Characterization of a biodegradable starch based film

Application on the preservation of fresh spinach

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Abstract

The goal of the present study was the characterization of a biodegradable thermoplastic starch based wrap film (TPS), produced from Mater-Bi®, and its application on the preservation of ready prepared fresh-cut spinach, in parallel with the non-biodegradable polyvinyl chloride (PVC) wrap film.

The TPS-based film presented a similar vapour adsorption (< 5%, dry basis) to the PVC film. In addition, the carbon dioxide and oxygen permeability was in the same range (TPS-based: $P_{CO2} = 34.6\text{x}10^{-17}$-$76.1\text{x}10^{-17}$mol.m/m^{2}sPa, $P_{O2} = 3.41\text{x}10^{-17}$ – $5.71\text{x}10^{-17}$ mol.m/m^{2}sPa; PVC: $P_{CO2} = 34.5\text{x}10^{-17}$ – $62.8\text{x}10^{-17}$mol.m/m^{2}sPa, $P_{O2} = 3.03\text{x}10^{-17}$ – $6.21\text{x}10^{-17}$ mol.m/m^{2}sPa), and was not significantly affected by the relative humidity. The major differences detected were in what concerns water vapour permeability, ($0.9\text{x}10^{-12}$ - $1.27\text{x}10^{-12}$ mol.m/m^{2}sPa and $3.64\text{x}10^{-12}$ – $4.43\text{x}10^{-12}$ mol.m/m^{2}sPa for TPS-based and PVC film) and strain at break (5.7 times higher for TPS-based under extension tests). TPS-based film showed a better transparency for white colour, although for green, yellow and red it was the PVC having better results. Both films revealed to have a similar performance in fresh-cut spinach preservation. The major difference was detected on the preventing weight loss, as PVC film showed to be more effective than TPS-based film due to its higher water vapour barrier. Based on the results obtained it can be concluded that the tested thermoplastic starch based wrap film is a strong substitute, ecological, to conventional PVC-based film.

Keywords: Thermoplastic starch based wrap film, Mater-Bi, polyvinylchloride, wrap film, spinach preservation.

Resumo

O presente estudo teve como objetivo a caracterização de uma película aderente à base de amido termoplástico (TPS), produzido a partir de Mater-Bi®, bem como a sua aplicação na conservação de espinafre de 4ª gama, comparativamente com a película aderente convencional de cloreto de polivinilo (PVC).

O filme à base de TPS apresentou uma adsorção ao vapor de água semelhante (< 5%, base seca) ao filme de PVC. Adicionalmente, os valores de permeabilidade ao dióxido de carbono e oxigénio apresentaram valores dentro da mesma gama (filme à base de TPS: P_{CO2} =$34,6x10^{-17}$-$76,1x10^{-17}$mol.m/m^2sPa, P_{O2} =$3,41x10^{-17}$ – $5,71x10^{-17}$ mol.m/m^2sPa; filme de PVC P_{CO2} = $34,5x10^{-17}$ – $62,8x10^{-17}$mol.m/m^2sPa, P_{O2} = $3,03x10^{-17}$ – $6,21x10^{-17}$ mol.m/m^2sPa), não sendo estes parâmetros significativamente afetados pela humidade relativa. As maiores diferenças foram detetadas na permeabilidade ao vapor de água ($0,9x10^{-12}$ – $1,27x10^{-12}$ mol.m/m^2sPa e $3,64x10^{-12}$ – $4,43x10^{-12}$ mol.m/m^2sPa para o filme à base de TPS e para o filme de PVC) e na deformação na rutura (5,7 vezes superior para o filme à base de TPS nos testes de extensão). O filme à base de TPS demonstrou uma melhor transparência para a cor branca, apesar de para o verde, amarelo e vermelho o PVC ter apresentado melhores resultados. Ambos os filmes revelaram um desempenho semelhante na conservação do espinafre fresco. A maior diferença foi detetada na prevenção de perda de massa, sendo que o filme de PVC mostrou ser mais eficiente do que o filme à base de PVC devido à sua elevada barreira ao vapor de água. Com base nos resultados obtidos pode ser concluído que o filme à base de TPS testado se apresenta como um forte substituto, ecológico, das películas aderentes convencionais, à base de PVC.

Palavras-chave: Película à base de amido termoplástico, Mater-Bi, cloreto de polivinilo, película aderente, conservação de espinafre.

Resumo alargado

A indústria dos plásticos remonta à década de 1860. Ao longo dos anos a complexidade e variedade de plásticos tem vindo a desenvolver-se, tendo como fonte o petróleo e sendo aplicados nas mais diversas áreas. A elevada disponibilidade e utilidade destes plásticos a baixo custo, confrontada com os problemas a nível ambiental e de sustentabilidade a que estão associados, levou à procura de alternativas que, obedecendo às mesmas exigências dos plásticos convencionais, respondessem igualmente às exigências ecológicas, cada vez mais presentes na sociedade. Surgiram assim os bioplásticos, a maioria biodegradáveis, e alguns com propriedades termoplásticas.

Atualmente os bioplásticos detêm uma parte significativa do mercado, com tendência crescente, existindo cada vez mais empresas ligadas à sua produção e à aposta na inovação dentro deste sector. No que respeita ao sector alimentar verifica-se uma utilização crescente de bioplásticos na composição das embalagens alimentares, tendo várias empresas adotado estas alternativas no sentido de contribuir para a diminuição da pegada ecológica.

Um material bastante utilizado na indústria alimentar é a película aderente, cujas características estão desenvolvidas para preservar a qualidade dos produtos alimentares durante um período de tempo mais alargado. As películas aderentes convencionais são produzidas a partir de polímeros não biodegradáveis, resultantes de combustíveis fósseis. Um desses polímeros é o cloreto de polivinilo (PVC), que apresenta uma adequada permeabilidade aos gases e uma boa resistência mecânica, fatores requeridos para a função em questão.

No âmbito deste trabalho é sugerida, como alternativa à utilização de peliculas aderentes de PVC, a utilização de uma película à base de amido termoplástico (TPS) comercial (Mater-Bi).

As propriedades das películas de amido termoplástico dependem do teor de amilose (sendo os amidos com maior teor de amilose os mais apropriados), temperatura de secagem, espessura, tempo e condições atmosféricas. A sua suscetibilidade à temperatura e humidade ao longo do tempo são os principais problemas associados à sua utilização, por afetarem a sua estrutura. Sendo um material hidrofílico o contacto com a água compromete bastante as suas características, contrariamente ao PVC, hidrofóbico. Outro problema associado ao amido termoplástico é a retrogradação da molécula de amido, que resulta igualmente na alteração das suas propriedades. A adição de certos aditivos permite uma melhoria destes fatores. Outra alternativa é a sua modificação química, por oxidação ou formulações com outros materiais, de modo a obter derivados mais estáveis.

O presente estudo focou-se na caracterização de uma nova película aderente à base de Mater-Bi®, um polímero constituído por TPS, celulose e óleos vegetais, produzido e comercializado pela empresa Novamont (Itália), e ao qual podem ser posteriormente adicionados outros componentes aquando do fabrico do produto final. Foi utilizada como padrão uma película convencional à base de PVC de modo a poder ser efetuada uma comparação entre as características de ambos. Fez parte da avaliação dos filmes o estudo da sua aplicação na conservação de espinafre de 4ª gama. A escolha deste produto deteve-se na elevada perecibilidade dos produtos vegetais, especialmente o espinafre devido à elevada taxa respiratória.

Numa primeira etapa, relativa à caracterização dos filmes, as suas propriedades foram avaliadas em função de diferentes graus de hidratação. Neste sentido, para amostras estabilizadas em condições de diferente humidade relativa, foi determinada a sua espessura; propriedades higroscópicas (capacidade de adsorção e absorção de água), propriedades barreira (ao dióxido de carbono, oxigénio e vapor de água), propriedades mecânicas (avaliação do comportamento aquando extensão e perfuração) e transparência.

Na segunda etapa, para avaliação da aplicação na conservação do espinafre, foi realizado o controlo de qualidade deste ao longo de 14 dias. Amostras de espinafre embaladas com ambos os filmes em teste, bem como um controlo que correspondeu a amostras de espinafre armazenadas sem película aderente, foram mantidas em ambiente refrigerado, com condições de temperatura (c.a. 4ºC) e humidade controladas. O controlo de qualidade ao longo do período estabelecido compreendeu uma avaliação do aspeto visual, perda de massa, taxa respiratória, composição atmosférica no interior da embalagem, firmeza, cor e teor de sólidos solúveis.

Os resultados obtidos na primeira etapa, nomeadamente no estudo das propriedades higroscópicas, revelaram não haver diferenças significativas entre os dois filmes, sendo que em ambos os casos existiu uma quantidade máxima de água adsorvida de 5% (base seca). Este resultado é bastante interessante, partindo do pressuposto que o filme à base de amido teria um carácter mais hidrofílico. A explicação para este facto reside na composição em óleos vegetais (hidrofóbicos) da matriz de Mater-Bi®. Em relação à adsorção de água líquida, o filme à base de TPS revelou uma capacidade de absorção de água 2,3 vezes superior ao filme de PVC, nomeadamente 17%, em contraste com 7% para o filme convencional.

O estudo das propriedades barreira ao O_2 e CO_2 demonstrou não haver igualmente diferenças significativas entre os dois filmes neste aspeto, nem influência do aw (atividade da água) de estabilização dos filmes nos resultados. Ambos os filmes apresentam maior permeabilidade ao CO_2 ($34,5\text{x}10^{-17}$ – $76,1\text{x}10^{-17}$ mol.m/m^{2}sPa) do que ao O_2 ($3,03\text{x}10^{-17}$ – $6,21\text{x}10^{-17}$ mol.m/m^{2}sPa), tendo uma seletividade de 15,3 e 11,2 respetivamente. Contrariamente ao observado nos testes de permeabilidade ao O_2 e CO_2, a permeabilidade ao vapor de água não só foi significativamente maior para o filme à base de TPS, como o aumento do a_w de estabilização deste filme foi acompanhado por um aumento do valor dos resultados obtidos. Este facto está relacionado com a maior hidrofilicidade associada ao polímero de amido.

Em relação às propriedades mecânicas, no teste de extensão, a resistência de ambos os filmes foi bastante semelhante, apresentando valores da ordem dos 23-35 MPa para a tensão na rutura e de 25-47 MPa para o módulo de Young. No entanto, o filme à base de TPS revelou ser mais extensível, apresentando valores de deformação na rutura de 12 em contraste com 1,5-3,0 no caso do filme de PVC. No caso do teste de perfuração, o filme à base de TPS apresentou uma menor resistência,

especialmente no caso de aw de estabilização entre 0,332 e 0,973, e tal como no teste anterior, uma maior extensibilidade (0,81 a 1,18 comparando com cerca de 0,7 para o filme de PVC).

Nos testes às propriedades óticas, o filme à base de TPS apresentou uma maior transparência à cor branca, apesar de para as restantes cores o filme de PVC ser mais transparente. Não se verificou relação entre a a_w de estabilização das amostras e os resultados obtidos.

Focando a segunda parte do estudo, relativa à aplicação dos filmes na conservação de espinafre foi demonstrado que a utilização de película aderente tem um efeito bastante significativo na conservação do produto. Comparando ambos os filmes, tanto o filme à base de TPS como o filme de PVC revelaram ter um desempenho semelhante na preservação das caraterísticas do produto, com exceção da prevenção da perda de massa. O filme à base de TPS, tendo uma permeabilidade ao vapor de água superior à do filme de PVC, permitiu uma perda de massa maior, que se refletiu num emurchecimento mais rápido das folhas.

Com base nos resultados obtidos pode ser concluído que o filme à base de TPS testado se apresenta como um forte substituto, ecológico, das películas aderentes convencionais, à base de PVC.

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ABS - Acrylonitrile butadiene styrene

CIE - Commission Internationale de l'Eclairage

EVOH - Polyvinyl Alcohol

HDPE – High density polyethylene

HP – Hydroxypropyl

IFPA – International Fresh-cut Produce Association

LDPE – Low density polyethylene

RH – Relative humidity

PA - Polyamide

PCL - Poly(ε-caprolactone)

PE – Polyethylene

PET - Polyethylene Terephtalate

PHA - poly-β-hydroxyalkanoate

PLA - Polylactic Acid

PP – Polypropylene

PS - Polystyrene

PVA - Polyvinyl Acetate

PVC – Polyvinil chlorine

TPS – Thermoplastic starch

VCM – Vinyl chlorine monomers

WVP – Water vapour permeability

1. BACKGROUNG AND MOTIVATIONS

Petrochemical based plastics have been increasingly used in packaging industry due to their large availability, low cost, functionality (Tharanathan, 2003), durability, resistance to solvents and to physical ageing (Frost, 2010). They have the characteristics required for a good packaging material such as good tensile and tear strength, good barrier properties to O_2 and aroma compounds and heat sealeability. However, they have very low water vapour permeability, are non-biodegradable (Tharanathan, 2003) and not sustainable as petroleum sources are limited (Matronha, 2015).

Conventional polymers from petroleum resources have a high resistance to degradation by micro-organisms imparting a long term global environmental concern in the way they remain in the environment for a very long time, being considered as a practically eternal waste (Frost, 2010).

Nowadays solid residues have three main problems associated:

- Increasing volume due to population and urbanization growth and increased use of disposable packaging (Frost, 2010; Instituto do PVC, 2014);
- Higher complexity, due to the use of new materials in their composition;
- Visual pollution, due to the increasing volume not being accompanied by an increased investment in landfield sites and treatment plants (Frost, 2010; Instituto do PVC, 2014).

Regarding to petrochemical plastics, one possible solution to reduce waste is recycling, which in fact has been increasing for these materials. However, recycled polymers are often contaminated, which results in inferior quality to feedstock created ones. This reduces plastics recycling desirability and, as consequence, economical viability of recycling. Other solution is incineration, but it produces harmfull gases and emissions, as furans and dioxins in the case of poly (vinyl chlorine) (PVC), and is becoming unfeasible given the current political climate on greenhouse emissions (Frost, 2010).

The limit of petroleum sources, the increase of consumers concerns on the environment and sustainability, the demand for non-toxic, economically and environmentally feasible products, as well as the waste disposable problems of synthetic polymers, lead to a demand for biodegradable materials from renewable resources (Bioplastics Council, 2012; Matronha et al, 2015).

Research has been made in order to find renewable biopolymers as polysaccharides, proteins and their composites, to substitute or complement their petrochemical-based counterparts, which are not biodegradable. In addition to being fully degradable in soil and environmentally friendly, these materials have the advantage of the possibility of being obtained from food processing industry wastes (Frost, 2010).

Thermoplastic starch biopolymers have a special growing interest within industry and society, being economically viable, biodegradable/compostable and having a wide range of native sources (Frost, 2010).

This dissertation aims contribute to the evolution of thermoplastic starch based materials market. Its specific objectives are:

- Characterization of a thermoplastic starch based wrap film, more specifically study its hygroscopic, barrier, mechanical and optical properties;
- Study the application of this film on packaging vegetables with high respiration rate, being in this case used fresh spinach;
- Evaluate in what extent the thermoplastic starch based wrap film tested could be a potential substitute for the conventional wrapping film of PVC, and in particular for packaging vegetables with high respiration rate.

2. LITERATURE REVIEW

2.1 Plastic films

2.1.1 Evolution of plastic films industry

The plastic industry in United States started with celluloid, a mix of cellulose nitrate and camphor, in the 1860s. After World War II, it began the research on the conversion of ethylene and propylene gases, which led to the discovery and transformation of hydrocarbons into plastics as polypropylene, high-density polypropylene (PP) and others. Some decades later, by the 1970s, low-density polyethylene (LDPE) was introduced and the market plastic consisted on plastic bags and thin films emerged on society (Darby, 2012).

Bioplastics production started on the 1980s and replaces traditional materials as PP, high-density polyethylene (HDPE), and acrylonitrile butadiene styrene (ABS). They can be biobased, biodegradable or both (Bioplastics Council, 2012; Darby, 2012) and, as plastics, they range from flexible to rigid and can be used for a wide variety of applications (Darby, 2012).

It is important to state that besides most biobased polymers be biodegradable, the reverse is not always true. While biobased polymers are defined as materials derived from renewable, natural resources, to be biodegradable it shall undergo deterioration and a decrease in molecular mass to form gases and other low molecular weight products, under influence of microorganisms (Flores, 2008).

Nowadays, composites are becoming more commonly used for packaging, food service and catering (Darby, 2012). These are matrix polymers that have a filler or secondary phase, such as glass particles, silica, carbon or natural fibers. When the dispersed filler is sized in the nano-domain, as silicon dioxide, these materials are called nano-composites (Frost, 2010).

The evolution of plastics is presented in Figure 1.

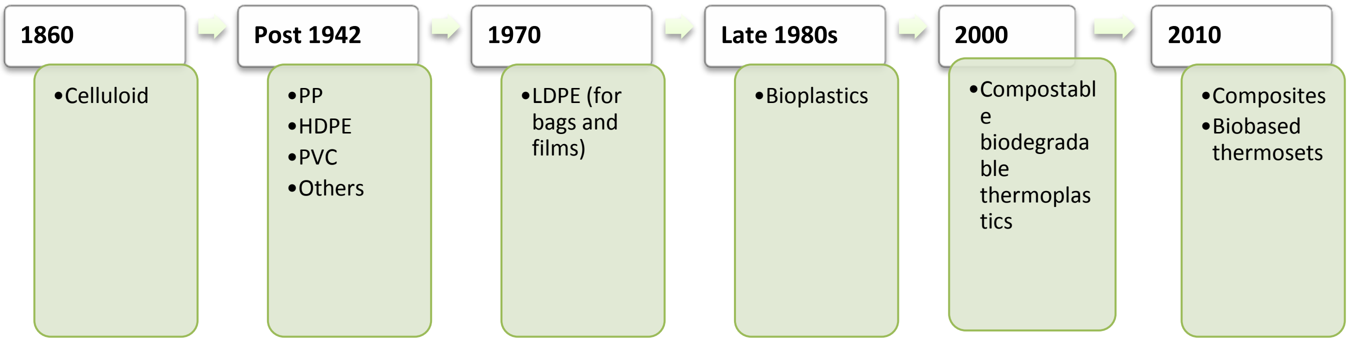

Figure 1 - Market Introdution of Plastics (Adapted from Darby, 2012)

Nowadays, plastics can be classified according to the representation on Figure 2.

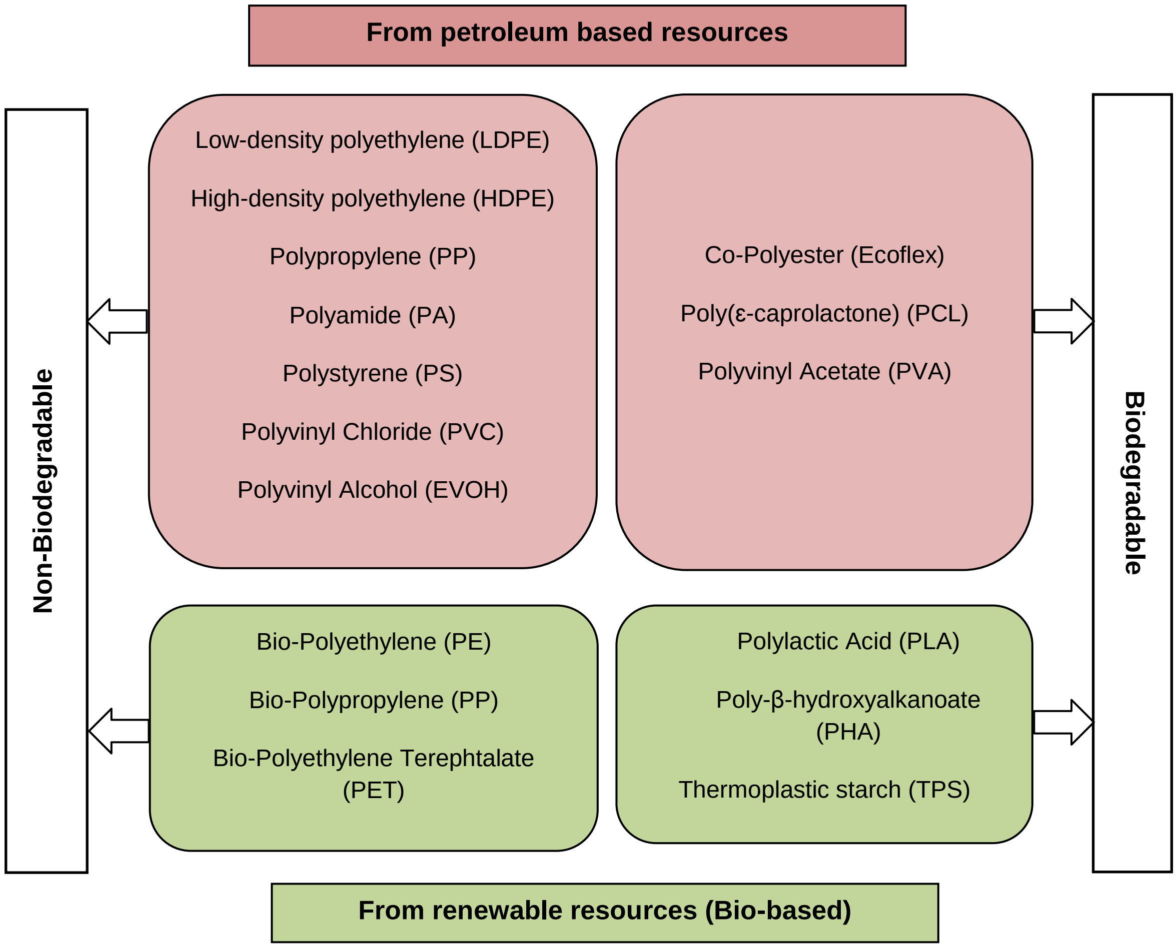

Figure 2 - Plastics classification according to based content and biodegradability (adapted from Darby, 2012)

European regulations require compostable or biodegradable to undergo 90% biodegradation in 180 days, while USA standard requires only 60% biodegradation for this period of time (Frost, 2010).

In what regards to bio-based polymers they can be divided into three main categories (Matronha, 2015):

- Directly extracted from biomass (e.g. starch, cellulose, casein)
- Synthesized from bio-derived monomers (e.g. PLA)
- Directly produced by microorganisms (PHA)

2.1.2 Bioplastics market overview

Global production of bioplastics reached 798.070 metric tons in 2010 and in 2015 it should reach 1.85 million metric tons, according to European Bioplastics Association (Bioplastics Council, 2012).

According to the same source, while in 2010 bioplastics represented less than 1% of global plastic production, in 2015 it should reach over 20%. These statistics were similar to the ones reported by Freedonia Group in its World Bioplastics Report in November 2011. In this report, Fredonia Group also noted that biodegradable plastics accounted for nearly 90% of the global bioplastics in 2010. Its market reached 932 million pounds in 2011 and is expected to reach nearly 2.6 million pounds in 2016, according to BCC Research Report (Darby, 2012).

Regarding to production capacity, the top five bioplastics in 2010 were bio-PE, biodegradable starch blends, PLA, PHA and biodegradable polyester. Bio-PE, PLA, PHA and biodegradable polyester are estimated to remain, together with bio-PET, in top five by 2015 (Bioplastics Council, 2012).

Figure 3 illustrates world bioplastics market demand by product sectors in 2010.

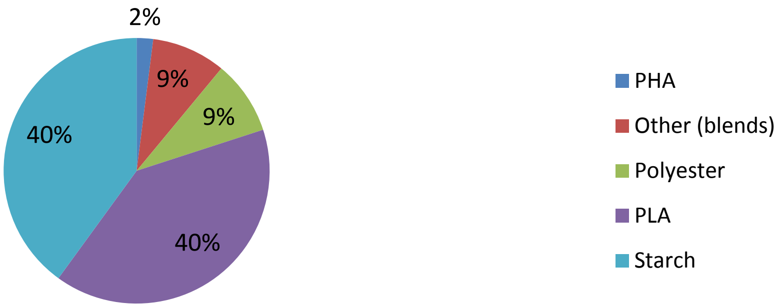

Figure 3 –World bioplastics market demand by product sector in 2010 (adapted from Darby, 2012)

Bioplastics are making inroads into a variety of markets as it can be seen in Figure 4 (Bioplastics Council, 2014). It is expected a significant increase in demand for compostable bags including in food service disposables area, which was about 22.5% in 2012. This increase is driven by lower bioplastics cost, their availability, growth in composting infrastructure, and consumer awareness (Darby, 2012).

Figure 4 – World bioplastics by market demand (adapted from Darby, 2012)

Non biodegradable biobased plastics

Since 2010, biobased polyethylene (bio-PE) has grown in production and market acceptance, being used by companies like Procter & Gamble, Tetra Pak and Shiseido (Darby, 2012).

Besides bio-PE, production of bio-based versions of other plastics, namely polypropylene (PP), polyvinyl chloride (PVC) and polyethylene terephthalate (PET) is growing. In the case of bio-PET its production has been supported by the big companies like Coca-Cola, Ford Motor Company, H.J. Heinz Company, Nike, Inc. and Procter & Gamble (Darby, 2012).

Biodegradable petroleum based polymers

BASF has been producing biodegradable aliphatic-aromatic copolyester ecoflex®, based on the monomers 1,4 butanediol, adipic acid and terephtalate, which can be blended to renewable raw materials (starch, cellulose, PLA, PHAs and Lignin) in order to produce compostable plastics. These have similar properties to LDPE, being used in trash bags or disposable packaging, and are able to be processed in the same blown film plants. An example is Ecovio®, which is a blend of this polymer with PLA (BASF, 2015).

Biodegradable biobased polymers

The large volume feedstock users of dextrose (the primary feedstock for production of bioplastics) are Ingeo polylactic acid (PLA) from NatureWorks (Cargill), Mater-Bi® starch-based resins from Novamont, and Telles' Mirel polyhydroxyalkanoate (PHA) (Darby, 2012).

Regarding Ingeo biopolymer, it composes 100% of SunChips packaging by Frito-Lay, a range of flavoured multigrain snacks, which fully be compostable in about 14 weeks in a hot, active composting pile. Ingeo was also incorporated by Target in the flexible multilayer packaging of its in-house Archer Farms brand of potato chips (Darby, 2012) (Figure 5).

Figure 5- SunChips 100% PLA packaging on the left side (Source: https://www.hornallanderson.com/casestudy/going-au-natural) and Archer Farms chips on right (Source: https://corporate.target.com/about/design-innovation/owned-brands/)

The current bioplastics market is growing, in the number of materials and products, manufacters, compounders, converters and end-users (Bioplastics Council, 2012; Darby, 2012). According to Bioplastics Council report (2012) the major players of bioplastics industry in North America were BASF, DuPont, Ecospan, Jamplast, Metabolix, Nature Works LLC, Nypro, PolyOne, Teknor Apex and UL.

The food service and catering sector are important key drivers for the development of bioplastics industry for the next years and the number of big companies adopting bioplastics is increasing. A development on production and properties drives to improvements on performance and lower costs (Darby, 2012). Regarding to EU, legal framework conditions and strategies also provides incentives to their use (Bioplastics Council, 2012).

2.2 Wrap film

Wrap film is also known as cling film, food wrap or saran wrap and consists in a thin transparent plastic film that adheres to surfaces and to itself. It is used in food packaging to keep product quality for a longer period of time (Malhotra et al, 2015).

For produce packaging specifically, wrap film should have the following characteristics (Tharanathan, 2003):

- To allow O_2 permeation and respiration of the produce, but in a slow and controlled mode
- To be a selective barrier to CO_2 and water vapour
- To maintain an internal gas composition, creating a modified atmosphere that regulates produce metabolism
- To present low lipid migration (in the case of high fat products)
- To maintain structural integrity by delaying chlorophyll loss
- To be a possible vehicle to incorporate food additives
- To prevent microorganisms development

The first wrap film for households was designed in 1953, and its commercial use started in 1949. It was made from polyvinylidene, which was accidentally discovered in 1933. It consists of long chains of polymerized vinylidene chlorine with monomers, unsaturated carboxyl groups and acrylic esters. Film results from copolymerization of molecules bound tightly together so that they have a very good barrier against gas, moisture and chemicals (Malhotra et al, 2015).

Nowadays wrap film is found to be safer because of phthalates (toxic chemical) are no longer used in its manufacture, so there is no risk of its migration into food and hence contamination (Malhotra et al, 2015).

The various synthetic polymers used in wrap films production include those presented in Table 1.

Table 1 - Synthetic polymer based wrap films (adapted from Malhotra et al, 2015)

Polymer	Properties
Low Density Polyethylene (LDPE)	Easy processing, strength and flexibility, toughness, easily sealable, moisture barrier
Polyethylene Terephthalate (PE)	Toughness and strength, effective gas and moisture barrier
High Density Polyethylene (HDPE)	Stiffness and toughness, moisture and gas permeability resistance
Polyvinylchloride (PVC)	Provides excellent oxygen gas and moisture blocking properties that are minimally affected by humidity

2.2.1 PVC

PVC was first commercially developed in the early 1930’s, being nowadays one of the world´s leading synthetic polymers (Leadbitter, 2003).

About 80% of this PVC polymer in the world results from polymerization of vinyl chlorine monomers (VCM), which are a result of a reaction between ethylene (C_2H_4) and chlorine atoms in a percentage of 57% and 43%, respectively (Instituto do PVC, 2014). These monomers are polymerised in water, in the presence of catalystics (Linpack, 2015) and the liquefied gas is solidified in PVC resin, a white powder, which is dried, sieved and packed (Leadbitter, 2003). This resin in then used in an extrusion process, coupled with additives, to produce PVC films (Leadbitter, 2003; Linpack, 2015). The additives consist on stabilisers, impact modifiers, processing aids, plasticizers, lubricants and pigments (Leadbitter, 2003).

Figure 6 represents both molecular form and chemical composition of PVC and other conventional plastics.

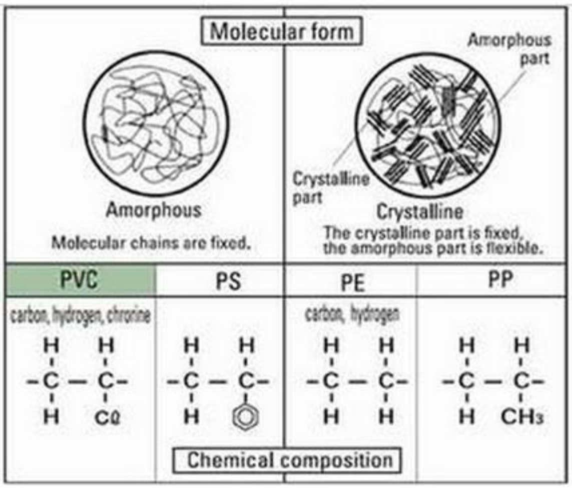

Figure 6 - Chemical composition and molecular structure of some conventional plastics (Source: http://www.pvc.org/en/p/pvcs-physical-properties)

Chlorine, being a halogen, provides a good chemic stability to PVC polymer (Instituto do PVC, 2014), what means an excellent combustion and chemical resistance to oxidation, acids, bases, oils and other hydrocarbons (Kent, 2012). This contributes to the high durability of this material (Instituto do PVC, 2014).

PVC has also a good mechanic resistance, having lower deformation than plastics as PE and PP (Instituto do PVC, 2014) and it is the most versatile of the thermoplastic plastics. It can be easily formulated by adding plasticizers and other additives in order to meet specific performance requirements (Kent, 2012), and easily processed by all commercially important processing techniques (Linpack, 2015).

2.2.2 Starch

Starch molecule

Starch is the major polysaccharide in plants functioning as an energy store (Singh et al, 2003; Bicudo, 2008). Together with cellulose, is one of the two most abundant polymers found in nature (Flores, 2008). It is renewable, relatively cheap and can be converted in valuable compounds for industries, as the case of thermoplastic starch. At commercial levels, it is extracted from cereals, roots and tubers (Bicudo, 2008).

In nature starch is present in the form of semi-crystalized granules, composed by two polymers, amylose and amylopectin, in helical coils (Dziedzic and Kearsley, 2012). While amylose represents the amorphous regions, the amylopectin content is the one responsible for crystallinity (Singh et al, 2003).

Amylose consists in a linear chain of D-glucose units linked by α- (1→ 4) (Dziedzic and Kearsley, 2012), with a polimerization degree of 200 to 3000, depending on the source of the starch (Bicudo, 2008). It represents about 20% of starch structure, being the water-soluble fraction, what endow flexibility and digestibility to the structure (Andrade, 2011).

On the other hand, amylopectin consists in a branched structure, composed also by D-glicose units, connected by α- (1→ 4) links, and α- (1→ 6) on the branches (Bicudo, 2008). This fraction represents about 80% of starch structure and is insoluble in water (Andrade, 2011).

The physicochemical and functional properties of starch depend on the percentages of amylose and amylopectin (Mali et al, 2010). These vary according to the source (Dziedzic and Kearsley, 2012), and ripening degree (Bicudo, 2008).

Being the primary structure of starch the continuous chains of amylose and amylopectin, the secondary structure, and the basis of crystallinity, is the double helices formed by adjacent amylopectin branches. Those are then associated into a tertiary structure of a superhelix (Shanks and Kong, 2012).

Starch's main property is gelatinization (Bicudo, 2008). Its granules, being insoluble in cold water due to hydrogen bridges between chains, when increasing temperature those bridges are broken being possible to absorb water that becomes linked by hydrogen bonding to the exposed hydroxyl groups of amylose and amylopectin (Singh, 2003; Frost, 2010). This induces swelling of the granules and a progressive loss of birefringence. Continuous swelling induces the exit of amylose molecules of the granules and therefore a rising of suspension viscosity. This viscosity starts then to weaken when granules lose their integrity (Bicudo, 2008).

After gelatinization, starch molecules can associate again through hydrogen bridges, resulting in an ordered structure that, when favourable conditions are present can form again a crystalline structure. This phenomenon is called starch retrogradation and it is responsible for film's structure loss, that is when they become firm and brittle (Mali et al, 2010; Frost, 2010). It is a re-crystalization process which extent depends on botanical origin, granule lipid content, hydration level and amylose:amylopectin ratio (Frost, 2010). High lipid content, as in rice and maize starches, contributes to their higher transition temperatures and lower retrogradation (Singh et al, 2003).

The main interest of using starch on film processing result from being an abundant and available resource all around the world, having several possibilities of chemical, physical and genetic modification, and enabling the production of resistant films which are biodegradable (Mali et al, 2010). This biodegradability comes from the enzymatic rupture of the glycosidic links of the starch structure, and utilization of the resultant monomer or smaller polymers by microorganisms (Andrade, 2011).

Thermoplastic starch (TPS) based films

Thermoplastics are polymers that can flow when heated above a melting or vitrification temperature. Due to their large molar mass they undergo plastic deformation, with entanglements and other interactions between polymer chains (Shanks and Kong, 2012).

Starch has a complex structure that is able to undergo plastic deformation. Unlike polymers as cellulose and various gums, it is applicable to thermoplastic processes as its microstructure contributes to multiphase transitions during thermal processing, when multiple chemical and physical reactions take place (Shanks and Kong, 2012).

TPS films need to have up to 70% starch content to be biodegradable or compostable (Frost, 2010). Its formation requires water and temperature (a concentration of 25% w/w of water gives a range of 60-70°C gelatinization temperature) and involves disruption of starch granules, dissociation of complexes with lipids and melting of crystals, meaning loss of crystallinity (Roz, 2013).

Amylose is more water-soluble than amylopectin because of its linear structure and lower molar mass (>10^6 g/mol against >10^7 g/mol of amylopectin) (Shanks and Kong, 2012). In solution, amylose molecules tend to become parallel orientated and, as consequence, become closer inducing the formation of hydrogen bonds between OH groups of adjacent polymer chains. This approximation between molecules results on reduced chemical affinity with water leading to an increase in viscosity until gelatinization (Mali et al, 2010).

Gelatinization consists in the formation of a strengthening amorphous gel structure by a continuous shear process (an extruder is used at commercial levels). After the extrusion process TPS is dried, wound into a coil for storage, transport or prior to further shaping by thermoforming. As starch is very susceptible to water biodegradation, starch based polymers has been processed mostly to packaging as films or sheets, thermoformed in order to assume several shapes, and is preferably used for packaging of dry products (Shanks and Kong, 2012).

An undesirable characteristic associated with TPS films is retrogradation, which is responsible by less flexibility, brittleness over time, and reduced shelf life and application potential. In order to prevent this and improve strength and stability hydrophilic polymers are added (Frost, 2010).

Factors influencing starch films properties

- **Amylose content**

Linear amylose chains are most mobile to recrystallize, and hence are associated with a more severe retrogradation than amylopectin (Frost, 2010; Rindlav-Westling et al 1998, Shanks and Kong, 2012). However, as starch based films production is based on the linearity on amylose molecules (Mali et al, 2010) starches with higher amylose content are more appropriated (Mali et al, 2012; Frost, 2010; Rindlav-Westling et al 1998; Shanks and Kong, 2012). They have better functional properties, regarding film strength and both gas and water barrier properties (Frost, 2010; Rindlav-Westling et al 1998).

Table 2 shows the amylose and amylopectin content of starch from different sources.

Table 2 - Typical amylose and amylopectin content of different starches

Starch	Amylose (%)	Amylopectin (%)
Corn	22.4 - 32.5[a] 29.4[b] 25[c]	67.5 - 73.6 70.6 75
Potato	20.1 – 31[a]	69 - 79.9
Wheat	18 – 30[a]	70 - 82
Rice	5 - 28.4[a]	71.6 - 95
Barley	27.5[b]	72.5
Oat	27.5 – 29.8[d]	70.2 - 72.5

[a]Singh et al, 2003, [b] Tester and Morrison, 1990, [c]Chinnaswamy and Hanna, 1998, [d]Tester and Karkalas, 1996

- **Drying temperature**

Drying starch films has a great impact on their mechanical and barrier properties. Higher drying temperatures (superior to 60°C) and lower relative humidities, results in more stable and resistant films (Mali et al, 2010) as it is expected a better molecular arrangement in the polymer structure (Berluzzi et al, 2012).

- **Time and atmospheric conditions**

Starch films are greatly affected by aging (Acosta, 2013). Time, temperature and humidity affect TPS structure, what is the major problem in what regards to use it in commercial applications. Being hydrophilic, it can absorb water from the atmosphere or high aw products, becoming softer. In the case of dry products the water is absorbed into them and the film turns dry and brittle (Shanks and Kong, 2012).

In what regards mechanical properties, in general they decrease upon exposure to water. Also retrogradation causes embrittlement and loss of optical clarity (Shanks and Kong, 2012).

Plasticisers

Plasticisers are substances with high melting point and low volatility (Matta, 2009). When added to film formulations they interact with the film molecules by hydrogen bridges, modifying their properties (Bertuzzi et al, 2012; Matta, 2009). The characteristics observed by adding a plasticiser in a film formulation depend on the plasticiser used as well as on its concentration (Mali et al, 2010).

Besides water, which is the main and best plasticiser, required for gelation (Bertuzzi, 2012; Frost, 2010), other plasticizers have been used, which have higher affinity for starch that does to water (Shanks and Kong, 2012). In regards to starch based films, the most common plasticisers used are polyols, as glycerol and sorbitol (Frost, 2010; Mali et al, 2010).

The addition of a plasticizer to films results in a lower glass transition temperature and higher flexibility (Bertuzzi et al, 2012; Mali et al, 2010; Matta, 2009), by producing a less ordered structure which becomes more able to move (Garcia et al, 2006). Regarding to starch they also prevent retrogradation by the complex they form with starch molecules by hydrogen bonding (Cirillo, 2015; Mali et al, 2010; Matta, 2009; Shanks and Kong, 2012).

Cirillo et al (2015) studied the effect of plasticisers in film formulations and, in respect to corn starch films, both glycerol and sorbitol addition improved starch barrier properties to CO_2, O_2 and water vapour. Other authors, namely Matta (2009) and Mali et al (2010), also state that plasticisers improve the water vapour barrier properties, characteristics of thermoplastic starch.

The addition of fatty acids is also required due to more severe retrogradation in high amylose starches (the ones used in TPS processing) in order to decrease opacity and strain, decrease tensile strength (Acosta et al, 2013; Shanks and Kong, 2012), decrease water vapour permeability (Acosta et al, 2013; Mali et al, 2010; Shanks and Kong, 2012), and decrease oxygen permeability (Gontard et al, 1996).

Blends, composites and chemically modified starch

These are the most common categories of thermoplastic starch materials.

A blend is a mixture of polymers of different molecular or monomer constitution. Starch blending with synthetic polymers reduces TPS hydrophilicity, improving its mechanical resistance (Corradini et al, 2015; Mano et al, 2003), processability and stability (Mano et al, 2003). Starch blends also show retarded retrogradation and slower degradation (Shanks and Kong, 2012). Common inclusions are PLA, PVOH, PE, and others (Frost, 2010).

Blending starch with synthetic polymers also enhances their biodegradability (Frost, 2010), but in the other hand, the polymer obtained is not completely biodegradable (Andrade, 2011). Though, starch blending with materials as natural fibers enhances TPS properties and don´t compromise the biodegradability of the final product (Corradini et al, 2015).

Composites, as referred before, are matrix polymers containing dispersed fillers. They have revealed enhanced barrier and mechanical properties (Acosta, 2013), optical clarity and restrained retrogradation (Frost, 2010; Shanks and Kong, 2012) and degradation (Shanks and Kong, 2012). However, they tend to be more brittle, which is a disadvantage. To arrive at optimum composition there are used combinations of composites with plasticisers. The most common mineral fillers include talc, clays, silica and cellulose fibers (Shanks and Kong, 2012).

Chemical modification is also used to improve starch properties (Frost, 2010; Mano et al, 2003; Shanks and Kong, 2012) and it includes grafting of molecules onto starch backbones and chemical cross-linking. The most important reactive process in starch film processing is hydropropylation. Hydroxypropyl (HP) starches have enhanced mechanical properties, low gelatinisation temperatures, low retrogradation and crystallinity. Regarding to cross-linking it improves mechanical properties, thermal stability and water resistance, by decreasing molecular chain mobility (Frost, 2010).

Oxidation is also a common process to chemically modify starch. It has been conducted using hypochlorite and influences the viscosity of the starch solution, increasing its solubility and decreasing gelatinization temperature. Oxidized TPS also showed higher modulus and lower elongation on break, though there is an increase in retrogradation (Shanks and Kong, 2012).

Stabilizers

Stabilizers, as calcium carbonate, are non-toxic additives that aim to decrease TPS susceptibility to moisture as well as fungus and bacteria action, increasing its stability to degradation (Shanks and Kong, 2012).

TPS *versus* conventional plastics

Using thermoplastic starch instead of conventional plastics has been a big focus in multiple utilizations, including food packaging. It production and commercialization is already conducted by some companies as Novamont (Italy) (Roz, 2003).

Novamont has developed, over twenty-five years of research, a family of biodegradable and compostable bioplastics called Mater-Bi®, which won the award of European Inventor of the year 2007 (Novamont, 2016). Mater-Bi® is obtained from renewable raw materials (starches, cellulose, vegetable oils and their combinations) under pioneering technologies, being produced in a granulated form (figure 7). It is then melt by manufacturing companies in order to be processed and used in a wide range of applications, being shopping bags one of the most common (Novamont, 2016). The basic matrix of Mater-Bi® can be blended with other polymers to achieve specific characteristics, and/or it can also be added plasticizers, stabilizers and other additives.

Beyond allowing optimal organic waste management and contribute to minimizing the environmental impact, Mater-Bi® has also the advantage of being suitable for processing by the most common technologies used for conventional plastics (Novamont, 2016).

Figure 7 – Mater-Bi® (Source: http://www.thisisitaly-panorama.com/business-news/novamont/)

Other available starch based polymers are Bio-P-TM®, produced by Bioenvelope (Japan) and BIOPAR®, made for potato starch and commercialized by Biop Biopolymer Technologies AG, Germany (Jiménez, 2012).

Besides the economic and environmental advantages of products made from thermoplastic starch, and production can be carried on using the same equipments used in conventional plastic processing, there are some limiting factors to their utilization at an industrial level (Roz, 2003), highlighting its high water sensibility and it weak mechanical resistance (Mano et al, 2003; Roz, 2003; Shanks and Kong, 2012). TPS has a typically high modulus, low and moisture dependant elongation at break, and brittle fracture compared to synthetic polymers (Shanks and Kong, 2012). This makes thermoplastic starch indicated for material with a short usage time and that doesn´t need a high mechanical resistance (Corradini et al, 2005).

Molecules transmission through TPS (oxygen, moisture or other volatiles) tend to be low compared with other polymers, what is related to density of hydrogen bonds and polarity that restrains diffusion of small molecules. This property is dependent on moisture content and any existing crystals. In what regards to moisture sorption, this is a problem on TPS, because of its hydrophilic character (Shanks and Kong, 2012).

2.3 Spinach

2.3.1 Botanic aspects

Spinach (*Spinacea oleracea L.*) is an annual culture, usually dioecious, grown in temperate regions (Rubatsky and Yamaguchy, 1999).

The harvest is made when spinach plant achieves marketable size, what can vary from 30 to 150 days (Rubatsky and Yamaguchy, 1999). For fresh market, plants are usually hand-cut, tied together in bunches and packaged, while processing crops are usually mechanical harvested. The plants used for processing are usually smooth or semisavoyed as they grow faster, yield more and are more easily washed. On the other hand, savoy plants have advantages for fresh market as they leaves resist compression during packing and allow better aeration, cooling and postharvest life (Rubatsky and Yamaguchy, 1999).

2.3.2 Nutritional composition

In spinach the part of the plant that is consumed are the leafs. It is, in a nutritional point of view, very interesting because of the vitamin content, especially ascorbic acid, riboflavin and carotene (vitamin A precursor). It is also a good source or folic acid and iron (Almeida, 2006).

Although its benefits, spinach has also some anti nutritional aspects, as the formation and accumulation of nitrates, which in excess is associated with the disease methemoglobinemia, and oxalic acid. The later combines with calcium to form insoluble calcium oxalate, reducing its availability as well as reducing dietary magnesium and iron availability. Nitrate and oxalic acid content varies among cultivar types (Rubatsky and Yamaguchy, 1999).

2.3.4 Spinach preservation

Spinach is traditionally consumed cooked, although it can be consumed raw, in salads or shakes. It can be found fresh, with no further process, minimally processed and also processed by canning and freezing.

Fruits and vegetables are highly perishable due to the presence of high amounts of moisture (about 95%), which is related to favouring the growth of micro-organisms. Bacteria and fungi, together with mechanical injuries during harvesting and processing, are the main causes of this products spoilage by inducing activities, biochemical reactions and/or pathogenic infection (Haq and Prasad, 2015)

Other issue involved in storage of fruits and vegetables is respiration rate, which consists in the oxidative breakdown of sugars and organic acids to CO_2 and H_2O, in order to produce energy for metabolic processes (Almeida, 2005). The overall reaction is (Lokke, 2012):

$$C_6H_{12}O_6 + 6CO_2 \longrightarrow 6CO_2 + 6H_2O + \text{energy} \qquad \textbf{(1)}$$

Respiration rate determines quality parameters of the produce, as firmness, sugar content, aroma and flavour. In general, commodities with higher respiration rate have shorter storage life, associated with the faster depletion of its respiration supplies (Almeida, 2005; Lokke, 2012).

Spinach is included in the range of vegetables with an extremely high respiration rate, with more than 33 ml CO_2 Kg^{-1} h^{-1} being highly susceptible to deterioration (Almeida, 2005).

Its potential storage time, at normal atmosphere conditions and optimal temperature and relative humidity (0°C, 90-100% HR), is less than 2 weeks. The main causes of quantitative and qualitative losses are yellowing, physical injury, high respiration rate, rot and water loss (Almeida, 2005; Pinto and Morais, 2015).

Yellowing is related with chlorophyll loss during senescence. Chlorophyll, the pigment responsible for the green colour of plants, when lost uncovers the underlying carotenoid pigments, which are responsible for the yellow/orange tonalities (Lokke, 2012).

Water loss results in withering, softening of the tissues, weight loss, and losses in nutritional value (Porte and Maia, 2001; Sandhya, 2010). The water rateloss depends on the product itself and environmental factors. Spinach, by having a high transpiration coefficient and a big surface/volume ratio (500-1000 $cm^2.cm^{-3}$), has a high water loss. Keeping low and stable temperatures (0-5°C in respect to fresh cut produce), high relative humidity conditions (by using water vapour barrier packaging), and low air velocity contributes to water loss decrease (Almeida, 2005).

Produce differs in the quantity of water that can be lost till there is a visual evidence of quality depreciation. Spinach has a very low allowable water loss. It can be visually noticed a whittered appearance if they lose more than 3% of it fresh weight (Almeida, 2005).

2.3.4.1 Minimally processed spinach

According to International Fresh-Cut Produce Association (IFPA) these products are defined as any fruit or vegetable or combination of both whose original form was physically modified, keeping however its freshness. These products, since their emergence in EUA around 1985, represent a rising market, supported by their convenience and consumer awareness about healthy choices (Gil, 2008). Minimally processed ready-to use fresh produce has higher respiration rate than intact produce (3-7 times higher) being more perishable (Porte and Maia, 2001). It is crucial to start with a high quality raw material and ensure that processing and storage requirements are strictly applied (Tudela et al. 2012).

Deterioration of fresh spinach can be reduced mainly by the following post-harvest factors:

- <u>Temperature</u>

Saenmuag et al (2010) states that shelf life of spinach is estimated to be less than 8, 6 and 4 days at 4°C, 10°C, and 20°C respectively, while at optimal conditions (0°C and 95-100% HR) it is 10 to 14 days. Low temperature conditions also reduce some enzymes activity, as tyrosinase and diphenol oxydase, contributing to preserve vegetables colour (Porte and Maia, 2001).
Mattos (2012) states that the maximal respiration rate for most fruit and vegetables undergoes a 4-6 fold increase from 0 to 15°C.

- <u>Atmospheric composition</u>

Studies have shown that low O_2 concentrations (0.8-3%) have benefits in modifying produce metabolism, allowing to reduce respiration rate, weight and antioxidant loss, as well as microbial contamination, particularly Pseudomonas (main spoilage agent) (Tudela et al, 2012, Ko et al, 1996, Frija, 2012). However O_2 concentrations below 0.8% (0.4% according to Saenmuang et al, 2011) lead to anoxia conditions (Tudela et al, 2012) and cells start anaerobic respiration, which has as consequences off flavours, structure loss and tissues darkening (Porte and Maia, 2001). Low O_2 concentrations are also responsible for the decrease of some specific enzymes activity as polyphenol oxydase, which are related to enzymatic browning (Porte and Maia, 2001).

The results achieved by Saenmuang et al (2011) shown that both the O_2 concentration and the initial amount of O_2 per unit mass of spinach have a strong influence on respiration rate. The depletion of O_2 with time, starting from different initial O_2 concentrations, caused a decrease in the respiration rate (O_2 consumption rates ranged from 70.4 to 32 mg $Kg^{-1}h^{-1}$ while the CO_2 production rates ranged from 154 to 79.2 mg $Kg^{-1}h^{-1}$). This decrease was specifically rapid in the case of lower void volume (O_2 consumption rates ranged from 81.6 to 1.6 mg kg^{-1} h^{-1} and CO_2 production rates ranged from 189 to 22 mg kg^{-1} h^{-1}).

High CO_2 concentrations also have benefits by decreasing respiration rate, pH of growth media and off-odours (Porte and Maia, 2001; Tudela et al, 2012).

- Physical stress – Physical stress increases respiration rate, and water loss and hence product degradation (Almeida, 2005).

Mattos (2012) states that it is important to ascertain the minimum O_2 required for aerobic respiration, as well as the potential hazards for each product, the permeability of the packaging films and the rate of respiration of the product, not forgetting that both are temperature dependent. The packaging film used shall control the gas transference and reduce water loss (Frija, 2012).

3. MATERIALS AND METHODS

3.1 Film characterization

3.1.1 Films stabilization

This part of the study aims to evaluate the TPS based wrap film properties when submitted to different conditions of relative humidity. This will simulate its behaviour when packaging products with different values of water activity (a_w) and when stored at different relative humidity conditions.

All tests were conducted using simultaneously the PVC wrap film in order to compare the performance of the TPS based film with the conventional wrap film used commercially.

Both TPS based (produced by extrusion from Mater-Bi) and PVC films were provided by Silvex – Transformadora de Plásticos e Papéis Lda (Benavente, Portugal).

The different relative humidity conditions tested were implemented by using desiccators with different saturated salt solutions (table 3). The films, when stored in these environments will modify their water content, till de equilibrium is reached where their weight will remain constant. This stabilization period was about three weeks.

As the water activity (aw) of the salt solution is influenced by temperature, the room temperature where the desiccators were kept was monitored over time.

Table 3 – Relative humidity conditions and salts used in each desiccator

Desiccator	HR (%)	aw	Saturated solution	
1	33.2	0.332	Magnesium chloride 6-hydrate	$MgCl_2.6H_2O$
2	53.4	0.534	Magnesium nitrate 6-hydrate	$Mg(NO_3)_2.6H_2O$
3	75.3	0.753	Sodium chloride	NaCl
4	90.1	0.901	Barium Chloride 2-hydrate	$BaCl_2.2H_2O$
5	97.3	0.973	Potassium sulphate	K_2SO_4

3.1.2 Thickness

The thickness of the films was measured by using a digital micrometer (Mitutoyo, Japan).

3.1.3. Hygroscopic properties

3.1.3.1 Water adsorption isotherms

Water vapour adsorption study was determined by a gravimetric method. Three samples were tested for each relative humidity, and they consisted in squares of 7 x 7 cm. As the wrap film is a difficult material to work with, the samples were attached on a acetate support, in order to keep their structure and ensure uniform drying and adsorption processes.

The samples were first dried for 24 hours in a vacuum chamber containing with silica gel, at 50ºC, and then weighed in order to determine their dried weight (m_1).

Those samples were then stored for three weeks in the desiccators prepared as referred previously, at room temperature (25ºC), being placed three replicates of each film (TPS and PVC) in each desiccator.

After that time the samples were weight again (m_2), as well as the plastic supports (m_3). The following equation gives the water absorbed per dry mass of each sample (X, %).

$$X = \frac{m_2 - m_1}{m_1 - m_3} \times 100 \qquad \textbf{(2)}$$

Adsorption isotherms are obtained by the plotting those values against the value of aw at which the samples were stabilized.

The samples were weighted using an analytic scale (Kern ABJ-NM/ABS-N).

3.1.3.2 Water absorption capacity

This test consisted in dipping circular film samples (7cm of diameter) in 50 ml of distilled water, during 12 hours liquid water at room temperature (25°C). Ten replicates of each film (TPS and PVC) were used. As in the water vapour adsorption evaluation procedure, the film samples were also supported by a plastic support.

After the 12 hours, the mass of the wet samples was measured (m_1), after which they were dried for 24 hours in a vacuum chamber, containing silica gel, at 50ºC. After this period the samples weighted again (m_2).

The water absorption capacity (%) corresponds to the maximum weight of water retained by the samples, in relation to it dried weight:

$$\frac{m_1-m_2}{m_2}\times 100 \tag{3}$$

The samples were weighted using an analytic scale (Kern ABJ-NM/ABS-N).

3.1.2 Barrier properties

3.1.2.1 Carbon dioxide and oxygen permeability

This study was carried out using an experimental set-up for gas permeability evaluation composed of two identical cylindrical compartments. Circular samples of 5 cm diameter were used, which were first stored for three weeks in the desiccators with different relative humilities, as described in section **3.1.1**, triplicates of each film (TPS and PVC) in each relative humidity, were evaluated.

Each sample, after the stabilization period, was placed between the two compartments of the gas permeability set-up. In the beginning of the test it was made a flushing of both compartments. In the feed compartment the flushing was made with the test gas (CO_2 or O_2) in order to achieve an ideal percentage of 100%. In the other compartment (permeate) the flushing was made with nitrogen (N_2), in order to ensure that it was eliminated every trace of test gas.

The experiments were conducted at atmospheric pressure and with the gas permeability set-up kept in a water bath at 25°C, in order to ensure a constant temperature (Figure 9).

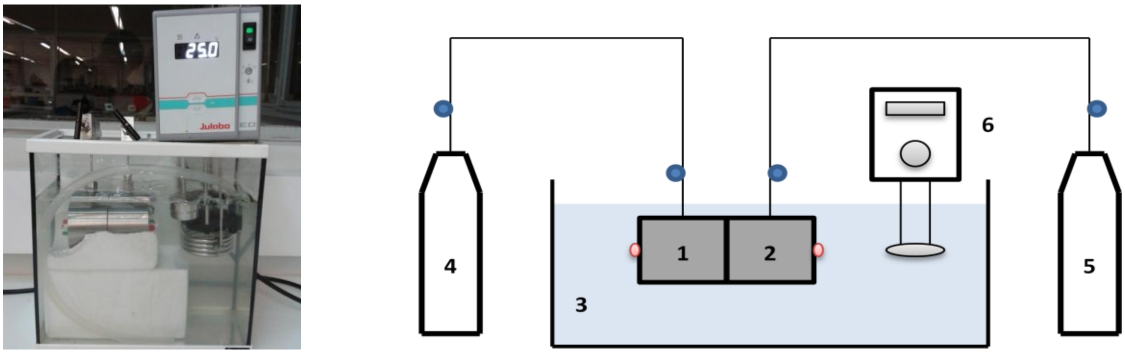

Figure 8 - Gas permeability set-up (1 - Permeate compartment; 2 - Feed compartment; 3 - Water bath; 4 - Nitrogen; 5 – Test gas; 6 – Resistance; ● - Valve; ● - Septa)

After a certain period of time, the gas composition (y_{gas}) of the permeate compartment was measured, using a gas sensor (Checkmate 9900, PBI Dansensor, Denmark). Preliminary tests were performed to evaluate the time needed to the test gas be detected in the permeate compartment. It was then fixed a time of 30 min to oxygen and 15 min to carbon dioxide.

The permeability of the film (P) (mol.m/m^2.s.Pa) is obtained from the test gas content in the permeate using the following equation (Zeman and Kubik, 2012):

$$P = \frac{p_{gas} \times V \times e}{R \times T \times t \times \Delta p, gas \times A} \quad \textbf{(4)}$$

Where p_{gas} is the pressure of test gas in permeate in the end of the test ($p_{gas} = y_{gas} \times p_{atmospheric}$) (Pa); V the volume of the permeate compartment (m^3); e the thickness of the film (m); R the gas constant (8,314 JK^{-1}mol^{-1}); T temperature (K); t time (s); and Δp is the increasing of the pressure of the gas transferred along the test.

$\Delta p, gas$ is calculated by:

$$\Delta p, gas = \frac{\Delta pi - \Delta pf}{\ln(\Delta pi / \Delta pf)} \quad \textbf{(5)}$$

being Δpi and Δpf the pressure difference of the test gas between both sides of the test film, in the beginning and in the end of the test, respectively.

In order to obtain the volume of the cell (V) it was used a standard polydimethyl silicone membrane (ref: SSP-M823), from Specialty Silicone Products, Inc., with a known permeability value (P_{O_2}=1.67x10^{-14} mol m/m^2 s Pa). The test was performed as for the test films, and the volume value was calculated using equation 4.

3.1.2.3 Water vapour

The water vapour barrier properties were studied using a gravimetric method in which a water vapour pressure difference is imposed between the two sides of a film sample, in order to induce water vapour transfer.

The samples consisted in film circles with 7 cm of diameter. They were first conditioned for three weeks at different relative humidity values as described in section **3.1.1**. Three replicates were tested for each film (TPS and PVC) at each relative humidity value.

The film samples were sealed with sealing wax at the top of Petri dishes with a diameter of 5 cm, containing 9 mL of a saturated solution with a aw value equal to the one at which the samples were stabilized. The petri dishes were placed in a desiccator containing a salt solution with a lower aw value, as indicated in Table 4, in order to have a similar water activity difference (∆aw) in all experiments.

Table 4 – Saturated salt solutions used inside and outside the petri dish in the water vapour permeability test

Inside the Petri dish		Outside the Petri dish		Δaw
Salt solution	aw	Salt solution	aw	
$Mg(NO_3)_2.6H_2O$	0.534	CH_3CO_2K	0.225	0.304
NaCl	0.753	K_2CO_3	0.432	0.321
$BaCl_2.2H_2O$	0.901	$Mg(NO_3)_2$	0.529	0.372
K_2SO_4	0.973	$NaNO_3$	0.743	0.230

The desiccator was equipped with a fan, in order to minimize mass transfer resistance outside the film, and with a thermo-hygrometer to measure the temperature and relative humidity conditions inside the desiccator (figure 10).

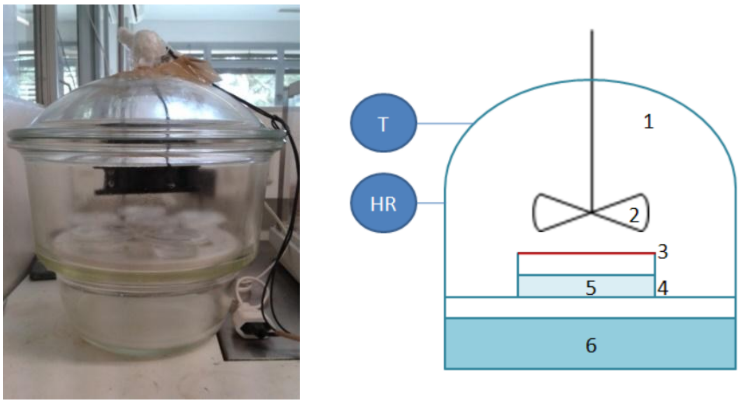

Figure 9 – Experimental set-up for water vapour permeability measurement. Legend: 1-Desiccator, 2-Fan, 3-Film sample, 4-Petri dish, 5-Saturated solution with the same aw at which film sample was stabilized, 6-Salt solution with lower aw

The Petri dishes were weighted every two hours in order to measure the water flux through the film samples using an analytic scale (Kern ABJ-NM/ABS-N). Water vapour permeability is calculated by the following equation (Alves et al, 2010):

$$WVP = \frac{N_W \times \delta}{\Delta P} \qquad \textbf{(6)}$$

where WVP is the water vapour permeability (mol.m/m^{2}sPa); N_w the water vapour molar flux (mol/m^2.s); δ the film thickness (m); and ΔP the water vapour difference between both sides of the film (Pa). ΔP is calculated by:

$$\Delta P = P_2 - P_3 \qquad \textbf{(7)}$$

Where P_2 is the water vapour pressure at the internal film surface and P_3 is the water vapour pressure outside the Petri dish. For P_2 determination, the following equation will be used, taking into account that in steady state conditions the water flux measured is equal to the water flux through the stagnant air layer inside the petri dish (Alves et al, 2010).

$$N_w = \frac{P}{RTZ} D_{w-air} \ln\left(\frac{P-P2}{P-P1}\right) \tag{8}$$

where P the atmospheric pressure; R the gas constant; T the temperature; Z the distance between the film and the salt solution; D_{w-air} the diffusion coefficient of water vapour in air; and P_1 the partial pressure of water vapor in contact with the surface of the liquid (Pa). P_1 is obtained by:

$$P_1 = a_{w1} \times P_w^* \tag{9}$$

where a_{w1} the saturated salt solution water activity and P_w^* the pure water vapour pressure (Pa)

The partial pressure of the water outside the Petri dish (P_3) was calculated using the relative humidity values measured, as following:

$$P3 = a_{w3} P_w^* = \%RH \times \frac{P_w^*}{100} \tag{10}$$

The water vapour permeability was also studied using the same water activity outside the Petri dish (aw =0.6, imposed by a saturated solution of sodium nitrate) for all film samples, with the different aw values inside the Petri dish indicated in Table 4.

3.1.4 Mechanical properties

Both mechanical tests, extension and puncture, were carried out using a texturometer TA-XT2 of Table Micro System, UK.

3.1.4.1 Extension tests

The samples used for this test corresponded to rectangles of 5x2 cm, stabilized for three weeks at the different relative humidity conditions described in section **3.1.1.**

The film samples edges were attached to tensile grips, with an initial effective sample length of 1cm, and stretched at a crosshead speed of 0.5 mm/s until rupture (Figure 11). Ten replicates were evaluated for each film sample.

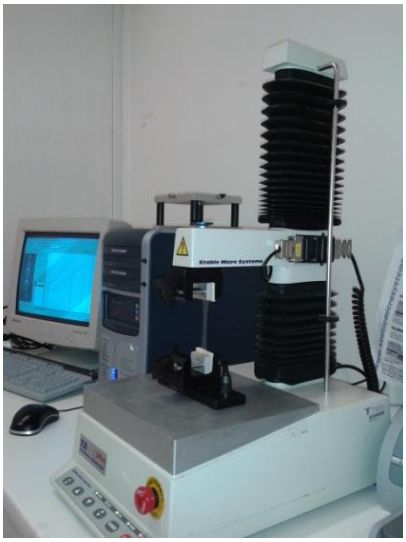

Figure 10 - Extension test

The results, given by the texturometer, consist in the force required to stretch the film by distance and time, being possible to obtain the stress at break (σ_e) and strain at break (ε_e).

Stress at break represents the maximum resistance to fracture, being the largest amount of tensile strength the polymer can achieve before breaking (Frost, 2010). It is calculated by the following equation.

$$\sigma_e = \frac{F_e}{S} \tag{11}$$

where σ_e is the stress at break (N/m^2), F_e the force applied upon rupture (N) and S the sectional area of the film where that force is being applied (m^2). S is the product of the thickness of the film and the width of the rectangle of sample used (m), which in this case was 0.02 m.

Strain at break is the length of the material upon breaking and corresponds to the relative increase in length to the initial length, given by the following equation.

$$\varepsilon = \frac{L_f - L_i}{L_i} \tag{12}$$

where L_f the maximum length; and L_i the initial length of the film.

From the plots of stress against strain it is possible to obtain the Young's Modulus (E). It gives a measure of the elasticity of the film and corresponds to the slope before the onset of plastic deformation.

3.1.4.2 Puncture tests

For puncture tests, the samples used consisted in squares of 3x3 cm, stabilized for three weeks at different relative humidity conditions.

The samples were fixed with adhesive tape to a platform with a 10 mm diameter opening and punctures by a 2 mm diameter probe, at a constant velocity of 1 mm/s, until rupture (Figure 12). Ten replicates were evaluated for each film sample.

Figure 11 - Puncture test

The results given by the texturometer consist in the force required to puncture the film as a function of the distance and time. As in extension tests, it is possible to obtain the stress at break (σ_p) and strain at break (ε_p). Stress at break is given by the following equation.

$$\sigma_p = \frac{F_p}{S_p} \quad \textbf{(13)}$$

where σ_p is the stress at break (N/m^2); F_p the force applied upon rupture (N); S_p the area of the film were the force is being applied (probe sectional area, m^2).

Strain at break is calculated by the following equation.

$$\varepsilon_p = \frac{L_f - L_i}{L_i} \quad \textbf{(14)}$$

where ε_p is the strain at break (-); L_f the maximum length; and L_i the initial length of the film. L_f is calculated by equation 15 taking into account Figure 13, where d is the distance moved vertically by the probe.

$$L_f = \sqrt{L_i^2 + d^2} \quad \textbf{(15)}$$

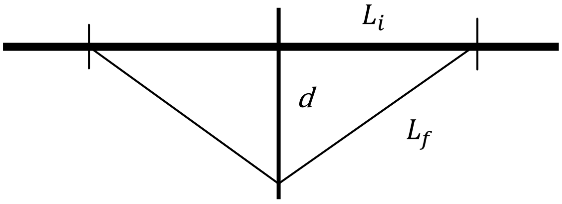

Figure 12 - Puncture test scheme

3.1.5 Optical properties

The samples consisted in 4 x 4 cm squares and were used 10 replicates of each film sample. It was used a colorimeter (Minolta CTR-300, USA), the CIELAB model, a white tile and three cardboards of different colours (yellow, green and red) (Figure 14).

Figure 13 – Colorimeter, white tile and yellow, green and red cardboards

The CIELAB model is supported by a model proposed by the Commision Internationale de L'Eclaraige (International Commission on Illumination), which is the body responsible for international recommendations for photometry and colorimetry. CIE Color Systems locate the colour in a colour space by three coordinates. These spaces include:

- CIE XYZ
- CIE L*a*b*
- CIE L*C*h°

To overcome the limitations of chromaticity diagrams related to CIE XYZ, the CIE recommended the other two colour scales. Those are based on the opponent-colors theory, which says that as two colours cannot be both green and red, nor blue and yellow, at the same time, can be used singles values to describe the red/green and yellow/blue attributes (X-Rite, 2007).

CIELAB model (Figure 15), expresses colour by the following parameters:

- L* - Lightness, where 0 and 1 corresponds to black and white respectively.
- a* - Associated with red/green
- b* - Associated with yellow/blue

From CIELAB it can be obtained CIELCH, that besides L* defines the colour by:

- C* - Chroma, which goes from -60 to +60
- h° - Denotes hue angle

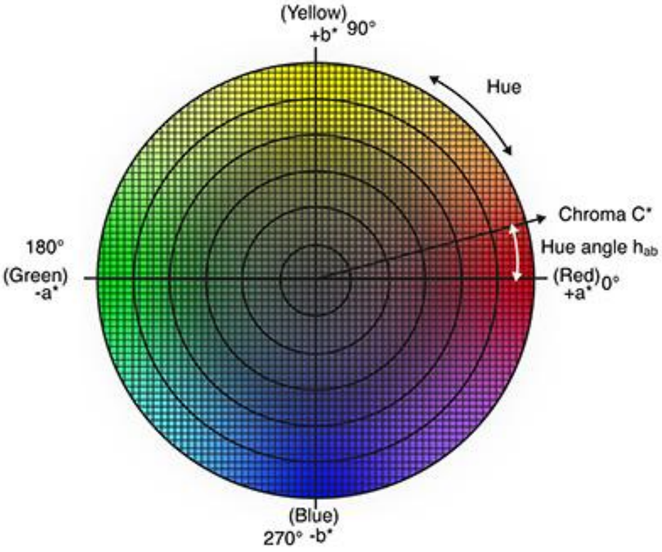

Figure 14 - CIELAB colour chart (Source: http://sensing.konicaminolta.us/)

Assessment of colour is usually, as in the current work, an assessment of the colour difference from a known standard, being specified as ΔE.

In order to determine the optical properties of the film samples, the colorimeter was first calibrated with a white pattern (the white tile). Then, the L*, a* and b* parameters were measured for each one of the four cardboards (white, yellow, green and red). Finally, each sample was placed on each standard, being those parameters measured and registered again with the colorimeter.

For each measurement, C* can be obtained by the following equation:

$$C^* = ({a^*}^2 + {b^*}^2)^{1/2} \qquad \textbf{(16)}$$

In addition, h* can be obtained by.

$$h^* = \text{arctan}\,(b^*/a^*) \qquad \textbf{(17)}$$

This equation requires however a correction:

- $\boldsymbol{h}° = \boldsymbol{arctan}\ (\boldsymbol{b}^*/\boldsymbol{a}^*)$ ×180/π, if a* > 0 and b*>0
- $\boldsymbol{h}° = \boldsymbol{arctan}\ (\boldsymbol{b}^*/\boldsymbol{a}^*)$ ×180/π + 180, if a* < 0
- $\boldsymbol{h}° = \boldsymbol{arctan}\ (\boldsymbol{b}^*/\boldsymbol{a}^*)$ ×180/π + 360, if a* > 0 and b*< 0

The difference between the colour of the cardboards covered by the sample films and the cardboards alone (ΔE), is obtained by the expression:

$$\Delta\text{E} = [(\Delta\text{L})^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \qquad \textbf{(18)}$$

Table 5 summarizes the evaluated colour parameters.

Table 5 - Colour parameters by CIELAB model

ΔL*	Difference in lightness/darkness value	+ = lighter - = darker
Δa*	Difference on red/green axis	+ = redder - = greener
Δb*	Difference on yellow/blue axis	+ = yellower - = bluer
ΔC*	Difference in chroma	+ = brighter - = duller
Δh*	Difference in hue	
ΔE*	Total colour difference value	

3.2 Preservation of minimally processed spinach

This part of the study consisted in monitoring the quality of minimally processed spinach during storage at 4ºC, packed both with TPS-based and PVC wrap films, being kept a control sample with no packaging.

The materials used comprised on:

- 15.6 kg of commercial minimally processed spinach leafs
- 78 polystyrene (PS) trays
- TPS-based wrap film (Mater-Bi®)
- PVC wrap film
- 6 silicon septa

The following procedure was applied:

1. The samples (packaged spinach) were prepared by placing 20 g of spinach leafs on each plastic tray
2. 34 trays were covered with TPS-based wrap film and other 34 with PVC wrap film, sealing them well. 31 trays were left unwrapped.
3. A septum was applied on the surface of the wrap film, in 3 trays of each type of film. This will be the samples for internal atmospheric composition analysis.
4. All samples were stored in a refrigerated chamber, at 4ºC and 70% HR until being analysed.
5. In the day after packaging (day 1), 3 trays of each type of packaging were taken for respiration rate measurement. This was repeated in the last day of the experiment (day 14)

6. In regular time intervals (days 1, 3, 5, 7, 9, 11 and 14), 3 trays from each type of packaging were taken and the spinach leafs were submitted to quality analysis (texture, colour and total soluble solids), after which were discharged.
7. At those days, it was also measured the internal gas composition and the weight loss of trays covered with each type of film. For these two measurements, the same trays were analysed over the entire experiment.

Table 6 summarizes the number of samples tested each day, for each type of test.

Table 6 - Representation of the test and the amount of trays tested each day

Day		Without wrap film	TPS wrap film	PVC wrap film
Quality tests (texture, colour and total soluble solids content)	1	3	3	3
	3	3	3	3
	5	3	3	3
	7	3	3	3
	9	3	3	3
	11	3	3	3
	14	3	3	3
Weight loss (every two days)		3	3	3
Atmosphere composition (every two days)		-	3	3

3.2.1 Quality control

3.2.1.1 Visual analysis

The visual analysis consisted in a visual inspection of the appearance of the product in a consumer point of view. This evaluation was focused on colour and texture.

3.2.1.2 Weight loss

The same triplicate of trays for each type of packaging was weighed every test day. The weight loss of the packages with time is representative of the moisture loss to the atmosphere. It was used a precision scale (Precisa BJ 1100D, Swiss) for samples weighting.

The weight loss between test days was calculated with the following equation:

$$\%\,weight\ loss = \frac{w_1 - w_2}{w_2} \times 100 \qquad \textbf{(19)}$$

where w_1 is the previous recorded mass and w_2 the current sample mass.

The total weight loss corresponds to the weight loss from the beginning of the experiment:

$$\%\,Total\ weight\ loss\ (TM)_n = \frac{w_i - w_n}{w_i} \times 100 \qquad \textbf{(20)}$$

where w_i the initial sample mass; w_n the mass at the storage day n.

3.2.1.3 Respiration rate

Respiration rate measurements were carried out by placing 20 g of spinach in a sealed glass flask and measuring the carbon dioxide composition in the beginning and after a specific period of time. In the first day of the study the period of time was 30 min and in the last day was 84 min.

For the carbon dioxide analysis a gas sensor (Checkmate 9900, PBI Dansensor, Denmark) was used. In the end of the gas analysis, all the flasks, with the spinach in it, were filled with water in order to determine it free volume.

The respiration rate R (ml CO_2 $Kg^{-1}h^{-1}$) is calculated by the following equation.

$$R = \frac{\Delta\%CO_2}{100} \times V_{free} \times \frac{1000}{m} \times \frac{60}{t} \qquad \textbf{(21)}$$

where $\Delta\%CO_2$ is the carbon dioxide variation; V_{free} the free volume inside the flask (ml); m the mass of spinach used (g); and t the time (min).

3.2.1.4 Atmosphere composition inside the packaging

This test was performed using always the same samples, the ones with the septa attached to the wrap film. O_2 and CO_2 composition inside the packaging was measured using a PBI Dansensor CheckMate 9900, Denmark, previously calibrated. The needle is introduced in the packaging from the septum, and after measuring the atmospheric composition, the septum will automatically seal again once the needle is removed (Figure 16).

Figure 15- Measurement of the internal atmosphere composition

3.2.1.5 Firmness

Spinach leafs firmness was measured by a puncture test, using a texturometer (TA-XT2 of Table Micro System, UK) equipped with a 2 mm diameter stainless steel probe and at a crosshead speed of 1 mm/s. A number of 10 leafs from each sample were analysed. Puncture was conducted on the front side of the leaf, on an area with no central or secondary veins (Figure 17).

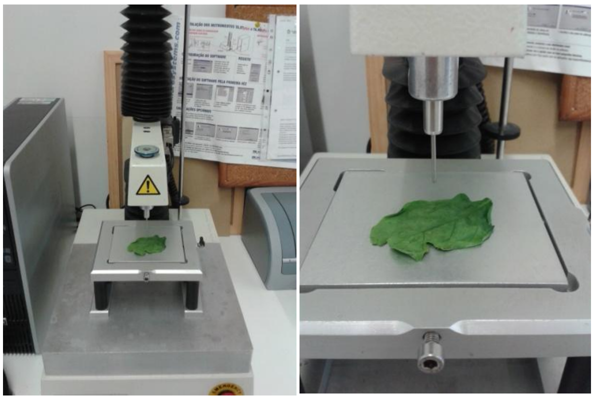

Figure 16 – Puncture test of spinach leafs

The results given by the texturometer consist in the force required to puncture the leaf as a function of distance and time, being possible to obtain the stress at break (σ) and the strain at break (ε). The determination on these values follows the same procedure stated at **3.1.4.2**, for the mechanical characterization of TPS-based and PVC films.

3.1.2.6 Optical properties

In order to determine optical properties, it was used a colorimeter (Minolta CTR-300, USA) and CIELAB model, obtaining the parameters L*, a* and b*, where L* defines lightness, a* denotes the red/green value and b* the yellow/blue value.

A number of 10 leafs from each sample were analysed, being the measurements performed in the backside of the leaf, on an area with no central or secondary veins (Figure 18).

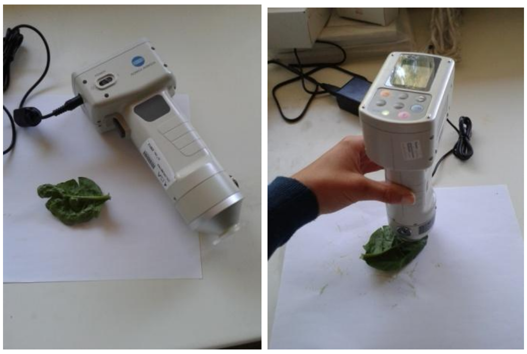

Figure 17 - Colour measurement of spinach leafs

The parameters C*, h* and ΔE were calculated as stated on **3.1.5** for determination of the optical properties of TPS and PVC films.

3.2.1.7 Total Soluble Solids content

Total soluble solids (TSS) or brix was determined using a Pocket Refractrometer Atago (0~85%). The spinach leafs of each tray was macerated with a waste grinder, squeezed through a cloth, to produce a liquid drop which was placed in the equipment (Figure 19).

Figure 18 - Preparation of the sample (left) and brix measurement on the refractometer (right)

The refractometer was calibrated before measuring each sample, and every first drop was rejected. It was measured a triplicate for each sample.

3.3 Statistical analysis

For the statistical analysis of the results it was used the Software Statistica$^{\text{TM}}$ v.7.0 from Statsoft (2004).

All the repetitions for each parameter studied were submitted to a basic statistical analysis, boxplot, in order to analyse the variation of each variable, reject outliers, and concentrate the most probable results. From these it was calculated representative mean value.

To evaluate the differences between each film, in the different parameters studied, there were performed post-hoc ANOVA tests (p-value<0.05) to compare the respective means obtained. For groups with similar number of samples, in the cases 3 or less number of samples for each parameter, it was used Fisher LSD test, and in the cases of more than 3 samples it was used Tukey HSD test. When the number of samples used for each parameter was higher than 3 and it dimension was quite different, it was used the Sheffé test.

4. RESULTS AND DISCUSSION

4.1 Films characterization

4.1.1 Thickness

The TPS-based film is thicker than the PVC one. While the first has a thickness of 11 µm, the second has a thickness of 8 µm.

4.1.2 Higroscopic properties

4.1.2.1 Water vapour adsorption isotherms

The influence of aw of stabilization of the films in water vapour adsorption is presented in Figure 20.

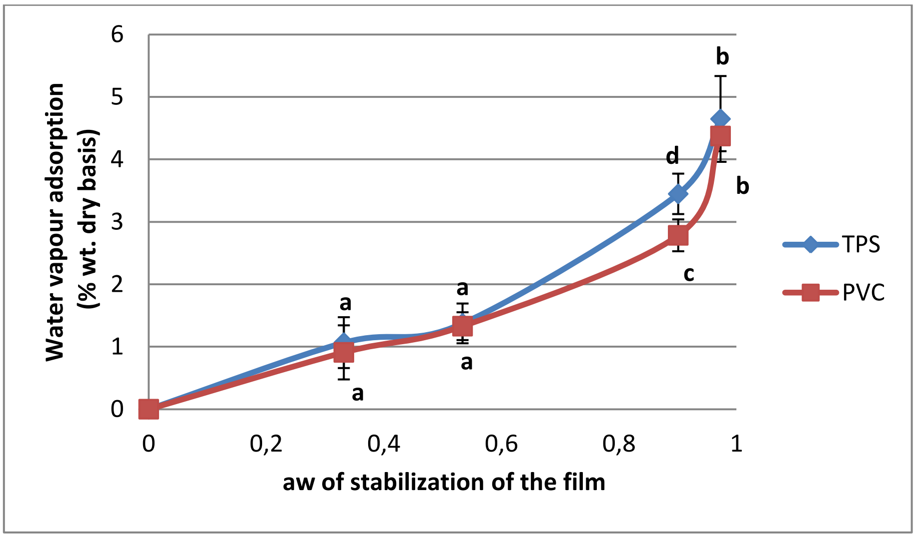

Figure 19 - Water adsorption isotherms for TPS-based and PVC films stabilized at different aw. Different letters indicate significantly different values (post-hoc Anova LSD test, α=0,05)

It can be perceived a slightly higher water adsorption for TPS-based films between aw of 0.6 and 0.8. Though, the results show that TPS-based and PVC films have similar water vapour adsorption behaviour.

The adsorption curves obtained indicate films with a low affinity to water vapour, since the maximum water vapour mass adsorbed was below 5%wt. (dry basis), even when the films were subjected to high aw values. These results for the new TPS-based film are interesting, since most of the starch based films referred in the literature are quite hygroscopic (Ayady et al., 2011; Mahieu et al., 2015). As example, Mahieu et al. (2015) reported values from around 10%wt (aw = 0.53) up to 50%wt (aw = 0.94) for wheat starch films plasticized with 20%wt glycerol. In the same work, the authors presented values from around 10%wt (aw = 0.53) up to 30%wt (aw = 0.94) for wheat starch/polycaprolactone/glycerol composite films.

The lower affinity of the TPS-based film tested can be related to the vegetable oils that are part of Mater-Bi® composition. According to Vieira et al (2011) and Jiménez et al (2012), lipids, being an excellent moisture barrier, increase the film´s hydrophobicity.

4.1.2.2 Water absorption capacity

The liquid water absorption capacity of both films is presented in Figure 21.

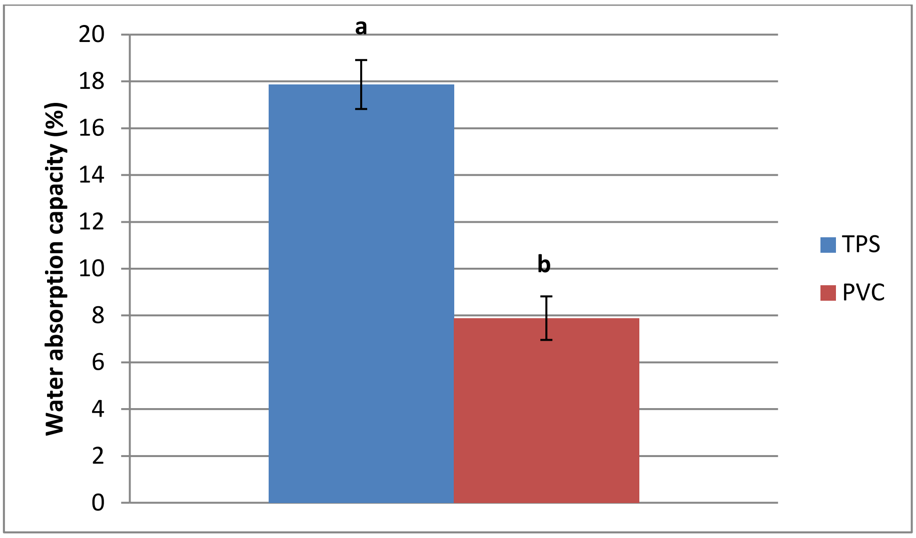

Figure 20 - Water absorption capacity of TPS-based and PVC films (T=26°C). Different letters indicate significantly different values (post-hoc Anova Tukey test, α=0,05)

The biodegradable TPS-based film has a significantly higher liquid water absorption capacity than PVC (about 2.3 times higher). This fact is related to the more hydrophilic character of TPS in contrast to PVC, which is hydrophobic.

4.1.3 Barrier properties

4.1.3.1 Carbon dioxide permeability

The results obtained for CO_2 permeability are presented in Figure 22.

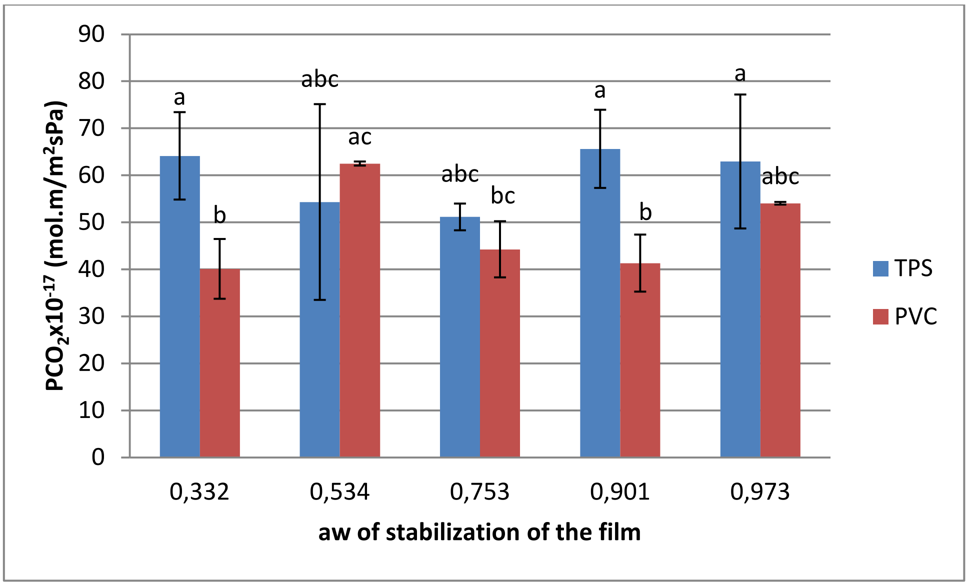

Figure 21 - CO_2 permeability (P_{CO2}), for TPS-based and PVC films stabilized under different aw conditions. Different letters indicate significantly different values (post-hoc Anova LSD test, α=0,05)

The results show that there are no significant differences between the films stabilized at different aw conditions. Between both types of films it can be noticed that in most of the cases (exception only for aw 0.534) TPS-based films tend to have a higher P_{CO2} than PVC films, although this difference is only significant at aw values of 0.332 and 0.901.

This higher permeability of starch based polymers to CO_2 than synthetic ones is in agreement to what was stated by Embuscado and Huber (2009) studies. However, the present results are different from the ones achieved by Gontard et al (1996) in a similar test, where not only it was found an increase of P_{CO2} with water content, but also the values obtained where about $2{,}64x10^{-14}$ mol.m/m^{2}sPa for starch films at 100% RH.

P_{CO2} of the TPS-based film studied was also lower than the one verified by Lehermeier et al (2001) for PLA, other biodegradable polymer in the market, which had values of $3.26x10^{-15}$ mol.m/m^{2}sPa at 25°C).

Regarding PVC, Brody et al (2010) states that permeability to CO_2 for plasticized PVC is in a range of $1.79x10^{-10}$ to $5.53x10^{-9}$ mol.m/m^{2}sPa, much higher than the ones obtained in this test.

4.1.3.2 Oxygen permeability

The results obtained for O_2 permeability are presented in Figure 23.

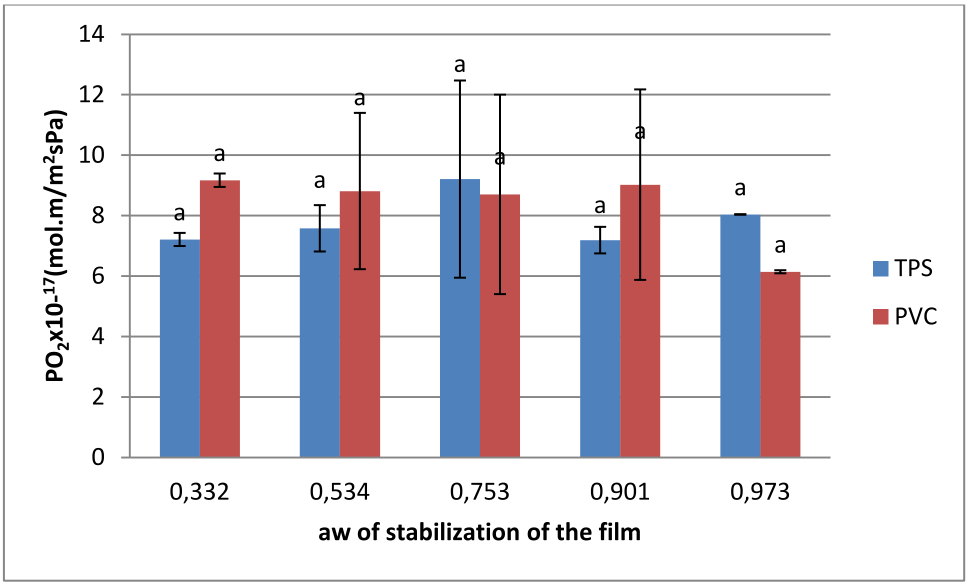

Figure 22 - O_2 permeability (P_{O2}), for TPS-based and PVC films stabilized under different aw conditions. Different letters indicate significantly different values (post-hoc Anova LSD test, α=0,05)

The results for O_2 permeability, as for CO_2, didn´t show significant differences between the films stabilized at different aw conditions, neither between both TPS-based and PVC films. This fact is in line to the low water vapour adsorption of these films, even for high aw values, presented in section **4.1.2.1**.

Comparing the values obtained with the ones of **4.1.3.1**, both films are more permeable to CO_2 than O_2, having a selectivity (CO_2/O_2) of 15.3 and 11.2 (TPS-based and PVC respectively). The higher solubility of CO_2 in the plastic polymers matrix than O_2 is confirmed by literature review (Embuscado and Huber, 2009; Gontard et al 1996; Stringer and Dennis, 2000).

Regarding RH influence, Gontard et al (1996) found an increase of P_{O2} with RH, what was not shown by this test. Again, this fact may be related to the low water adsorption capacity of these films (<5% dry basis), even at high values of aw.

The values obtained for TPS-based films O_2 permeability are higher to the ones referred by Ferreira et al (2014). According to this source, O_2 permeability of starch films at 57% RH was in a range of 0.14 $x10^{-18}$ – 6.2 x 10^{-18} mol.m/m^{2}sPa. However, AlPlastics (2013) stated an higher permeability to O_2 for Mater-Bi polymer than the one achieved. According to this source, P_{O2} for these polymers is 7.46x10^{-16} – 2.4x10^{-14} mol.m/m^{2}sPa.

In comparison to other biodegradable films in the market, as in the case of P_{CO2}, the TPS-film tested showed lower values of $P_{O2.}$ In the case of PLA it were stated values of 1.05x10^{-15} mol.m/m^{2}sPa at 25°C (Lehermeier et al, 2001), while for 100% PCL had results of 4.73x10^{-16} mol.m/m^{2}sPa, at 35°C and 0% RH (Myllymaki et al, 1998).

Regarding PVC, the results obtained are much lower than the ones recorded by other studies. According to Sandhya (2010) the permeability of plasticized PVC to O_2 is 2.37×10^{-12} to 1.46×10^{-10} mol.m/m^{2}sPa. SoarnolTM states that PVC's O_2 permeability is about 2×10^{-11} mol.m/m^{2}sPa. Finally, Brody et al (2010) gives a range of 1.8×10^{-11} to 3.6×10^{-9} mol.m/m^{2}sPa to plasticized PVC.

The discrepancy between the values given by the studies referred and the ones obtained in the present study, for both O_2 and CO_2 can be explained by differences in several factors including film density, thickness, crystallization degree, and formulation (polymers blended, type of plasticiser and other additives used). The results also depend on the methods of determination applied and/or in the equipment used. For example, the application of pressure associated with most gas permeability determination methods may lead to different from the ones achieved if it is used atmospheric pressure.

All these factors have strong influence on the film behaviour and it variation can be reflected on a great fluctuation of the results.

4.1.3.3 Water vapour permeability

The results obtained for water vapour permeability are presented in Figure 24.

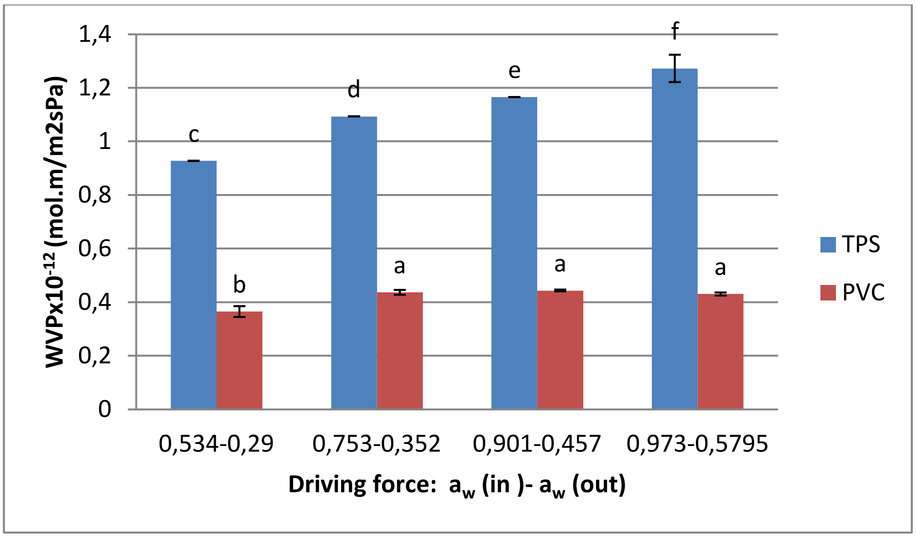

Figure 23 – Water vapour permeability (WVP) of TPS-based and PVC films at 29°C, for different values of a_w, between both sides of the film. Different letters indicate significantly different values (post-hoc Anova Fisher LSD test, α=0,05)

Besides it couldn´t be accomplished the situation of exactly same driving force (Δa_w) for every sample tested in every situation (Δa_w = 0.244, 0.401, 0.444 and 0.394 respectively), the results show that for TPS-based film there is a slight rise of water vapour permeability with increasing the hydration degree, while for PVC film this influence is not verified.

It can also be seen that the TPS-based film is about 3 times more permeable to water vapour than PVC. This higher permeability is a result of the hydrophilic nature of the starch polymers (Rindlav-Westling et al, 1998), which has a higher affinity to water than to the synthetic PVC. This suggests that

PVC would be a more effective water vapour barrier for all Δa_w tested, including those that may correspond to the preservation products at high relative humidity values.

The results obtained by AlPlastics for Mater-Bi polymer were similar to the ones achieved by this test. According to this source it permeability ranges from 1.45x10^{-12} to 2.1x10^{-11} mol.m/m^{2}sPa.

Other studies about WVP properties of starch based films show different results. Talja (2007) obtained WVP of 1.16x10^{-12} and 1.79x10^{-12} mol.m/m^{2}sPa (Δaw = 0.33 and 0.54, respectively) for potato starch films without any plasticizer addition. By adding a plasticizer water vapour barrier was improved, as stated in literature review. At the same testing conditions, the film with a 40% xylitol addition had WVP of 2.12x10^{-13} and 6.42x10^{-13} mol.m/m^{2}sPa.

Garcia et al (2006) found a WPV for corn starch films of 0.98x10^{-11} mol.m/m^{2}sPa (Δa_w = 0.75), which also decreased with the addition of glycerol (0,2856/g starch at a dry basis) to 0,48x10^{-11} mol.m/m^{2}sPa.

The lower WVP values of the TPS-based film in the present study, even at lower Δa_w, can be explained by the hygroscopic vegetable oils in Mater-Bi® composition.

Regarding other biodegradable films, those from EVOH have a water vapour permeability of 0.9x10^{-12} mol.m/m^{2}sPa (Δa_w = 0,9) (Ferreira et al, 2014), in the same order of that of the TPS based film of the present study. Still, it is difficult to compare WVP values with the literature since they are dependent on many factors, such as driving force, type and amount of additives added and films thickness.

In the situation of trying to mimic the humidity conditions of a commercial surface (Figure 25), although the testing humidity achieved (60%) was not as high as the one planned (around 70%), it can be confirmed the lower permeability to water vapour of PVC film in relation with the TPS-based, already concluded in the previous analysis.

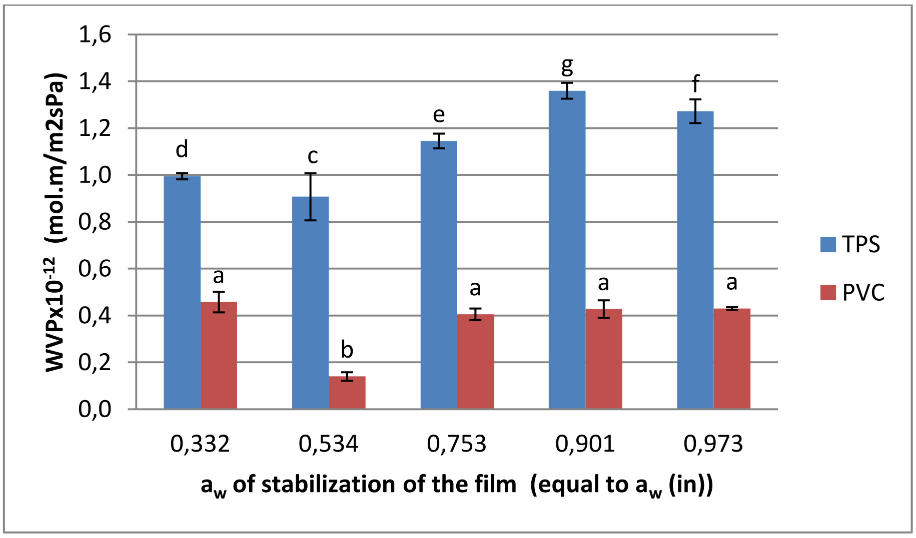

Figure 24 - Water vapour permeability (WVP) of TPS and PVC films, at 25.9 °C and similar exterior a_w (0.6)

In addition, as the interior aw was increased from 0.332 to 0.534, a decrease of permeability was observed, due to a decrease of the driving force (difference aw condition between both sides of the film is lower). At these interior aw values, the water flux takes place from the exterior to the interior of the Petri dish. For the other inner aw values tested, a slight rise of WVP was perceived as the driving force becomes higher. For these range of aw values, the water flux takes place from the inside to outside of the petri dish.

These results indicate that for high aw food products stored at RH values around 60%, the PVC film would be a more effective water barrier than the TPS-based films.

4.1.4 Mechanical properties

4.1.4.1 Extension

The values of stress and strain at break for TPS-based and PVC films, stabilized at different relative humidity values, are presented in Figures 26 and 27.

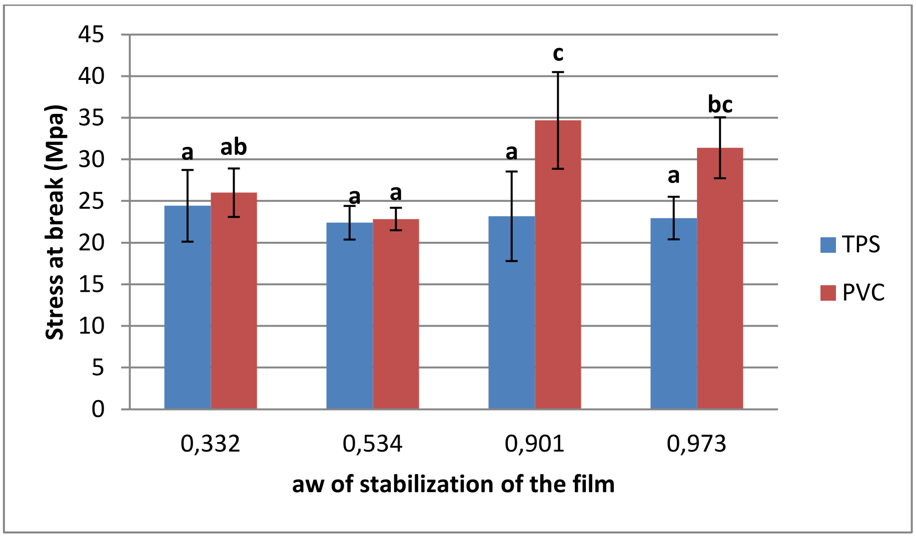

Figure 25 - Stress at break on extension test, for TPS-based and PVC films stabilized under different aw conditions. Different letters indicate significantly different values (post-hoc Anova, Tukey test, α=0.05)

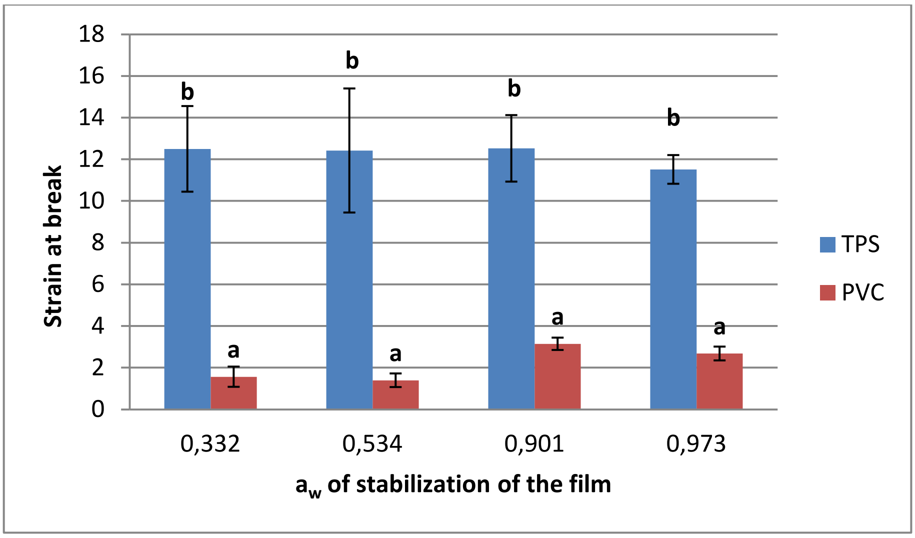

Figure 26 - Strain at break on extension test, for TPS-based and PVC films stabilized under different aw conditions. Different letters indicate significantly different values (post-hoc Anova, Tukey test, α=0.05)

The value of aw under which the films were stabilized didn´t affect neither stress nor strain at break values of both films tested, as they were not statistically different for all aw conditions tested.

Comparing both films regarding stress at break, it only can be seen a significant difference between TPS-based and PVC films at aw of 0.901 and 0.973. For these values of aw, PVC films have higher stress at break values, which means that is necessary an higher force to break them when extended, in other words, they are slightly stronger. In what concerns the strain at break, TPS-based films present a quite higher value than the PVC ones (about 5.7 times superior), what may be an advantage for its application as wrapping material.

The values of stress at break of PVC films obtained are in accordance with the ones stated by Kent (2012) for flexible PVC, which ranged from 14 to 31 MPa. However, higher values (55.5 to 55.6 MPa) were reported by Bhunia et al. (2013).

Bertuzzi et al (2012) it their studies on the mechanical properties of a high amylose content corn starch based film, got the same range of values for tensile strength than the present study, in extension test, for TPS-based films with any plasticizer addition. However, Rindlav-Westling et al (1998) found slightly different values in their studies. They state that stress at break for high amylose corn starch (50% and 55% amylose) with no glycerol addition was 50 and 38 MPa, respectively. The same films had values of strain at break of 0.025 and 0.09 respectively.

Han (2014) states a stress and strain at break of 31.8 MPa and 0.041 for corn starch films with 23.9% amylose content. On another study, of Garcia et al (2006), corn starch films (at a concentration of 5%) with no plasticizer addition had stress and strain at break values of 47.4 MPa and 0.036. With the addition of glycerol (0.2856 glycerol/g starch at a dry basis) the values changed to 7.1 and 0.225, as a result of a more flexible network provided by the plasticizer.

The values of the present study, being slightly lower for stress at break and much higher for strain at break, than the ones of the literature, can be explained by the lipid component of Mater-Bi® matrix which has an influence on the mechanical properties of the film, providing a higher strain and toughness, low tensile strength, and low modulus (Vieira et al, 2011). The values obtained, when compared with the studies mentioned, also suggests that the starch based film tested has plasticizers in it formulation, providing a less rigid matrix. However, when comparing the results the ones achieved by different sources, it is important to take into account that different formulations and films characteristics, as well as the testing methods followed have a big influence on the results achieved.

Focusing other biodegradable polymers in the market, the values of stress and strain at break for those from PLA according to Alavi et al (2015) are 21-60 MPa and 0.025-0.06. For PLC films the same source stated values of 21-42 MPa and 3-10. Regarding polyolefin PVOH films they present values of 25 MPa and 0.5 (Olabisi and Adewable, 2016).

Young's Modulus was calculated under the elastic region of the extension curves (Figure 28), as shown by Annex I. The results show that there is no significant dependence on the water content for TPS-based films, while for the PVC ones it can be seen a small decrease on Young's modulus values as the aw at which the samples were stabilized increases.

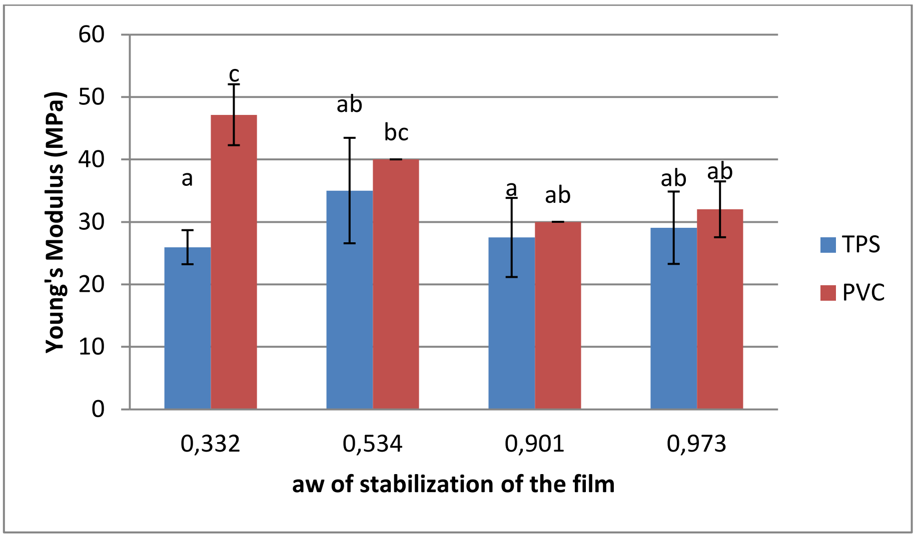

Figure 27 - Young's Modulus on extension test, for TPS-based and PVC films stabilized under different aw conditions. Different letters indicate significantly different values (post-hoc Anova, Tukey test, α=0.05)

The Young's modulus of PVC films is also significantly higher than TPS-based ones, when both were stabilized at low aw (aw = 0.332). Since this is a measure of the elasticity of a material, it can be concluded that at those values of aw PVC films are slightly more resistant to deformation than TPS-based films. At higher values of aw (> 0.534), although the values are still higher for PVC films, the difference is not significant.

The TPS-based film tested has lower Young’s Modulus than those from PLA and PVC. According to Alavi et al (2015) the Young´s Modulus of these polymers is 400-4000 MPa and 200-500 MPa, respectively.

4.1.4.2 Puncture

The stress and strain at break values under puncture tests are presented in Figures 29 and 30, respectively.

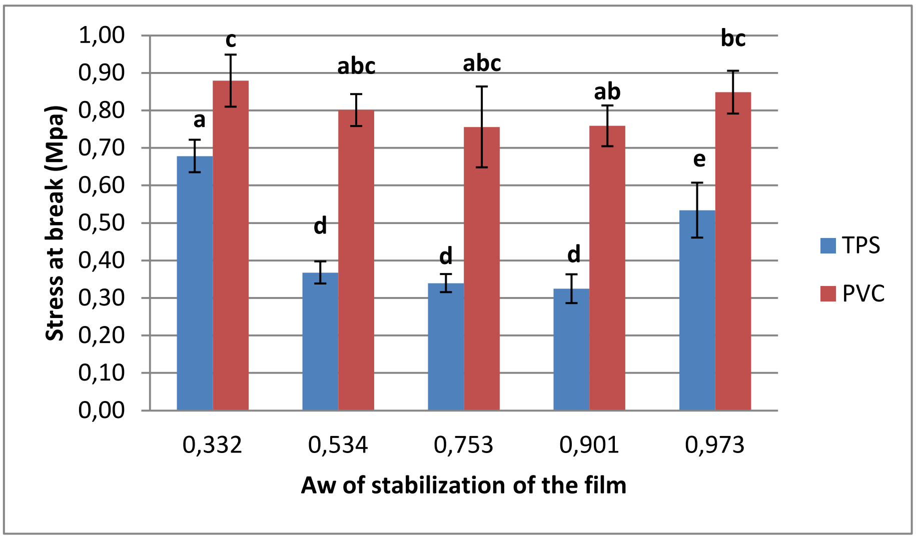

Figure 28 - Stress at break on puncture test for TPS-based and PVC films stabilized under different aw conditions. Different letters indicate significantly different values (post-hoc Anova, Tukey test, α=0.05)

For PVC films, there is no significant influence of aw of stabilization on the stress at break. However, TPS-based films show a significant decrease of stress at break after aw of stabilization of 0.332, and then a significant increase for aw=0.973. This fact may be attributed to the more hydrophilic character of TPS.

It can also be seen a significant difference between the stabilization aw for both types of films tested, in the way that the TPS-based has a lower stress at break.

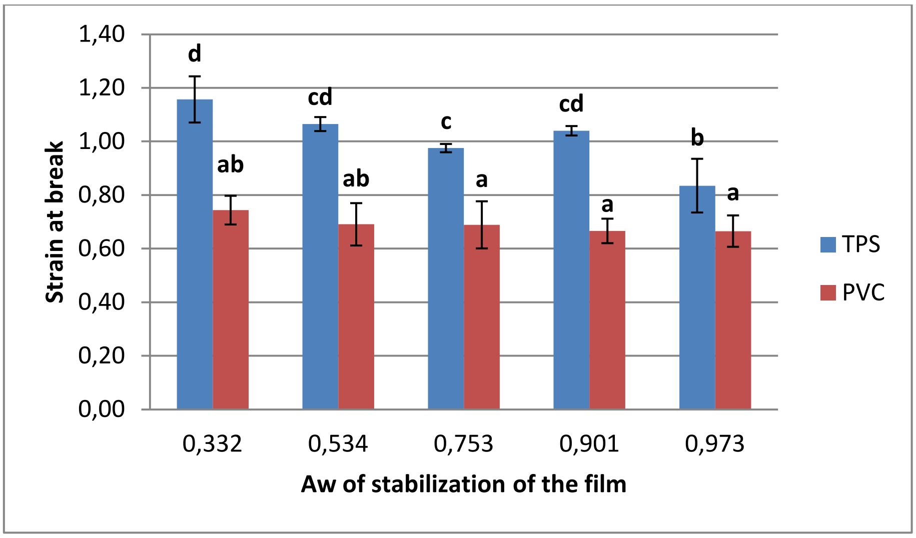

Figure 29 - Strain at break on puncture test for TPS-based and PVC films stabilized under different aw conditions. Different letters indicate significantly different values (post-hoc Anova, Tukey test, α=0.05)

In what regards strain at break, the results show that for all stabilization aw tested, the TPS-based films are the ones presenting higher values. In addition, they are also the ones whose strain at break values are more influenced by the values of aw stabilization. For PVC films, the results show that there is not a significant influence of the aw of stabilization in this parameter. This properties may be a result of the more hydrophilic character of TPS compared to PVC.

These results from puncture tests are in line with the ones from extension tests, in the way that suggest that the TPS-based film is more deformable than PVC, when subjected to a certain force.

4.1.5 Optical properties

The colour differences induced by the application of both types of films on coloured paper sheets are presented in Figures 31, 32, 33 and 34. For all colours tested, the colour differences observed were not influenced by the values of aw at which both film types were stabilized.

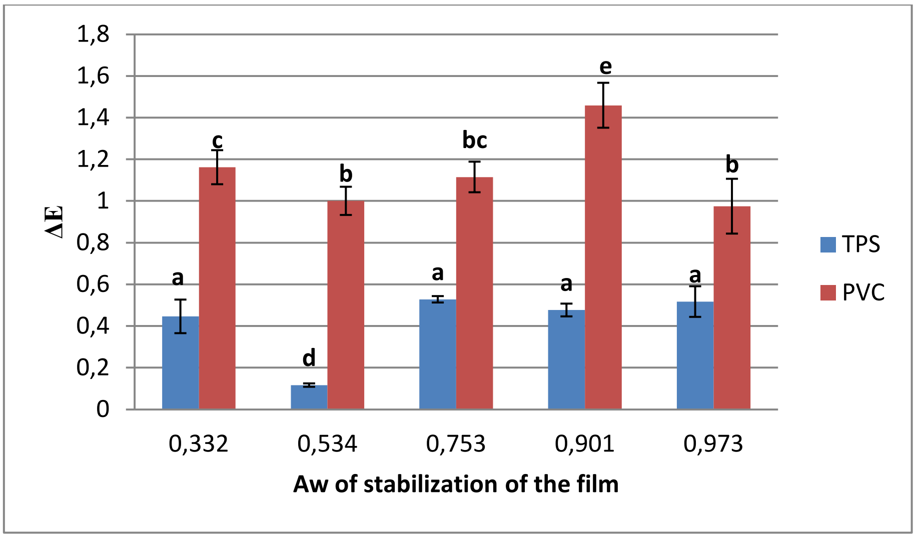

Figure 30 – ΔE values for white, for TPS-based and PVC films stabilized under different aw conditions. Different letters indicate significantly different values (Anova, Fisher LSD test, α=0.05).

For the white colour (Figure 31), both films present low ΔE values and hence a good transparency for white. Though, it´s important to highlight a significantly lower ΔE for the biodegradable film, which indicates that this film would be a better choice to pack white products, as the case of fresh mushrooms or leek steams.

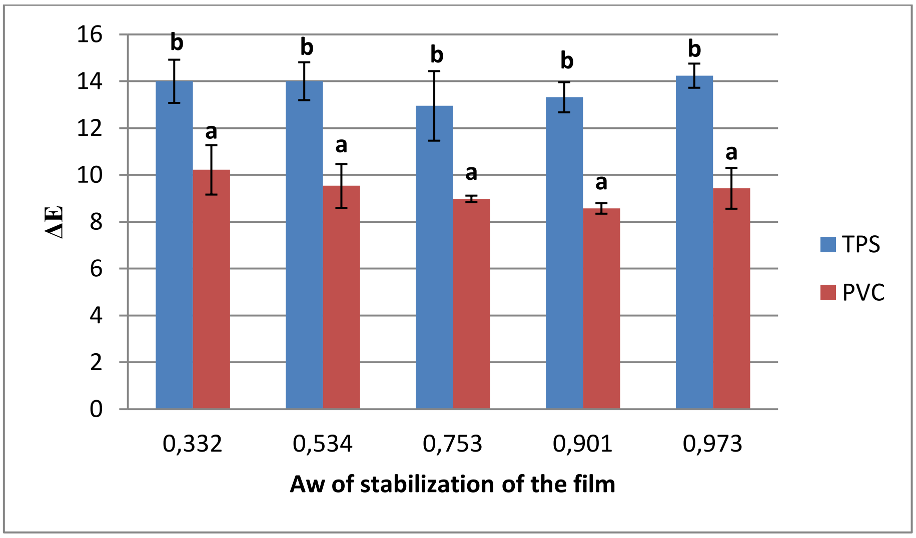

Figure 31 - ΔE values for green, for TPS-based and PVC films stabilized under different aw conditions. Different letters indicate significantly different values (Anova, Fisher LSD test, α=0.05)

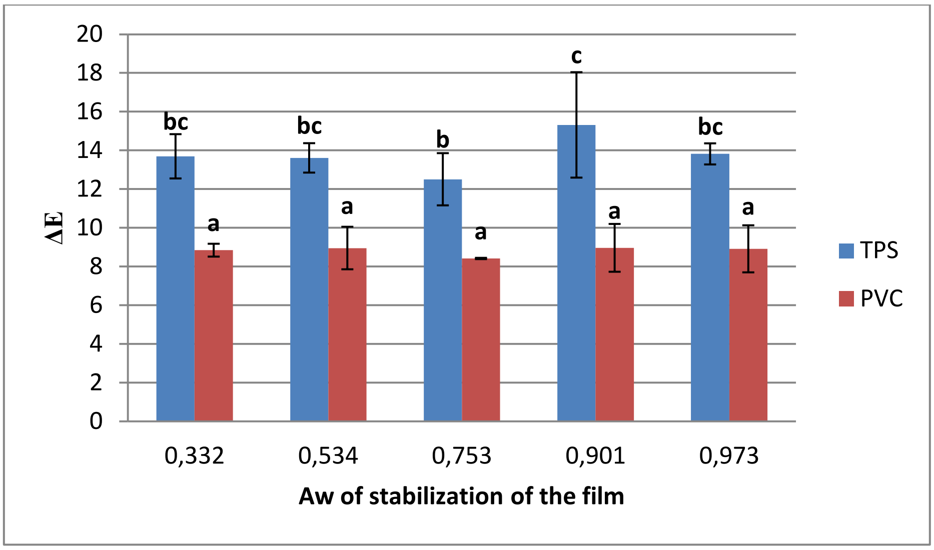

Figure 32 - ΔE values for yellow, for TPS-based and PVC films stabilized under different aw conditions. Different letters indicate significantly different values (Anova, Fisher LSD test, α=0.05)

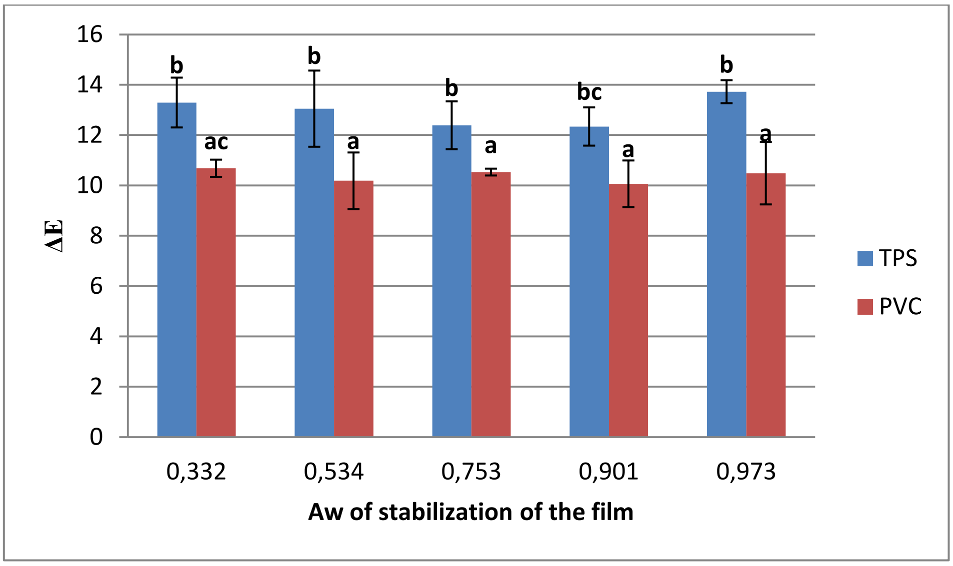

Figure 33 - ΔE values for red, for TPS-based and PVC films stabilized under different aw conditions. Different letters indicate significantly different values (Anova, Fisher LSD test, α=0.05)

For yellow, green and red (Figures 32, 33 and 34), comparing with white colour, ΔE values are much higher, meaning that both films change more significantly the instrumental colour parameters measured. In these cases the main factor responsible for this isn´t the difference in lightness/darkness (ΔL) but the difference between yellow/blue axis (Δb*).

For green, yellow and white, in contrast with white, are the PVC films the ones showing a higher transparency, which means they would be better to pack colourful products.

4.2 Application on the preservation of fresh spinach

4.2.1 Visual analysis

The appearance of the samples of spinach without packaging, packed with TPS-based film and packed with PVC film at different days of testing is presented in Figures 35 to 38.

Figure 34 – Samples of spinach without packaging, packed with TPS-based film and packed with PVC film (from left to right respectively), at day 7

Figure 35 – Samples of spinach without packaging, packed with TPS-based film and packed with PVC film (from left to right respectively), at day 9

Figure 36 – Samples of spinach without packaging, packed with TPS-based film and packed with PVC film (from left to right respectively), at day 11

Figure 37 – Samples of spinach without packaging, packed with TPS-based film and packed with PVC film (from left to right respectively), at day 14

While it could be noticed visual modifications in the spinach without packaging just after 12 hours of storage, the visual changes on the spinach packed with films were way slower.

Comparing both films, the spinach packed with the TPS-based film started to present slight changes on its appearance before the spinach packed with PVC film. Those changes started to be noticed around 9 days after packaging, while for PVC it took 2 days more.

In the last day of testing (14th day) the entire samples didn´t look adequate for consumption.

4.2.2 Weight loss

Mass loss over time for spinach without packaging, packed with TPS-based film and packed with PVC film is presented in Figure 39.

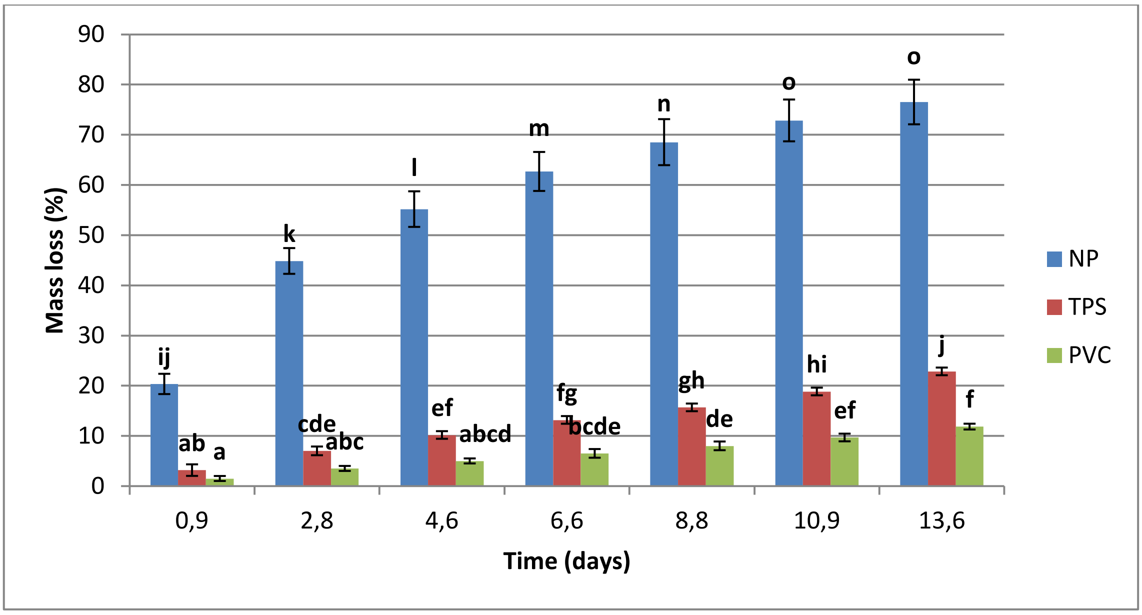

Figure 38 – Mass loss over time for spinach without packaging (NP), packed with TPS-based film (TPS) and packed with PVC film (PVC). Different letters indicate significantly different values (Anova, Fisher LSD test, α=0.05).

As it can be seen, there is a significantly higher mass loss (due to water loss) for spinach without packaging. Water is a product released by both respiration and transpiration processes. While without packaging this water is immediately lost to the atmosphere, in the cases were the product is packed the film retains part of the water released inside the packaging. On the other hand, the limited availability of oxygen provided by the film induced a modification of the metabolic processes of the product, including a reduction of respiration rate and transpiration. This will consequently reduce the amount of water released from the product itself.

Comparing both films, TPS-based film show a moisture loss of about 2 times higher than that of PVC. This fact is related to the higher water vapour permeability of TPS showed in **4.1.3.3.**, which leads to a higher amount of water transferred to the exterior per unit of time.

The lower water loss associated with the PVC films represents an advantage to the TPS-based in packing high moisture products, as fruits and vegetables. Moisture loss, besides being associated to a decrease of quality, it also means an overall weight loss and thus a direct economic loss to the grower or producer (Sandhya, 2010).

4.2.3 Respiration rate

The respiration rate values of the spinach packed with the different types of packages, measured at days 1 and 14 after starting the preservation test, are shown in Figure 40.

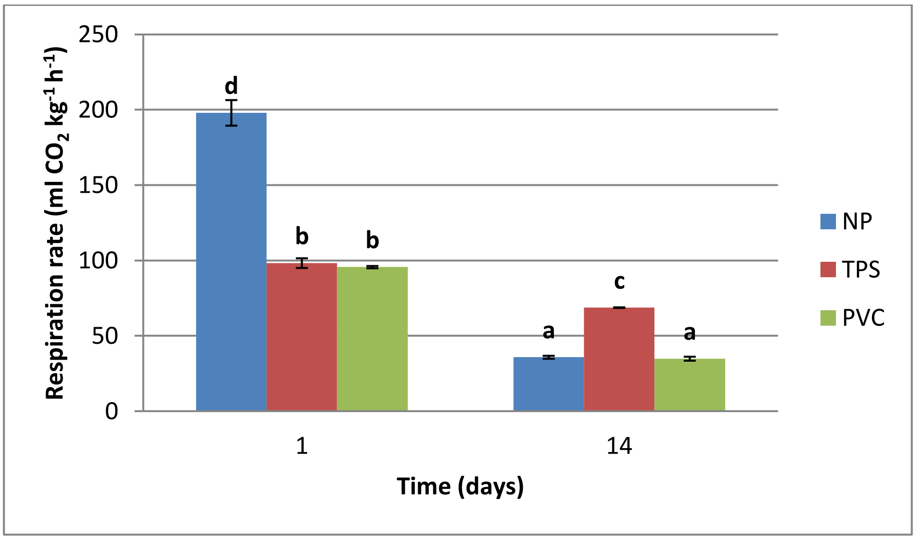

Figure 39 – Respiration rate over time for spinach packed with TPS-based wrap film (TPS), with PVC wrap film (PVC) and with no packaging (NP). Different letters indicate significantly different values (Anova, Fisher LSD test, α=0.05)

It can be seen that after 24 hours the respiration rate of the spinach without packaging is significantly higher than the produce packed with films. This is explained by the availability of oxygen in the first case, allowing the spinach to keep a high respiration rate, already accelerated by processing. In the case of the spinach packed with film, the respiration rate was reduced as a consequence of the reduced availability of oxygen.

At the last day of testing, it can be perceived a significant decrease of respiration rate for all samples. On one hand, since respiration consists in the process of energy production by oxidative decomposition of the substrate present in cells, this reduction may be related to the depletion of substrate. On the other hand, in the case of the spinach packed with film, the exposure to an atmosphere with lower oxygen concentration along the test period, induced changes in respiration metabolism, more specifically a reduction in respiration rate. As the test was performed on the spinach right after being removed from the packaging, at regular atmosphere, it can be concluded that this effect was permanent.

Also regarding the last day of testing, the results show that for both spinach without packaging and spinach packed with PVC film, the respiration rate is lower than for spinach packed with TPS-based film. In the case of the spinach without packaging, the lower value may be explained by the fact that the product was already in a high degree of senescence. By keeping a high respiration rate along all the test period it resulted on a faster depletion of substrate supplies for respiration process to occur.

Though the values of respiration rate obtained confirm that this product has a high respiration rate (more than 33 ml CO_2 Kg^{-1} h^{-1} according to Almeida, 2005), they are higher than the ones achieved by other studies:

- Gil (2008) states that the respiration rate of spinach, at 4°C and free atmosphere conditions is 31.81 ml CO_2 $kg^{-1}h^{-1}$;
- Gomez-Lopez (2012) determined a respiration rate of baby spinach leafs stored for 3 days at 5°C was 18.9 – 24.2 mg CO_2 Kg^{-1} h^{-1}: 9.6 – 12.3 ml CO_2 Kg^{-1} h^{-1}.

This is related to the temperature at which the test was performed and the degree of processing of the product. As the present test was performed at a higher temperature (25°C) and the product was minimally processed it could explain the increase of respiration rate.

4.2.4 Atmospheric composition inside the packaging

The results obtained for the changes in atmospheric composition inside the packages of spinach packed with TPS-based film and PVC film along storage period are presented in Figures 41 and 42.

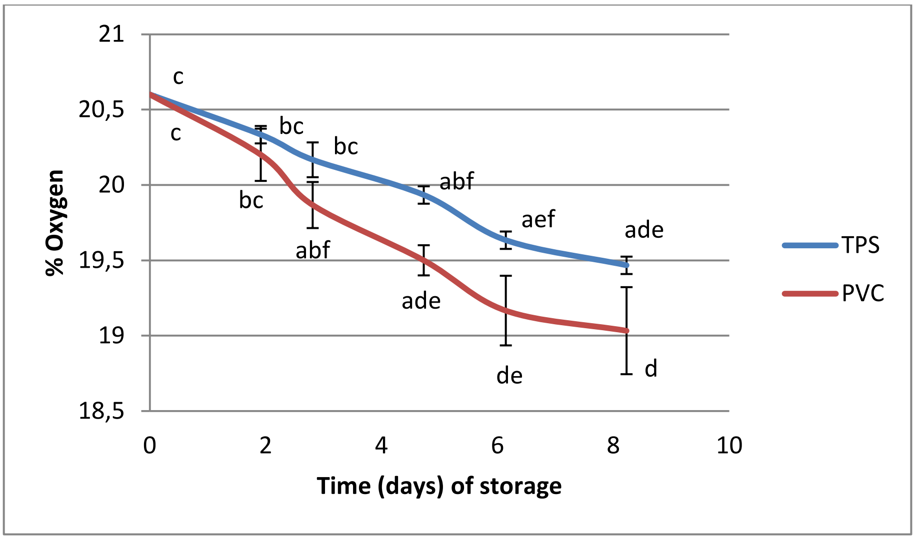

Figure 40 – Changes in oxygen composition inside the packages of spinach packed with TPS based film and PVC film, along storage period. Different letters indicate significantly different values (Anova, Sheffé test, α=0.05).

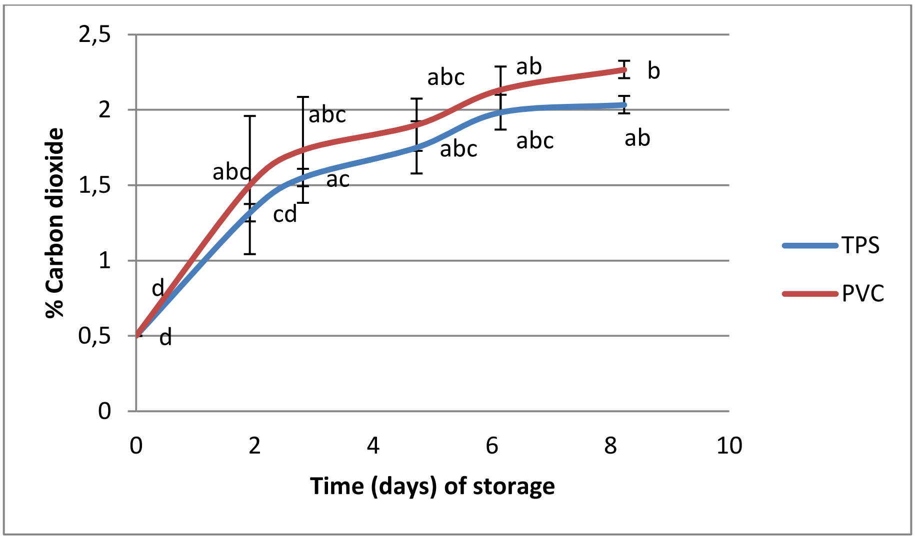

Figure 41 – Changes in carbon dioxide composition inside the packages of spinach packed with TPS-based film and PVC film, along storage period. Different letters indicate significantly different values (Anova, Sheffé test, α=0.05).

In the beginning of the test, the gas composition inside both type of packaging was similar to the atmospheric one. On the following days it can be seen a decrease of oxygen content simultaneously with an increase of carbon dioxide content. This is a consequence of the respiration process that, as explored in literature review, consumes oxygen and produces carbon dioxide.

Along the test period it can be seen that in the first days (until around day 5), both oxygen decrease and carbon dioxide increase rates are higher, being these rates reduced in the following days. The packages gas composition is tending to reach a stationary stage.

Comparing both films, the packages with TPS-based films tend to present a higher oxygen content and lower carbon dioxide concentration than PVC films, during all the test period. This suggests that

TPS-based films are more permeable to both gases than PVC, in the way it would let more O_2 to diffuse into the packaging for respiration process, and would at the same time let a greater amount of CO_2 to be released to the atmosphere. Still, the differences encountered are not statistically different.

This suggestion is supported by gas permeability test (**4.1.3.1** and **4.1.3.2)**. Although the difference between both films is not statistically significant, it can be noticed that, for the films stabilized at 97.3 RH, TPS-based films tend to have a higher permeability to O_2 than PVC ones, and in the case of CO_2 there is not much difference in the permeability of both films.

4.2.5 Firmness

Both stress and strain at break variation with time, on puncture test, for spinach without packaging, packed with TPS-based film and packed with PVC film is presented in Figures 43 and 44.

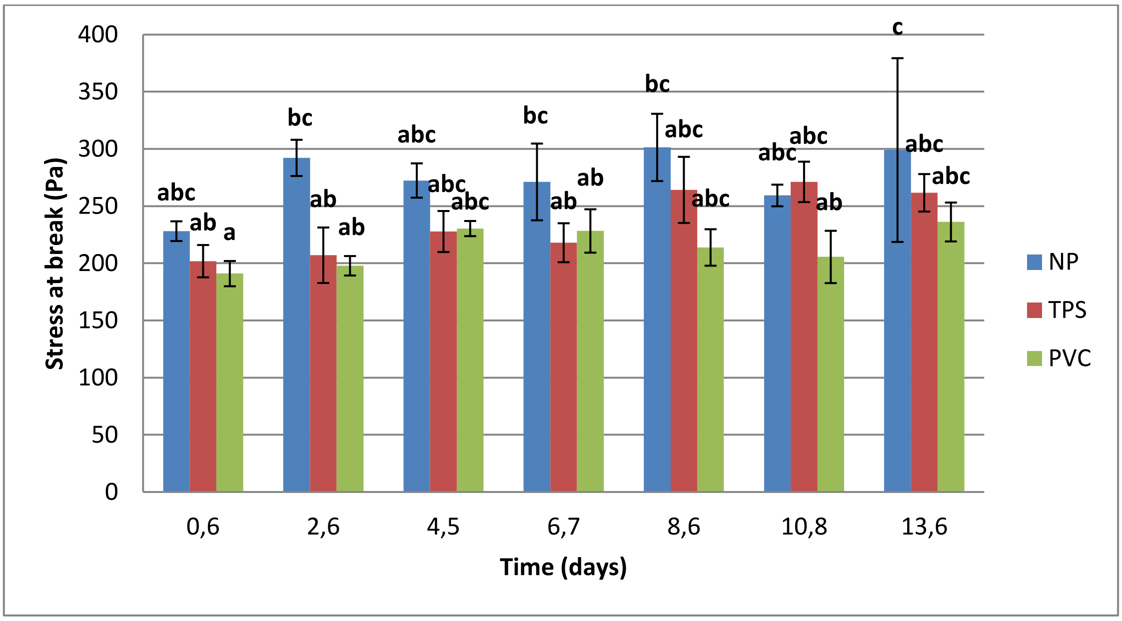

Figure 42 – Stress at break variation with time, on puncture test, for spinach without packaging (NP), packed with TPS-based film (TPS), and packed with PVC film (PVC). Different letters indicate significantly different values (Anova, Sheffé test, α=0.05).

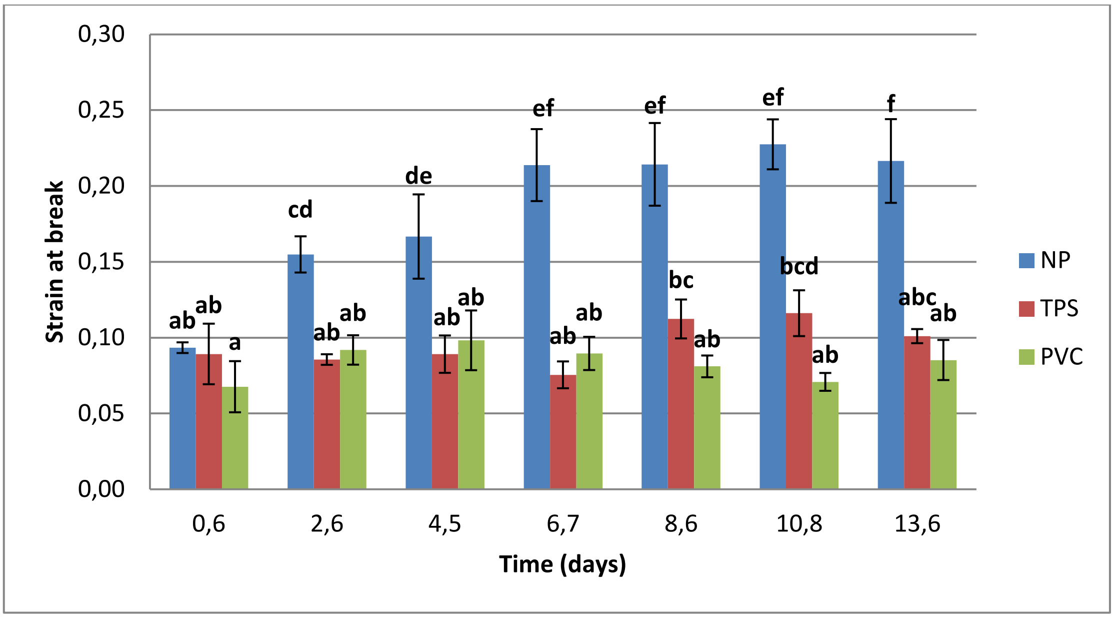

Figure 43 - Strain at break variation with time, on puncture test, for spinach without packaging (NP), packed with TPS-based film (TPS), and packed with PVC film (PVC). Different letters indicate significantly different values (Anova, Sheffé test, α=0.05).

The results show that the stress at break did not change significantly over time for all the samples. In addition, were no statistical significant differences in the stress at break between the produce packed without packaging and with the two types of films.

Regarding strain at break, it increased significantly over time for the samples with no packaging until around 7 days. After that, it remained unchanged. These results are explained by the higher amount of moisture loss associated with the spinach without packaging, discussed on **4.2.2**. The dehydrated tissues lose the original structure becoming softer and more elastic, as it could be seen by visual analysis (**4.2.1**). As such, a higher force is needed to break them (higher stress at break) and they achieve a higher strain till reach the breakage point (higher strain at break). After the sixth day it seems that the water loss has no longer a significant expression on the mechanical properties.

In contrast to the samples with no packaging, the samples packed had a more effective preservation of its physical characteristics and there was no evident difference between the two types of films.

4.2.5 Optical properties

Chroma, hue, and colour variations with time for spinach without packaging, packed with TPS-based film and packed with PVC film are presented in Figures 45, 46 and 47 respectively.

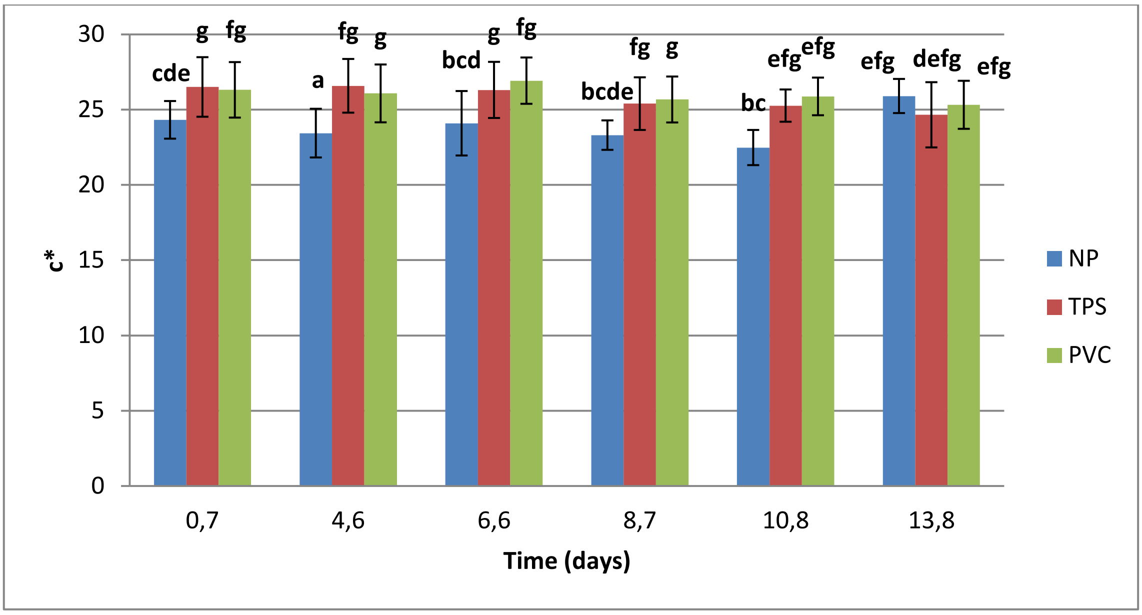

Figure 44 – Chroma variation with time for spinach without packaging (NP), packed with TPS-based film (TPS), and packed with PVC film (PVC). Different letters indicate significantly different values (Anova, Tukey HSD test, α=0.05).

For each sample, the results for chroma do not show significant changes along the test period. On the other hand, comparing the three types of packaging tested, the samples without packaging have significant lower values than the samples packed. This is related to the faster senescence of the samples without packaging and the consequent higher water loss. These samples, more dehydrated, lose their bright colour and became duller, which will then influence chroma values.

Regarding hue variation (Figure 46), there are no significant changes with time for every sample, neither the type of package show having a significant influence on this parameter.

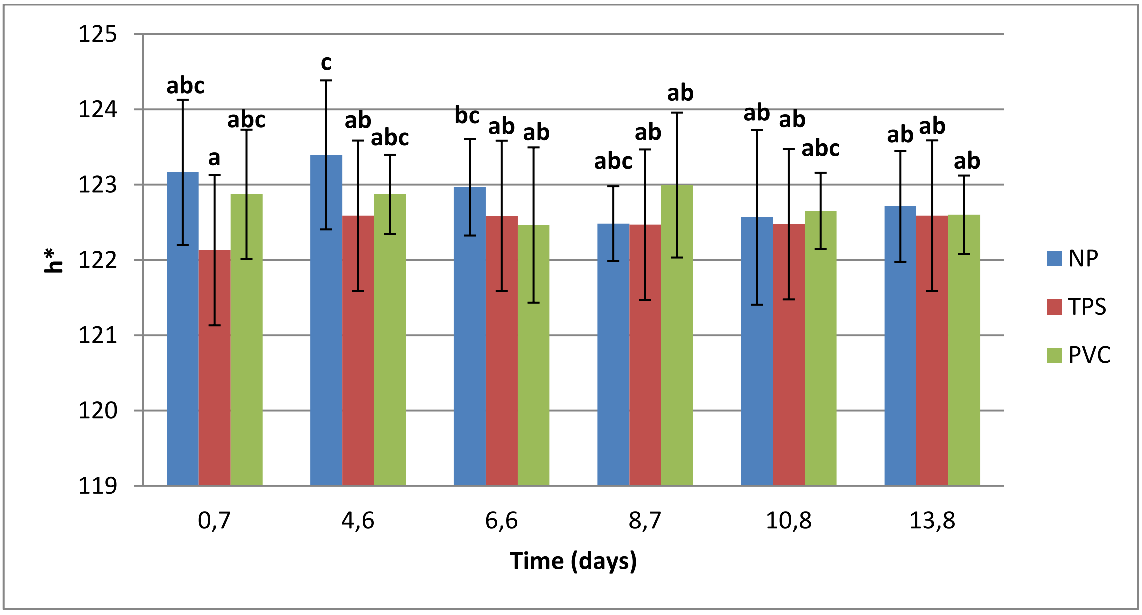

Figure 45 - Hue variation with time for spinach without packaging (NP), packed with TPS-based film (TPS), and packed with PVC film (PVC). Different letters indicate significantly different values (Anova, Tukey HSD test, α=0.05).

The results for the global colour variation (Figure 47) show that there are only significant changes in the colour with time for the spinach without packaging, although this is only noticed between the first and the fifth day.

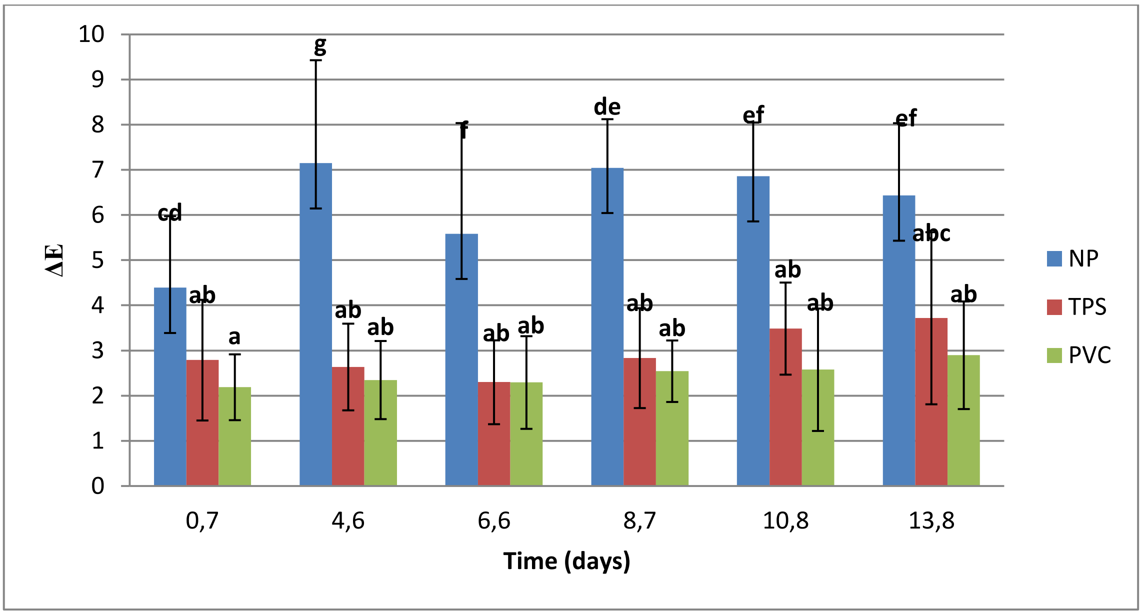

Figure 46 - Colour variation with time for spinach without packaging (NP), packed with TPS-based film (TPS), and packed with PVC film (PVC). Different letters indicate significantly different values (Anova, Tukey HSD test, α=0.05).

Comparing the three types of samples tested, the colour variation of the spinach without packaging is significantly higher than the ones packed with film. In addition, both films have no significant differences in colour change values between them.

On Annex II, it can be seen that L* parameter is the one that present the greater variation, which means lightness changes are the main factor associated with colour change.

Annex II also show a greater variation for b* that a*. Since b* is a measure of yellow/blue axis, the results are explained by the physiologic alterations associated with senescence, which include chlorophyll loss and consequent exposure of underlying carotenoid pigments. This is responsible for the characteristic green of spinach leafs acquire a yellower tonality.

4.2.6 Total Soluble Solids content

Brix variation with time for spinach without packaging, packed with TPS-based film, and packed with PVC film is presented in Figure 48.

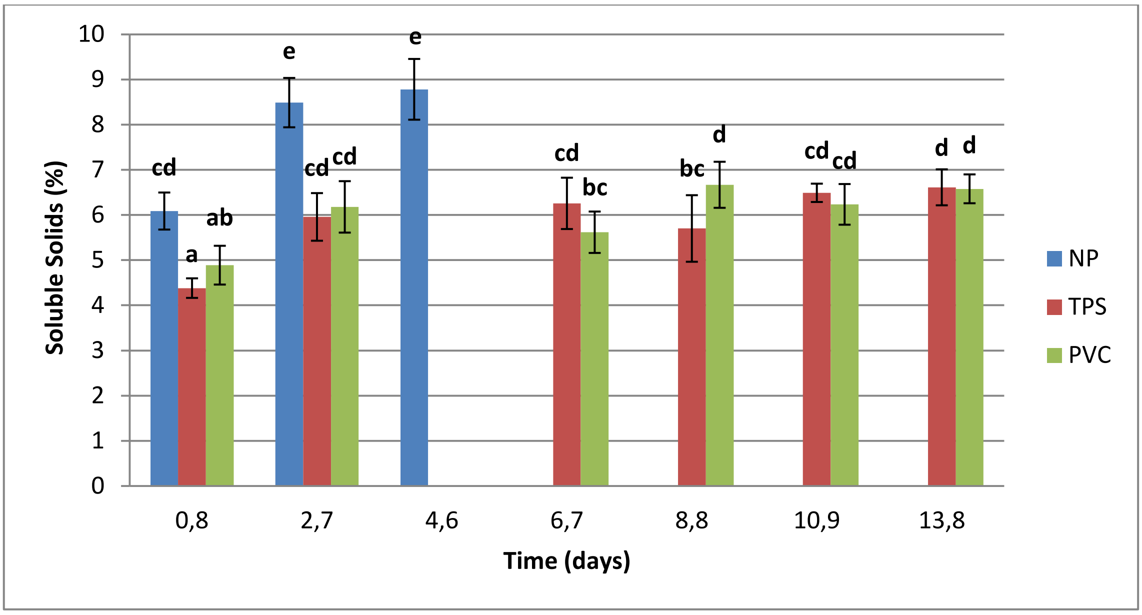

Figure 47 – Brix variation with time for spinach without packaging (NP), packed with TPS-based film (TPS), and packed with PVC film (PVC). Different letters indicate significantly different values (Anova, Tukey HSD test, α=0.05).

It can be seen that brix variation of the spinach without packaging was significantly faster than for the spinach packed with film. By the seventh day it was not even possible to extract a drop from the samples, to test it in the refractometer. This is explained by the faster drying rate of the spinach when not protected with a moisture barrier (as seen at **4.2.2**). In both other cases, the film retarded the moisture loss and the brix variation of the spinach was lower.

Regarding both films there is no difference between them in the way they affect brix values, at this level of significance.

5. CONCLUSIONS AND FUTURE WORK

In this work, a new wrapping film (TPS-based) produced by extrusion from Mater-Bi, a thermoplastic material based on thermoplastic starch, cellulose and vegetable oils, was characterized. Its properties where compared to a wrapping film produced from polyvinylchloride (PVC), one of the most used non-biodegradable petrochemical based polymers for this purpose.

The evaluation of the colour alterations induced by white, green, yellow and red showed that TPS-based film is better than PVC film for packaging white colour products, while the changes due to PVC films for green, yellow and red were lower.

Both films presented a similar and low water vapour adsorption, as the maximum value was around 5% (dry basis) for a relative humidity of 97%. In what concerns liquid water absorption, the TPS-based film has shown a higher value (17%) than the PVC one (7%). Still, both water absorption and water vapour absorption of the TPS-based film are much lower than those reported in the literature for other TPS-based films.

Regarding mechanical properties, TPS-based film is slightly less resistant than the PVC film, as it showed lower values of stress at break on both extension and puncture tests, and a lower Young's Modulus on extension test. However, TPS-based film showed a much higher strain at break on both mechanical tests performed, which may be an advantage for its application as wrapping material.

The barrier properties to gases (O_2 and CO_2) were similar for both films, and were not significantly dependent on the relative humidity at which the films were conditioned before the test. In what concerns the permeability to water vapour, not only it was higher for the TPS-based film, but also it slightly increased as the driving force imposed shifted for higher relative humidity values.

On the fresh-cut spinach preservation case study, both films revealed to have a similar performance in most of the tested quality parameters (colour, texture and total soluble solids). The major difference was detected on the preventing water loss, as PVC film showed to be more effective than TPS-based film, as it showed to be a better water vapour barrier.

According to all the results achieved by this study, it can be concluded that the TPS-based film studied has a good potential to be a direct substitute of the conventional PVC wrapping film. However it would be interesting to address in further studies, the performance of this novel TPS-based film in packing products with different characteristics, as for example with lower aw, like dates and nuts. It would also be relevant the performance of the same study, but using a polyethylene wrap film instead of PVC.

6. REFERENCES

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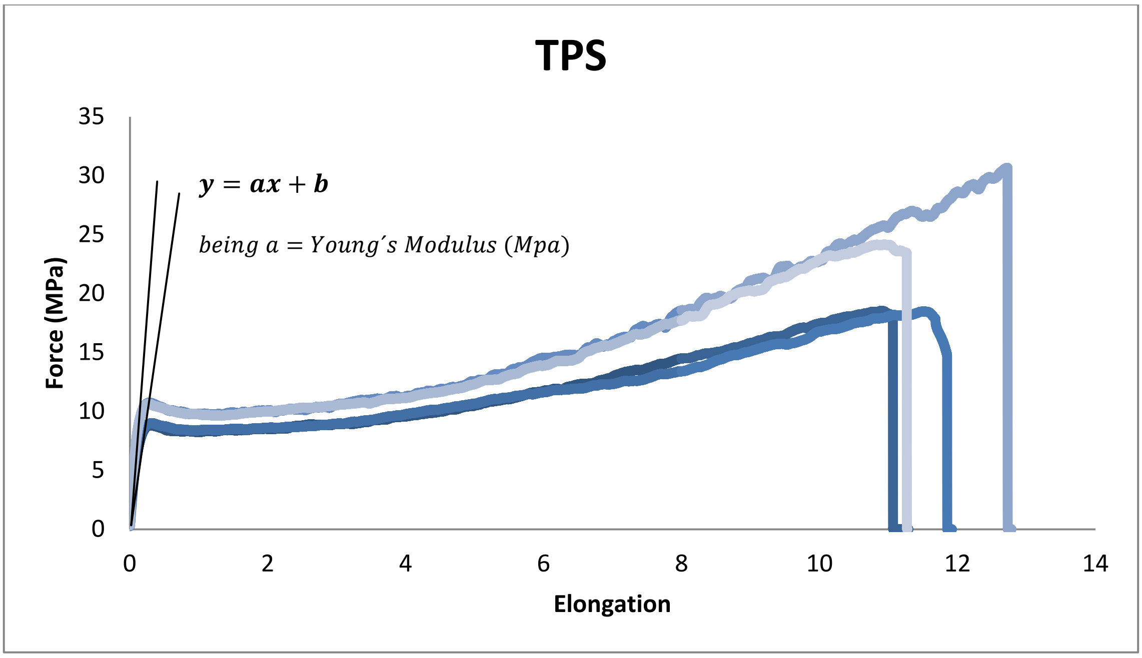
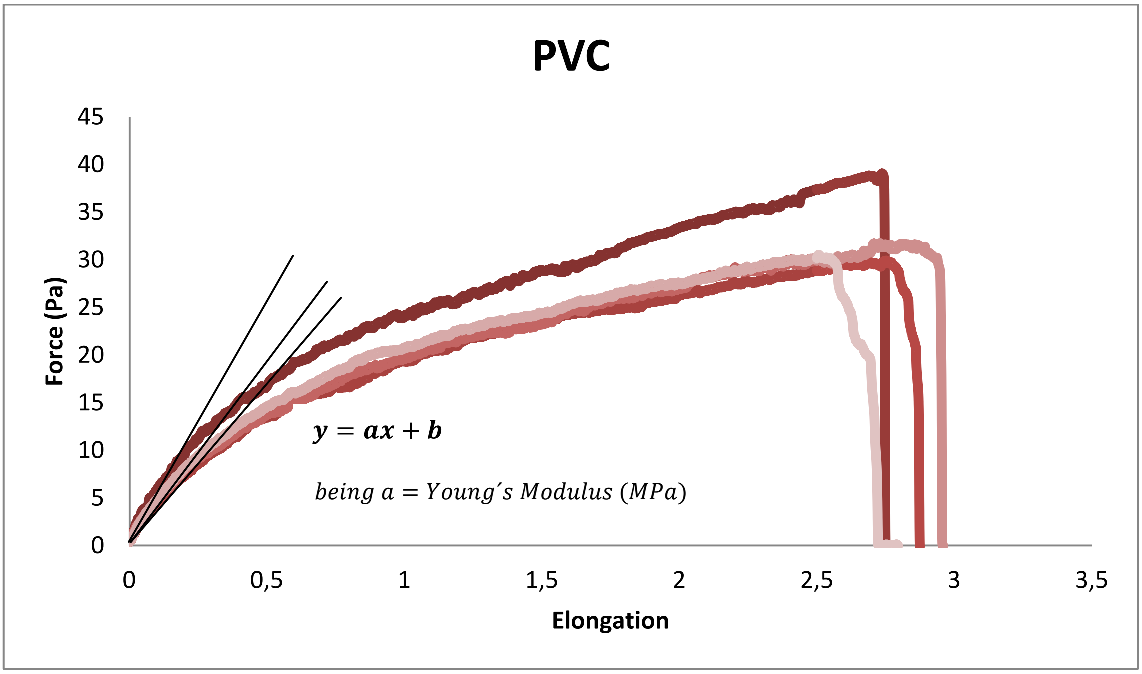
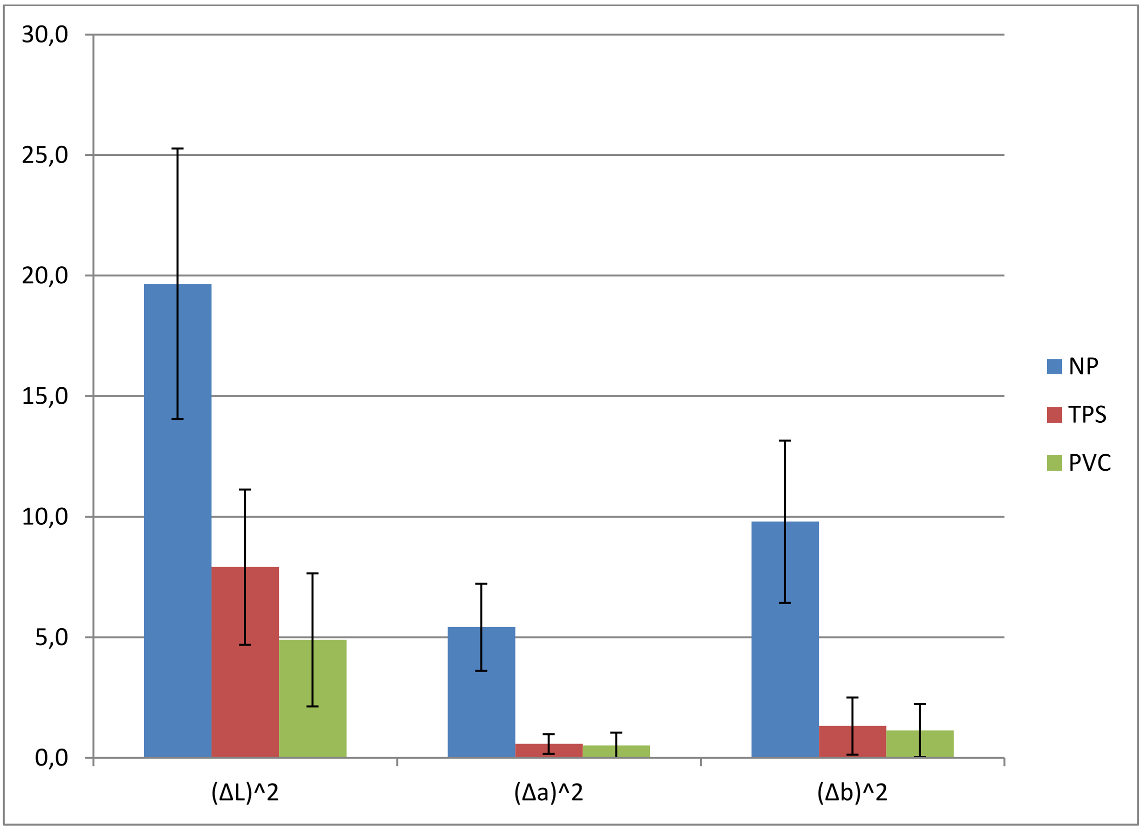
7. ANNEXES

Annex I – Extension curves of TPS-based and PVC films stabilized at 97.3% RH





Annex II – L*, a* and b* parameter changes between the first and last day of testing, for spinach with different type of packaging



NP – No packaging; TPS – Thermoplastic Starch; PVC – Polyvinyl Chlorine