Figure 6.31), increasing with the increase of carrageenan, due to its plasticizing effect. The increase of application speed at maximum plasticizer content led to a decrease in elongation. The tensile strength and elongation at break are a measure of the real resistance of films for practical uses. A better diagnosis of film structure can be obtained examining the Young's modulus. This modulus is measured in the linear region of response where no irreversible changes in structure can be found (Tapia-Blácido et al., 2005). Higher Young's modulus (stiffer films) was obtained, at high carrageenan concentration and application speed and low plasticizer content (Figure 6.32). The results obtained for Young's modulus presented the same behaviour showed for the tensile strength results, increasing with the increase of carrageenan and application speed and decrease of plasticizer content.

In spite of the fact that all the regression effects expressed by the models of Equations 6.1 to 6.3 are not truly statistically significant ($p>0.05$), the results obtained in these experiments allowed the optimization of the film production process and to obtain films with good mechanical properties. The best results were obtained for both concentration of carrageenan used in this study (30% and 50%) and for an application speed of 300 mm/s.

6.5.6. Conclusions

The effect of carrageenan and plasticizer contents and application speed on the mechanical properties of starch-carrageenan based films were studied using a 2^3 full factorial design. The results obtained showed that with the increase of carrageenan in the mixture, the films presented better mechanical properties. Tensile strength and Young's modulus improvement confirms the results of section 6.1. Due to its hydrophilic character and plasticizing effect, the addition of carrageenan also increased elongation. Increasing the application speed resulted in a general improvement in all the mechanical properties of the films. The statistical methodology helped to find optimal specifications in terms of carrageenan content and application speed to produce films with good mechanical properties, which is required to protect the product during the processing, handling and storage.

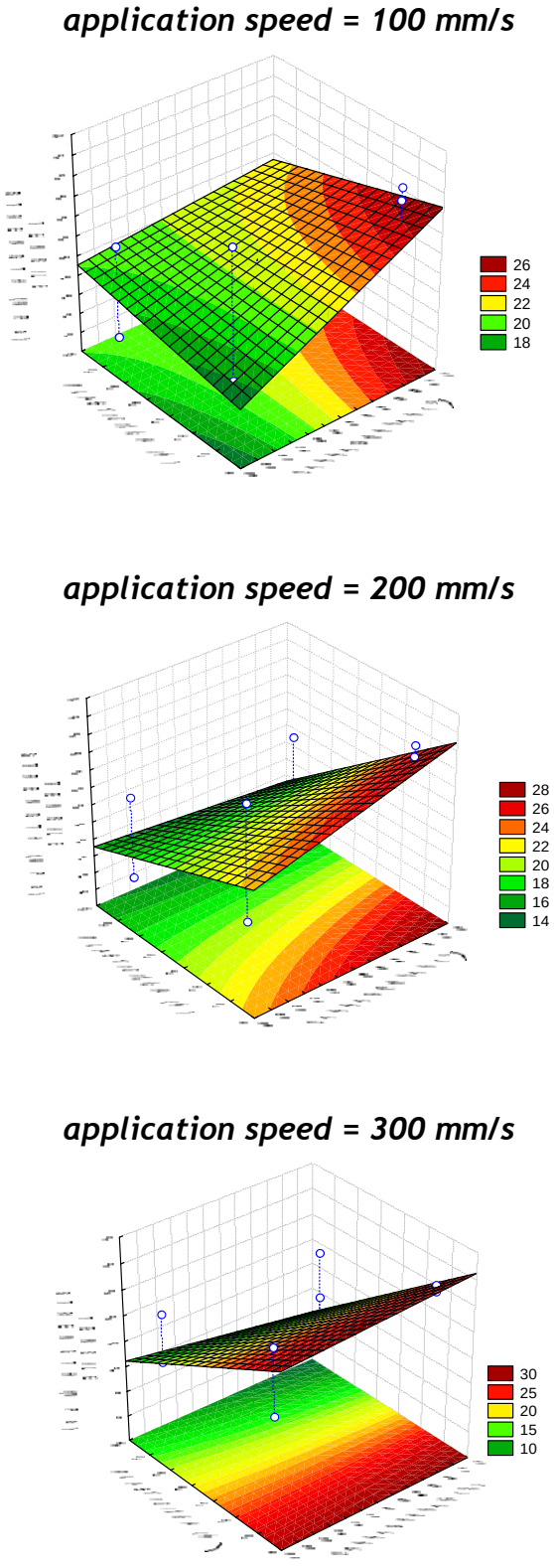

Figure 6.30 – Tensile strength (MPa) of starch-carrageenan based films as a function of carrageenan and plasticizer concentration, at an application speed of 100, 200 and 300 mm/s.

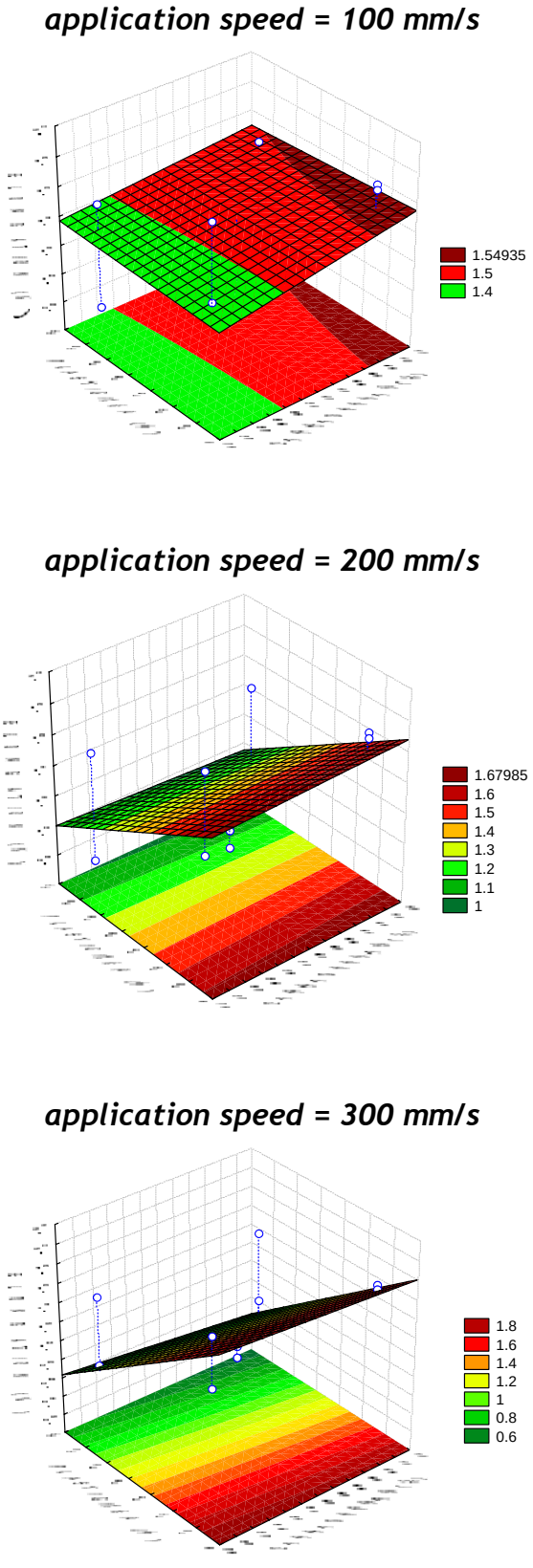

Figure 6.31 – Elongation (%) of starch-carrageenan based films as a function of carrageenan and plasticizer concentration, at an application speed of 100, 200 and 300 mm/s.

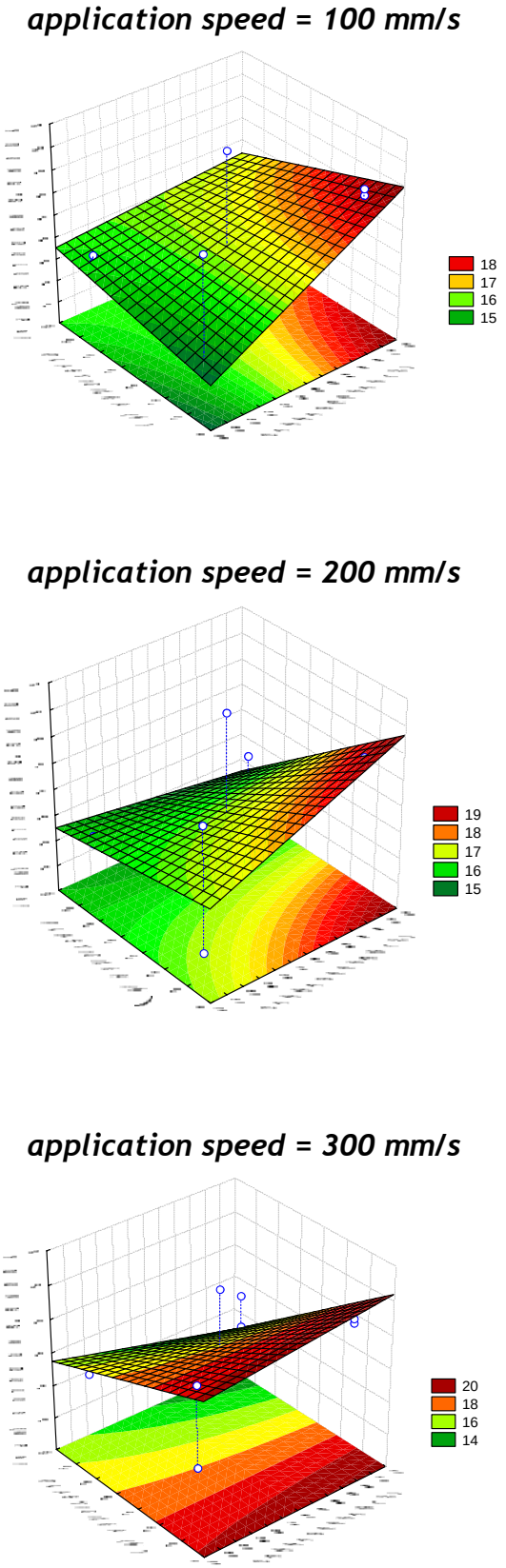

Figure 6.32 – Young's Modulus (MPa) of starch-carrageenan based films as a function of carrageenan and plasticizer concentration, at an application speed of 100, 200 and 300 mm/s.

6.6. κ/ι-hybrid carrageenan films

The use of a κ/ι-hybrid carrageenan obtained from *Mastocarpus stellatus*, an underexploited seaweed of the Portuguese coast, for the production of films was a major objective of this study. The effect of different plasticizers, one hydrophilic (glycerol) and one hydrophobic (triethyl citrate), to improve film's functional properties (hygroscopicity, mechanical, water barrier and optical). was evaluated.

6.6.1. Materials

κ/ι-hybrid carrageenan extracted from *Mastocarpus stellatus* (Hilliou et al., 2006) was used as the film forming component to provide a continuous matrix of the film. Glycerol (GLY) (Merck, Germany) was used as hydrophilic plasticizer and triethyl citrate (TEC) (Fluka, Germany) was used as hydrophobic plasticizer.

6.6.2. Preparation of κ/ι-hybrid carrageenan based films

κ/ι-hybrid carrageenan extracted from *Mastocarpus stellatus* was mixed with 0, 10, 20 and 40% (with respect to the mass of carrageenan) of glycerol (GLY) and triethyl citrate (TEC) and dispersed in distilled water. The total concentration of polymers in water was 4% (carrageenan + plasticizer), leading to optimal solution viscosity to use with the knife coating technique. The samples were codified as follows: κ/ι-hybrid carrageenan (M) and plasticizers (P), glycerol (G) and triethyl citrate (T) followed by the corresponding amount (%), as for example M:P(G)-90:10 was the formulation with 90% of κ/ι-hybrid carrageenan and 10% of plasticizer, in this case glycerol. Films were produced by the knife coating method, as described in Chapter 5. The average thickness of film samples was 15 ± 3 μm.

6.6.3. Film characterization

Film samples were characterized as described in Chapter 5. Film properties evaluated were: moisture sorption isotherms, mechanical properties, water vapour permeability and optical properties (colour and light transmission). A rheological characterization of the film-forming solution was also performed.

6.6.4. Results and discussion

Homogeneous, thin, flexible and transparent films were obtained from κ/ι-hybrid carrageenan by the knife coating technique, using glycerol and triethyl citrate as plasticizers. All film samples were easily removed from the acrylic plate and showed smooth surfaces. The films were very sensible to water, being easily dissolved in distilled water. The films plasticized with the hydrophobic plasticizer (triethyl citrate) presented a little water exudation.

6.6.4.1. Moisture sorption isotherms

Moisture sorption isotherms of the film samples obtained from carrageenan, varying the plasticizer type and its relative content are presented in Figure 6.25, together with the GAB model fitted for each sample. The GAB model parameters and the correlation coefficients are presented in Table 6.13. The values of k (<1) and the correlation coefficient ($r > 0.98$) show that GAB equation gives a good fit to experimental values, except for the data sets corresponding to films plasticized with triethyl citrate.

Films of κ/ι-hybrid carrageenan plasticized with the hydrophobic plasticizer (triethyl citrate) presented the lowest monolayer values (X_0) (around 0.06 g water/g solids), while the highest value (0.097 g water/g solids) was observed when the maximum content of glycerol was incorporated in the

film (M:P(G)-60:40). The monolayer value indicates the maximum amount of water that can be adsorbed in a single layer per gram of dry film and it is a measure of number of sorbing sites (Strauss et al., 1991). The addition of hydrophilic plasticizers provides more active sites by exposing its hydrophilic hydroxyl groups in which the water molecules can be adsorbed (Mali et al., 2005).

The *C* parameter decreased with increased hydrophilic plasticizer and glycerol containing films showed the lower values (Table 6.13). This was according to findings of others authors, who also stressed that *k* parameter is effectively independent of composition, whilst X_0 increases and C decreases with increased glycerol concentration (Coupland et al., 2000).

In general, the moisture sorption isotherms for the films studied showed a sigmoid shape and were influenced by the concentration and by type of plasticizer (Figure 6.33). Higher levels of hydrophilic plasticizer increased the films moisture affinity and these results could be attributed to the hydrophilicity of the plasticizer, which presented hydroxyl groups capable to interact with water by hydrogen bonds. Similar results were reported by Karbowiak et al. (2006) (Figure 6.33a). In general, glycerol films showed a higher capacity to adsorb water at all concentrations and at all water activities conditions.

The films with hydrophobic plasticizer showed a similar behaviour (sigmoid shape) in terms of water sorption, but the moisture content adsorbed was lower than for the film of pure carrageenan and for the carrageenan-glycerol films.

Table 6.13 - GAB equation parameters for moisture sorption isotherms of κ/ι-hybrid carrageenan films at different plasticizers levels and type.

Film formulation	X_0	C	k	r
M:P-100:0	0.068	10.900	0.921	0.993
M:P(G)-90:10	0.077	5.819	0.961	0.997
M:P(G)-80:20	0.086	3.410	0.958	0.999
M:P(G)-60:40	0.097	2.912	0.971	0.998
M:P(T)-90:10	0.060	4.500	0.946	0.974
M:P(T)-80:20	8.004	0.020	0.572	0.969
M:P(T)-60:40	0.063	301.520	0.924	0.965

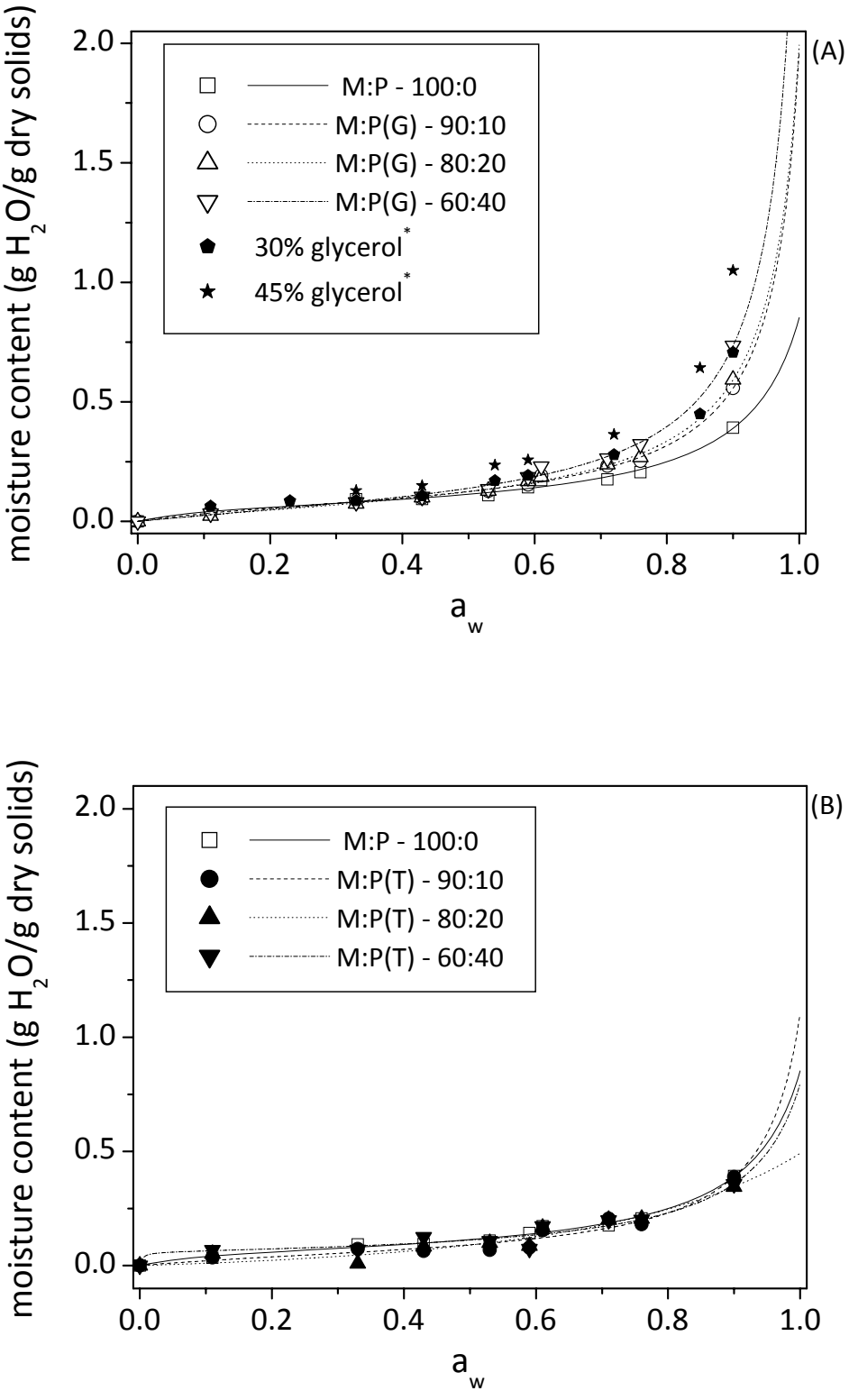

Figure 6.33 – Experimental data and GAB fittings for moisture sorption isotherms of the carrageenan based films varying the amount and type of plasticizer: (A) films plasticized with glycerol (GLY) (*data noted with a star correspond to films of iota-carrageenan with 30% and 45% obtained by Karbowiak et al. (2006)); (B) films plasticized with triethyl citrate (TEC).

6.6.4.2. Mechanical properties

As expected, the increase in plasticizer concentration in the film-forming solution produced films that were less stiff and rigid and more extendible; the inclusion of the plasticizer caused a reduction on tensile strength and an increase of the elongation at break of films (Figures 6.34-36). These trends were probably due to the reduction in interactions between the biopolymer chains. This effect of plasticizer concentration on the mechanical properties is well known and broadly discussed in the literature (Gontard et al. 1993; Parris et al., 1995; Cuq et al., 1997; Lourdin et al, 1997; Arvanitoyannis and Biliaderis, 1998; Sobral et al., 2001; Paschoalick et al., 2003; Laohakunjit and Noomhorm, 2004; Mali et al., 2005; García et al., 2006).

Increasing plasticizer content resulted in films with lower Young's modulus and then more flexible (Figure 6.36). This behaviour could be related to the structural modifications of carrageenan network when plasticizer was incorporated, the matrix of the film become less dense and under stress movements of polymer chains were facilitated (Mali et al., 2005).

The tensile properties of κ/ι-hybrid carrageenan without plasticizer show the typical pattern of brittle materials, because they exhibited high values of tensile strength (Figure 6.34) and low values of elongation at maximum breaking force (Figure 6.35). Films plasticized with glycerol and triethyl citrate presented similar values of mechanical properties for the concentrations of 10 and 20%. For the concentration of 40%, films with glycerol present better results than the films with triethyl citrate. Glycerol films were more affected in its mechanical properties than the triethyl citrate films, indicating a more effective plasticization. Water is also an effective plasticizer for carrageenan films as shown before in section *6.1.4.6* (Figure 6.11) where the T_g of carrageenan films was affected by the relative humidity; thus adsorbed water influenced and enhanced the plasticizing effect of added glycerol.

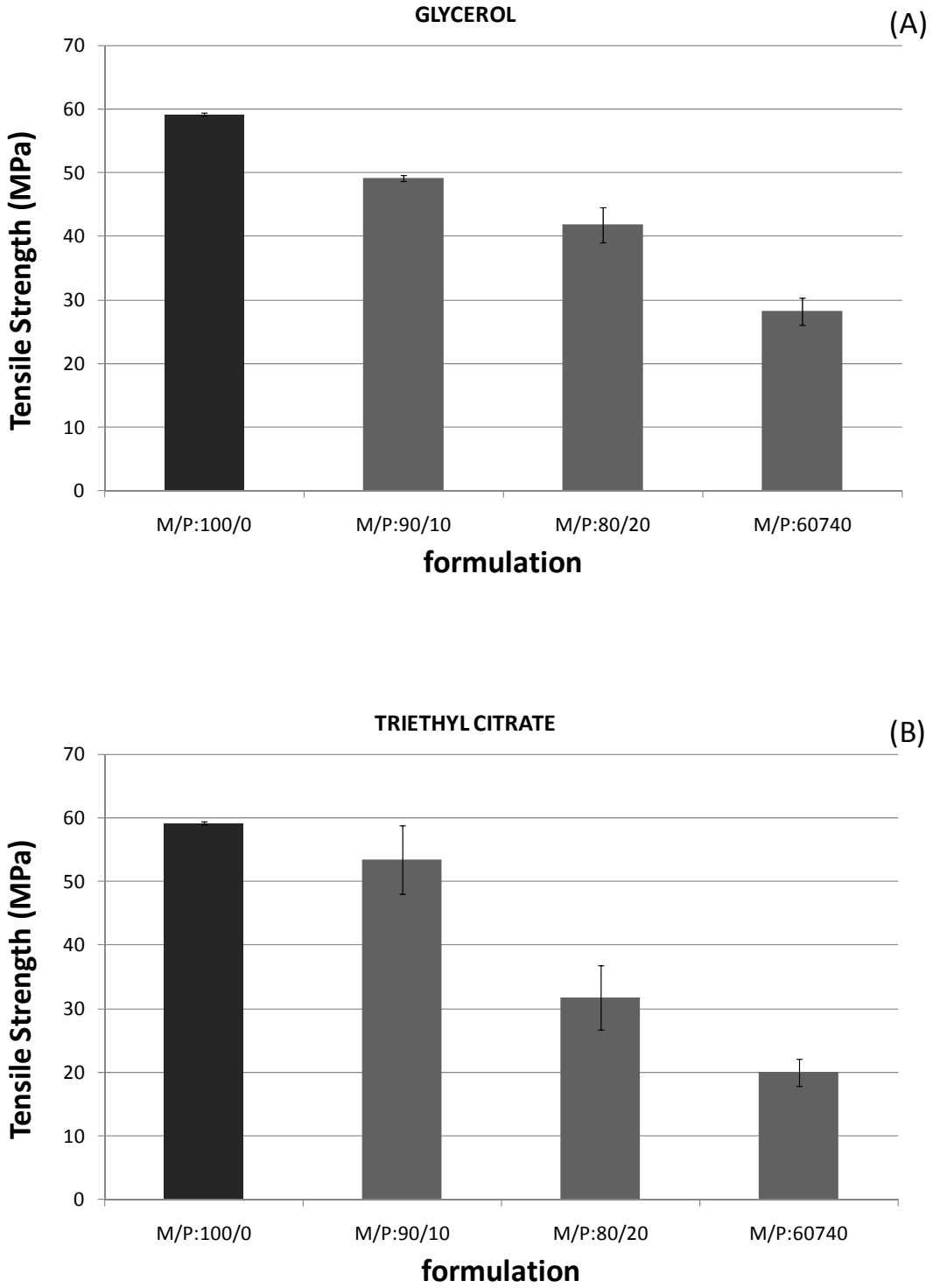

Figure 6.34 – Tensile strength for the starch-carrageenan based films plasticized with: (A) glycerol; (B) triethyl citrate. (The code M/P means the proportion carrageenan/plasticizer in the film forming solution).

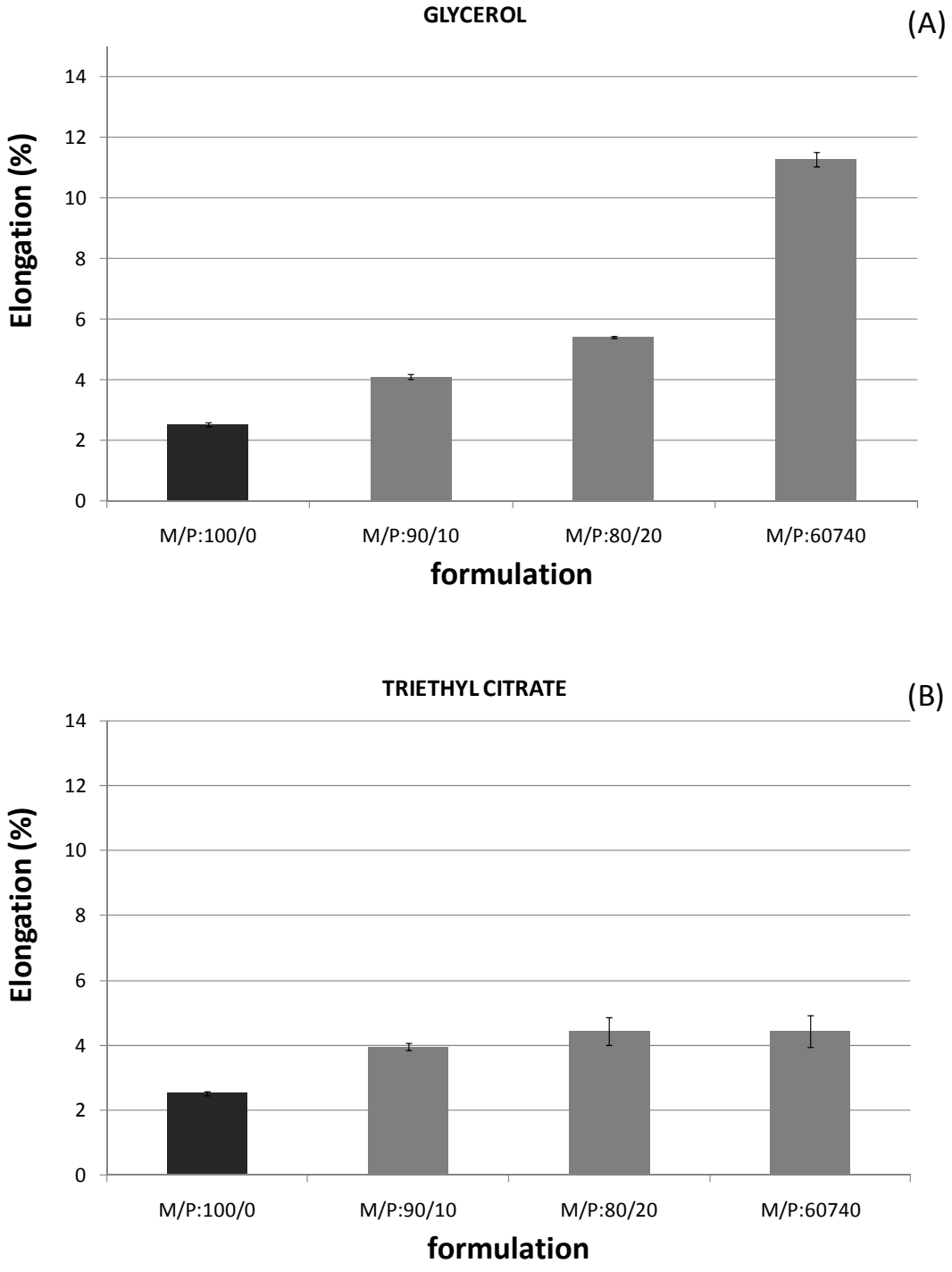

Figure 6.35 – Elongation at break for the starch-carrageenan based films plasticized with: (A) glycerol; (B) triethyl citrate. (The code M/P means the proportion carrageenan/plasticizer in the film forming solution).

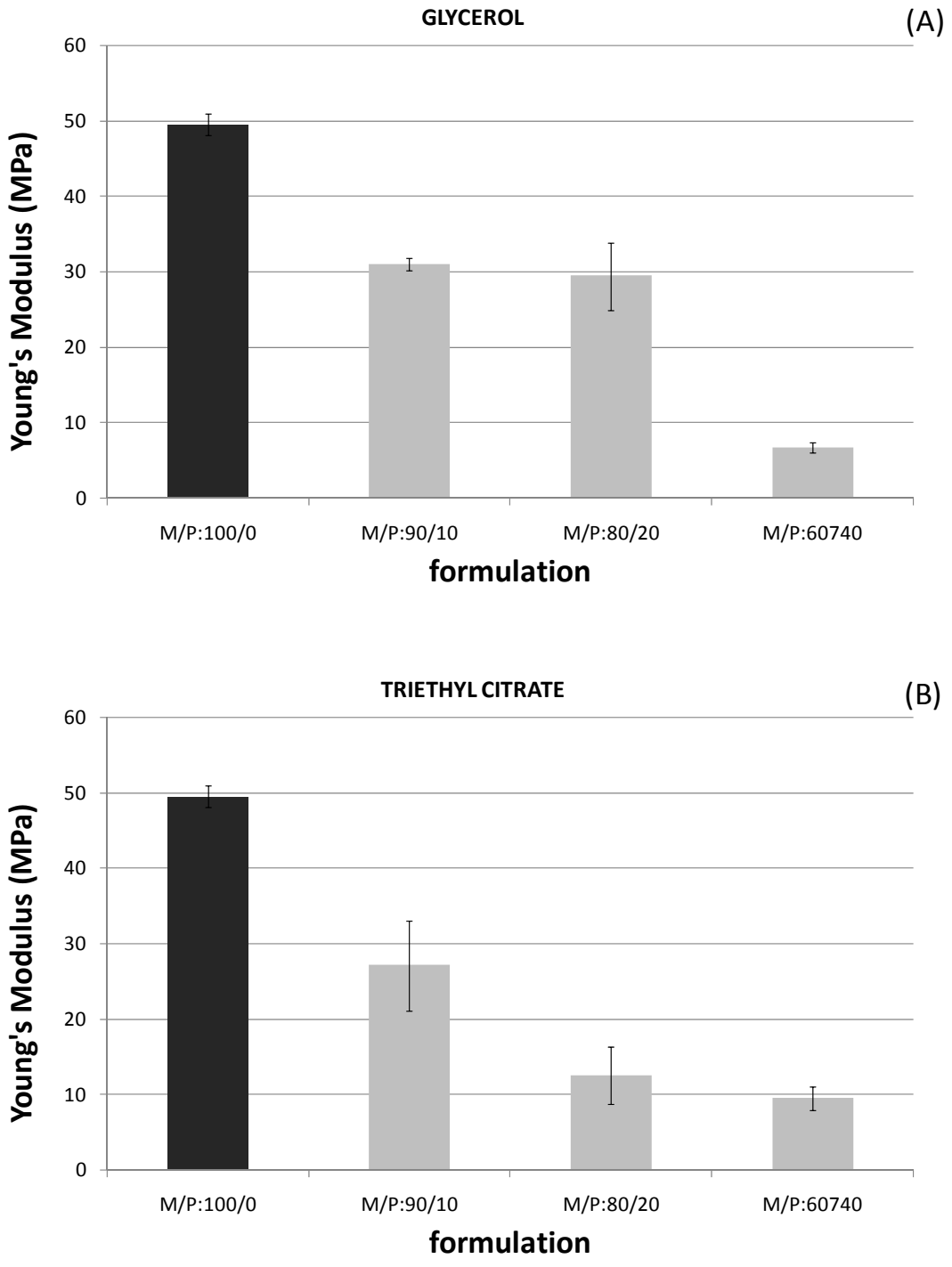

Figure 6.36 – Young's modulus for the starch-carrageenan based films plasticized with: (A) glycerol; (B) triethyl citrate. (The code M/P means the proportion carrageenan/plasticizer in the film forming solution).

6.6.4.3. Water vapour permeability (WVP)

Since a frequent important function of a food packaging is to avoid or at least to decrease moisture transfer between the food and the surrounding atmosphere or between two components of a heterogeneous food product, water vapour permeability of the barrier film should be as low as possible (Gontard et al., 1992).

Figure 6.37 shows WVP of κ/ι-hybrid carrageenan films with varying amount and type of plasticizer. WVP values ranged between 0.72 and 1.49 x 10^{-10} g m^{-1} s^{-1} Pa^{-1}. It was observed that WVP increases with the addition of plasticizer, when compared to pure κ/ι-hybrid carrageenan (films with 0 and 10% of plasticizer). as was reported in previous works for other hydrophilic films (McHugh et al., 1994; Buttler et al., 1996; Cuq et al., 1997; Arvanitoyannis et al., 1997; Sobral et al., 2001; Mali et al., 2002; Mali et al., 2004; Laohakunjit and Noomhorm, 2004). This behaviour could be related to structural modifications of the polymer network that might become less dense, with the addition of a plasticizer. At lower contents of κ/ι-hybrid carrageenan in the formulation WVP decreases, despite the increase of plasticizer (films with 20 and 40% of plasticizer), pointing out that all the films were formulated using a total components concentration of 4%. This result indicates that the relative amount of carrageenan has a great influence on WVP. The films with hydrophobic plasticizer showed lower values of WVP compared with the hydrophilic plasticizer.

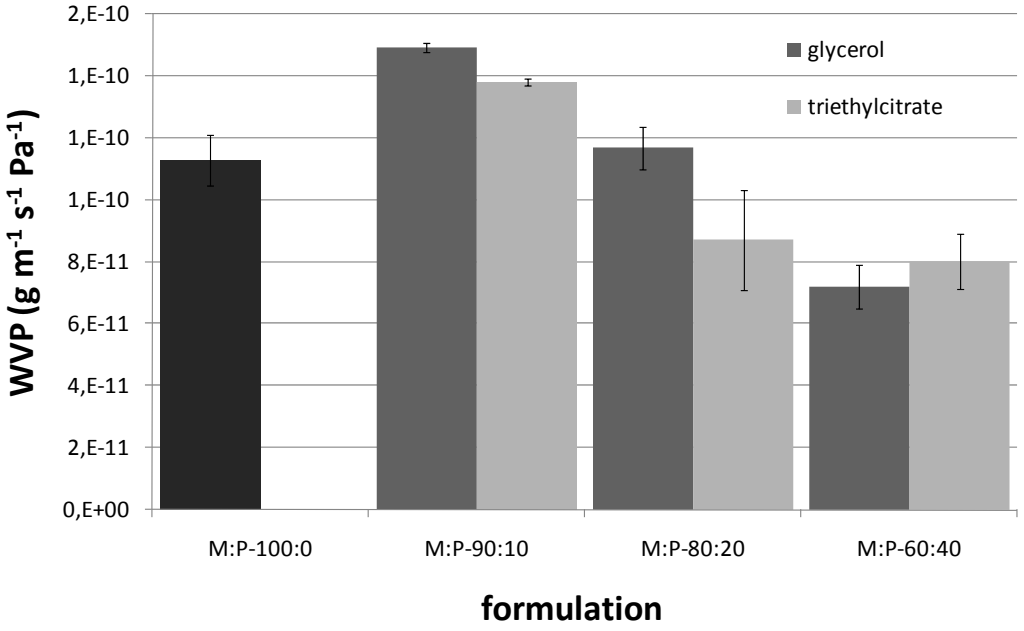

Figure 6.37 – Water vapour permeability of films formulated with: κ/ι-hybrid carrageenan, κ/ι-hybrid carrageenan plasticized with glycerol and with triethyl citrate. Films were formulated with 4% of components in solution.

6.6.4.4. Oxygen permeability (OP)

The effect of plasticizer concentration on oxygen permeability was studied by several authors (McHugh and Krochta, 1994; Maté and Krochta, 1996; Miller and Krochta, 1997; Laohakunjit and Noomhorm, 2004; Ghanbarzadeh et al., 2007), reporting that oxygen transmission rate increases with the increase of plasticizer content, due to an increased mobility of polymer chains caused by the plasticizer resulting in a reduced resistance of the film to gas or water vapour transmission. Figure 6.38 shows OP of κ/ι-hybrid carrageenan films varying the amount of plasticizer. It was not possible to measure the oxygen permeability of the films plasticized with triethyl citrate (TEC) because the films obtained were very fragile and they broke when were fixed in the diffusion cell. OP values ranged between 3.26 and 4.43 x 10^{-12} ml m m^{-2} s^{-1} Pa^{-1}. Increasing the plasticizer content to 10% decreased the OP when compared to the OP of the pure κ/ι-hybrid carrageenan film, but this difference was not significant ($p>0.05$). This occurred probably due to an increase of association between polymer chains

at low levels of plasticizer. Increasing glycerol content to 20 and 40% increased OP values and this could be attributed to an increase of biopolymer chain mobility and creation of void spaces in the film matrix.

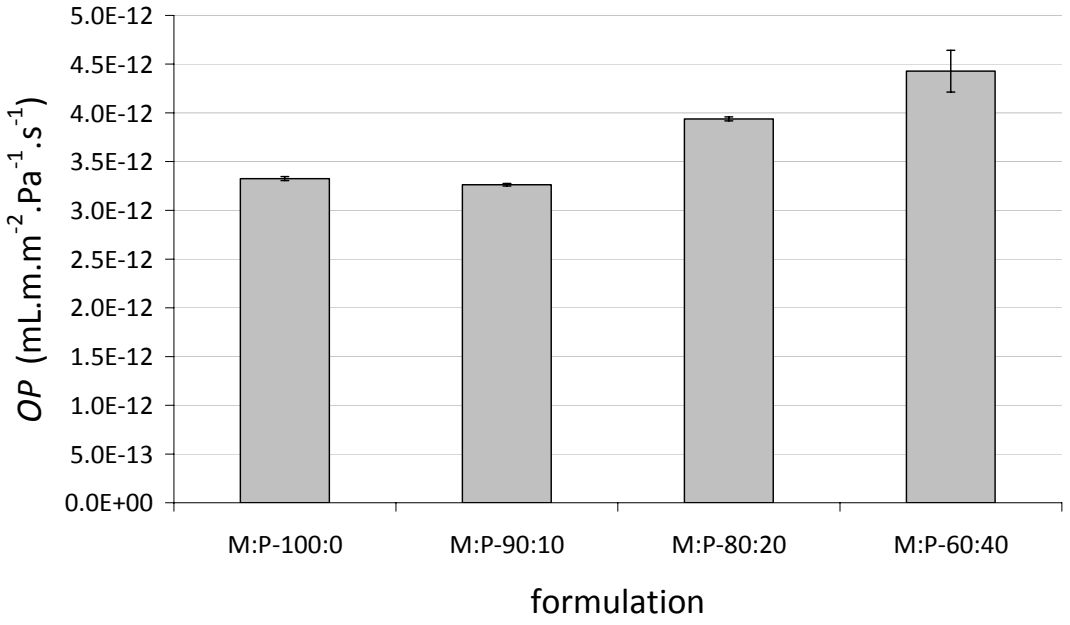

Figure 6.38 – Oxygen permeability of films formulated with κ/ι-hybrid carrageenan plasticized with glycerol. Films were formulated with 4% of components in solution.

6.6.4.5. Optical properties

Visually, all the films had a slightly yellow appearance. Instrumental colour determination performed on films showed significant differences ($p<0.05$) in the colour parameters (*L*, a* and b**) values for all assayed films (see Table 6.14). Colour difference (*ΔE**) increases with the addition of plasticizer, decreasing with higher values of plasticizer concentration, in the case of glycerol, possibly due to the effect of dilution of glycerol, which is a colourless substance (Sobral et al., 2005). For the triethyl citrate, the colour difference is significantly different from unplasticized film, but did not show the same behaviour of glycerol.

Table 6.14 - Colour standards of κ/ι-hybrid carrageenan films plasticized with glycerol and triethyl citrate.

Film samples	Lightness *(L*)*	Chromaticity parameter *a**	Chromaticity parameter *b**	Colour differences *(ΔE*)*
M:P-100:0	94.74 ± 0.22	-0.34 ± 0.03	5.96 ± 0.25	4.83 ± 0.32
M:P(G)-90:10	93.75 ± 0.47	-0.63 ± 0.03	8.31 ± 0.28	7.40± 0.41
M:P(G)-80:20	94.24 ± 0.23	-0.53 ± 0.08	7.26 ± 0.27	6.23 ± 0.33
M:P(G)-60:40	95.08 ± 0.23	-0.33 ± 0.05	5.55 ± 0.14	4.32 ± 0.10
M:P(T)-90:10	94.95 ± 0.23	-0.43 ± 0.06	6.17 ± 0.23	4.94 ± 0.15
M:P(T)-80:20	95.03 ± 0.53	-0.31 ± 0.03	5.83 ± 0.37	4.59 ± 0.56
M:P(T)-60:40	94.52 ± 0.07	-0.46 ± 0.05	6.92 ± 0.24	5.79 ± 0.24

* CIELab standards for the white standard, used as the film background were: L_s^* (97.10), a_s^* (0.05) and b_s^* (1.76).

Table 6.15 lists the light transmission and transparency of κ/ι-hybrid carrageenan films plasticized with glycerol and triethyl citrate, at 600 nm that is commonly used wavelength for the film transparency (Han and Floros, 1997; Fang et al., 2002; Shiku et al., 2003). The addition of plasticizers decreased the transparency of κ/ι-hybrid carrageenan films. Increasing the plasticizer content decreased the transparency and the films plasticized with triethyl citrate were less transparent than the films plasticized with glycerol. The same result was obtained comparing hydrophilic and hydrophobic plasticizers, in section *6.4.4.3* (Table 6.9).

The incorporation of plasticizers to the κ/ι-hybrid carrageenan films decrease the light transmission at 10% of glycerol in the mixture and the increasing of this hydrophilic plasticizer increased the light transmission. For the films plasticized with triethyl citrate, the addition and content increasing, decreasing the light transmission rate of carrageenan films, blocking most light in the wavelength of 600 nm (Table 6.15).

Table 6.15 – Light transmission (%, T_{600}) and transparency (A_{600}/mm) of κ/ι-hybrid carrageenan films plasticized with glycerol (GLY) and triethyl citrate (TEC).

Film	Light Transmission (%, T_{600})	Transparency (A_{600}/mm)
M:P-100:0	84.8	4.8
M:P(GLY)-90:10	78.6	5.8
M:P(GLY)-80:20	80.2	7.4
M:P(GLY)-60:40	88.3	4.1
M:P(TEC)-90:10	72.6	12.6
M:P(TEC)-80:20	61.5	10.5
M:P(TEC)-60:40	48.1	21.2

Figure 6.39 shows the absorbance of light in the range of 200 to 600 nm. All the films showed good barrier properties to UV light in the 280nm region, increasing the absorbance of light when compared with the initial wavelength of 200 nm. The increasing of triethyl citrate increases the values of absorbance in all the wavelength range measured, while the increase of the glycerol decreases the values of absorbance. The results obtained for the κ/ι-hybrid carrageenan films plasticized with glycerol and triethyl citrate indicate that they had excellent barrier properties to UV light (in the 200-280 nm region), suggesting that these films can protect food systems from the UV light. These results of absorbance comparing glycerol (hydrophilic) and triethyl citrate (hydrophobic) are in agreement with the results presented in section *6.4.4.3* (Figure 6.29).

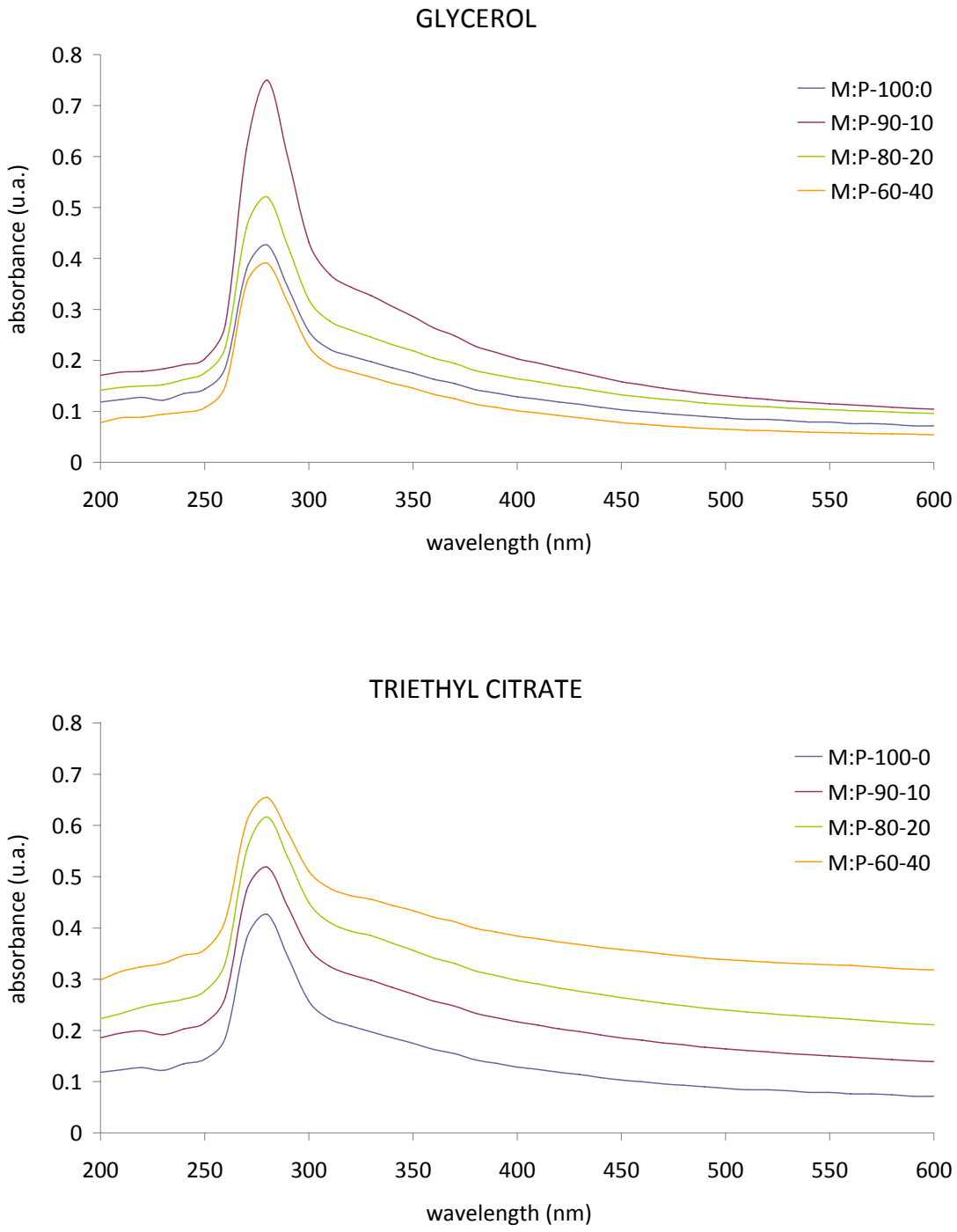

Figure 6.39 – Light transmission spectra (absorbance) of κ/ι-hybrid carrageenan films varying the content and type of plasticizer: (A) glycerol; (B) triethyl citrate.

6.6.4.6. Rheological analysis of the film forming solution

In order to evaluate the effect of plasticizer addition (0, 10, 20 and 40% with respect to carrageenan mass) on the viscosity of the film-forming solution, rheological tests was carried out with all solutions as described in section 5.2.9, keeping a total weight of material (carrageenan and plasticizer) in solution of 4%. The viscosity of the film forming solution and its dependence with respect to the velocity of deformation are important parameters to produce the films by the knife coating process, and allows properties as solution spreadability to be quantified. The possible orientation of the macromolecules by the knife coating during film forming process was also studied.

An initial characterization of κ/ι-hybrid carrageenan solution was performed to evaluate the pure carrageenan viscoelastic properties in distilled water. Small amplitude oscillatory shear experiments were firstly carried out. Figure 6.40 shows the storage (G') and loss (G") moduli and *tan* δ (G"/G') as function of temperature. Figure 6.40A shows a cooling temperature ramp (80 to 5ºC) and it can be observed that in a temperature close to 5ºC a liquid-solid transition occurred (G'=G", *tan* δ = 1), which could correspond to the formation of a weak gel. Figure 6.40B shows a heating temperature ramp (5 to 80ºC), carried out after the cooling ramp, that shows a melting temperature close to 18ºC. Both temperatures (gelling and melting) were below the temperature of film processing, which is around 25-30ºC. The crossover between G' and G" observed in Figure 6.40A, indicate a solid behaviour (G'>G") at a frequency of 1 Hz and a temperature of 5ºC. Another process that can be observed in the Figures 6.40A, where a change in the otherwise monotonic increase of viscosity with decreasing the temperature is observed, is referred in the literature as a coil-helix transition (van de Velde et al., 2005). In order to verify if the solid behaviour is valid for other frequencies, a frequency sweep was performed at 5ºC. In the Figure 6.41 can be observed that at low frequencies (< 1 Hz), G'>G" and the solution of

κ/ι-hybrid carrageenan can be considered a viscoelastic solid or a weak gel at 5ºC, with a storage modulus (G') around 2 Pa.

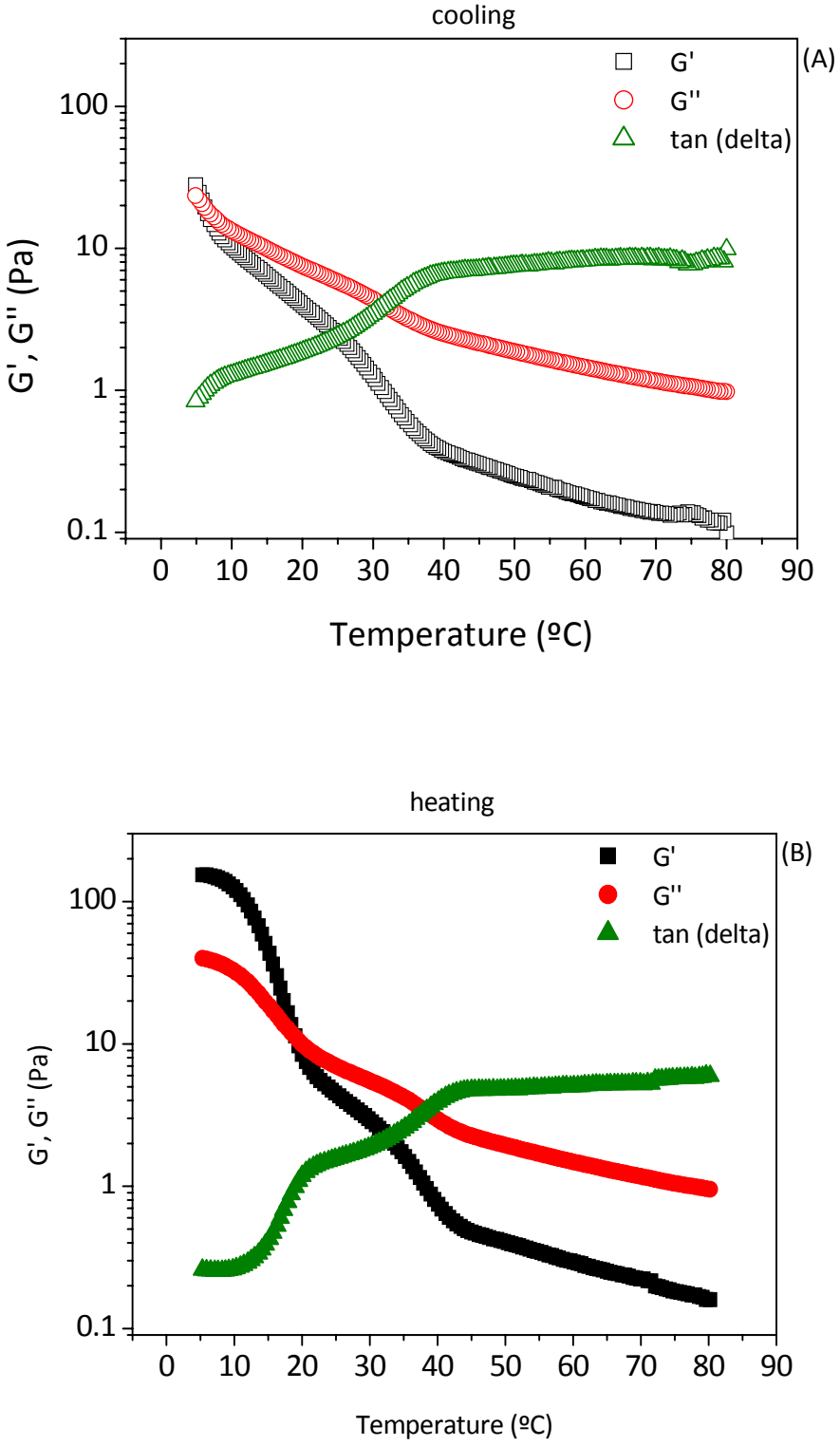

Figure 6.40 – G', G" and *tan* δ as function of temperature of a solution of 4% of κ/ι-hybrid carrageenan in distilled water.

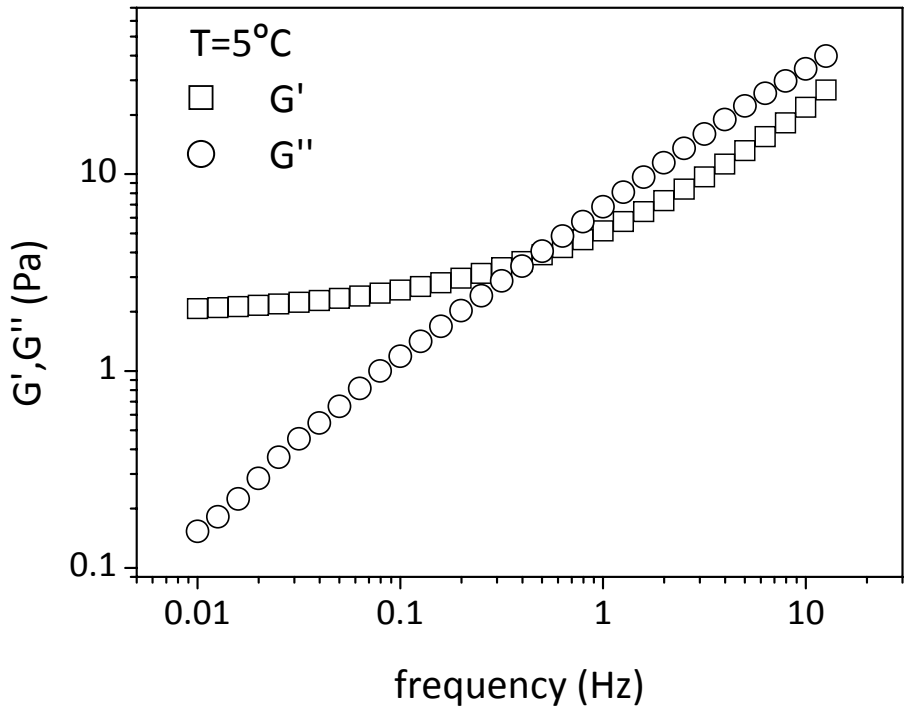

Figure 6.41 – Mechanical spectra of a solution of 4% of κ/ι-hybrid carrageenan in distilled water at a temperature of 5ºC and strain amplitude of 5%.

At 25ºC, the solution of 4% of κ/ι-hybrid carrageenan in distilled water can be considered a viscoelastic solution (Figure 6.42A). At lower frequencies (<0.1 Hz) G'>G" and the solution could be considered as elastic, but as G' does not reach a plateau, the solution is considered like a pre-gel or non-dilute entangled polymer solution (polymer entanglements give rise to the observed elasticity), that presented a slow relaxation time > 100 s (0.01 Hz) and can not be measured precisely because of the limit of sensitivity of the rheometer. To further study the viscoelasticity of the carrageenan solution, steady shear experiments were then carried out. The solution of 4% of κ/ι-hybrid carrageenan in distilled water showed thixotropy (Figure 6.42B). The solution presented an abrupt decrease in the viscosity in the shear rate between 0.1 to 1 s^{-1} when the experiment was carried out increasing the shear rate from 0.1 to 500 s^{-1}, which was not reproducible when the shear rate ranged from 500 to 0.1 s^{-1} characterizing the thixotropic behaviour.

This occurred because the weak gel suffered an irreversible breaking when the shear rate was increased, for the time scale used. Alternatively, the initial strong shear thinning is related to non steady state data as more shearing time is needed before collecting the viscosity data plotted in Figure 6.42B. This shear thinning behaviour presented by the carrageenan solution is good for the film formation process because the viscosity decreases with the increase of shear rate and at the shear rate that the solution is spreaded (300 s^{-1}) the solution is a liquid.

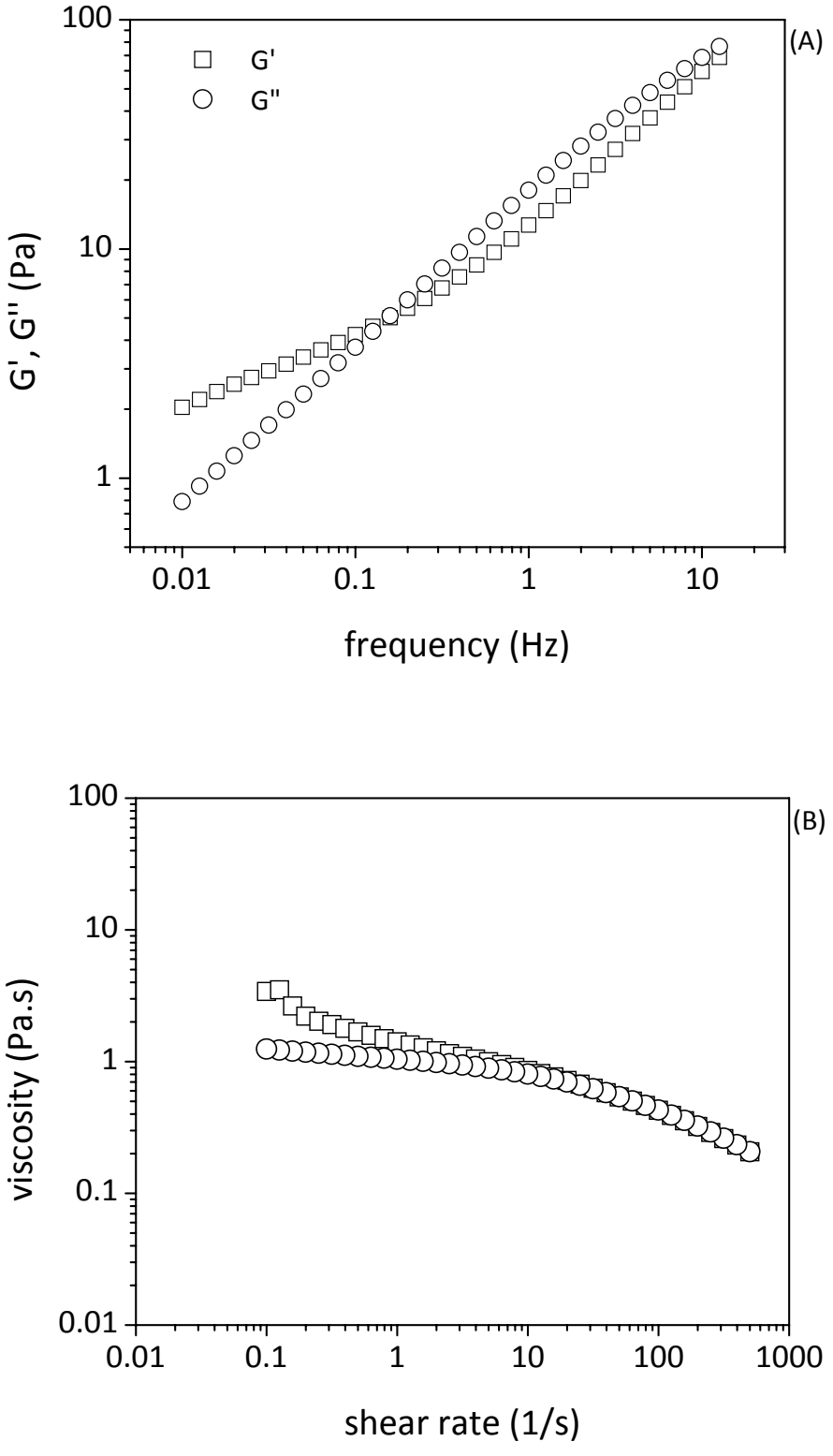

Figure 6.42 – Rheological analysis of a solution of 4% of κ/ι-hybrid carrageenan in distilled water at a temperature of 25ºC: (A) Frequency sweep; (B) Steady state flow (each data point corresponds to a steady state viscosity: less than 5% variation in the time dependence of the measured viscosity; or to a viscosity measured after 1 min of shearing at the corresponding shear rate).

The decrease of viscosity with the increase of shear rate can be related to a polymer orientation. In order to verify if could occur a polymer orientation when the film was spread over the support to produce the film, a time sweep (small amplitude oscillatory shear) was performed at 25ºC, 1 Hz, 5% of strain for 10 minutes. Figure 6.43 shows that the polymer solution needed about 500 s to return to the equilibrium state and the film drying process during more than 5000 s, 10 times slower than the relaxation process, which indicates that the orientation did not occur. It can be concluded that the film formed from an aqueous solution of κ/ι-hybrid carrageenan show no macromolecular orientation, no optical and mechanical anisotropy and that is a homogeneous structure.

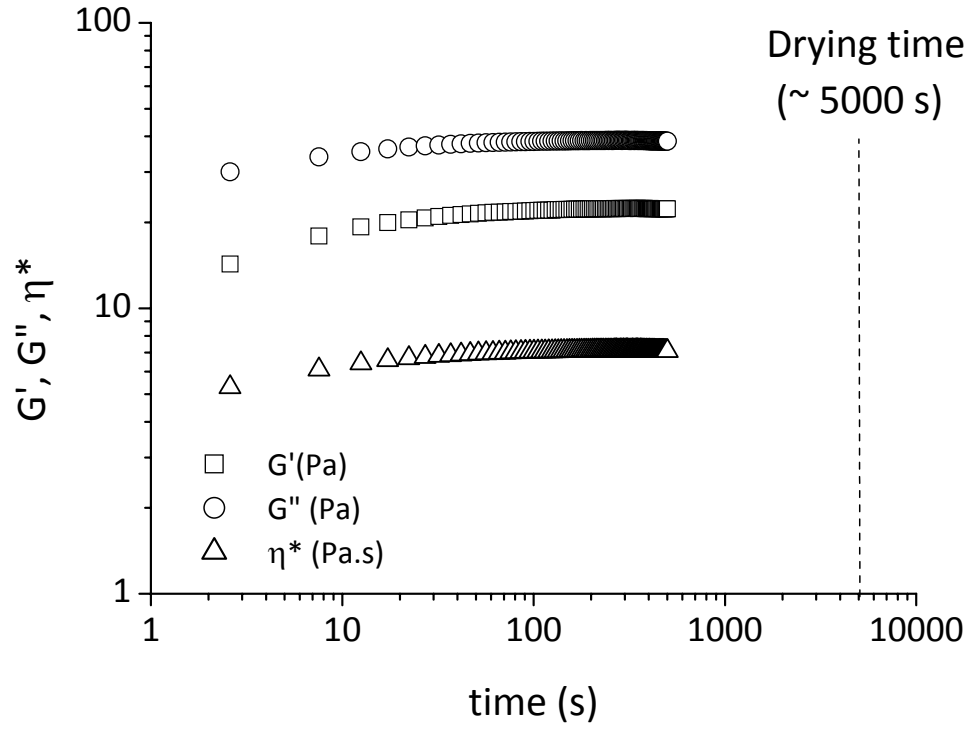

Figure 6.43 – G', G" and η* variations as a function of time for the solution of 4% of κ/ι-hybrid carrageenan in distilled water at 25ºC, 1 Hz and 5% of strain amplitude.

In order to verify the effect of plasticizer addition on the film forming solution viscosity (η_0), all the samples were prepared maintaining a total weight of material (carrageenan and plasticizer) in solution of 4%. The same thermal history was performed for all samples (cooling rate of 5ºC/min and 10 min of rest time at 25ºC). Viscosity (η_0) was defined as the pseudo viscosity plateau after sweeping down the shear rates, because how it was observed before (Figure 6.42B), it was not possible to define η_0 in the shear rate sweeping up curves and it was not reproducible. Figure 6.44 displays the viscosity (η_0) in function of the concentration of film forming solution. Various dilute solutions of carrageenan were prepared to measure η_0 and in Figure 6.44A can be observed that for dilute solutions, the slope of the fitted points was 1 and for solutions more concentrated (> 1.5% concentration) the slope changed for a value equal 3 and the solution is called semi-dilute entangled solution. This behaviour is a signature of a polymer solution (liquid). In other words, the solution spread by the knife coating technique at 300 s^{-1} is a relaxed semi-dilute entangled polymer solution. Assuming that the major viscous contribution in the solution come from the carrageenan polymer, then the plasticizers can be considered as solvent molecules and all formulations (with or without plasticizers) should exhibit the same dependence of viscosity with respect to carrageenan concentration. Such hypothesis is tested in Figure 6.44B. Data reported in Figure 6.44B show that the film-forming solution viscosities are virtually unchanged by the presence of plasticizer. As a consequence, these solutions containing a plasticizer can be spread using the previous machine parameters (application speed of 300 $mm.s^{-1}$).

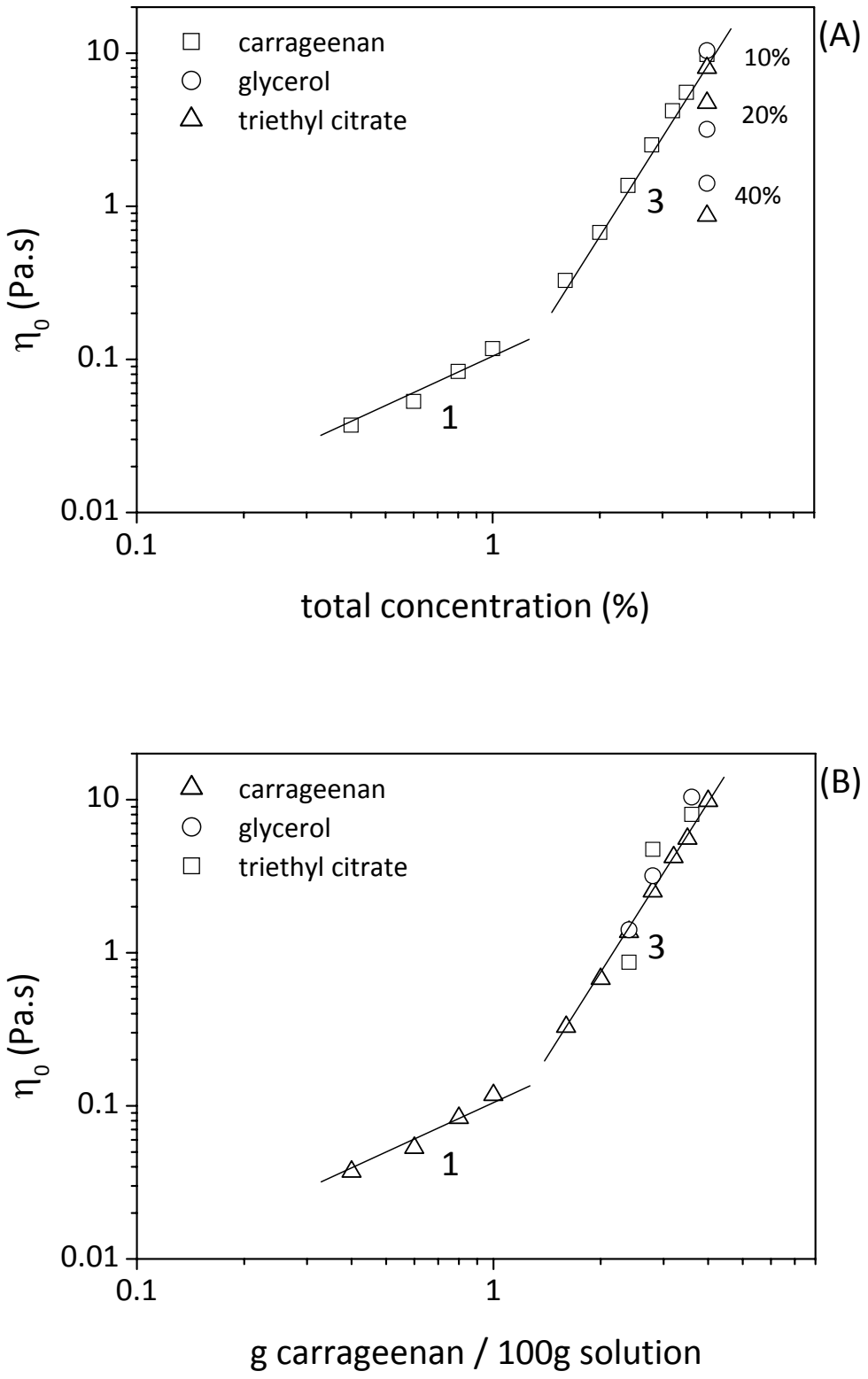

Figure 6.44 – Viscosity (η_0) as function of the concentration of film forming components in solution: (A) total concentration of components (carrageenan + plasticizers); (B) concentration of carrageenan in solution.

6.6.5. Conclusions

A major component of edible films is the plasticizer, as well as, the film-forming polymer. The addition of a plasticizer agent to edible films is required to overcome film brittleness, caused by high intermolecular forces. Plasticizers reduce these forces and increase the mobility of polymer chains, thereby improving flexibility and extensibility of the film.

Practically, all studied film properties were affected by plasticizer concentration. Hydrophilicity of plasticizer and its concentration were found to be important factors in determining moisture affinity of carrageenan based films. GAB model was useful to fit sorption isotherm data. Glycerol (hydrophilic) films adsorbed more water than the triethyl citrate (hydrophobic) and pure carrageenan films. The presence of plasticizers resulted in lower tensile strength and Young's modulus values and glycerol films were more affected in its mechanical properties, indicating that glycerol exerted a more effective plasticization than triethyl citrate. WVP was more affected by carrageenan content than the plasticizers, decreasing with the decrease of carrageenan and consequently increase of plasticizer. The addition of plasticizer maintaining a high content of carrageenan (90%) has the expected effect that is well documented by several authors, that with the addition of plasticizers results in an increase in WVP. In terms of optical properties, the films presented a slightly yellow appearance, due to the κ/ι-hybrid carrageenan. All the films showed excellent barrier to UV-light and films plasticized with triethyl citrate presented better results than the glycerol films. It must be pointed out that the films plasticized with triethyl citrate were difficult to work in some experiments, because they were not so uniform due to its moisture exudation. The rheological analysis showed that the plasticizers did not affect the viscosity of the film-forming solution and that with the conditions of film processing used it is not possible to perform some orientation in the films, because their relaxation time were about 10 times faster than the drying process.

6.7. Films based in acorn starch-carrageenan mixtures

The use of acorn starch extracted from cork oak (*Quercus suber* L.) and κ/ι-hybrid carrageenan obtained from *Mastocarpus stellatus* for the production of films was studied. The effect of glycerol in the functional properties (hygroscopicity, mechanical, water barrier and optical) was also studied.

6.7.1. Materials

Commercial κ-carrageenan (SKW Biosystems, France) and κ/ι-hybrid carrageenan extracted from *Mastocarpus stellatus* (Hilliou et al., 2006) and acorn starch extracted from cork oak (*Quercus suber* L.) were used as the film forming component to provide a continuous matrix of edible film. Glycerol (GLY) (Merck, Germany) was used as plasticizer in the film formulation.

6.7.2. Preparation of acorn starch-carrageenan based films

Acorn starch and carrageenan were utilized to prepare starch-carrageenan mixtures, with a total concentration of polymers in water of 7% (5% starch and 2% carrageenan). Different amounts of glycerol (0, 10, 20, 30 and 40% of total polymer concentration) were added to the mixture solution. The mixtures with commercial κ-carrageenan were codified as SKW and the mixtures with κ/ι-hybrid carrageenan were codified as MST. The glycerol content is indicated as a number after the carrageenan code (for example: MST40 means that film contains κ/ι-hybrid carrageenan and 40% of glycerol). Films were prepared by the knife coating method, as described in Chapter 5. The average thickness of MST film samples was 51.52 ± 6.01μm and that of the SKW film samples was 35.68 ± 6.79μm.

6.7.3. Film characterization

Film samples were characterized as was described in Chapter 5. Film properties evaluated were: moisture sorption isotherms, mechanical properties, water vapour permeability and optical properties (colour).

6.7.4. Results and discussion

Homogeneous, thin, flexible films were obtained from acorn starch-carrageenan mixtures by the knife coating technique (semi-continuous process), using as plasticizer glycerol at different concentrations. Films formed without plasticizer was very brittle and broke easily when peeled of from the support. All the plasticized films were easily removed from the acrylic plate and showed smooth surfaces. The films showed a yellow appearance (Figure 6.45).

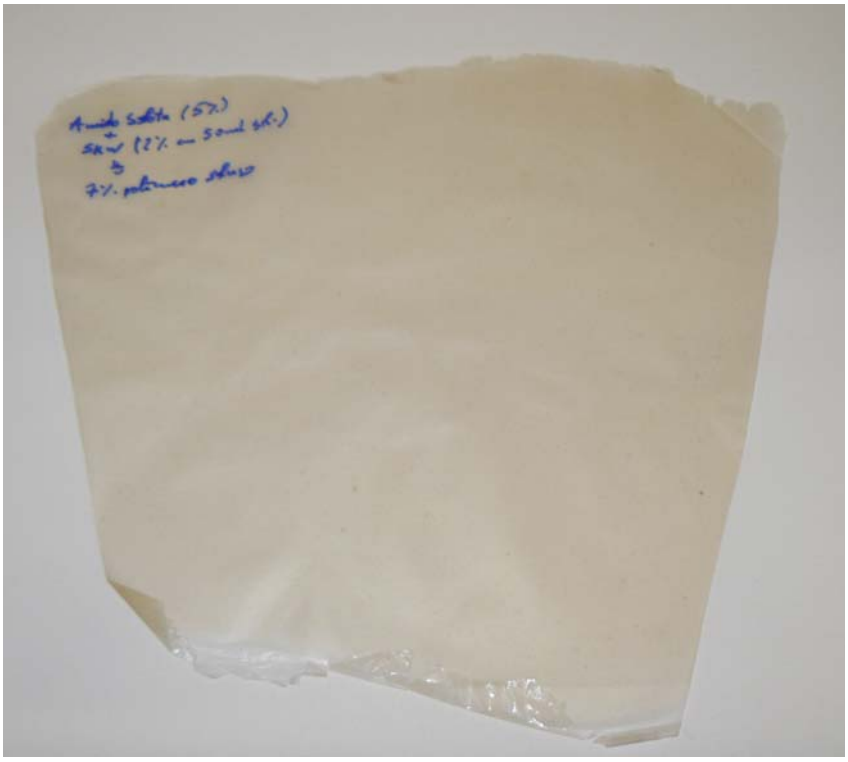

Figure 6.45 – Acorn starch-carrageenan film.

6.7.4.1. Moisture sorption isotherms

When an edible film is exposed to an environmental atmosphere, the moisture content of the film will not equilibrate with the environmental RH since the RH of atmosphere will continuously vary. Continuously varying environmental RH would alter the mechanical properties of films ceaselessly by moisture sorption and desorption (Cho and Rhee, 2002). The study of moisture adsorption of starch-carrageenan films is helpful in understanding the performance of films under varying RH conditions.

Moisture sorption isotherms at 25ºC for starch-carrageenan based films containing 0, 10, 20, 30 and 40% of glycerol (w/w total polymer concentration) are presented in Figure 6.46, together with the GAB model (van der Berg, 1984) fitted for each sample. The GAB equation parameters and the correlation coefficients are presented in Table 6.16. The values of k (<1) and the correlation coefficient ($r>0.98$) show that GAB equation gives a good fit to experimental values.

Because of their hydrophilic nature, hydrocolloid film properties are highly dependent on environmental conditions such as relative humidity and temperature (Karbowiak et al, 2006). As presented in Figure 6.39, the water content of starch-carrageenan based films is more sensitive to relative humidity when glycerol content was increased. Higher levels of plasticizer increased the film moisture affinity and these results could be attributed to the hydrophilicity of the plasticizers, which presented hydroxyl groups capable to interact with water by hydrogen bonds. In general, glycerol films showed higher capacity to absorb water at all concentrations and at all RH conditions. Glycerol molecules are small and present high capacity to interact with polymer chains, enhancing the molecular mobility and increasing free volume in the film matrix (Sothornvit and Krochta, 2001).

The isotherms curves presented a sigmoid shape and indicate that the equilibrium moisture content increases slowly with increasing environmental

a_w up to 0.7, exhibiting a less slope of the curve. With the increase in a_w the slope increased rapidly and an increase in the moisture content in film samples was observed. The behaviour of MST and SKW films was similar in terms of moisture adsorption, independent of plasticizer content.

The sorption isotherms obtained from experimental data result in an estimation of equilibrium moisture content, which is necessary to predict the properties of films in different environments pertinent to their application (Jangchud and Chinnan, 1999).

Table 6.16 – GAB equation parameters for moisture sorption isotherms of acorn starch-carrageenan films at different glycerol contents.

Film formulation	X_0	C	k	r
MST-0	0.054	19.714	0.850	0.990
MST–10	0.048	10.099	0.963	0.997
MST–20	0.062	2.562	0.971	0.996
MST-30	0.072	4.009	0.978	0.998
MST-40	0.089	2.860	0.969	0.999
SKW-0	0.043	4.759	0.976	0.997
SKW-10	0.063	1.248	0.961	0.998
SKW-20	0.102	0.733	0.945	0.997
SKW-30	0.081	1.135	0.975	0.998
SKW-40	0.047	27.887	0.883	0.991

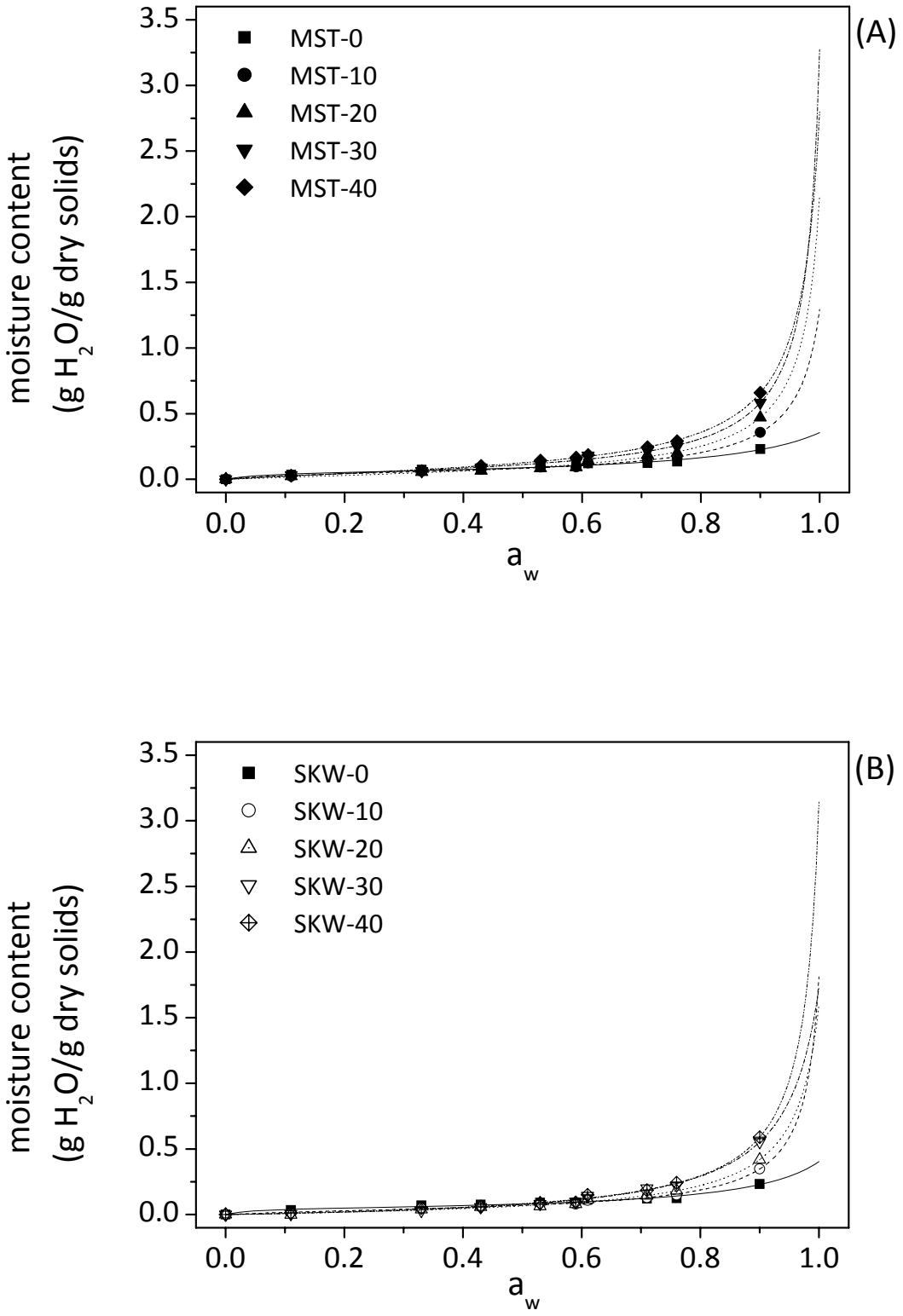

Figure 6.46 – Experimental data for moisture sorption isotherms of the starch-carrageenan based films varying the plasticizer content and the respective fitted GAB curves: (A) films with κ/ι-hybrid carrageenan (MST); (B) films with κ-carrageenan (SKW).

6.7.4.2. Mechanical properties

As shown in Figure 6.47, a decrease in tensile strength and an increase in elongation were evidenced when glycerol content increased. As shown before, the films containing higher amounts of glycerol presented higher equilibrium moisture content, which exerted a plasticizing effect. Water exerted a plasticizing effect acting as a mobility enhancer; its low molecular weight leads a large increase in molecular mobility of amorphous and partially crystalline polymers due and increased free volume (van de Berg, 1991).

Values of Young's modulus decreased with increasing of glycerol content with a concurrent increase in elongation of films at break (Figure 6.47). Both increased glycerol and water content increased elongation of film at break with decreased tensile strength. The increasing of plasticizer decreased significantly ($p<0.05$) the tensile strength and Young's modulus and increased significantly ($p<0.05$) the elongation at break of starch-carrageenan based films. Tensile strength decreased from 38.3 to 3.8 MPa for MST films and from 43.4 to 7.9 MPa for the SKW films, when glycerol content was 40%. Elongation increased from 2.8 to 44.4% and 2.2 to 22.0%, for MST and SKW films respectively, with the addition of 40% of plasticizer. For tensile strength and Young's modulus, MST and SKW films differed significantly ($p<0.05$) and for elongation MST and SKW did not differ significantly ($p>0.05$). This result showed the influence of glycerol and water absorbed by the films in the plasticization effect, mainly in the film deformation.

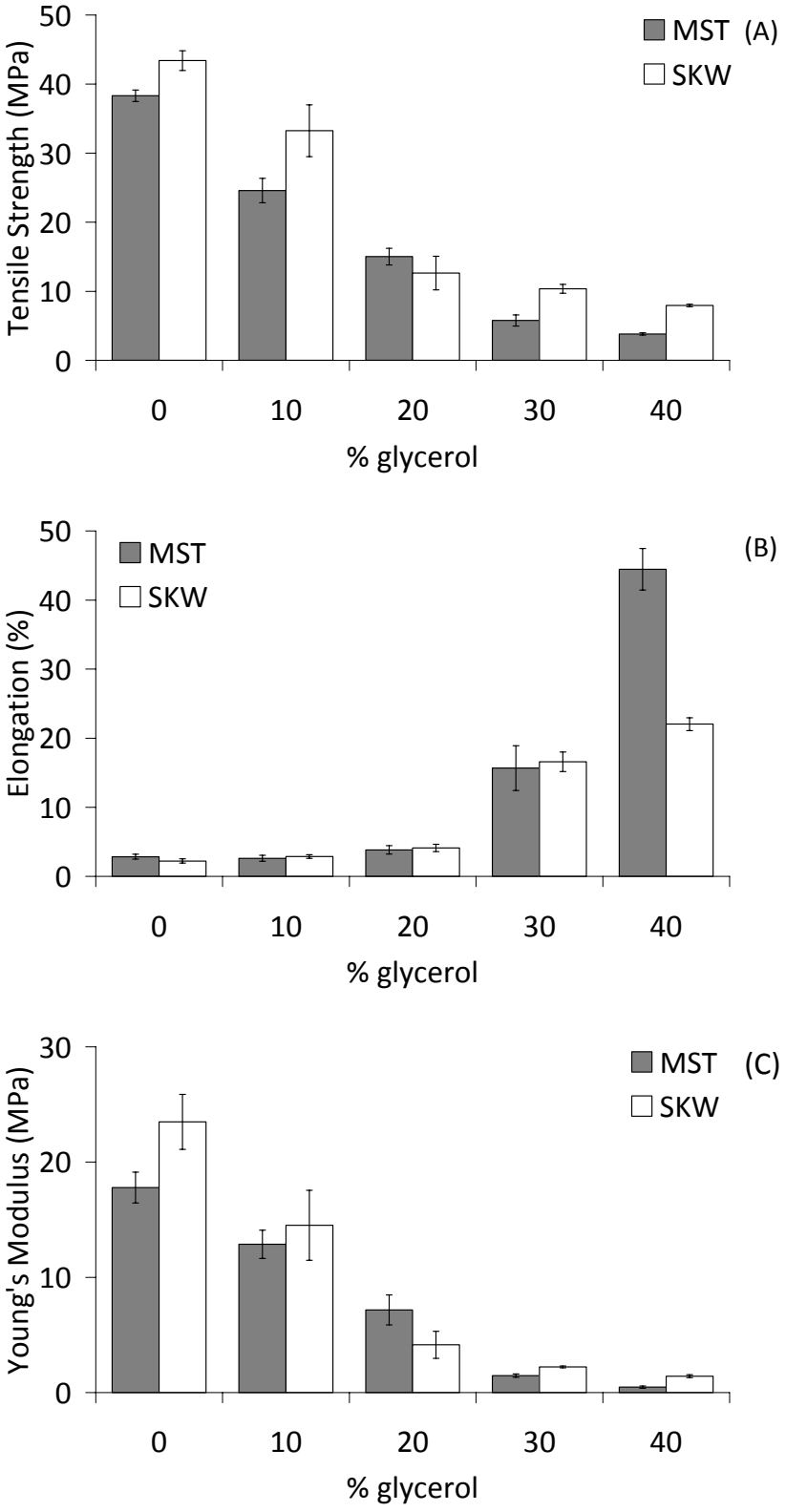

Figure 6.47 – Mechanical properties ((A) tensile strength, (B) elongation and (C) Young modulus) of the acorn starch-carrageenan films varying the amount of glycerol (0, 10, 20, 30 and 40%) and type of carrageenan (MST and SKW).

6.7.4.3. Water vapour permeability (WVP)

Barrier and mechanical properties of films can be controlled by altering plasticizers and their concentrations (McHugh and Krochta, 1994; Park et al., 1994). Water vapour permeability results can be useful to understand possible mass transfer mechanisms and solute and polymer interactions in edible films. According to the thermodynamic of irreversible process, water chemical potential difference is the driving force of the water transfer through a film. When the process occurs at constant temperature and pressure, the water chemical potential difference results proportional to water vapour concentration difference between the two faces (Morillon, Debeaufort, Blond, & Voilley, 2000).

WVP was measured at 25ºC and with a 2-100% RH gradient through the films. As expected, the use of glycerol causes the film to have higher water vapour permeability. Glycerol is a low molecular weight plasticizer, so it easily penetrates the starch-carrageenan film structure and since it has a hydroxyl group on each carbon this renders the films very hydrophilic, favouring more water absorbing into the polymer and increasing the WVP through the specimens (Martelli et al., 2006).

Another explanation could be related to structural modifications of the polymer network that might become less dense, with the addition of a plasticizer. This behaviour was reported in previous works for other hydrophilic films (McHugh et al., 1994; Buttler et al., 1996; Cuq et al., 1997; Arvanitoyannis et al., 1997; Sobral et al., 2001; Mali et al., 2002; Mali et al., 2004; Laohakunjit and Noomhorm, 2004). The consequences of the plasticizing action of glycerol are favourable to the adsorption and absorption of water molecules to the film, so the WVP increased (Irissin-Mangata et al., 2001).

Figure 6.48 shows WVP of acorn starch-carrageenan films varying the amount of plasticizer. WVP values ranged between 2.99 and 3.85 x 10^{-10} g m^{-}

^{1} s^{-1} Pa^{-1} for the MST films and for SKW films ranged from 1.65 and 2.41 x 10^{-10} g m^{-1} s^{-1} Pa^{-1}, increasing the glycerol content from 0 to 40%. The WVP values were higher for the films produced with MST, because their thicknesses were higher than the films produced with SKW. The film thicknesses are presented in Table 6.17. The thickness influence on WVP was discussed in section *6.2.4.2*.

Table 6.17 – Thickness of starch-carrageenan based films produced by knife coating varying the plasticizer content for the WVP measurements.

Composition (glycerol concentration)	Thickness (μm)	
	MST	SKW
0%	46.5 ± 0.7	28.4 ± 0.1
10%	56.8 ± 0.6	36.6 ± 6.2
20%	53.2 ± 0.2	41.9 ± 6.4
30%	52.5 ± 7.5	38.1 ± 7.2
40%	48.6 ± 11.6	33.4 ± 8.8

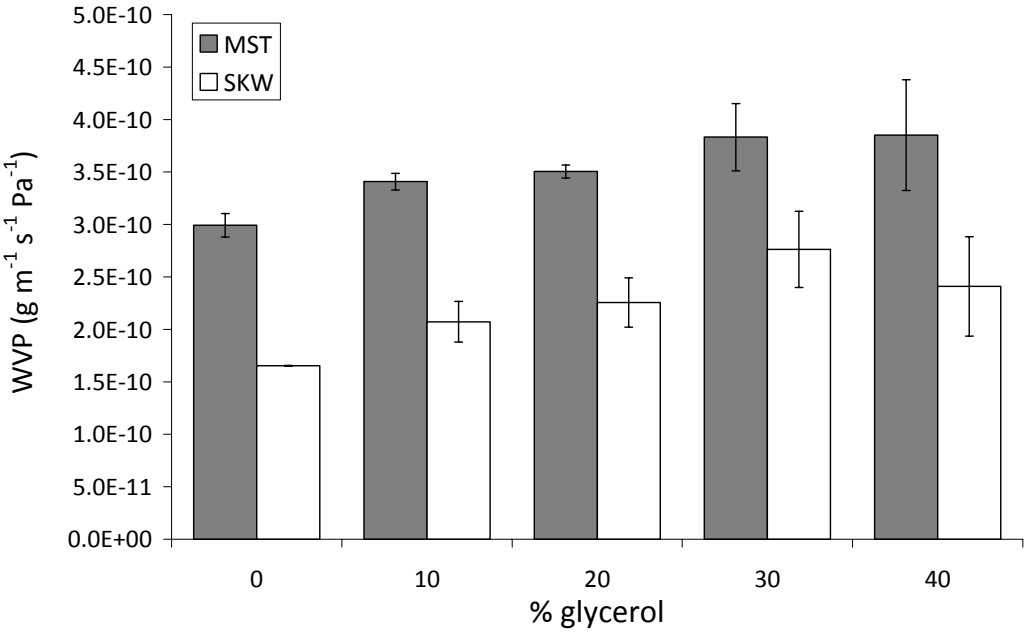

Figure 6.48 – Water vapour permeability of acorn starch-carrageenan (MST and SKW) based films plasticized with glycerol at different amounts (0, 10, 20, 30 and 40%).

6.7.4.4. Optical properties

Visually, all the films had a yellow appearance (Figure 6.45), due to the beige coloration of acorn starch. Instrumental colour determination performed on films showed significant differences ($p<0.05$) in the colour parameters (*L* and b**) values for all assayed films (Table 6.18). Colour difference (*ΔE**) were higher for MST samples than the SKW films, due to the κ/ι-hybrid carrageenan extracted in laboratory did not suffer a bleaching process. The yellow appearance of all the films studied, represented by the *b** parameter was the parameter with highest contribution to colour difference (*ΔE**) showed a similar trend. The increasing of glycerol concentration in the film formulation did not show great influence in the colour properties.

Table 6.18 – Colour standards of acorn starch-carrageenan films plasticized with glycerol.

Film samples	Lightness *(L*)*	Chromaticity parameter *a**	Chromaticity parameter *b**	Colour difference *(ΔE*)*
MST-0	76.51	1.99	19.92	21.11
MST–10	74.75	2.45	22.76	24.48
MST–20	78.7	1.81	16.52	17.08
MST-30	77.64	1.92	18.34	19.18
MST-40	76.13	2.39	20.22	21.61
SKW-0	80.31	0.95	16.32	16.09
SKW-10	78.33	1.67	17.08	17.73
SKW-20	78.63	1.82	17.66	18.11
SKW-30	78.38	2.07	17.3	17.94
SKW-40	79.04	1.72	16.86	17.19

* CIELab standards for the white standard, used as the film background were: L_s^* (97.10), a_s^* (0.05) and b_s^* (1.76).

6.7.5. Conclusions

Homogeneous, flexible and thin films were obtained from the mixture of acorn starch and carrageenan (commercial and extracted from *Mastocarpus stellatus*). In general, films showed good mechanical and barrier properties. The addition of glycerol in the mixture improved the film deformation and workability and decreased the tensile strength and Young's modulus, as was expected and well reported in the literature. The acorn starch-carrageenan films presented good values of water vapour permeability, comparable to the obtained by García et al. (2006) for corn starch films. Films formulated with commercial carrageenan and carrageenan extracted in the laboratory showed similar results, in all properties studied and these results confirmed the results obtained in the section 6.1 of this chapter. In terms of colour, the films did not have good appearance due to the colour of acorn starch, which did not suffer a purification process. Acorn starch extracted from cork oak (*Quercus suber* L.) showed to be an interesting alternative to produce edible films and to give a new application for this low cost national resource. Additional studies and an extraction of a more purified starch are however needed to verify the real capacity of this renewable material.

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Chapter 7
Application of a coating to fresh cherries

In this chapter, the results of the application of a biopolymer coating made of κ/ι-hybrid carrageenan extracted from *Mastocarpus stellatus*, to extend the shelf life of fresh cherries are presented.

7.1. Introduction

Sweet cherry (*Prunus avium* L.) is one of the most appreciated fruit by consumers since it is an early season fruit with an excellent taste, texture and visual appeal. For this, the skin colour, which is related to fruit ripening and affected by anthocyanin concentration (Serrano et al, 2005) plays a decisive role to attract consumer attention. Fruit firmness comes next as a very important quality attribute, directly related to enhancing its storability potential by inducing a greater resistance to decay and mechanical damage (Barret and González, 1994).

Sweet cherries are highly perishable, non-climateric fruits. With a high respiration rate, their shelf life is very short, as manifested by browning and drying of the stems, darkening of fruit colour, shriveling and decay (Alonso and Alique, 2004). Sweet cherry fruit deteriorate rapidly after harvest and in some cases do not reach consumers at their best optical quality, after transportation and marketing. The main causes of sweet cherry deterioration are weight loss, colour changes, softening, surface pitting, stem browning and loss of acidity, while small variations occur in total soluble solids (Bernalte et al., 2003).

Coating of fresh fruits has several purposes: (i) to reduce transpiration and control weight loss (Drake et al., 1988; Sümnü and Bayoindirli, 1995); (ii) to slow down ripening and extend shelf life (Yaman and Bayoindirli, 2001; Jiang and Li, 2001; Togrul and Arslan, 2004); (iii) to reduce symptoms of fruit injury; (iv) to reduce browning and rotting (Zhang and Quantick, 1997); (v) to give the fruit a glossy or matte finish (Bai et al., 2003).

Edible coatings are traditionally used to improve food appearance and conservation. They act as barriers during processing, handling and storage and do not solely retard food deterioration enhancing its quality, but may a role in improving safety either through a natural biocide activity or by the incorporation of antimicrobial compounds (Petersen et al., 1999). Different compounds are already used as edible coatings to prevent commodity weight loss, including wax, milk proteins, celluloses, lipids, starch, zein and alginate (Cha and Chinnan, 2004). In the recent literature, there is some evidence of the use of edible coatings in sweet cherry. Semperfresh™ is used to reduce weight loss and softening, extending shelf life (Yaman and Bayindirh, 2002), in some cases with added fungicides to avoid fungal spoilage (Yaman and Bayindirh, 2001). Derivatives of fatty acids and polysaccharides decreased sweet cherry fruit respiration rate and weight loss (Alonso and Alique, 2004). In addition, edible coatings based on chitosan alone or in combination with hypobaric treatments reduced postharvest decay in sweet cherry (Romanazzi et al., 2003). Other edible

coatings used to maintain the quality of cherries are *Aloe vera* (Martínez-Romero et al., 2006), zein and carnauba wax emulsion (Carvalho Filho et al., 2006) and milk protein concentrate- and sodium caseinate-based coatings (Certel et al., 2004).

The aim of this study was to evaluate the effect of κ/ι-hybrid carrageenan extracted from *Mastocarpus stellatus*, applied as an edible coating, on the change in physicochemical parameters related to fruit quality during cold storage and shelf life in sweet cherry.

7.2. Preliminary study

7.2.1. Materials and methods

7.2.1.1. Material

Sweet cherries (*Prunus avium* L. cv. Burlat) were harvested in June 2006 and purchased at the Gross Supplier Market of Porto (Porto, Portugal). At the laboratory, fruits of homogeneous colour and size, without injuries and health greenish stems were selected, washed and air-dried at room temperature.

7.2.1.2. Coating application

The selected fruits were divided into two groups: control and coated fruits. The edible coating used was an aqueous solution of κ/ι-hybrid carrageenan (2.0 g/100 ml solution) with the addition of glycerol (30 g/100 g κ/ι-hybrid carrageenan). The solution was heated at 80ºC under stirring during 30 minutes for total solubilization of carrageenan. After solubilization it was cooled to room temperature for the coating application. Coating was applied by dipping the cherries (hanged in an acrylic support as described in Figure 7.1a) into the carrageenan solution for about 1 minute. The control

fruits were immersed in distilled water for the same time of the coated fruits (Figure 7.1b). After the immersion in the coating solution, the fruits were allowed to drain (by hanging them by the stems) (Figure 7.1c) and then dried at room temperature with the aid of a fan (Figure 7.1d), in order to hasten drying and also to allow a film layer to form on the fruits. The fruits were then placed in polystyrene trays and kept in storage at 5 ± 2ºC and 80-85% RH. The fruits were divided in 14 lots (7 for control and 7 for coated fruits). Weight loss and colour were checked daily and firmness was measured every three days.

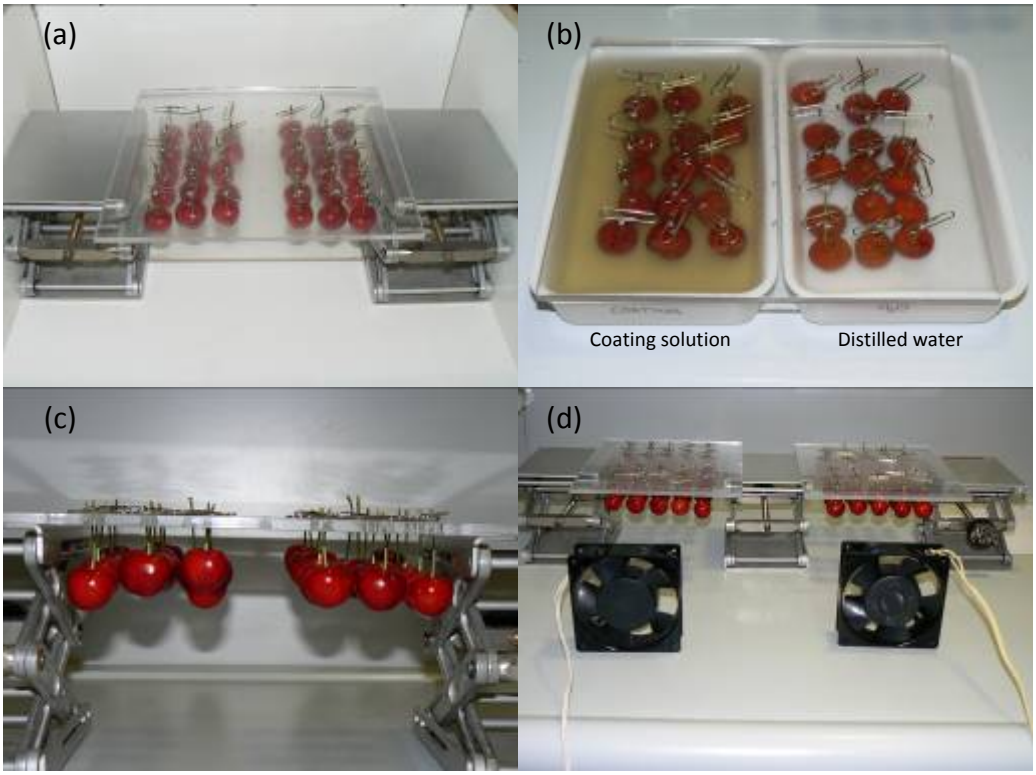

Figure 7.1 – Coating application process: (a) support for hanging and dipping the fruits; (b) dipping process (~1 minute); (c) fruit draining; (d) air-drying of the coated fruits.

7.2.1.3. Weight loss

Weight of individual lots was recorded following treatment (day 0) and every day until the end of the experiments (day 18). Cumulative weight loss was expressed as percent of original weight (n=5). The weight loss was calculated by the equation 7.1:

$$weight \cdot loss(\%) = \frac{W_0 - W_f}{W_0} \times 100 \quad (7.1)$$

where W_0 is the initial weight and W_f is the final weight.

7.2.1.4. Firmness

Texture analysis was performed using a TA.XT2 Stable Micro Systems texture analyzer (Surrey, England). The system was equipped with a load cell of 5 kg and a cylindrical probe of 2 mm in diameter, moving at 0.1 mm/s until 5% of sample deformation. Firmness was measured as the maximum penetration force (N) reached during tissue breakage. The fruits were peeled in the region of probe penetration to avoid the influence of the peel or coated peel in the tissue firmness (Figure 7.2). A number of 5 cherries per treatment were used for each storage time, which were 0, 3, 6, 9, 12, 15 and 18 days.

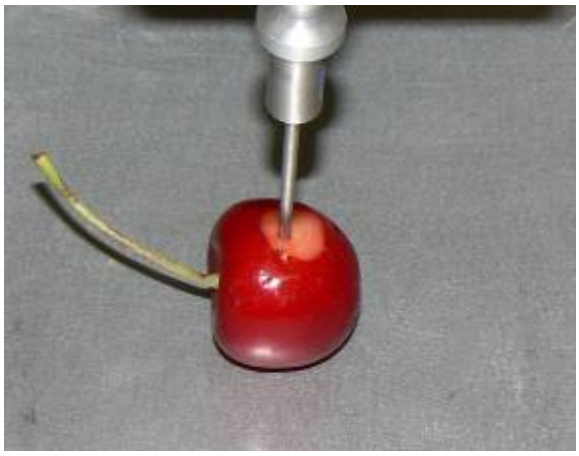

Figure 7.2 – Scheme of fruit flesh firmness measurement.

7.2.1.5. Surface colour development

Cherry surface colour was evaluated with a Minolta colorimeter CR300 series (Tokio, Japan). *L** (lightness), *a** (redness) and *b** (yellowness) were registered following treatment (day 0) and at each day until the end of the experiments (day 18). As the ratio of chromaticity parameters (*a*/*b*) has been commonly accepted for describing colour changes on postharvest fruits and vegetables (McGuire, 1992), it was also calculated and reported.

7.2.2. Results and discussion

7.2.2.1. Visual inspection

The coatings were well adhered to the cherry surfaces. Comparing the control and coated cherries at 0, 3, 6, 15 and 18 days of storage (Figure 7.3), all the cherries shrank and the control fruits lost their gloss (at day 3) whereas the coated fruits kept at least a slight gloss until day 18. By visual analysis it was possible to verify that good quality was maintained until the day 15 for the cherries coated, whereas for the control was the day 9. This observation can be an indicative of coating efficiency to delay the senescence process of cherries and extend their shelf life.

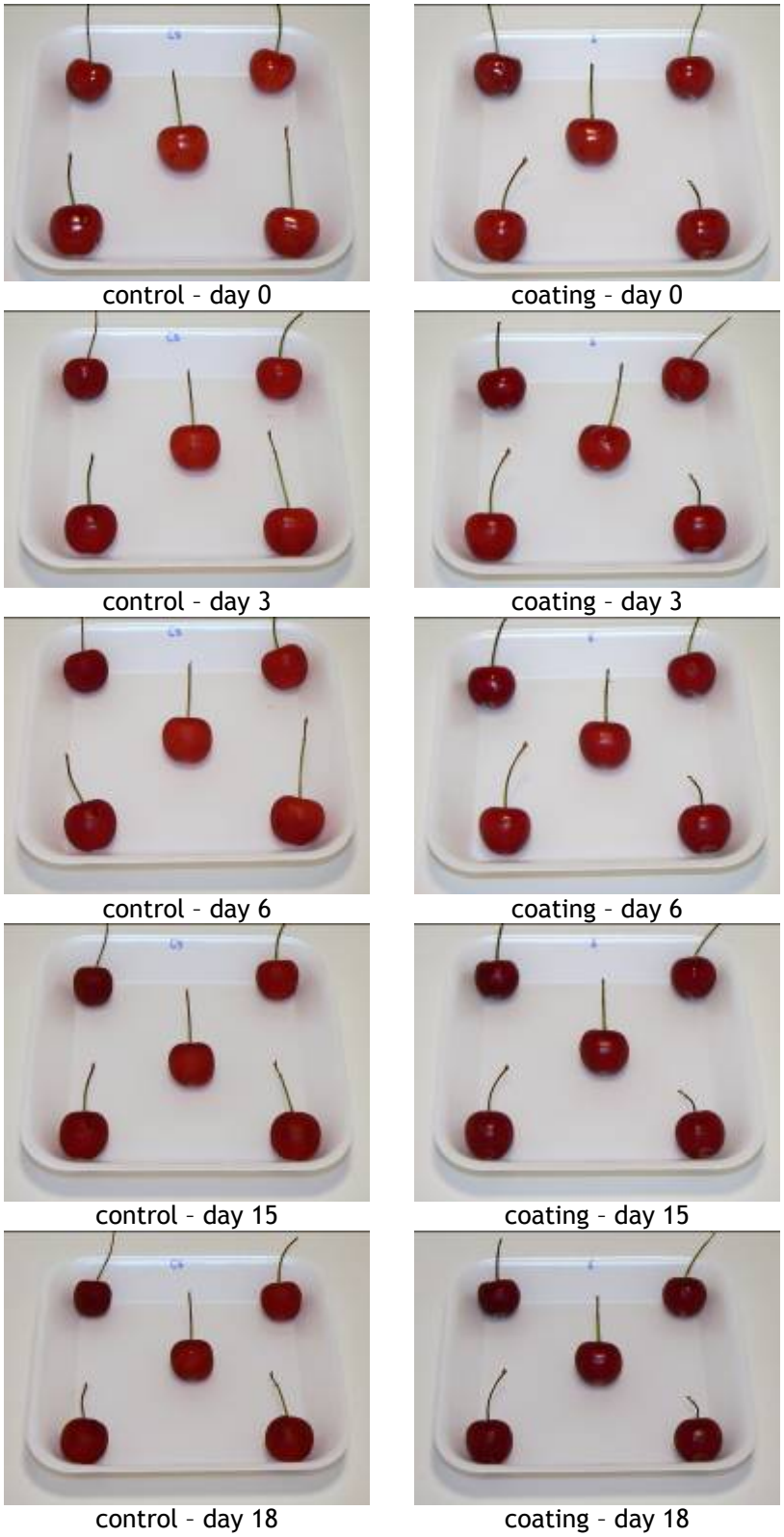

Figure 7.3 – Cherries coated and not coated (control) with κ/ι-hybrid carrageenan (2.0 g/100 ml solution) with the addition of glycerol (30 g/100 g κ/ι-hybrid carrageenan), at 0, 3, 6, 15 and 18 days of storage at 5 ± 2ºC and 80-85% RH.

7.2.2.2. Weight loss

Microbial growth, texture loss and changes in appearance are the most important modifications occurring in fruits and vegetables during storage and they determine postharvest storage life of the product. Normally, the weight loss occurs during fruit's storage due its respiratory process, the transfer of humidity and in some cases an oxidation process (Ayranci and Tunc, 2003). The effect of the κ/ι-hybrid carrageenan coating on the weight loss of the cherries during storage at 5 ± 2ºC and 80-85% RH are presented in Figure 7.4. Weight loss increased progressively with storage time. The carrageenan coating reduced significantly ($p<0.05$) the weight loss of cherries during the storage period, compared to the control. In this way, the film formed on the surface of fruits delayed moisture migration form the fruits into the environment, thus reducing weight loss during the storage. After 18 days of storage, control fruits lost 21% of their initial weight, whereas the coated fruits suffered a weight loss of 15%, which is 27% lower than the control fruits. Yaman and Bayindirh (2002) working with sweet cherry coated with a solution of 20 g/l of Semperfresh™ found a reduction of weight loss of 22%, for fruits stored at 30ºC during 15 days and 25% for fruits stored at 0ºC during 33 days, comparing with the control fruits (uncoated). Martínez-Romero et al. (2006) applied a coating solution based in *Aloe vera* in sweet cherries and found a reduction of weight loss of 42% for fruits stored at 1ºC during 16 days. The reduction of weight loss of coated fruits was probably related to the water vapour barrier properties of the coating; Avena-Bustillos et al. (1997) working on apple and celery treated with caseinates and acetylated monoglycerides coatings, found an increase of 75% in the resistance to water vapour transfer and consequently a decrease in weight loss of coated vegetables compared to the control ones.

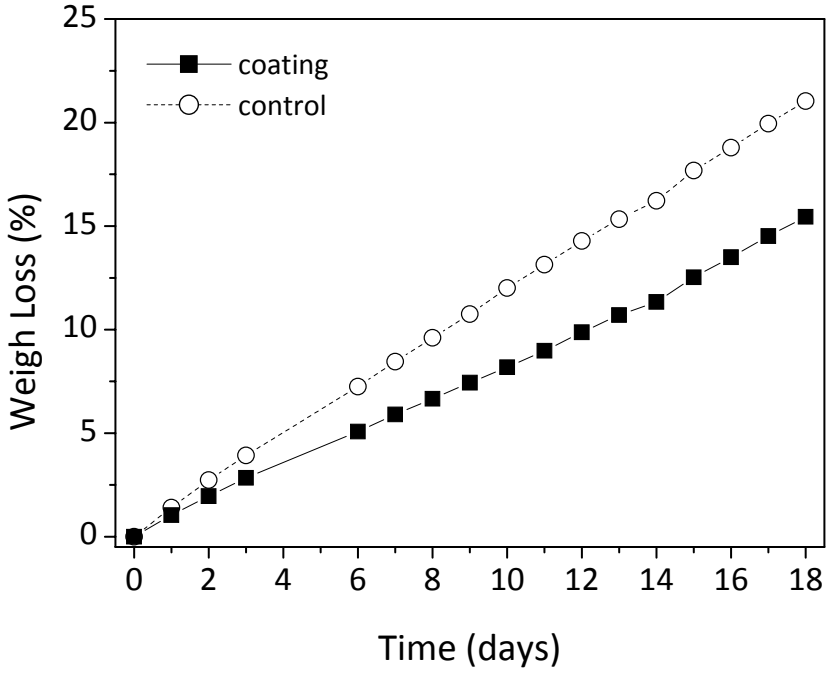

Figure 7.4 – Effect of carrageenan coating on weight loss of fresh sweet cherries stored at 5 ± 2ºC and 80-85% RH.

7.2.2.3. Firmness

Texture loss and change in appearance are the most noticeable changes occurring in fruits and vegetables during extended storage and are normally related to metabolic changes and water content. The rate and extension of firmness loss during ripening of soft fruits, such as cherries, is one of the main factors of decreasing fruit quality, limiting their postharvest shelf life. According to Manning (1993), fruit softening is attributed to the degradation of cell wall components, mainly pectin due to the action of specific enzyme activity such as polygalacturonase.

The softening process of sweet cherries has been reported to be dependent on the increase in polygalacturonase, ß-galactosidase and pectinmethylesterase activities (Batisse et al., 1996; Gerardi et al., 2001; Remón et al., 2003), being responsible for fruit quality loss. For both control

and coated fruits breaking force decreased as a function of storage time (Figure 7.4). Values of initial firmness of the control and coated samples was similar; thus, symbols in Figure 7.5 are overlapped. Firmness values were similar for control and coated samples ($p>0.05$) during the storage time. The coated cherries only lost 43% of their initial firmness, whereas the control fruits shown a 58% decrease of their firmness, at the day 18 of storage. The reason for the sweet cherry firmness maintenance may be related to the lower weight losses, as has been reported in strawberry (Mali and Grossmann, 2003; Del-Valle et al., 2005), apple (Moldão-Martins et al., 2003) and sweet cherry treated with different edible coatings (Yaman and Bayoindirli, 2002).

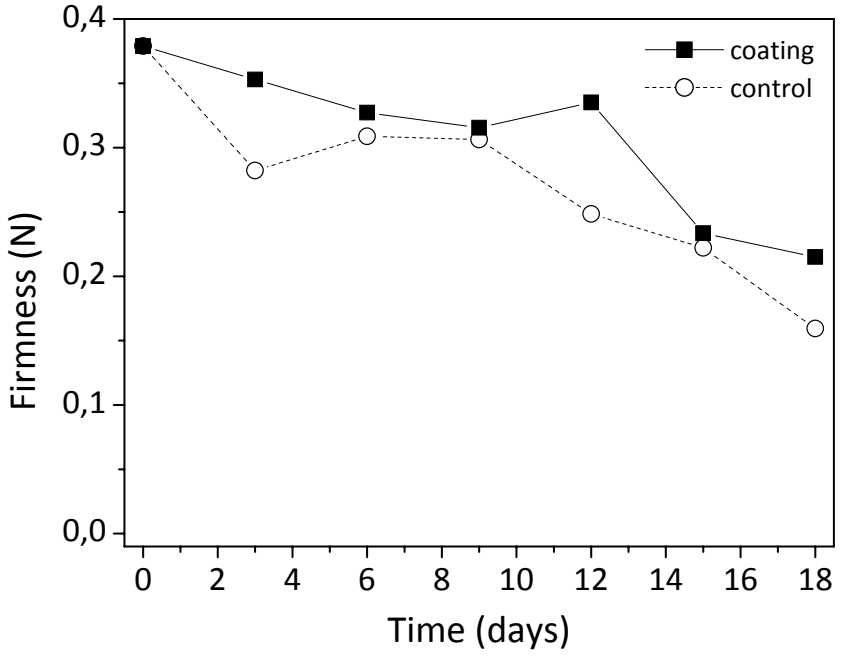

Figure 7.5 – Effect of carrageenan coating on firmness of fresh sweet cherries stored at 5 ± 2ºC and 80-85% RH.

7.2.2.4. Surface colour development

Cherry colour tended to darken in the course of this storage at 5ºC. This entailed a fall in the "*Lab*" parameters, which was especially significant in the case of parameter *a* (green/red) (Alonso and Alique, 2004).

Lightness values (*L**) of cherries showed a general decreasing trend during the cold storage for the control and coated fruits (Figure 7.6). Similar results were obtained by Yaman and Bayoindirli (2002). *L** values were not significantly different ($p>0.05$) comparing control and coated fruits. The colour changes of the coated cherries and control fruits were evaluated by the chromaticity parameters (*a*/*b*) ratio in function of storage time (Figure 7.7). As may be observed in the Figure 7.7, the control and coated fruits presented similar behaviour and values ($p>0.05$), which indicate that it was not evidenced a senescence delay, showed by an decrease in the colour changes comparing the control fruits with the coated cherries.

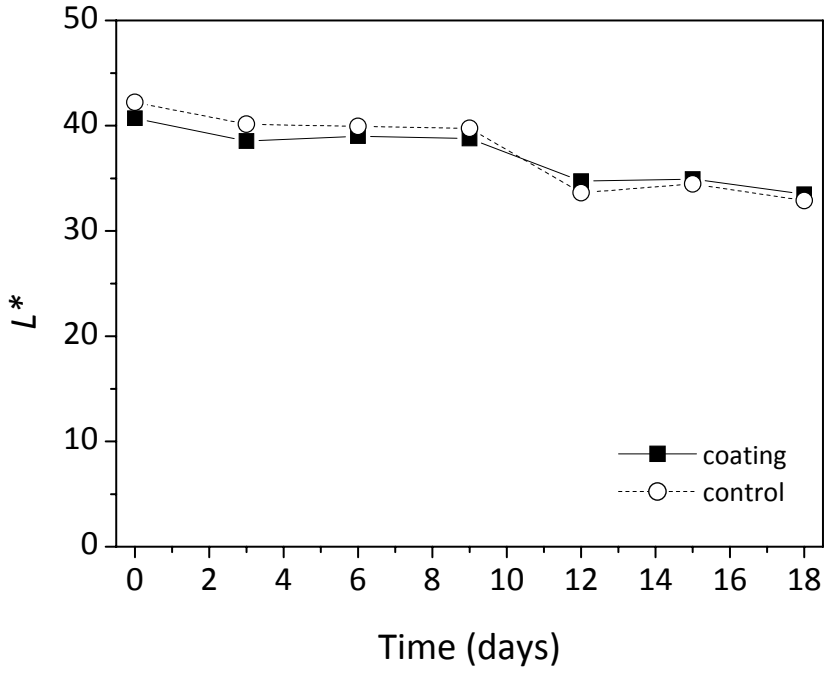

Figure 7.6 - Effect of carrageenan coating on the Lightness value (*L**) of fresh sweet cherries stored at 5 ± 2ºC and 80-85% RH.

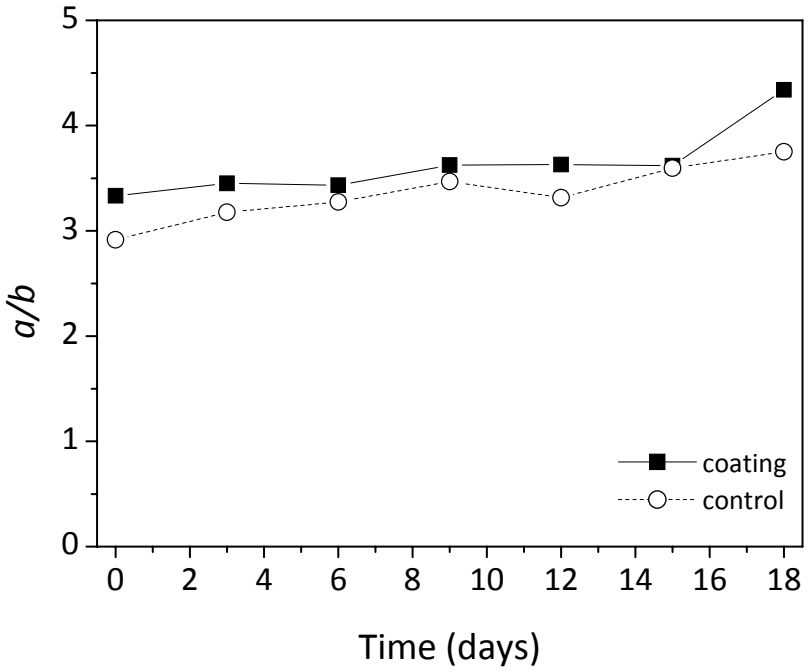

Figure 7.7 - Effect of carrageenan coating on the surface colour development (*a*/*b* ratio) of fresh sweet cherries stored at 5 ± 2ºC and 80-85% RH.

7.2.3. Conclusions

The application of a coating made of κ/ι-hybrid carrageenan and glycerol to fresh cherry fruits proved to be beneficial to extend their shelf life. This coating reduced the weight loss in about 27% comparing to the uncoated fruits and was effective in retaining the firmness of the stored cherries.. Visual inspection of the fruits shown that the control fruits lost their gloss whereas the coated fruits kept a light gloss up to the end of the test. Thus, this coating of κ/ι-hybrid carrageenan proved to be an interesting material for acoating formulation in line with the actual trends in to use natural materials for edible films and coatings.

7.3. Effect of glycerol on the coating of cherries

In general, plasticizers are added to overcome brittleness and improve some relevant mechanical properties, such as flexibility and extensibility, of films and coatings. Plasticizers must be compatible with film-forming polymers, reduce intermolecular forces and increase the mobility of polar polymer chains. Hydrophilic compounds such as polyols (glycerol, sorbitol and poly(ethylene glycol)) are commonly used as plasticizers in hydrophilic film formation. Glycerol improves film extensibility but reduces the elasticity and water vapour barrier properties (Gontard et al., 1992). The use of plasticizers in coating formulations may be necessary to maintain film and coating integrity and avoid pores or cracks (García et al., 2001).

The aim of this study was to evaluate the effect of the glycerol on the coatings formulated from κ/ι-hybrid carrageenan extracted from *Mastocarpus stellatus*, on the change in physicochemical parameters related to fruit quality during cold storage and shelf life in sweet cherry.

7.3.1. Materials and methods

7.3.1.1. Material

"Picota" sweet cherries (*Prunus avium* L.) (Figure 7.8) were harvested in June 2007 and purchased at Gross Supplier Market of Porto (Porto, Portugal). At the laboratory, fruits of homogeneous colour and size, without injuries and healthy greenish stems were selected, washed and air-dried at room temperature.

Figure 7.8 - "Picota" sweet cherries (*Prunus avium* L.).

7.3.1.2. Coating application

The selected fruits were divided into three groups: one of control fruits and two of coated fruits. The edible coatings used were aqueous solution of pure κ/ι-hybrid carrageenan (2.0 g/100 ml solution) codified as coating MST and aqueous solution of pure κ/ι-hybrid carrageenan (2.0 g/100 ml solution) with the addition of glycerol (30 g/100 g κ/ι-hybrid carrageenan) codified as coating MST-G. The process of preparation of coating solutions and coating application were described in the section 7.2.1.2. The fruits were divided in 21 lots (7 for control and 7 for each coating) and the analysis of weight loss and colour were carried out daily and firmness was carried out at each 3 days.

7.3.1.3. Quality parameters

Weight loss, firmness and surface colour development was previously described in sections *7.2.1.3*, *7.1.2.4* and *7.1.2.5*, respectively.

7.3.2. Results and discussion

7.3.2.1. Visual inspection

All coatings were transparent and improved fruit surface gloss, becoming more attractive in appearance after coating. The coatings were well adhered to the fruits as revealed by the smooth surface of the cherries. Comparing the control and coated cherries at 0, 5, 10, 14 and 20 days of storage (Figure 7.9), the control fruits lost their gloss after day 5 whereas the coated fruits kept a light gloss until day 20. All the cherries suffered a slight shrinkage due to weight loss. By visual inspection it was possible to verify that good quality was maintained until the day 16, for the coated cherries, whereas the control lost its gloss after day 10. This observation is an important indication of the coatings effectiveness to extend the shelf life of the cherries during storage.

Figure 7.9 – Cherries not coated (control) and coated with κ/ι-hybrid carrageenan (coating MST) and κ/ι-hybrid carrageenan with the addition of glycerol (coating MST-G), at 0, 5, 10, 14 and 20 days of storage at 5 ± 2ºC and 80-85% RH.

7.3.2.2. Weight loss

The effect of the κ/ι-hybrid carrageenan coatings on the weight loss of the cherries during storage at 5 ± 2ºC and 80-85% RH are presented in Figure 7.10. Weight loss increased progressively with storage time. Water is lost by transpiration due to differences in the vapour pressure of water between the atmosphere and the cherry surface (Park et al., 1994). Surface coatings, by providing differential changes in skin permeance to water vapour, O_2 and CO_2, can create different levels of water loss and modification of fruit internal atmosphere, depending on the chemical nature (Hagenmaier and Shaw, 1992; Hagenmaier and Baker, 1993), thickness (Hagenmaier and Baker, 1993) and character of surface cover (Banks et al., 1993). As other edible coatings, carrageenan prevented moisture loss and controlled respiratory exchange. In general, this positive effect of edible coatings is based on their hygroscopic properties, which enables formation of a water barrier between the fruit and the environment and thus avoiding its external transference (Morillon et al., 2002). Edible coatings can help to prevent water loss by acting as water loss barriers, causing high relative humidity in the surrounding atmosphere of the fruits and thus reducing the gradient to the exterior (Olivas et al., 2003). The carrageenan coatings reduced significantly ($p<0.05$) the weight loss of cherries during the storage period, compared to the control. After 20 days of storage, control fruits loose 16% of their initial weight, whereas the coated fruits had a weight loss of 11% for the MST coating and also 11% for the MST-G coating. These values for the coatings are 31% lower than the control fruits. The results of the plasticized coating were not significantly different from the unplasticized coating and the effect of glycerol was not evident. This may have occurred because of the low concentration of glycerol in the mixture, about 0.6g/100 ml of coating solution, although the glycerol proportion relative to carrageenan was 30g/100 g carrageenan. García et al. (2001) obtained significantly reduction of weight loss of strawberries coated with plasticized coatings using 2g/100 ml of coating solution.

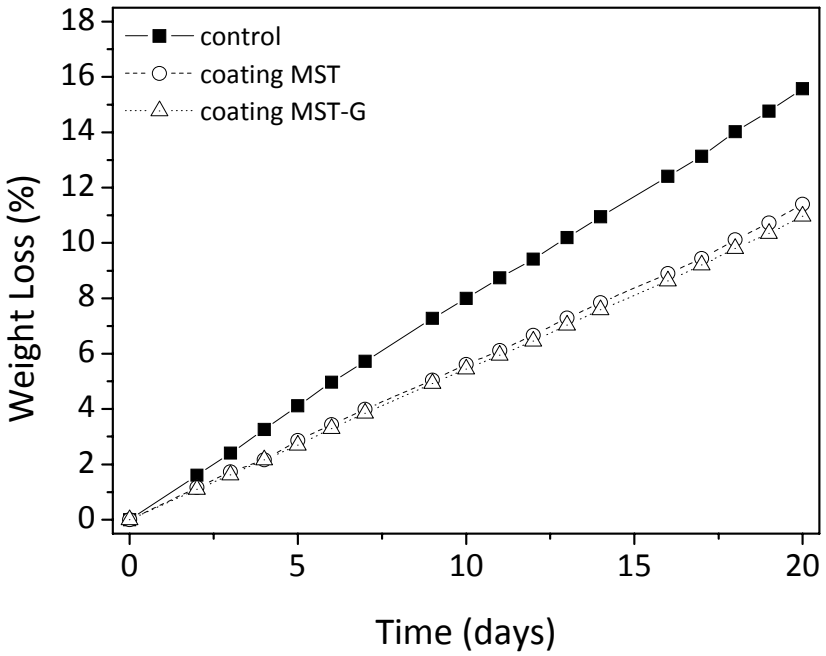

Figure 7.10 – Effect of carrageenan coatings on weight loss of fresh sweet cherries stored at 5 ± 2ºC and 80-85% RH.

7.3.2.3. Firmness

Texture is an important attribute required by consumers and most of the time is responsible for fruit acceptability. The texture of fruits and vegetables is affected by traits such as cellular organelles and biochemical constituents, water content or turgor and cell wall composition. Thus, any external factor affecting these traits can modify texture and can therefore lead to changes in final product quality (Sams, 1999). The rate and extension of firmness loss during storage are the main factors determining fruit quality and postharvest shelf life (Valverde et al., 2005). Changes in the firmness between control and coated fruits during the storage time at 5 ± 2ºC and 80-85% RH are shown in Figure 7.11. Initial firmness values were similar for control and coated samples ($p>0.05$), becoming significantly different ($p<0.05$) during the storage time. Increasing the storage time, the firmness decreased for both control and coated fruits and the results were similar for coating MST and MST-G ($p>0.05$) during all the period of storage.

In fact, carrageenan coatings reduced the firmness losses during storage and subsequent shelf life, loosing 13% and 14% of their initial firmness for MST and MST-G coatings, respectively, whereas losses of 36% were detected in control fruits after 20 days of storage. The firmness maintained by coated fruits could be related to their lower weight loss when compared with the control fruits. The similar results observed for plasticized and unplasticized coatings may be due the the low plasticizer concentration used in the coating formulation, as was commented in the section *7.3.2.2.*

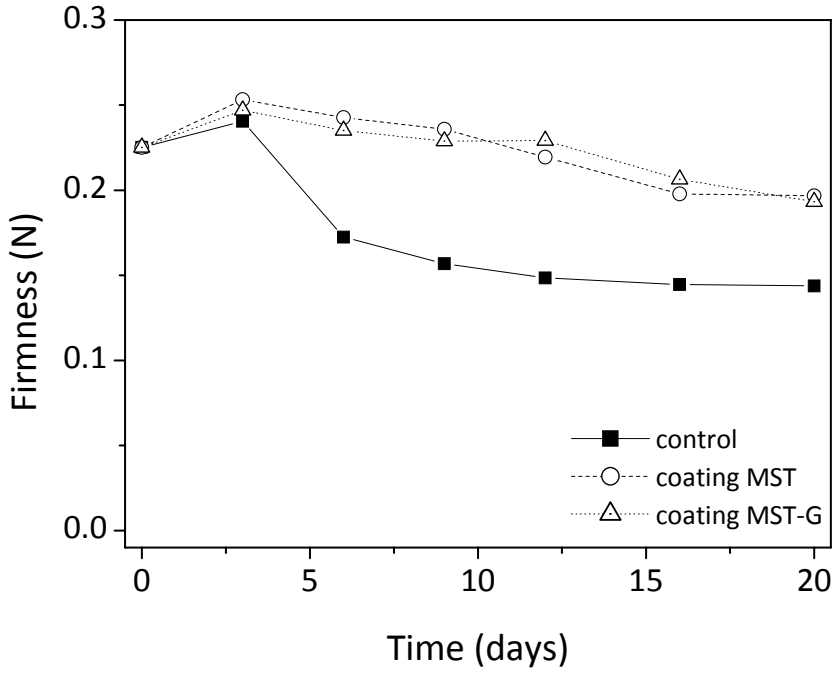

Figure 7.11 – Effect of carrageenan coatings on firmness of fresh sweet cherries stored at 5 ± 2ºC and 80-85% RH.

7.3.2.4. Surface colour development

Lightness values (*L**) of cherries showed a general decreasing trend during the cold storage for the control and coated fruits (Figure 7.12) and *L** values were lower for the control fruits. *L** values were significantly different ($p<0.05$) comparing control and coated fruits, from the day 6. Similar results were obtained by Yaman and Bayoindirli (2002). The colour changes of the

coated cherries and control fruits were evaluated by the chromaticity parameters (*a*/*b*) ratio in function of storage time. As can be observed in the Figure 7.13, the control and coated fruits presented similar behaviour and values ($p>0.05$), which indicate that it was not evidenced a senescence delay, showed by an decrease in the colour changes comparing the control fruits with the coated cherries.

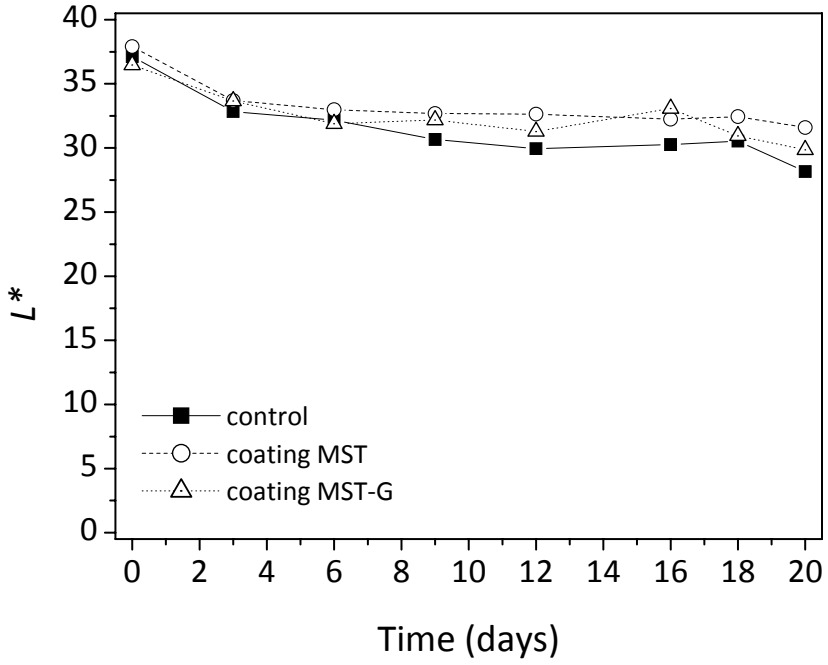

Figure 7.12 - Effect of carrageenan coatings (MST and MST-G) on the Lightness value (*L**) of fresh sweet cherries stored at 5 ± 2ºC and 80-85% RH.

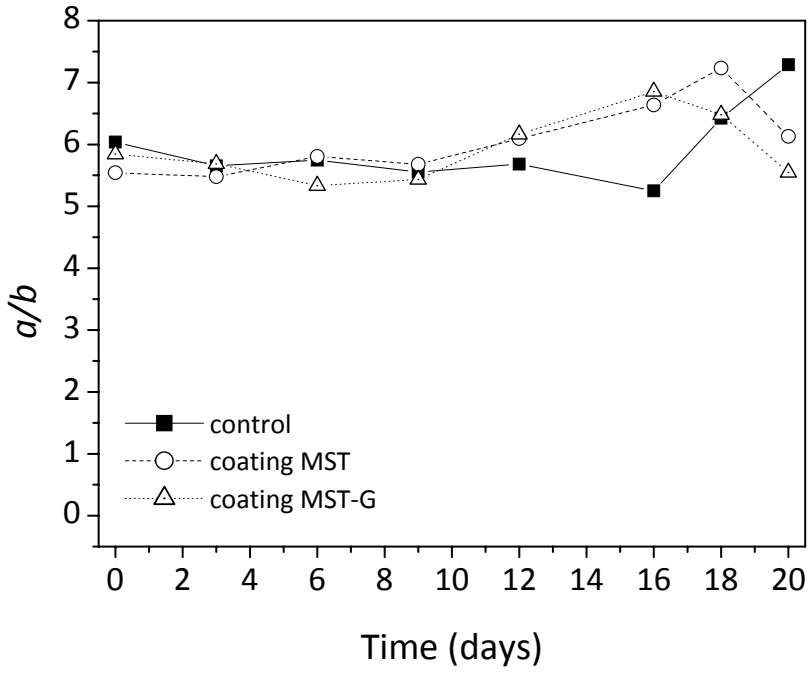

Figure 7.13 - Effect of carrageenan coatings (MST and MST-G) on the surface colour development (*a/b* ratio) of fresh sweet cherries stored at 5 ± 2ºC and 80-85% RH.

7.3.3. Conclusions

The application of the coatings made of κ/ι-hybrid carrageenan (MST) and of κ/ι-hybrid carrageenan with the addition of glycerol (MST-G) to cherry fruits was shown to be beneficial in extending their shelf life. These coatings reduced the weight loss in about 27% and 30%, for MST and MST-G respectively, comparing to the uncoated fruits and was effective in retaining the firmness of the refrigerated cherries. The external colour changes were not evidenced in this study, but in a visual characterization the control fruits lost their brightness whereas the coated fruits kept the brightness until the day 20 of storage. The effect of the glycerol on the coatings formulation was not so evidenced in the results, due the low concentration of glycerol used. κ/ι-hybrid carrageenan plasticized with glycerol showed a little better performance than the unplasticized coating, but both showed characteristics to be a very interesting material for coatings formulation to extend the shelf life of fruits and vegetables.

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Chapter 8
Conclusions

The main objective of the present work was to develop improved technologies to produce edible coatings and biodegradable films from low value renewable raw materials available in Portugal, assessing the extraction procedure, optimizing the formulations and developing a new continuous technique to produce biodegradable films.

In the search for new sources of renewable materials, two interesting raw materials were found in Portugal as potential sources of biopolymers. The first was the macroalgae *Mastocarpus stellatus*, present in the northern coast of Portugal, easily available and underexploited nowadays. This red seaweed is a source of carrageenan, a biopolymer extensively used in food and pharmaceutical industries as gelling and stabilizing agent. Carrageenan has the ability to form excellent gels and produces aqueous solutions with good film forming properties. The carrageenan obtained from *Mastocarpus stellatus* was characterised a κ/ι-hybrid carrageenan with good properties to produce biodegradable films, similar or even better than the commercial κ-carrageenan.

The second interesting source of biopolymer identified in this work was the acorn, the fruit from cork oak (*Quercus suber* L.) very rich in starch. Portugal has the highest surface of cork oak stands in the world that is responsible for more than half of the world cork production. Acorn from cork oak, a non-conventional source of starch, is economically less important than the cork itself and is usually used as a feed source for free-ranging wild animals, particularly pigs. Starch is a biopolymer that is becoming widely used in technical applications and has recently gained interest as a renewable and biodegradable plastic. Although the starch extraction procedure from acorn used still requires some optimization and an additional purification step, starch samples obtained from cork oak acorn presented good characteristics for the production of films.

Concerning the techniques for film production, casting is the most popular among the majority of researchers studying these films. It is a simple method and the films obtained by casting are in general adequate as testing structures for determination of barrier, mechanical and other relevant properties offered by a certain biopolymer material. The disadvantage of the casting lies on the fact that it is a batch method no able to be replicated industrially, which does not allow an easy control on the sample film thickness and uniformity, and rarely allows samples larger than 100 to 200 cm^2. An alternative to produce biodegradable films in a semi-continuous process and consequently in a larger-scale, was studied in this work. This semi-continuous method uses a knife coating technique over an adequate non-adhering support. The technique allows a very strict control over the thickness and application speed. The width of the film layer depends only on the width of the knife used, which is maintained at a fixed gap over the support. In the case of the applicator used, the length of the coating on the support can be adjusted up to 0.4 m. To convert the technique into a continuous one, a movable flexible support could be used under a stationary knife. Suitable supports may be selected for each biopolymer solution used. For the properties under consideration, films produced by both methods, knife coating and casting, did not show significant differences. However,

the films produced by knife coating were much more uniforms in terms of thickness and surface than the films produced by casting. This characteristic led to more uniform results, with improved reproducibility for all the film properties measured, particularly in tensile tests. Another advantage of the films produced by the knife coating technique was the required drying time, which was reduced to about two hours whereas the films produced by casting took about one to two days to dry. Based on the results obtained and their good reproducibility, the use of the knife coating has definitively a great potential to be converted into a continuous industrial scale technique for these films. This is a major result of this study with great economic potential.

Films produced with κ/ι-hybrid carrageenan extracted from *Mastocarpus stellatus* showed excellent properties in general. The films presented good optical properties in terms of transparency and UV-light barrier, in spite of its yellowness appearance. Water vapour permeability of carrageenan based films had an intermediate value, comparable to other films based in proteins and polysaccharides reported in literature. With regard to synthetic polymers, carrageenan films have permeability values similar to those of cellophane. Mechanical properties of these films were excellent in terms of tensile strength, which reached 60 MPa for pure carrageenan films. This value of tensile strength is higher than that for polyethylene films (LDPE and HDPE) and for other biopolymer films (corn starch, wheat starch, polylactic acid (PLA)).

The carrageenan based films showed, however poor elongation properties and a brittle nature. These two aspects were improved by the addition of a plasticizer at a cost of a decrease of tensile strength. Hydrophilic plasticizers and hydrophobic plasticizers were studied. The best results were obtained for glycerol, of the hydrophilic type, in terms of almost all properties. Films with glycerol became more deformable and easier to work; besides the hygroscopicity of the films was increased. The films with all hydrophobic plasticizers presented moisture exudation, a negative quality

factor for usual applications. The only positive aspect detected was the improvement of barrier properties to UV light, when compared with glycerol films.

Films produced from acorn starch extracted from cork oak (*Quercus suber* L.) were very brittle as well and difficult to remove from the support without breaking. The alternative explored was to mix starch with carrageenan. The results obtained were good in terms of mechanical properties. The acorn starch-carrageenan films showed some yellowness appearance and opacity, due to the non-purification of starch. In general, acorn starch showed to be an interesting alternative to produce films with good properties and further studies is necessary in the extraction, purification and a better characterization of the starch.

As a practical application for the carrageenan extracted from *Mastocarpus stellatus* fruit coating with this biopolymer was studied. Cherries were coated with an aqueous solution of carrageenan, with and without the addition of a plasticizer (glycerol); and stored at 5ºC. The results indicate that with the coating application, the fruits lost about 30% less weight than the uncoated fruits. The coating application was effective in retaining the firmness of the refrigerated cherries. Visual inspection of the coated and uncoated fruits has shown that the latter lost their gloss after about one half of the storage period while the coated fruits kept the gloss until the day 20 of storage. Coating based in κ/ι-hybrid carrageenan solution showed to be an interesting alternative to protect and extend the shelf life of fruits. Further studies are necessary in order to perform a sensory analysis to evaluate the consumer acceptance of the coated fruits compared to the uncoated fruits.

Both biopolymers extracted and studied showed to be promising materials for the production of edible coatings and biodegradable films, with good properties. Improvements in the extraction method and additional purification will certainly allow the enhancement of their capabilities for

such end use. The new technique to produce these biofilms in a semi-continuous way showed to be a good alternative to produce homogeneous and uniform films, with good thickness control and allowing its scale-up to a continuous industrial scale.

Faced with these encouraging results, some other possibilities for improvement may be considered, namely the incorporation of active additives in the film formulation like, for example, antimicrobials and antioxidants to produce an active packaging that interacts with the food; surfactants, emulsifiers and cross-linkers to obtain films with even better mechanical properties. In terms of the film production technique, one aspect requiring additional research for a future scale-up to an industrial scale is its efficient drying, fast enough without damaging the surface.