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Propriedades mecânicas e de barreira, solubilidade e microestrutura de filmes de farinha de amaranto modificada com epicloridrina ou misturada com poli(vinil álcool).

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Dedico
Às pessoas que tem me acompanhado
a cada momento da minha vida,
brindando-me seu apoio e
amor incondicional... **a minha família**.
À razão da minha felicidade...
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“A aventura da vida é aprender
A natureza da vida é mudar
O objetivo da vida é crecer
O segredo da vida é ousar
O desafio da vida é superar
A alegria da vida é amar”.

William Arthur

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RESUMO GERAL

O amaranto apresenta-se como uma matéria prima interessante para a elaboração de filmes biodegradáveis por seu conteúdo em amido, proteína e lipídeos. Embora o amaranto não seja nativo do Brasil, seu cultivo está sendo desenvolvido nos solos dos cerrados brasileiros com bons resultados. Isto incentiva o desenvolvimento de alternativas para o uso comercial do amaranto. Filmes elaborados a partir de amaranto da variedade *caudatus* (TAPIA-BLÁCIDO, 2003) resultaram em materiais com excelentes propriedades de barreira, entretanto apresentaram propriedades mecânicas pobres. Estudos desenvolvidos com a variedade que está sendo implantada no Brasil (*Amarantus cruentus*) com adição de ácido esteárico apresentaram as mesmas características (COLLA, 2004). Assim, o objetivo desta pesquisa foi o estudo do processo de elaboração de dois tipos de filmes biodegradáveis: (1) filmes de farinha de amaranto (*A.cruentus*) reticulados com epicloridrina e (2) a partir de blendas de farinha de amaranto (*A.cruentus*) com poli (vinil álcool) (PVA); assim como a avaliação do efeito das duas estratégias (reticulação e PVA) sobre as características mecânicas e de barreira dos biofilmes. O efeito da reação de reticulação (concentração de epicloridrina (1,6-18,4 g/100g farinha), temperatura (39,8-90,2ºC) e tempo de reação (12,8-147,2 minutos)) sobre as propriedades mecânicas e de barreira, foram estudados utilizando a metodologia de superfície de resposta. Para a caracterização dos biofilmes, foram realizadas as seguintes análises: testes mecânicos, de solubilidade em água, de permeabilidade ao vapor de água, microscopia eletrônica de varredura e espectroscopia de infravermelho com transformada de Fourier (FT-IR).

Para os filmes de farinha de amaranto reticulados com epicloridrina, de acordo com a análise estatística das superfícies de resposta, foi observado que a reação de reticulação teve um efeito plasticizante sobre os filmes de farinha de amaranto, ao diminuir a resistência e aumentar a flexibilidade dos mesmos. Além disso, foi evidenciado o forte impacto que tem a temperatura de reação sobre a solubilidade dos filmes, já que ao aumentar a temperatura, aumentou a solubilidade em água, e diminui o pH da solução filmogênica. Foi assim como foram realizadas uma série de reações com temperatura (39,8ºC) e tempo (120 min) fixos, e diferentes concentrações de epicloridrina (1,6-18,4 g/100g farinha). Nestas condições os filmes reticulados não apresentaram diferença significativa ($p<0,05$) nas propriedades mecânicas em relação ao filme de farinha de amaranto não modificado. Mesmo assim, a reticulação melhorou as propriedades de barreira do filme, com uma diminuição considerável da permeabilidade ao vapor de água ($PVA_{média}$ = 2,3 x 10^{-10} g / m s Pa). Além disso, a reticulação reduziu a solubilidade em água ($S_{média}$ = 29%) e manteve a integridade dos filmes durante o teste. A análise do FT-IR mostrou evidência de modificações químicas nos filmes de farinha de amaranto reticulados com epicloridrina. Além disso, as imagens de MEV deram indícios do efeito que a reação de reticulação com epicloridrina teve sobre a microestrutura dos filmes de farinha de amaranto.

Para os biofilmes a partir de blendas de farinha de amaranto com PVA, foram testados seis tipos de PVA com diferentes graus de hidrólise e peso molecular, foi escolhido o PVA 325 por apresentar as melhores propriedades mecânicas (Tensão (TS) 10,21MPa, Elongação (ELO) 89,76%, Força (PF) 9,40N e Deformação (PD) 16,3%). Quando foram avaliadas várias proporções da mistura, farinha de amaranto – PVA (10-50%) foi observado que as propriedades mecânicas, tanto a resistência quanto a flexibilidade, foram aumentadas ao incrementar a concentração de PVA na blenda, os valores foram pelo menos dobrados. A solubilidade em água foi diminuída ao aumentar a proporção de PVA na mistura, até atingir 44% de matéria solúvel para o filme com iguais proporções de farinha – PVA. A análise do FT-IR confirmou a formação de pontes de hidrogênio entre os componentes dos filmes a partir de blendas de farinha de amaranto e poli(vinil álcool).

SUMMARY

The amaranth is an interesting material for biodegradable films production because it contains starch, protein and lipids. Although the amaranth is not native of Brazil, its culture is being developed in Brazilian ground with good results. This stimulates the development of commercial use of amaranth alternatives. Films elaborated from amaranth of the *caudatus* variety (TAPIA-BLÁCIDO, 2003) had resulted in materials with excellent barrier properties, but presented poor mechanical properties. Studies developed with the Brazilian cultivated variety (*Amaranthus cruentus*) with addition of stearic acid had presented the same characteristics (COLLA, 2004). Therefore, the research objective was to study the elaboration process of two types of biodegradable films: (1) Amaranth (*A.cruentus*) flour films crosslinked with epichlorohydrin, and (2) Amaranth (*A.cruentus*) flour films blended with poly(vinyl alcohol) (PVA); as well as to evaluate the effect of both strategies (crosslinking and PVA addition) on the mechanical and barrier characteristics of the films. The effect of the crosslinking reaction (epichlorohydrin concentration (1.6-18.4 g/100g flour), temperature (39.8-90.2ºC) and time (12.8-147.2 minutes)) on the mechanical and barrier properties was studied using the response surface methodology (RSM). For the biofilms characterization, the following essays were carried out: mechanical tests, solubility in water, water vapor permeability, scanning electron microscopy (SEM), and Fourier transformed infrared spectroscopy (FT-IR).

The statistics analysis (RSM) for amaranth flour films crosslinked with epichlorohydrin, showed that the crosslinking reaction provided a plasticizing effect on the *A.cruentus* flour films, as it reduced the resistance and increased the flexibility of crosslinked films. Moreover, it was evident the strong impact that the reaction temperature had on the films solubility: at high reaction temperatures, the solubility in water increased, and the pH of the solution decreased. Thus, a series of crosslinking reactions with fixed temperature (39.8ºC) and time (120 min), and different epichlorohydrin concentrations (1.6-18.4 g/100g flour) were developed. Under these conditions the crosslinking reaction did not improve the mechanical performance of the *A.cruentus* flour films. However, it considerably reduced the solubility in water ($S_{average}$ = 29%) and the water vapor permeability ($WVP_{average} = 2.3 \times 10^{-10}$ g / m s Pa) of the crosslinked films. It also maintained the film integrity during solubility test. FT-IR analysis showed evidence of chemical modification in the crosslinked *A.cruentus* flour films. SEM micrographs demonstrated the effect of the crosslinking reaction on the film microstructure.

Six types of PVA with different hydrolysis degrees and molecular weight were tested for biofilms from amaranth flour blended with PVA. PVA 325 was chosen due to it superior mechanical performance (TS 10.21MPa, ELO 89.76%, PF 9.40N and PD 16.3%). When blended films of amaranth flour - PVA (10-50%) were evaluated, all mechanical properties (TS, ELO, PF and PD) were enhanced as the PVA 325 content was increased, values were at least doubled. The solubility in water (S) of the blended films decreased as the PVA content increased; reaching 44% of soluble mater for equal proportion blended film. The formation of hydrogen bonds between the blend components was confirmed by the FT-IR spectra analysis.

INTRODUÇÃO GERAL

Quase todos os alimentos, sejam frescos ou processados, requerem algum tipo de embalagem. A embalagem é utilizada para preservar e proteger o alimento da deterioração oxidativa e microbiana, e consequentemente aumentar sua vida de prateleira. O uso de plásticos sintéticos, como as poliolefinas e poliésteres, como material de embalagem, é grande por sua disponibilidade em grandes quantidades e baixo custo, assim como por suas características funcionais, como as propriedades mecânicas, barreira aos gases e compostos aromáticos, e a selagem térmica (THARANATHAN, 2003).

No entanto, o uso crescente dos polímeros sintéticos preocupa pelos problemas de contaminação ambiental decorrentes, por não serem biodegradáveis. Além disso, a sua reciclagem consome grandes quantidades de energia térmica (PARRA et al., 2004). O interesse de manter, ou melhorar, a qualidade dos alimentos, e ao mesmo tempo reduzir o desperdício de embalagens, tem encorajado a exploração de novos materiais de embalagens, como os filmes biodegradáveis a base de recursos renováveis (OLABARRIETA, 2005).

Os materiais mais comuns usados na produção de filmes e coberturas comestíveis são os polissacarídeos, as proteínas e os lipídeos. Muitos desses materiais têm boas propriedades de formação de filmes. Os filmes a base de polissacarídeos (amido, celulose, gomas) são hidrofílicos e provêem uma barreira eficiente aos óleos e lipídeos, mas apresentam pouca barreira ao vapor de água. Os filmes a base de proteínas (gelatina, glúten, zeína) são altamente interessantes, já que conferem mais propriedades funcionais potenciais. Os filmes a base de lipídeos têm excelentes propriedades de barreira ao vapor de água, mas podem causar problemas organolépticos e de textura devido a sua oxidação e sabor de cera (GUILBERT et al., 1997).

O amaranto apresenta-se como uma matéria prima interessante para a elaboração de filmes biodegradáveis por seu conteúdo em amido, proteína e lipídeos. O interesse crescente pelas espécies de amaranto é resultado da resistência da cultura a condições ambientais adversas, sua utilidade como fonte de nutrientes e sua grande tolerância a solos áridos e pobres, não aptos para o cultivo de outros cereais (CALZETTA et al., 1999). Embora o amaranto não seja nativo do Brasil, o seu cultivo está sendo desenvolvido nos solos dos cerrados brasileiros com bons resultados (variedade “BRS

Alegria", do *Amaranthus cruentus*). Isto incentiva o desenvolvimento deste estudo que tem como objetivo central apresentar alternativas de uso comercial do amaranto.

Filmes elaborados a partir de farinha de amaranto da variedade *caudatus* (TAPIA-BLÁCIDO, 2003) apresentaram excelentes propriedades de barreira, entretanto com propriedades mecânicas pobres. Filmes desenvolvidos com uma variedade que está sendo implantada no Brasil (*Amarantus cruentus*) apresentaram as mesmas características, mas com solubilidade menor, pela adição de ácido esteárico (COLLA, 2004).

Existem várias estratégias para melhorar as propriedades dos filmes, entre elas o uso de blendas, a reticulação e a adição de plastificantes (OLABARRIETA, 2005). A preparação de filmes compostos é uma alternativa para se melhorar as propriedades mecânicas dos biofilmes. Um exemplo é a adição do poli (vinil álcool) (LAWTON, 1996; SIDDARAMAIAH et al., 2004; RAJ et al., 2003; YOOH et al., 2006b; PARK et al., 2005; JAYASEKARA et al., 2004; FOLLAIN et al., 2005b; SREEDHAR et al., 2005; YIN et al., 2005; IMAM et al., 2005; KIM et al., 2002). A incorporação do poli (vinil álcool) (PVA), um polímero biodegradável, cristalino e solúvel em água, altera as propriedades termodinâmicas do amido por modificar a estrutura do polímero a nível molecular e morfológico. O PVA é amplamente utilizado por sua flexibilidade e boa capacidade de formar filmes (SREEDHAR et al., 2005). Os filmes compostos de amido-PVA são uns dos mais populares plásticos biodegradáveis, amplamente usados em embalagens e no setor agrícola (SREEDHAR et al., 2006).

Uma outra alternativa para melhorar as propriedades dos filmes é a modificação da estrutura do polímero por reticulação das cadeias do mesmo. A presença de grupos reativos no polímero torna possível a reticulação de cadeias adjacentes por três métodos:

(a) *Tratamento químico*: reticulação de amidos por epicloridrina (RIOUX et al., 2004; DELVAL et al., 2004; LLOYD & KIRST, 1963; SREEDAHAR et al., 2006; KIM et al., 2002; KIM & LEE, 2002); bórax (SREEDHAR et al., 2005), glutaraldeído (PARRA et al., 2004), hexametoximetilmelamina (IMAM et al., 2005) e ácido bórico (YIN et al., 2005).

(b) *Tratamento enzimático*: reticulação de proteínas com a enzima transglutaminase (OH et al., 2004; KOLDZIEJSKA et al., 2006; DI PIERRO et al., 2006; CHAMBI & GROSSO, 2006; DI PIERRO et al., 2005; LIM et al., 1998; LARRE et al., 2000; LIM et al., 1999).

(c) *Tratamentos físicos*: reticulação de amido e proteína de soja por tratamento térmico e a radiação UV e gama (FOLLAIN et al., 2005b; SABATO et al., 2001; LE TIEN et al., 2000).

A reticulação química se apresenta como a opção mais viável por ser a mais econômica e com diversidade de alternativas, sendo a reticulação com epicloridrina (ECH) a mais usada para o amido. A reação do amido com ECH tem sido amplamente estudada (KARTHA & SRIVASTAVA, 1985a e 1985b; KUNIAK & MARCHESSAULT, 1972; HAMERSTRAND et al., 1960) e apresenta-se como um método relativamente simples para obter amidos modificados (DELVAL et al., 2004).

Dessa forma, o presente estudo teve como objetivo empregar duas estratégias para melhorar as propriedades mecânicas e de barreira de biofilmes a base de farinha de amaranto plasticizados com glicerol: (1) a reticulação química do amido com epicloridrina na solução filmogênica, e (2) a adição de poli (vinil álcool) (PVA).

REVISÃO BIBLIOGRÁFICA

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2.1 Amaranto

O amaranto (*Amaranthus*) é uma planta dicotiledônea da família das *Amaranthaceae*, originário dos Andes e do planalto mexicano. Esta planta, apesar de ter características de cereal, não pode ser classificada como tal, por possuir aspectos botânicos (presença da inflorescência tipo panícula) e características nutricionais (alto teor de proteína, aminoácidos sulfurados e lisina) e por isto é considerado como pseudo-cereal. As partes comestíveis do amaranto envolvem as folhas e os grãos, sendo estes últimos os mais explorados econômica e cientificamente (AULER, 2002).

O amaranto tem grande capacidade de adaptação climática, podendo ser cultivado desde o nível do mar até em altitudes de 3500 metros. As espécies *A.caudatus, A.cruentus* e *A.hypochondriacus* têm sido objeto de estudos em diversas partes do mundo, principalmente por suas características nutricionais e requerimentos para produção (SPEHAR & TEIXEIRA, 2004).

Em relação ao cultivo do amaranto no Brasil, a Embrapa Cerrados-DF lançou a variedade “BRS Alegria”, do *Amaranthus cruentus*. Esta variedade possui um ciclo curto e rápido crescimento, apresentando duas utilidades principais, proteção do solo e produção de grãos e forragem na entressafra. Constitui-se também em boa opção para rotação de culturas, pois possibilita quebra do ciclo milho/soja. Pode ser cultivada em sucessão a qualquer dessas culturas, uma vez que não é gramínea nem leguminosa, nem suscetível às pragas dessas culturas (SPEHAR et al., 2003).

O componente mais importante do grão de amaranto é o amido, perfazendo 48 a 69% (base seca) do grão. Seus grânulos são pequenos (0,75–3,0 µm de diâmetro) e sua temperatura de gelatinização varia na faixa de 70 - 90℃, com propriedades funcionais únicas em aplicações para alimentos, como substitutos de gordura, espessantes de alimentos e plásticos biodegradáveis (HOOVER et al., 1998). A maioria das variedades cultivadas de amaranto contém amido ceroso, com alto conteúdo de amilopectina (98-100%).

O amaranto apresenta uma composição balanceada de aminoácidos, com alto conteúdo de lisina e aminoácidos sulfurados. As pontes intramoleculares de dissulfeto proporcionam rigidez e estabilidade na estrutura das proteínas, fazendo com que estes sejam capazes de formar espumas, filmes e emulsões, sistemas nos quais as propriedades de superfície são desenvolvidas (KINSELLA & PHILLIPS, 1989).

SALCEDO-CHÁVEZ et al. (2002) indicam que a maior solubilidade das proteínas de *A.cruentus* está em pH superior a 10.

Os lipídeos perfazem 6 a 8% do peso total do grão, sendo que 75,44% dos ácidos graxos são insaturados, ricos em ácido linoléico (ESCUDERO et al., 2004).

Por seu conteúdo em amido, proteína e lipídeos, o amaranto apresenta-se como uma matéria prima interessante para a elaboração de filmes biodegradáveis.

2.2 Farinha de amaranto

A farinha de amaranto pode ser obtida por moagem seca ou úmida. No primeiro caso, os grãos de amaranto secos são moídos até a obtenção de pó ligeiramente amarelado. Na moagem úmida, a farinha é produzida utilizando-se uma solução de NaOH 0,25% para a maceração do grão. Após a moagem, o material é peneirado para separar o líquido que contém o amido, proteínas e lipídeos suspendidos, das fibras. O líquido é então neutralizado e centrifugado antes da secagem e obtenção da farinha sem fibras (TAPIA-BLÁCIDO, 2003).

A composição da farinha de amaranto da variedade brasileira (*A.cruentus*) obtida por moagem úmida (COLLA, 2004) é: 15,72% de proteína, 10,81% de lipídeos, 70,53% de amido (15,70% amilose e 84,30% amilopectina), 2,94% de cinzas e 6,10% de umidade.

De acordo com AULER (2002) a composição da farinha de amaranto (*A.cruentus*) obtida por moagem seca foi de 14,64% de proteína, 6,65% de lipídeos, 66,40% de amido, 3,67% de cinzas e 7,64% de umidade. Segundo ESCUDERO (2004), a farinha de *A.cruentus* preparada por moagem seca dos grãos de amaranto (45ºC por 48h) e peneirados (200µm) apresentou a composição seguinte: 16,6% de proteína, 10,30% de umidade, 8,77% de lipídeos, 3,35% de cinzas, 9,83% de fibra e 61,45% amido, sendo os valores expressos em base seca.

De acordo com os diferentes trabalhos acima citados, o componente mais importante da farinha é o amido com baixo conteúdo de amilose (tipo ceroso). Entretanto, o conteúdo de proteínas e lipídeos na farinha proporcionam boas propriedades de barreira. A farinha de amaranto comporta-se como um sistema complexo que é o resultado das interações dos diversos biopolímeros: amido e proteína, e a fase lipídea (TAPIA-BLÁCIDO, 2003).

2.3 Biofilmes

Os biofilmes são materiais de fina espessura, preparados a partir de macromoléculas biológicas, que agem como barreira a elementos externos (umidade, gases e óleos) e conseqüentemente, protege o produto e aumentam a sua vida de prateleira (KROCHTA & MULDER-JOHNSTON, 1997).

Geralmente são utilizados dois tipos de biomoléculas na preparação de filmes biodegradáveis, os hidrocolóides e os lipídeos. Os hidrocolóides, como polissacarídeos (amido, celulose, gomas) ou proteínas (gelatina, glúten, zeína) por serem hidrofílicos apresentam uma pobre barreira à umidade, sendo essa propriedade compensada se adicionar lipídeos, os quais apresentam uma boa barreira à umidade (THARANATHAN, 2003).

Cada tipo desses materiais utilizados na formulação dos filmes tem suas vantagens e desvantagens. Dessa forma, muitas coberturas e filmes atualmente são produzidos através da combinação dos mesmos (BALDWIN et al., 1997).

Os filmes biodegradáveis são preparados geralmente pelo método “casting”, onde a solução aquosa é depositada numa superfície apropriada e secada posteriormente. A formação do filme envolve ligações inter e intramoleculares, ou a reticulação das cadeias dos polímeros, formando assim uma rede tridimensional semi rígida que retém e imobiliza o solvente. O grau de coesão depende da estrutura do polímero, do solvente usado, da temperatura e a presença de outras moléculas, como os plastificantes (THARANATHAN, 2003).

Qualquer que seja o processo de produção, a transformação da solução filmogênica em filmes ou coberturas é conseqüência de interações intermoleculares, que se traduz em forças estruturais (CARVALHO, 1997).

2.3.1 Biofilmes de farinha de amaranto

Uma tendência na área de elaboração de biofilmes é a combinação de diferentes materiais biológicos (polissacarídeos, proteínas e lipídeos) para melhorar as propriedades mecânicas e de barreira (PARK, 1999). A farinha de amaranto se apresenta como uma mistura natural desses componentes. TAPIA-BLÁCIDO et al. (2005) elaboraram filmes a partir da farinha de amaranto *(A.caudatus)* com 4% de farinha, 22,5% de glicerol, pH 10,7 e temperatura de processo igual a 82℃. Os filmes apresentaram uma coloração amarelada, moderada opacidade, alta flexibilidade, baixa resistência mecânica, solubilidade intermédia (42,25%) e excelente propriedade de barreira ao vapor de água.

Observou-se que as interações amido – proteína – lipídeos influenciaram as características finais dos filmes, já que os filmes obtidos a partir do amido de amaranto apresentaram maior solubilidade. COLLA et al. (2006) estudaram a elaboração de biofilmes a partir de farinha de amaranto de *Amarantus cruentus*, variedade que está sendo implantada no Brasil, com adição de ácido esteárico. As melhores propriedades de barreira foram obtidas para os filmes contendo 10% de ácido esteárico e 26% de glicerol, sob condições de agitação de 12000 rpm. Além disso, a solubilidade foi reduzida a 15,2%.

2.4 Propriedades de filmes biodegradáveis e / ou comestíveis

2.4.1 Barreira ao vapor de água

A migração de vapor de água é um dos principais fatores de alteração da qualidade sensorial e da estabilidade da estocagem dos alimentos (GONTARD et al., 1994). A transferência de água em materiais poliméricos ocorre por difusão molecular. O processo de difusão em sistema polímero – solvente depende do tamanho, natureza química, polaridade e configuração da molécula penetrante e do movimento molecular da cadeia do polímero na matriz do filme (KESLER & FENNEMA, 1986).

A permeabilidade ao vapor de água é definida pela ASTM E96-90 como a taxa de transmissão de vapor de água por unidade de área através do filme, de espessura conhecida, induzida por um gradiente de pressão entre duas superfícies específicas, de temperatura e umidade relativa especificada (ASTM, 1990).

O coeficiente de permeabilidade não é só função da estrutura química do polímero, também depende de fatores como a densidade, cristalinidade, reticulação, plastificante, sensibilidade à umidade e temperatura. Por isso, é preciso manter as condições do teste o mais similares possível para não afetar o resultado (OLABARRIETA, 2005).

Na Tabela 2.1 apresentam-se alguns valores de permeabilidade ao vapor de água para filmes biodegradáveis.

Tabela 2.1 Permeabilidade ao vapor de água (PVA) de biofilmes

Material	PVA ($\times 10^{-10}$ g / m s Pa)	Referência
Farinha de amaranto (*A.caudatus*) com glicerol (22.5%)	0,74	TAPIA-BLÁCIDO et al. (2005)
Farinha de amaranto (*A.cruentus*) com glicerol (26%) e ácido esteárico (10%)	0,89	COLLA et al. (2006)
Amido de mandioca (*M.esculeta*)	0,26 – 1,36	VICENTINI, et al. (2005)
Proteína muscular de tilapia com glicerol	1,11 – 2,50	PASCHOALICK et al. (2003)
Gelatina bovina e suína com sorbitol	0,56 – 1,11	SOBRAL, et al. (2001)
Gelatina com misturas de glicerol - sorbitol	1,50 – 2,66	THOMAZINE, et al. (2005)
Gelatina com plasticizantes (10-30%)	0,74 – 1,63	VANIN et al. (2005)

2.4.2 Propriedades mecânicas

Um filme com propriedades de barreira adequadas pode ser ineficiente se as propriedades mecânicas não permitem a manutenção da integridade do filme durante o processo de manipulação, empacotamento e transporte. Os biofilmes devem ser resistentes à ruptura e à perfuração, fazendo que o alimento não perca a sua proteção por manuseio ou armazenamento (SARMENTO, 1999). As propriedades mecânicas estão diretamente relacionadas com a natureza do material filmogênico utilizado e com a coesão da estrutura da matriz polimérica, que está relacionada com a distribuição e concentração de ligações inter e intramoleculares das cadeias do polímero (CUQ et al., 1998).

As principais propriedades mecânicas dos filmes são a resistência à tração, que expressa a tensão máxima desenvolvida pelo filme durante um teste de tração, e a elongação, que é a capacidade do filme em esticar. Segundo GONTARD (1994), uma alta resistência à tensão é requerida, enquanto que o valor da elongação depende do tipo de aplicação do filme, já que para manter a sua integridade e propriedades de barreira, um filme deve tolerar a tensão normal encontrada durante a sua aplicação, além do transporte e manuseio.

Os testes utilizados para avaliar as propriedades mecânicas são o teste de tração, onde podem ser derivadas as propriedades de resistência à tração e elongação na ruptura; além do teste de perfuração, do qual se pode obter valores de força e deformação na ruptura. Os testes mecânicos são geralmente conduzidos de acordo com métodos padrão para determinação de propriedades mecânicas de filmes plásticos finos (ASTM, 1997). Valores de propriedades mecânicas (Tensão, TS; Elongação, ELO; Força, PF e Deformação, PD) de alguns biofilmes são mostrados na Tabela 2.2

Tabela 2.2 Propriedades mecânicas de biofilmes

Material	TS (MPa)	ELO (%)	PF (N)	PD (%)	Referência
Farinha de amaranto (*A.caudatus*) com glicerol (22.5%)	0,3 – 3	12 – 168	0,5 – 4	3,4 – 37	TAPIA et al. (2005)
Farinha de *A.cruentus* com glicerol (26%) e ácido esteárico (10%)	0,8 – 3	74 – 620	1 – 2		COLLA et al. (2006)
Amido de mandioca (M.esculeta)			1,6 – 22	0,6 – 1	VICENTINI, et al. (2005)
Gelatina com misturas de glicerol – sorbitol	7 – 48	6 – 127	8 – 27	2 – 18	THOMAZINE, et al. (2005)
Gelatina com plasticizantes (10-30% glicerol)			9 – 27	1 –7	VANIN et al. (2005)
Queratina com glicerol (1 - 9%)	2 – 17	1,7 – 32			MOORE (2006)
Polietileno de baixa densidade (LDPE) com amido de batata	9 – 15	175 – 253			KIM & LEE (2002)
Amido com PVA, reticulado com bórax.	15 – 30	5 – 30			SREEDHAR et al. (2005)
Amido (HSPAN) com PVA, reticulado com epicloridrina.	12 – 35	2-13			KIM et al. (2002)
Amido de milho com PVA e acido cítrico	5 - 20	20 - 150			PARK et al. (2005)
Amido em diferentes umidades relativas	10 – 60	1 - 400			LAWTON (1996)
Amido (70% amilose) reticulado com epicloridrina	15 - 50	2 – 12			RIOUX et al. (2002)

2.4.3 Solubilidade em água

A solubilidade em água de filmes biodegradáveis é de grande importância, uma vez que a grande maioria dos filmes elaborados a partir de carboidratos e proteínas possuem grande afinidade com a água (VEIGA-SANTOS, 2004).

Além disso, a solubilidade é uma propriedade importante dos filmes no que se refere ao seu emprego, pois algumas aplicações requerem insolubilidade para manter a integridade do produto, como nos casos de utilização como proteção de alimentos onde a atividade de água é alta, ou quando o filme é submetido ao contato com água durante o processamento do alimento embalado (GONTARD et al., 1992).

A solubilidade também influencia a propriedade de barreira ao vapor de água dos filmes. Para se obter uma baixa permeabilidade ao vapor de água (dentro de uma grande faixa de umidade relativa), torna-se necessária a utilização de material insolúvel ou pouco solúvel em água (FAKHOURI, 2002).

Valores de solubilidade de alguns biofilmes são mostrados na Tabela 2.3. Pode-se observar que a maioria dos valores são altos, o que limita o uso dos biofilmes. Ainda em alguns casos verifica-se a desintegração do filme devido a sua solubilização. A manutenção da integridade do filme ao estar em contato com água é de grande importância nas possíveis aplicações dos biofilmes como material de embalagem.

Tabela 2.3 Solubilidade em água de biofilmes

Material	Solubilidade (%)	Referência
Farinha de amaranto (*A.caudatus*) com glicerol (22.5%)	40 – 63	TAPIA et al. (2005)
Farinha de amaranto (*A.cruentus*) com glicerol (26%) e ácido esteárico (10%)	15	COLLA et al. (2006)
Queratina com glicerol (1 - 9%)	31 – 51	MOORE et al. (2006)
Amido (HSPAN) com PVA, reticulado com epicloridrina.	5 – 37	KIM et al. (2002)
Amido de milho com PVA e acido cítrico	20 – 55	PARK et al. (2005)

2.4.4 Microscopia eletrônica

A microestrutura dos alimentos e dos seus componentes está sendo reconhecida agora como um pré-requisito necessário para compreender as suas propriedades. Todo pesquisador que têm interesse em descrever, predizer e controlar o comportamento dos materiais no alimento percebe a importância do conhecimento profundo da maneira em que os componentes estão organizados (AGUILERA & STANLEY, 1990).

Duas técnicas utilizadas no estudo da microestrutura dos filmes biodegradáveis são a microscopia eletrônica de transmissão (MET) e a microscopia eletrônica de varredura (MEV).

2.4.4.1 Microscopia eletrônica de transmissão (MET)

A disponibilidade do microscópio eletrônico de transmissão (MET) fez centrar a atenção na organização estrutural dos materiais biológicos e produz uma mudança no paradigma da maneira em que tais materiais foram pensados.

O MET tem sido a ferramenta a mais importante no estudo da estrutura biológica. Na sua forma mais simples, o MET assemelha-se a um microscópio ótico (MO) invertido, onde um injetor de elétrons (filamento de tungstênio) é aquecido e emite um feixe de elétrons estreito que viaja a alta velocidade. A voltagem aplicada para conseguir esta aceleração é da ordem de 40-100 kV. O feixe de elétrons toma o lugar da lâmpada no MO e age como a fonte de iluminação.

A diferença principal entre o microscópio ótico e eletrônico é que os elétrons precisam de vácuo elevado (10^{-4} - 10^{-5} torr) a fim de viajar as distâncias necessárias na microscopia eletrônica. O requisito de um ambiente a vácuo, junto com a necessidade de que um poderoso feixe de elétrons passe através do material observado, limita profundamente os tipos de espécimes que podem ser examinados: devem estar completamente secos, ser bastante fortes para resistir aos danos do feixe e para ser cortados finamente. Assim, a integridade da amostra é comprometida a fim de aproveitar as vantagens da elevada ampliação (300.000x) possível com o MET (AGUILERA & STANLEY, 1990).

2.4.4.2 Microscopia eletrônica de varredura (MEV)

A análise de microscopia mais utilizada para avaliar a microestrutura de filmes biodegradáveis é a microscopia eletrônica de varredura (VEIGA-SANTOS, 2004).

A microscopia eletrônica de varredura (MEV) faz possível ver um amplo espectro da organização estrutural dos materiais biológicos. Em várias formas, este instrumento combina as melhores características do microscópio ótico e o de transmissão eletrônica.

A preparação da amostra é mais fácil e introduz poucos artefatos. Tanto as características superficiais quanto as internas podem ser estudadas, dependendo das técnicas de preparação utilizada. É possível usar uma larga escala de ampliações (20x - 100000x) e a MEV pode atingir uma profundidade de campo 500 vezes maior que a do microscópio ótico, em ampliações equivalentes (AGUILERA & STANLEY, 1990).

Apesar das múltiplas vantagens, os inconvenientes do MEV permanecem, já que as amostras precisam ser desidratadas totalmente para serem expostas ao vácuo elevado, e o material é bombardeado por um feixe de elétrons que pode danificá-lo (AGUILERA & STANLEY, 1990). Uma alternativa para diminuir a degradação do material é utilizar um feixe de elétron de intensidade bem baixa, 5 a 10 kV. Para uma melhor visualização da imagem é necessário metalizar a amostra com uma fina camada de ouro, já que a maioria dos materiais biodegradáveis é a base de carbono (VEIGA-SANTOS, 2004).

2.4.5 Espectroscopia de infravermelho com transformada de Fourier (FT-IR)

A radiação infravermelha na faixa aproximada de 10000 a 100 cm^{-1} converte-se, quando absorvida em energia de vibração molecular. O processo é quantizado, porém o espetro vibracional costuma aparecer como uma série de bandas, são as bandas de vibração-rotação que ocorrem entre 4000 e 400 cm^{-1}, as que se utilizam para a identificação dos compostos. A frequência ou o comprimento de onda de uma absorção depende das massas relativas dos átomos, das constantes de força das ligações e da geometria dos átomos. As intensidades das bandas podem ser expressas como transmitância (T) ou absorbância (A) (SILVERSTEIN & WEBSTER, 2000).

A espectroscopia com transformações de Fourier (FT-IR), tem várias vantagens sobre os instrumentos de dispersão. Radiação contendo todos os comprimentos de onda de interesse (5000-400 cm^{-1}) é separada em dois feixes. Um deles percorre uma distância fixa e o outro uma distância variável (espelho móvel). A transformação de

Fourier em posições sucessivas do espelho dá origem ao espectro completo de infravermelho da amostra (SILVERSTEIN & WEBSTER, 2000).

Existem vantagens no uso de transformações de Fourier. Como não se usam monocromadores, a totalidade da faixa de radiação passa simultaneamente pela amostra com enorme ganho de tempo. Isto permite resoluções extremamente altas (< 0,001 cm^{-1}). Além disso, como os dados sofrem conversão analógico-digital, os resultados são manipulados fácilmente. O resultado de várias varreduras é combinado para diminuir o ruído, e espectros excelentes podem ser obtidos com pouca amostra (SILVERSTEIN & WEBSTER, 2000).

Com o advento dos espectroscópios de FTIR e as suas vantagens concomitantes da velocidade e sensibilidade, a espectroscopia de absorção no infravermelho tem se tornado uma das ferramentas importantes para a caracterização e identificação de grupos estruturais, por meio das interações que ocorrem a nível molecular (FERRARO & KRISHNAN, 1990).

O espectro infravermelho do amido tem mostrado que é sensível às mudanças na estrutura a nível molecular, como a conformação da cadeia do amido, a cristalinidade e o processo de retrogradação, assim como o conteúdo de água. A espectroscopia FTIR relacionada a métodos de amostragem, como a reflexão total atenuada, e a possibilidade de melhorar a resolução por deconvolução, tem ampliado os campos de aplicação da informação espectral do infravermelho (VAN SOEST et al., 1995).

A técnica conhecida como reflexão total atenuada ou espectroscopia de reflexão interna permite a obtenção de espectros qualitativos de sólidos, independentemente da espessura. Esta técnica se baseia no fato de um feixe de luz refletido internamente pela superfície de um meio transmissor penetra uma pequena distância além da superfície refletora e retorna ao meio transmissor durante o processo de reflexão (SILVERSTEIN & WEBSTER, 2000).

2.5 Estratégias para melhorar as propriedades dos biofilmes

Existem várias estratégias para melhorar as propriedades dos filmes. A maioria dessas são modificações no pré-tratamento, onde as mudanças são feitas na solução filmogênica. Os métodos mais comuns são: a adição de plastificantes, o uso de blendas e a reticulação (OLABARRIETA, 2005).

2.5.1 Plastificante

Os agentes plastificantes são substâncias de baixo peso molecular que reduzem as interações intermoleculares ao acoplar-se entre as cadeias da rede polimérica. A composição, tamanho, e forma da molécula do plasticizante influenciam a sua habilidade de interagir com as cadeias dos polímeros e ligar-se às moléculas de água, causando assim um maior efeito plasticizante, já que a água é um plasticizante efetivo nos filmes de biopolímeros hidrofílicos (SOTHORNVIT & KROCHTA, 2001). Os mais comuns são os polióis, como o polietilenoglicol, o glicerol e o sorbitol. O mais usado é o glicerol, que é um plastificante com alto ponto de fusão, solúvel em água, polar, não-volátil, usado como adoçante na indústria de alimentos (OLABARRIETA, 2005). A adição de plastificantes à solução filmogênica reduz a fragilidade do filme, e aumenta a flexibilidade e extensibilidade do mesmo. A incorporação desse agente modifica a organização molecular tridimensional da rede polimérica, diminuindo as forças de atração intermoleculares e aumentando o volume livre do sistema. A rede torna-se menos densa devido à redução das forças entre as cadeias, ocorrendo assim, a diminuição da temperatura de transição vítrea (Tg) e o aumento da mobilidade da estrutura polimérica (MALI et al., 2002).

Porém, a adição de plastificante pode aumentar a permeabilidade do filme, dependendo do tipo e quantidade adicionada. Esse aumento na permeabilidade é indesejável para a qualidade dos alimentos. Por isso é preciso otimizar o uso do plastificante para oferecer um balanço correto entre as propriedades mecânicas e de barreira do filme. Em resposta aos problemas causados pelos plastificantes de baixo peso molecular, é possível substitui-los com compostos de peso molecular maior, que sejam hidrofóbicos. Substâncias estudadas como substitutos são os anfifílicos, incluindo os ácidos graxos (ácido láurico, esteárico e oléico) (OLABARRIETA, 2005).

2.5.2 Blendas

A preparação de filmes a base de blendas é um método que procura combinar as vantagens de cada um dos componentes da blenda, para obter propriedades melhores daquilas esperadas para cada substância individualmente (OLABARRIETA, 2005). Misturas de amido com polímeros têm sido testadas amplamente, e mais especificamente com altos conteúdos de poliésteres biodegradáveis ou poli (vinil álcool) (PVA). A tensão e elongação na ruptura geralmente são melhoradas para misturas com mais de 25% de polímero sintético na sua formulação. É possível diferenciar três sistemas que têm sido desenvolvidos: (a) misturas de amido com polímeros incompatíveis (poliolefinas), onde é preciso a adição de um agente compatibilizante, (b) mistura de de amido com polímeros mais ou menos compatíveis (poliésteres biodegradáveis) onde o polímero atua como recheio, e (c) misturas de amido com polímeros hidrossolúveis (PVA) que é o único caso que têm demonstrado a capacidade de melhorar tanto a tensão quanto a elongação dos filmes a base de blendas (FOLLAIN et al., 2005a).

2.5.2.1 Poli (vinil álcool) (PVA)

O PVA ($(H_2C{=}CH\text{-}OH)_n$) é um polímero biodegradável, cristalino e solúvel em água, que muda as propriedades termodinâmicas do amido ao modificar a estrutura do polímero a nível molecular e morfológico. O poli(vinil álcool) é utilizado por sua flexibilidade e boa capacidade de formar filmes (SREEDHAR et al., 2005).

O PVA é produzido a partir do monômero de acetato de vinilo num processo de várias etapas. No início, o monômero é polimerizado em poli (vinil acetato) para logo ser convertido em poli (vinil álcool) ao ser hidrolisado com NaOH. Os tipos de PVA variam tanto em seu peso molecular (grau de polimerização), quanto em seu grau de hidrólise. O peso molecular é expresso geralmente em termos da viscosidade da solução, e segundo o fabricante, ao aumentar a viscosidade, aumenta-se a resistência do filme. O grau de hidrólise é expresso em porcentagem de moles hidrolisados, e esta propriedade afeta a sensibilidade à água dos filmes, aumentando a resistência à água com o incremento do grau de hidrólise devido ao aumento nas pontes de hidrogênio (CELANESE, 2002). Na Tabela 2.4 apresentam-se as propriedades dos tipos de PVA usados no presente estudo.

Tabela 2.4 Propriedades dos tipos de poli (vinil álcool) em solução aquosa a 4%, 20ºC

Tipo de PVA	Grau de hidrólise (%)	Viscosidade (cps)	pH
Celvol 107	98,0-98,8	5,5-6,6	5,0-7,0
Celvol 325	98,0-98,8	28,0-32,0	5,0-7,0
Celvol 350	98,0-98,8	62-72	5,0-7,0
Celvol 205	87,0-89,0	5,2-6,2	4,5-6,5
Celvol 523	87,0-89,0	23,0-27,0	4,5-6,5
Celvol 540	87,0-89,0	45,0-55,0	4,5-6,5

Fonte: Celanese, 2002

ABD EL-KADER & ABDEL HAMIED (2002) e ABD EL-KADER et al. (2002) estudaram o efeito do peso molecular sobre as propriedades mecânicas dos filmes comparando-os com filmes de polietileno comerciais. Foi observado que ao aumentar o peso molecular do poli (vinil álcool), diminuiu-se a resistência mecânica dos filmes por causa do aumento no grau de desordem das cadeias (diminuição da cristalinidade), resultado que contradiz o observado pelo fabricante do PVA. Além disso, esses autores observaram que os filmes de PVA (5000 g/mol) apresentaram forças na ruptura e módulos de Young maiores, em duas ordens de magnitude, que os filmes de polietileno comerciais, e observando o comportamento espectroscópico (FT-IR) dos filmes de PVA com diferentes pesos moleculares concluíram que os espectros não apresentaram diferenças pela similaridade das estruturas químicas dos polímeros, que contém os mesmos grupos funcionais (ABD EL-KADER & ORABI, 2002).

Os filmes de PVA podem ser plasticizados com glicerol ou com moléculas de baixo peso molecular que atuam como umectantes ao reter água no filme. O poli (vinil álcool) (C.A.S. 25213-24-5) é amplamente usado em materiais em contato com os alimentos, incluindo adesivos para embalagens de alimentos e coberturas para papel. Os filmes de PVA apresentam um interesse crescente pelos seus atributos: solubilidade em água, biodegradabilidade, propriedades de barreira ao oxigênio, resistência a solventes e óleos, inocuidade e não contaminação do meio ambiente (CELANESE, 2002).

2.5.2.2 Filmes compostos de amido com poli (vinil álcool)

Tanto o amido quanto o PVA são polímeros polares. Por isso, é de se esperar que a blenda desses dois compostos produza materiais com excelentes propriedades mecânicas, pela formação de pontes de hidrogênio entre as duas moléculas (SIDDARAMAIAH et al., 2004). Na Figura 2.1 observa-se a possível interação entre as moléculas de amido e o poli (vinil álcool).

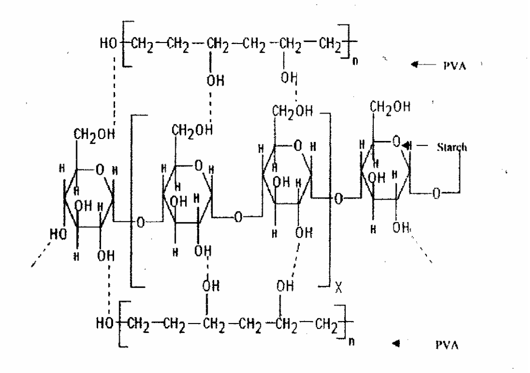

Figura 2.1 Formação de pontes de hidrogênio entre o amido e o PVA nas blendas (SIDDARAMAIAH et al., 2004)

SIDDARAMAIAH et al. (2004) avaliaram o efeito da adição de 1-10% de amido de milho em filmes de PVA com 99% de hidrólise e grau de polimerização de 17000-18000, e observaram que a incorporação do amido não afetou as propriedades mecânicas dos filmes de PVA. RAJ et al. (2003) fizeram a modelagem das isotermas de sorção de umidade dos filmes de PVA com amido de milho (10-50%) concluindo que os filmes são apropriados para a embalagem de alimentos.

YOON et al. (2006b) e PARK et al. (2005) avaliaram o efeito de aditivos com diferentes grupos funcionais (hidroxila e carboxila) nas propriedades físicas dos filmes de amido de milho com PVA (99% de hidrólise, PM: 89000-98000 g/mol) na mesma proporção. Os plasticizantes avaliados foram: glicerol, sorbitol e ácido cítrico (AC). Os autores observaram que as melhores propriedades foram atingidas com a adição de ácido cítrico, devido a que o AC contém grupos hidroxila e carboxila que aumentaram as interações inter e intramoleculares do amido através das ligações de hidrogênio. As melhores propriedades foram obtidas com agitação da blenda com AC por 50 min a 1500 rpm. Os filmes apresentaram as seguintes características: tensão na ruptura 20,12 MPa, elongação 36,98% e 19% de solubilidade.

A adição de PVA também foi estudada em filmes de amido de trigo (60%), PVA (20%) e glicerol (20%), os filmes foram analisados por espectroscopia de infravermelho com transformada de Fourier (FTIR) e não evidenciou-se a formação de ligações entre os componentes individuais (JAYASEKARA et al., 2004).

LAWTON (1996) estudou o efeito de diferentes tipos de amido nas propriedades de filmes a base de blendas de amido (41%) e PVA (41%) com adição de glicerol (15%) e ácido polietilen-co-acrilico (EAA) (3%). O EAA foi adicionado para melhorar a compatibilidade entre o PVA (99% de hidrólise, PM: 85000-146000 g/mol) e o amido, já que estes não são miscíveis em altas concentrações. Sem a presença do EAA as fases se separam durante a secagem. Quando se analisou a influência do tipo de amido, observou-se que os filmes com alto conteúdo de amilose possuem as melhores propriedades mecânicas, estáveis ao longo do tempo, entretanto os filmes com amido ceroso apresentaram pobre desempenho mecânico comparado com os outros filmes, devido a sua estrutura com alto conteúdo de amilopectina. O EAA não consegue se complexar com a amilopectina tão bem quanto com a amilose (SHOGREN et al., 1991).

Além das blendas do PVA com amido, existem estudos de filmes compostos de PVA com proteínas e outros polissacarídeos como a quitosana. SABATO et al. (2001) reportaram que a adição de PVA em filmes a base de proteína de soja aumentou a força de perfuração, mas não provocou redução na permeabilidade ao vapor de água. WANG et al. (2005) estudaram as propriedades dos filmes a base de blendas de quitosana e PVA para medicamentos de liberação controlada, caracterizaram os filmes com FT-IR, microscopia electrônica de varredura (MEV), e avaliaram a influência dos factores (proporção quitosana-PVA) na velocidade de liberação de um medicamento modelo. Os resultados indicaram que o filme é sensível ao pH e força iônica do meio, e o tempo de reticulação influenciou as propriedades de liberação do medicamento. Pelo que o filme tem aplicação para a liberação de medicamentos no intestino (WANG et al., 2005).

2.5.3 Reticulação

Uma outra alternativa para melhorar as propriedades dos filmes é a modificação da estrutura do polímero através da reação de reticulação. Segundo BELITZ & GROSCH (1988), a reação do amido com grupos poli funcionais produzem compostos com temperatura de gelatinização mais ou menos incrementada e capacidade espessante mais ou menos diminuída, em função do agente reticulante utilizado. Também a estabilidade frente ao cisalhamento e valores extremos de pH é incrementada. Os amidos reticulados são utilizados em produtos onde é preciso uma grande estabilidade, como nos biofilmes poliméricos naturais (BELITZ & GROSCH, 1988). A reticulação envolve a formação de ligações intermoleculares entre macromoléculas adjacentes do polímero. O aumento no grau de reticulação torna o material mais rígido, ao incrementar o número de ligações covalentes na estrutura do polímero, reduzindo assim a sua mobilidade (DELVAL, 2004), e melhorando a resistência do filme. A presença de grupos reativos no polímero torna possível a reticulação das cadeias por três métodos: tratamento químico, tratamento enzimático e o tratamento físico.

2.5.3.1 Tratamento físico

O tratamento físico pode ser térmico (usado para formar ligações intermoleculares nos filmes de proteína) e a radiação gama (γ) ou UV. FOLLAIN et al. (2005b) reticularam filmes a base de blendas de amido de trigo nativo e PVA com radiação UV, usando benzoato de sódio como foto sensibilizador, observando que a modificação na distribuição molecular do amido, por adição de PVA e a reticulação com radiação UV, produz um aumento na tensão na ruptura do filme.

SABATO et al. (2001) formularam filmes com isolado de proteína de soja e caseína com adição de PVA e carboximetilcelulose (CMC). Os filmes foram reticulados com radiação gama, observando-se que a força e deformação na ruptura aumentaram ao reticular os filmes de proteína de soja e caseína. Entretanto, a adição de PVA, mais do que a adição do CMC, provocou o aumentou da força na ruptura dos filmes de proteína de soja e caseína (SABATO et al., 2001).

LE TIEN et al. (2000) formularam filmes de proteína de soro de leite reticulados por tratamento térmico e radiação gama, obtendo filmes insolúveis com boas propriedades mecânicas e alta resistência ao ataque das enzimas proteolíticas.

2.5.3.2 Tratamento enzimático

OH et al. (2004) reticularam com transglutaminase, filmes de proteína de leite e filmes de hidrolisado de zeína. A elongação do filme de caseína foi aumentada em 41% pela ação da transglutaminase. No entanto, a reticulação enzimática não afetou significativamente a solubilidade nem a permeabilidade ao vapor de água dos filmes. CARVALHO e GROSSO (2004) diminuiram a solubilidade em 20% e a permeabilidade ao vapor de água em 35% de filmes de gelatina ao reticular com transglutaminase.

A reticulação com transglutaminase tem se aplicado na formulação de grande variedade de filmes, como biofilmes a base de gelatina de peixe e quitosana (KOLDZIEJSKA et al., 2006), quitosana e proteína de soro de leite (DI PIERRO et al., 2006), gelatina e caseína (CHAMBI & GROSSO, 2006), pectina e farinha de soja (DI PIERRO et al., 2005), proteína de clara de ovo (LIM et al., 1998), glúten deamidado (LARRE et al., 2000), e gelatina com glicerol (LIM et al., 1999).

2.5.3.3 Tratamento químico

A modificação química do amido é baseada na alteração dos grupos hidroxilas por reações químicas (esterificação, eterificação ou oxidação). A presença de três grupos hidroxilas por unidade glucosídica, mostra que o amido atua fundamentalmente como um álcool, embora a natureza química da macromolécula do amido não permite que seja considerado como um álcool (FLECHE, 1985).

A reticulação química tem sido usada para limitar a hidrofilicidade do amido (FOLLAIN et al., 2005a).

A reação inicia na quebra da ligação O-H para formar o amido aniônico ($St\text{-}O^-$). O resumo do mecanismo de reação pode ser descrito com as Equações 2.1 e 2.2, onde o St representa o amido e R o agente reticulante. O mecanismo da reação de reticulação é facilitado pela presença de reagentes alcalinos (NaOH, KOH).

$St\text{-}O\text{-}H \rightarrow St\text{-}O^- + H_2O$ Equação 2.1

$St\text{-}O^- + R^+\text{-}X^- \rightarrow St\text{-}O\text{-}R + X^-$ Equação 2.2

A reação de reticulação é desenvolvida em presença de reagentes bifuncionais que possam reagir com dois grupos hidroxilas. Entre os agentes reticulantes mais usados estão: a epicloridrina (ECH), o metafosfato trisódico (STMP), o oxiclorato de fósforo ($POCl_3$) e o formaldeído, nos três primeiros reagentes é preciso o meio alcalino para que a reação ocorra, entretanto para os aldeídos é requerido o meio ácido (FLECHE, 1985).

A Tabela 2.5 apresenta o resumo dos resultados obtidos nos diferentes estudos onde a reticulação química foi utilizada como uma estratégia para melhorar as propriedades dos filmes.

Tabela 2.5 Resumo dos resultados obtidos por diferentes autores ao utilizar a reticulação como estratégia para melhorar as propriedades dos biofilmes.

Material	Reticulante	Resultado	Referência
Amido (70% amilose)	Epicloridrina (1-10g ECH/100g polímero)	Ao aumentar o grau de reticulação, diminuiu o grau de cristalinidade do amido. Os valores máximos das propriedades mecânicas foram dos filmes com graus moderados de reticulação.	RIOUX et al. (2002)
Farinha com alto conteúdo de amido	Epicloridrina	O aumento na concentração de ECH (de 10 a 70 mL) tornou o material mais rígido. Devido ao aumento no número de ligações covalentes na rede do polímero, reduzindo assim a mobilidade da matriz.	DELVAL et al. (2004)
Amido de milho ceroso e com alto conteúdo de amilose	Epicloridrina	A tensão dos filmes aumentou com o aumento no conteúdo de amilose. Filmes de amido ceroso mostraram elongações menores às observadas nos filmes com amilose. Os filmes de amido reticulado mostraram uma variação moderada das propriedades mecânicas (tensão e elongação).	LLOYD & KIRST (1963)
Amido (20% amilose) e PVA	Epicloridrina	Foi observada uma queda na intensidade do pico –OH (3237 cm^{-1}) ao aumentar o grau de reticulação (FTIR). A reticulação do filme produz temperaturas de transição vítrea (T_g) menores.	SREEDHAR et al. (2006)
Amido de batata e LDPE	Epicloridrina (0,1-2,0g ECH/100g amido)	Filmes de LDPE contendo 5-10% de amido reticulado apresentaram melhores propriedades mecânicas (tensão e elongação na ruptura) que os filmes de LDPE com amido de batata nativo.	KIM & LEE (2002)

Tabela 2.5 Resumo dos resultados obtidos por diferentes autores ao utilizar a reticulação como estratégia para melhorar as propriedades dos biofilms. (Continuação)

Material	Reticulante	Resultado	Referência
Amido de milho (HSPAN) e PVA	Epicloridrina	Foi melhorada a compatibilidade entre HSPAN/PVA pela reticulação com ECH, e assim as propriedades mecânicas dos filmes. A solubilidade em água dos filmes diminuiu com o aumento de ECH. E foi observada uma reticulação simultânea do ECH com os grupos hidroxilas do amido e do PVA.	KIM et al. (2002)
Isolado de proteína de amendoim	Epicloridrina glutaraldeído	A reticulação dos filmes de isolado de proteína de amendoim com ECH melhorou as propriedades dos mesmos, ao diminuir sua permeabilidade ao vapor de água e aumentar a tensão na ruptura.	FENG et al. (2005)
Quitosana	Epicloridrina	Os filmes de quitosana reticulados com epicloridrina apresentaram propriedades mecânicas (tensão na ruptura) melhoradas.	ZHENG et al. (2000)
Amido de mandioca	Glutaraldeído	Ao aumentar o conteúdo de glutaraldeído diminuiu a permeabilidade ao vapor de água dos filmes de amido de mandioca, devido ao efeito da reticulação.	PARRA et al. (2004)
Quitosana e PVA	Formaldeído glutaraldeído glioxal	A reticulação dos filmes compostos de quitosana e PVA com três tipos de aldeídos diminuiu a permeabilidade ao vapor de água e aumentou a tensão e elongação na ruptura.	CHEN et al. (2005)

Tabela 2.5 Resumo dos resultados obtidos por diferentes autores ao utilizar a reticulação como estratégia para melhorar as propriedades dos biofilms. (Continuação)

Material	Reticulante	Resultado	Referência
Isolado de proteína de soja e zeína	Formaldeído glutaraldeído glioxal cinamaldeído	A adição de formaldeído, glutaraldeído, glioxal e cinamaldeído como agentes de reticulação em filmes de isolado de proteína de soja e zeína, melhorou as propriedades físicas dos filmes. Em particular, o cinamaldeído foi considerado o melhor agente porque é seguro em alimentos.	LEE et al. (2005)
Amido de mandioca	Diacrilato tetraetileno glicol	Após a reticulação a solubilidade em água do amido diminuiu a 9-16%. A reação de reticulação foi confirmada pela caracterização no FT-IR.	MARQUES, et al. (2006)
Amido de milho, fibra lignocelulósica e PVA	Hexametoxi metilmelamina (HMMM)	A reticulação química dos filmes de amido, fibra e PVA (todos eles polímeros com grupos hidroxila) com hexametoximetilmelamina (HMMM) aumentou a resistência à água dos filmes.	IMAM et al. (2005)
Amido (21% amilose), PVA, polietilenglicol	Bórax	Os filmes reticulados apresentaram maior estabilidade e menor Tg que as blendas de amido-PVA sem adição de bórax. A tensão na ruptura dos filmes aumentou e a elongação diminuiu ao aumentar a concentração de bórax adicionada.	SREEDHAR et al. (2005)
Amido de milho, PVA e glicerol	Ácido bórico	Os filmes obtidos a partir de amido de milho com PVA, glicerol e ácido bórico apresentaram boas propriedades mecânicas e resistência à água.	YIN et al. (2005)

A reticulação química apresenta-se como a opção mais viável por ser a mais econômica e com diversidade de alternativas, já que a reticulação enzimática envolve um custo elevado e o tratamento com radiação requer equipamentos especiais.

Dentre os agentes químicos, os aldeídos (formaldeído, glutaraldeído, glioxal, hexametoximetilmelamina) são geralmente utilizados para reticular proteínas, com bons resultados, mas apresentam efeitos indesejáveis ao meio ambiente, e seu uso encontra-se regulado. Igualmente ocorre com o ácido bórico e o bórax, sendo seu uso restrito como aditivo indireto aos alimentos (FDA, 2005).

A epicloridrina (C.A.S. 106-89-8) é o agente de reticulação mais usado para o amido (DELVAL et al., 2004).

2.5.3.4 Reação de reticulação com epicloridrina

A epicloridrina (C_3H_5ClO) (Figura 2.2) é um dos agentes de reticulação bifuncionais permitido pela lei americana. O FDA regula o uso da epicloridrina como aditivo para uso direto em alimentos (na elaboração de amidos modificados) estabelecendo uma dosagem máxima de 0,3% (FDA, 2005).

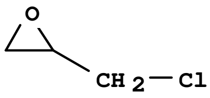

Figura 2.2 Estrutura química da epicloridrina

A ECH reage com os grupos hidroxilas do amido para formar mono e diéteres. Os diéteres podem ser inter ou intramoleculares (HAMERSTRAND et al., 1960). A reação de amido reticulado por epicloridrina é amplamente documentada e apresenta-se como um método relativamente simples para obter amido modificado. No polímero reticulado existem duas estruturas: o amido reticulado pela ECH, e a epicloridrina homo polimerizada ao reagir com ela mesma.

A estrutura do polímero reticulado é complexa (Figura 2.3), com muitas unidades de glicose interconectadas, formando uma rede tridimensional (DELVAL et al., 2004).

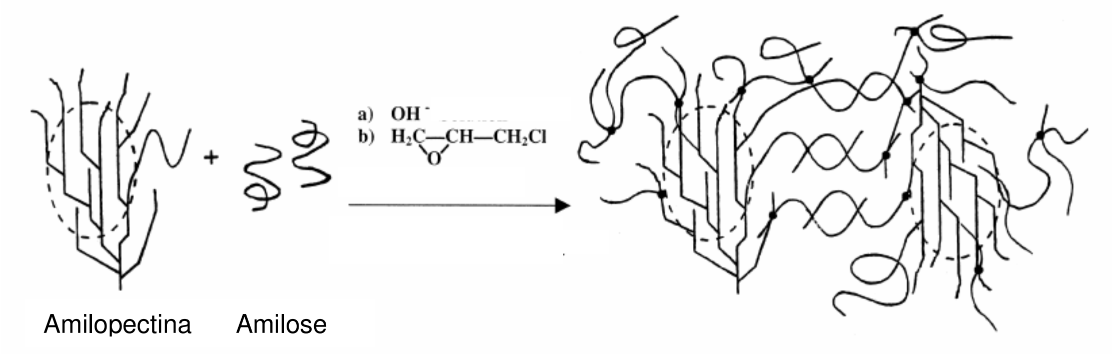

Figura 2.3 Formação da rede polimérica por reticulação do amido com a epicloridrina (SHIFTAN et al., 2000)

Existem duas formas de se promover a reação de reticulação com ECH: a forma heterogênea e a homogênea. Na reação heterogênea, o amido é mantido na forma granular ao adicionar uma baixa concentração de NaOH (0,3-0,5%) e reagindo com a epicloridrina a temperaturas baixas (20-30ºC) por tempos longos (16-24h). Desta forma, é possível recuperar o amido após a reação para a secagem. Na reação homogênea, o amido é disperso numa solução alcalina, com uma concentração de NaOH mais elevada (2-20%), e a ECH é adicionada sob agitação contínua. A temperatura da reação pode ser elevada (40-50ºC) e o tempo será consideravelmente menor, cerca de 1 a 2 horas (KUNIAK & MARCHESSAULT, 1972).

Tanto LLOYD & KIRST (1963), quanto KIM & LEE (2002), desenvolveram a reticulação heterogênea, no primeiro caso com um tratamento de 16 horas a 23ºC, e no segundo caso por 24 horas a temperatura ambiente, com um pH inicial de 10,5, sendo ajustado no final a pH 5,5 com acido acético para deter a reação.

Reações do tipo homogêneo foram desenvolvidas por KIM et al. (2002) e SREEDHAR et al. (2006), sendo nos dois casos a 40ºC por 2 horas, com um pH inicial de 10 e com concentrações de epicloridrina que variavam entre os 5 e 20% (m/m). Para a formação de filmes de amido com alto conteúdo de amilose (70%), a reticulação do amido com ECH (1-10%) foi realizada a 50ºC por uma hora, com posterior ajuste do pH com acido acético (RIOUX et al., 2002).

KARTHA & SRIVASTAVA (1985a, 1985b) estudaram a cinética e o mecanismo da reação do amido com a epicloridrina, observando que a reação segue uma cinética de segunda ordem. Baseado nas evidências encontradas na cinética da reação, esses autores propuseram os mecanismos da reação conforme descrito na Figura 2.4.

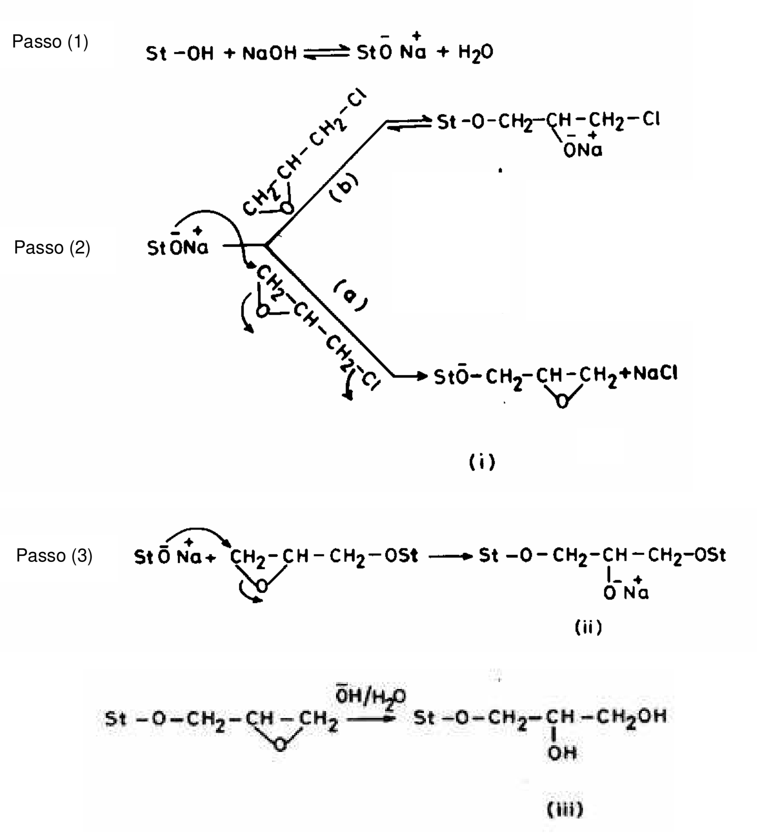

Figura 2.4 Mecanismo da reação de amido com epicloridrina em meio alcalino (KARTHA & SRIVASTAVA, 1985b)

Em presença de álcali, ocorre a formação do amido sódico, mostrado no passo (1) do mecanismo. No passo (2) o amido aniônico ataca a molécula da epicloridrina, e o anel epóxido é aberto, resultando na formação de um novo epóxido (i). A formação de (i) pode se dar pelo mecanismo (a) ou em duas etapas pelo mecanismo (b). No passo (3) outra molécula de amido sódico reage de maneira similar ao passo (2) dando como resultado o produto reticulado (ii).

Entretanto, o produto (i) pode reagir com os OH^- do meio para formar o subproduto mono glicerol éter (iii) numa reação lateral. A formação de (ii) e (iii) é resultado da competição entre StO^- e OH^-/H_2O por (i). A formação preferencial de um ou outro produto dependerá da concentração destas espécies. No caso de reações sob condições homogêneas, tem sido observado que a formação de (iii) é aumentada com o incremento da razão água/amido. Outro fator que pode influenciar a formação de (ii) e (iii) é a razão amido/ECH.

Sob condições idênticas, maiores razões amido/ECH vão favorecer a formação de (ii), que é o produto reticulado. Portanto, altas concentrações de NaOH e ECH favorecem a formação de mono glicerol éter (iii), produto não desejado na reação.

Na Figura 2.5 mostra-se a formação de produtos de reações secundárias com altas concentrações de NaOH e ECH.

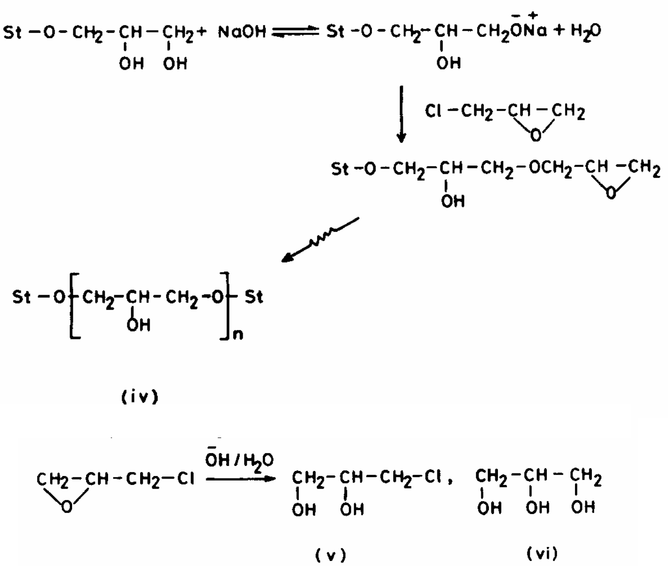

Figura 2.5 Mecanismo da reação secundária do amido com epicloridrina em meio alcalino (KARTHA & SRIVASTAVA, 1985b)

Em condições de abundancia de NaOH e ECH no meio, a reação de reticulação envolve mais de uma unidade de epicloridrina, formando-se o produto (iv). Outra reação lateral importante é a hidrólise da ECH, a cloridrina (v), e posteriormente o glicerol (vi). Nas reações sob condições homogêneas, observou-se que ao se aumentar o conteúdo de água no sistema, incrementa-se a formação de (v) e (vi) por hidrólise da epicloridrina (KARTHA & SRIVASTAVA, 1985b).

A reação do amido com a epicloridrina é de natureza complexa. É difícil relacionar o rendimento do produto reticulado com a eficiência química da reação de reticulação, já que tanto a reticulação quanto as reações secundárias encontram-se envolvidas no processo. HAMMESTRAND et al. (1960) tentou determinar a extensão da reação de reticulação do amido com ECH ao determinar a quantidade de epicloridrina que não reagiu por analise química.

KUNIAK & MARCHESSAULT (1972) reportaram que dependendo das condições de tempo, temperatura e razão molar dos reagentes, de 5 até 25% da epicloridrina pode ser ligada ao amido para formar mono glicerol éter (iii). Esses autores observaram que a maior eficiência da reação de reticulação foi atingida ao usar altas concentrações de amido (140-360g/L) e uma baixa razão molar de NaOH/amido, entre 0,5 e 1,0, onde as temperaturas (25-50°C) não afetam o rendimento da reação, mas sim o tempo da mesma; sendo recomendadas temperaturas baixas.

KARTHA & SRIVASTAVA (1985b) recomendaram para condições heterogêneas de reação (onde se mantêm a estrutura granular intacta), uma concentração de NaOH que não supere 1% (m/v), com temperatura inferior a 50°C e com uma razão amido/água de 1:2. Sob estas condições o tempo de reação foi de 16-24 horas, onde 70-85% do reagente é consumido.

2.6 Propriedades de filmes a base de blendas de amido com poli (vinil álcool) reticulados

O PVA também pode ser reticulado através dos seus grupos hidroxilas para aumentar a resistência à umidade e a resistência mecânica. Os agentes reticulantes mais comuns são os aldeídos, como o glioxal. Mas, com o interesse de usar agentes sem aldeídos por fatores ambientais, é recomendado o uso de compostos como a epicloridrina, que apresenta bons resultados na reticulação do PVA (CELANESE, 2002).

Diversos autores (FOLLAIN et al., 2005b; SREEDHAR et al., 2005; YIN et al., 2005; IMAM et al., 2005 e YOON et al., 2006a) estudaram filmes a base de blendas de poli (vinil álcool) com amido, usando diferentes formas de reticulação química e física, obtendo melhoras nas propriedades mecânicas dos filmes, e demonstrando assim a boa compatibilidade que existe entre esses dois polímeros. KIM et al. (2002) reportaram que a compatibilidade entre o amido (amido-g-PAN hidrolisado, HSPAN) e o PVA foi melhorada pela reticulação com a epicloridrina (ECH), devido à reticulação simultânea dos grupos hidroxilas do amido e do PVA através de epicloridrina.

SREEDHAR et al. (2006) avaliaram as características térmicas e de superfície dos filmes compostos de amido (21% amilose) e PVA em proporção 2:8, reticulados com epicloridrina, e observaram que a incorporação de PVA no amido melhora as propriedades térmicas e mecânicas dos filmes, já que modifica a estrutura do polímero tanto no nível molecular quanto no morfológico.

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CAPÍTULO 1

Amarantus cruentus flour films: Effect of crosslinking with epichlorohydrin on the solubility, mechanical and barrier properties of the films.

Amarantus cruentus flour films: Effect of crosslinking with epichlorohydrin on the solubility, mechanical and barrier properties of the films

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Abstract

Crosslinked films of *Amaranthus cruentus* flour were prepared at different epichlorohydrin concentration (1.6-18.4 g/100g flour), reaction temperature (39.8-90.2ºC) and time (12.8-147.2 minutes). The effect of these parameters on the mechanical and barrier properties was evaluated using the response surface methodology (RSM). According to the statistical analysis, the crosslinking reaction provided a plasticizing effect in the *A.cruentus* flour film, and the reaction temperature had a strong impact under the film solubility and the pH solution. A series of reactions were performed with fixed temperature (39.8ºC), time (120 min) and different ECH concentration (1.6-18.4 g/100g flour), without pH adjustment. The *A.cruentus* flour film crosslinked under these conditions (39.8ºC, 120 min) presented no significant difference from the non-modified film. However, it showed an improved barrier performance with low solubility in water ($S_{average}$ = 29%) and water vapor permeability ($WVP_{average} = 2.3 \times 10^{-10}$ g / m s Pa). Moreover, the crosslinking reaction prevented the film disintegration during solubility test. FT-IR analysis showed evidence of chemical modification in the crosslinked *A.cruentus* flour films, and SEM micrographs demonstrated the effect of the crosslinking reaction on the film microstructure.

Keywords: biofilms; *Amaranthus cruentus* flour; crosslinking; epichlorohydrin; mechanical properties; solubility.

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3.1 Introduction

In recent years, biodegradable films have increased their commercial importance because of environmental problems of non-biodegradable and non-renewable polymeric materials (*1*). The major biopolymers used for biofilms production are the polysacharides and proteins, with addition of plasticizers. Other ingredients normally used are lipids, acids, among others. The interest in combining these ingredients in biofilm production is due to the advantages and disadvantages of these components. The use of natural blends of protein, polysaccharides, and lipids directly obtained from agricultural sources takes advantage of each component in the original system and appears to be a new opportunity for material in the area of biofilms (*2*).

Amaranth seeds contain a significant proportion of starch (62%), protein (14-17%) and fat (5-9%) (*3*). Thus, amaranth flour can be an interesting source of raw material for biofilm production, following the actual trends in the use of natural mixtures of starch, protein and lipids to reach the desired properties for biofilms. Amaranth is a pseudo cereal of rapid growth, with a high tolerance to arid conditions and poor soils where traditional cereals cannot be grown. The main cultivars used are *Amaranthus hipochondriacus, A. cruentus* and *A. caudatus*. Amaranth seeds contain a significant proportion of starch (62%), protein (14-17%) and fat (5-9%) which make the flour a promising material for film formation (*3*).

A. caudatus flour films have been developed, producing films slightly yellowish with moderate opacity, good flexibility and barrier properties, although mechanical resistance was relatively low (*3*). Besides, *A. cruentus* flour films produced with stearic acid addition, the incorporation of stearic acid into the polymeric matrix provided an additional water vapor barrier to the film (*2*).

The attempts to improve the properties of biopolymer based films are made in several ways. Chemical crosslinking has been proposed as a strategy of improving biofilms properties, since it reinforces the intermolecular binding with the introduction of covalent bonds to supplement natural hydrogen bonds of the polysaccharide macromolecules (*6*). Crosslinking of starch with epichlorohydrin (ECH) is the most common method used in polysaccharide chemistry. Epichlorohydrin is a bifunctional agent that is used for crosslink starch by forming the glycerol diether linkage through the reactions with hydroxyl groups of polysaccharide (*7*). The crosslinked starch has more carbon chains than native starch, which causes the polymer to be more hydrophobic (*8*).

The crosslinking reaction with epichlorohydrin could be developed in two different conditions. First, when the starch granules are maintained in low NaOH concentration (0,3-0,5%) solutions, low temperatures (20-25ºC) and long times (16-24h), this condition is called heterogeneous reaction (*5,8*); and when the starch is gelatinized, with higher NaOH concentration (2-20%) solutions, enhanced temperature (40-50ºC) and lower reaction time (1-2h), this condition is called the homogeneous reaction (*1,6-7*).

Some researchers studied both crosslinking reactions with epichlorohydrin. Kartha and Srivastava (*9, 10*) reported the kinetics and mechanism of the starch-epichlorohydrin reaction. Hamerstrand *et al.* (*11*) determined the extent of crosslinking reaction by the amount of unreacted ECH. Kuniak and Marchessault (*12*) reported that depending upon conditions of time, temperature and mole ratio of the reactants, 5-25% of the applied ECH is bound in the starch as glycerol monoether derivative, which is an undesired side reaction, just like the hydrolysis of ECH to glycerol in presence of water excess (*10*).

The characterization of films based on starch crosslinked with ECH has been reported for several authors (*1,4-8*). Nevertheless, a lack of information about the crosslinking of starch in a complex mixture such as flours exists. Therefore, the purpose of this work was to develop films based on *Amaranths cruentus* flour using the epichlorohydrin as a starch crosslinker, and to optimize this process production using a response surface methodology, in terms of the ECH concentration, the reaction temperature and time. The studied responses were the mechanical properties and solubility in water of films.

3.2 Materials and methods

3.2.1 Materials

Amaranth flour was obtained from *Amaranthus cruentus* seeds (cultivar BRS Alegria provided by Embrapa Cerrados, Brazilian Company of Agricultural Research, Federal District, Brazil) by the alkaline wet milling method described elsewhere (*2*), with a yield of 57%. The *A. cruentus* flour samples were analyzed following the standard methods of the AOAC (1997) and the results were (on dry basis): 72.5% starch (7.8 ± 0.2 g amylose/100 g flour), 11.9 ± 0.3% protein, 7.7 ± 0.8% lipids, 2.2 ± 0.6% ash and 5.6 ± 0.2% moisture. Amylose content was determined using a colorimetric method (*13*), modified by Martínez and Cuevas (*14*).

The epichlorohydrin (C_3H_5ClO) 99.0%, with molecular weight of 92.53 g/mol and density 1.179-1.181 g/mL at 20ºC; glycerol, NaOH, and other chemicals were analytical reagent grade, purchased from Synth (São Paulo, Brazil), and used without further purification.

3.2.2 Film preparation and conditioning

Amaranth flour crosslinked films were made by the casting technique (Figure 3.1). The amaranth flour film forming solutions were made as established by Tapia-Blácido (*3*) from amaranth flour (4g/100g solution) dispersed in deionized water. The solution was mixed for at least 2 hour with a magnetic stirrer, and the pH was adjusted to 10.7, for protein solubilization. The solution was heated up to 70°C, then glycerol (20g glycerol/100g flour) was added. The mixture was gelatinized at 80°C for 15 minutes with constant stirring in a jacketed beaker connected to a water bath (Tecnalise, Brazil).

The film forming solution crosslinking was performed under homogeneous conditions, with predetermined epichlorohydrin concentration, reaction temperature and time. At the end of the reaction, the pH of the solution was measured and adjusted to 10.7, to ensure the protein solubility. Then the solution was defoamed under ultrasonic waves (Ultrasonic Cleaner / Unique, Model USC 1450) for 5 minutes. Afterward, the film forming solution was poured and evenly spread over Teflon plates (18x21 cm). The weight of filmogenic solution was controlled to obtain a constant thickness of 80±2µm. The casting solutions were dried at controlled temperature of 37ºC and relative humidity of 55% for 12-14h, until the equilibrium moisture was reached (13.5±1.7%).

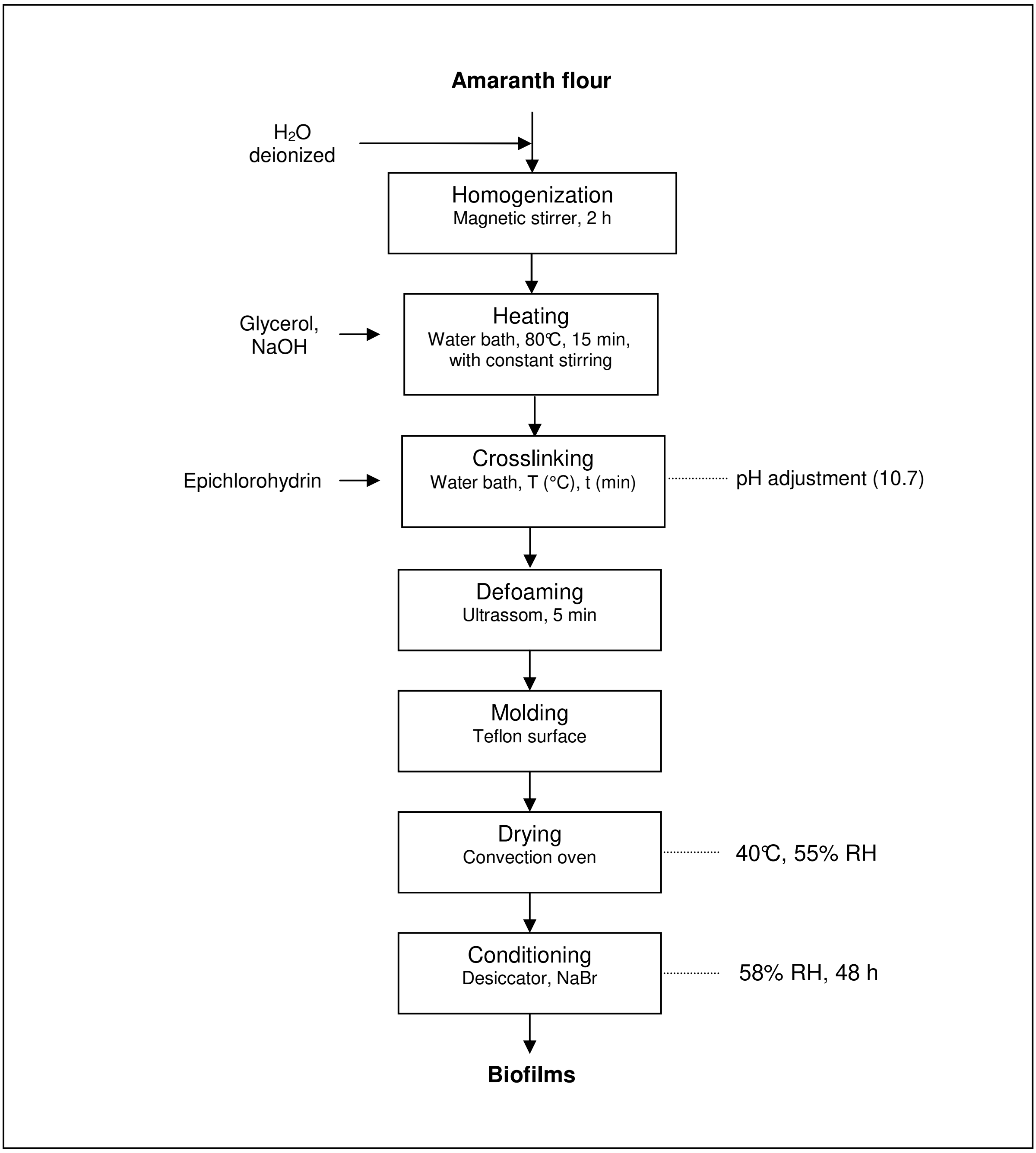

Figure 3.1 Flowchart for the elaboration of *A.cruentus* flour films crosslinked with epichlorohydrin

Dried films were peeled off from the casting surface and cut into adequate samples. The films were conditioned at 25°C and 58% RH in desiccators containing a saturated solution of NaBr for 72h prior to mechanical tests and water vapor permeability determination. For solubility test and FTIR analysis, the films were conditioned in desiccators with silica gel (0% RH) for 48 hours and 1 week, respectively.

3.2.3 Film characterization

Thickness was measured using a micrometer (model FOW72-229-001, Fowler, U.S.A.) with a range of 0-1in and an accuracy of 0.0001in. The mean thickness of the films was determinated from the average of 15 measurements made at five different locations.

Moisture content of films was determinate by the method of the AOAC (1997) drying the sample (0.200±0.001g) at 105°C for 24h, in an air oven. This determination was made by triplicate.

Mechanical properties were determined by puncture tests: puncture force (PF) and puncture deformation (PD); and tensile tests: tensile strength (TS) and elongation at break (ELO), both tests using a TA-XT2i Stable Micro Systems texture analyzer.

For puncture tests, circular sample films were fixed in a 34mm diameter cell, and then they were perforated by a 3mm diameter cylindrical probe, moving at 1mm/s (*15*). These tests were done in triplicate. The puncture deformation was calculated as follows, equation 1:

$$PD = \Delta l / l_o = [(D^2 + l_o^2)^{1/2} - l_o] / l_o \qquad \text{Eq.1}$$

where l_o is the initial disc radius (17 mm) and D the distance penetrated at the break point.

Tensile tests were performed according to the ASTM standard method D882-97 (16). Dumbbell shaped samples were cut for each film with 115mm length and 6mm wide in the middle. The initial grip separation was set at 80 mm and the crosshead speed at 1.0 mm/s. At least four samples from each film were evaluated. The elongation at break was obtained with equation 2:

$$ELO = (\Delta L / L_o)100 \qquad \text{Eq.2}$$

where ΔL is the elongated distance at break and L_o the initial distance between the grips (80mm). Care was taken to remove the samples just before they were tested to minimize the chance of samples drying out or picking up moisture due to the change in humidity from the desiccator to the testing room. The TS (MPa), ELO (%), PF (N) and PD (%) were calculated using Texture Expert version 1.15 (SMS).

Solubility in water was determinated according to the method described by Gontard et al. (*17*) with modifications. Three dried film discs (2 cm diameter) were weighed (w_i) and immersed in 50mL deionized water (with sodium azid, 0.02%) at 25℃ for 24h, with constant agitation (50 rpm) in a shaker (Tecnal, TE-420). Then the films were recovered by vacuum filtration on filter paper with known weight, dried at 105℃ for 24h and weighed (w_f). The solubility (S) was calculated as the percentage of dissolved film, equation 3:

$S = [(w_i - w_f) / w_i]\ 100$ Eq.3

Water v*apor permeability (WVP)* tests were conducted using ASTM method E96-95 (*18*), considering the modifications proposed by Gontard et al. (*19*). Each film sample was sealed over the circular opening of a permeation cell containing silica gel. These cells were placed on desiccators with distilled water kept at 25℃. The cell weight was measured each hour, for 10 hours, and then 12 hours later, using an analytic balance (A200S, Sartorius Analytic). The WVP was calculated as equation 4:

$WVP = (w / t)\ (x / \Delta P\ A)$ Eq. 4

where x is the average thickness of the amaranth flour films, A is the permeation area (9.07×10^{-4} m^2), ΔP is the difference between the partial pressure of the atmosphere over silica gel and over pure water (3.168 kPa, at 25 ℃), and the term w/t was calculated by linear regression ($R^2 > 0.99$) using data of weight gain as a function of time. All tests were performed in triplicate.

Fourier transformed IR spectroscopy (FTIR). Infrared spectra of dry films were recorded between 4000 and 600 cm^{-1} at 4 cm^{-1} resolution, with a Spectrum One (Perkin Elmer) spectrometer, supplied with a universal attenuated total reflectance (UATR) accessory. Each sample was scanned 16 times and averaged to produce each spectrum (*24*).

Scanning electron microscopy (SEM) analyses were performed using a Leica (Cambridge, England) model LEO440i scanning electron microscope operating at 10 kV. Film samples were maintained in a desiccator with silica gel for 7 days and then randomly broken to investigate the cross section of the samples. A cylindrical aluminum stub cut like a straight chair, upon which the film was fixed using a double-sided cupper tape, was used in a specific way to observe the morphology of the cross section and the surfaces. Further, the stubs with films were coated with gold in a VG Microtech (Cambridge, England) model SC7620 sputter coater for 180 s at 4 mA.

3.2.4 Preliminary tests were tested

Four sets of preliminary tests were developed in order to determine the parameters of the crosslinking reaction with epichlorohydrin. For these tests, the amount of glycerol added to the films was varied from 22.5 to 20g/100g flour, and the reaction temperature from 60 to 80ºC. Two epichlorohydrin concentration (5 and 10g/100g flour) were tested, at three different reaction times (30, 60 and 90 min, or 40, 80 and 120 min). In all cases, the pH of the solution was adjusted to 10.7 at the end of the reaction. The crosslinked films were prepared as described before, with the following conditions:

Table 3.1 Parameters of the preliminary tests for the *A.cruentus* flour films crosslinked with epichlorohydrin

Set	Reaction temperature (ºC)	Glycerol (g/100g flour)	Reaction time (min)	Epichlorohydrin (g/100g flour)
1	60	22.5	30, 60, 90	5, 10
2	70	20.5	40, 80, 120	5
3	80	21.5	40, 80, 120	5
4	80	20.0	40, 80, 120	5, 10

Mechanical properties (TS, ELO, PF and PD) were measured for all films, and the solubility in water was determined for the 4th set. For statistical analysis, mean values of the mechanical properties and solubility were analyzed by Tukey honest significant difference (HSD) test, performed using Statistica 5.0 software (Statsoft Inc., U.S.A.).

3.2.5 Experimental Design

A 2^3 full factorial central composite design (star configuration) with 6 axial and 3 central points (triplicate only at the central point), resulting in 17 experiments, was performed. This experimental design was used to obtain a second-order model for prediction of mechanical properties (by puncture and tensile tests) and solubility (dependent variable) as a function of three independent variables: epichlorohydrin concentration (x_1), reaction temperature (x_2) and reaction time (x_3). The crosslinking reaction conditions were: epichlorohydrin concentrations of 1.6, 5, 10, 15 and 18.4g/100g of flour; reaction temperatures of 39.8, 50, 65, 80 and 90.2ºC, and reaction times of 12.8, 40, 80, 120 and 147.2 minutes.

The statistical design and the coded and real values of these variables are given in Table 3.2. All experiments were performed randomly, and data were treated with the aid of Statistica 5.0 software (Statsoft Inc., U.S.A.)

Table 3.2 Central composite design matrix with coded and real values of the variables for the crosslinking reaction with epichlorohydrin.

Essay	ECH (x_1) (g/100gflour)	Temperature (x_2) (ºC)	Time (x_3) (min)
1	5 (-1)	50 (-1)	40 (-1)
2	15 (+1)	50 (-1)	40 (-1)
3	5 (-1)	80 (+1)	40 (-1)
4	15 (+1)	80 (+1)	40 (-1)
5	5 (-1)	50 (-1)	120 (+1)
6	15 (+1)	50 (-1)	120 (+1)
7	5 (-1)	80 (+1)	120 (+1)
8	15 (+1)	80 (+1)	120 (+1)
9	1.6 (-1.68)	65 (0)	80 (0)
10	18.4 (+1.68)	65 (0)	80 (0)
11	10 (0)	39.8 (-1.68)	80 (0)
12	10 (0)	90.2 (1.68)	80 (0)
13	10 (0)	65 (0)	12.8 (-1.68)
14	10 (0)	65 (0)	147.2 (+1.68)
15	10 (0)	65 (0)	80 (0)
16	10 (0)	65 (0)	80 (0)
17	10 (0)	65 (0)	80 (0)

3.2.6 Crosslinking reactions without pH adjustment

Another set of experiments were conducted with varied epichlorohydrin concentration, at constant temperature and time. The temperature was fixed at the lowest condition of the experimental design (39.8ºC), and the reaction time was 120 min. The epichlorohydrin concentration varied from 1.6 to 18.4g/100g flour, at the same five levels used before. The crosslinked films were prepared as described in section 3.2.2, but without pH adjustment at the end of the crosslinking reaction.

Mechanical properties (TS, ELO, PF and PD), solubility in water and water vapor permeability were determined for the crosslinked *A.cruentus* flour films. FTIR analysis was also performed for three samples, 1.6, 10 and 18.4g/100g flour. For statistical analysis, mean values of the mechanical properties and solubility were analyzed by Tukey honest significant difference (HSD) test, performed using Statistica 5.0 software (Statsoft Inc., U.S.A.).

3.3 Results and discussion

The study of *A.cruentus* flour films crosslinking reaction with epichlorohydrin was carried out in three stages. (a) The determination of the reaction parameters by a series of preliminary tests; (b) the development of an experimental design to assess the effect of three variables (ECH concentration, reaction temperature and time) on the mechanical and barrier responses; and finally (c) the study of the ECH concentration effect on the mechanical and barrier properties of the crosslinked *A.cruentus* flour films without pH adjustment.

3.3.1 Preliminary tests

3.3.1.1 Mechanical properties of *A.cruentus* flour films crosslinked with epichlorohydrin (5 and 10%) at 60ºC with glycerol (22.5%)

Figure 3.2 shows the results of the tensile tests as function of reaction time for two epichlorohydrin concentrations. It was observed that the non-crosslinked film was the less resistant (lowest TS). There was no significant difference between the crosslinked films, due to the high variability of the results, but there is a tendency of increasing TS with increased reaction time, for films crosslinked with 5% ECH.

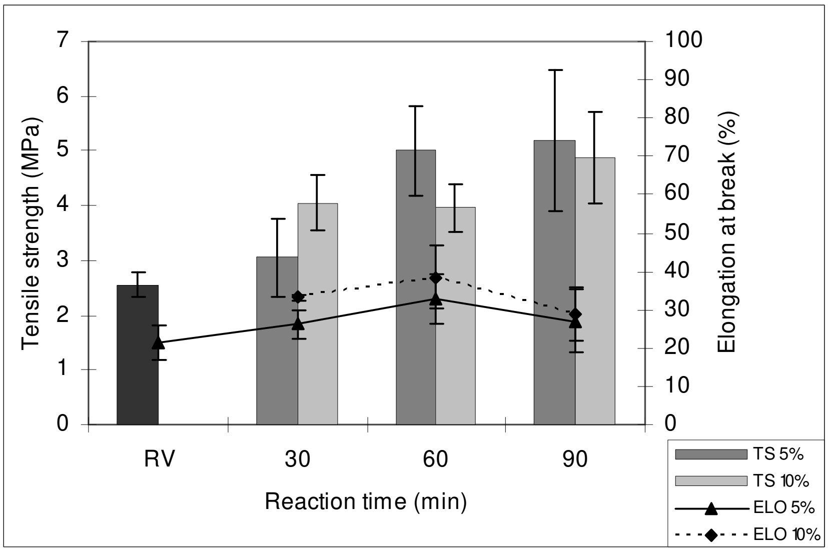

Figure 3.2 Tensile strength (MPa) and elongation at break (%) of *A.cruentus* flour films crosslinked with epichlorohydrin (5 and 10 %) at 60ºC and different reaction time, with 22.5g/100g flour glycerol. Reference value (RV) without crosslinking (0% ECH).

There was no significant difference between the flexibility of the films, but the elongation at break tends to be higher at intermediate reaction time (60 min). The response values and significant differences are shown in Table A.1.

Figure 3.3 shows the results of the puncture test where there was no significant difference among the puncture force of the crosslinked films and the non-treated amaranth flour film. Just the puncture deformation (PD) of the non-crosslinked film was higher than the other crosslinked films, with PD = 5.30%.

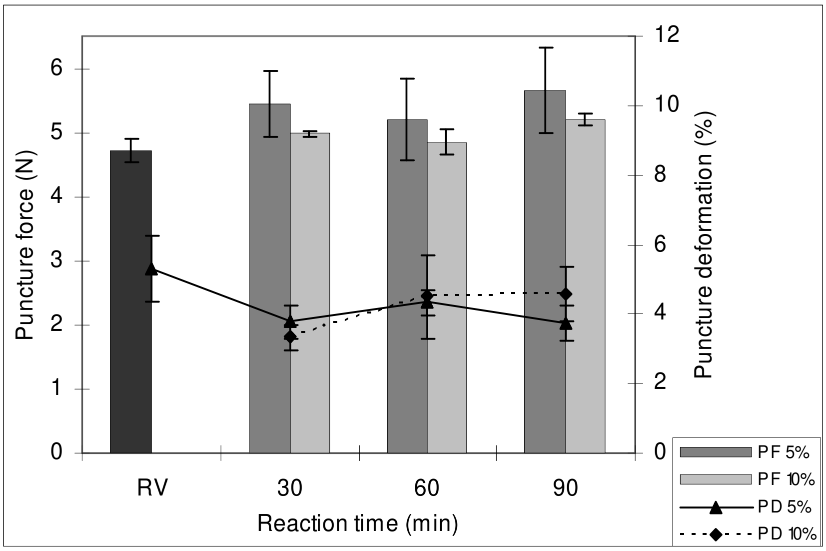

Figure 3.3 Puncture force (N) and deformation (%) at break of *A.cruentus* flour films crosslinked with epichlorohydrin (5 and 10 %) at 60ºC and different reaction time, with 22.5g/100g flour glycerol. Reference value (RV) without crosslinking (0% ECH).

From these results, it was decided to increase the reaction time up to 120 minutes, since it seems to be a trend of increasing resistance with time. Higher reaction temperatures were tested in order to assess the possibility of performing the crosslinking reaction at the amaranth starch gelatinization temperature (70 - 80ºC), as the gelatinization was carried out just before the crosslinking step. Also the amount of glycerol added to the solution was reduced based on the results obtained from Tapia-Blácido (*21*) in the optimization of the *A.cruentus* flour films with glycerol addition.

3.3.1.2 Mechanical properties of *A.cruentus* flour films crosslinked with epichlorohydrin (5%) at 70 and 80ºC; 20.5 wt% and 21.5 wt% glycerol respectively

Figure 3.4 shows the effect of two reaction temperatures and glycerol concentration as function of reaction time. It was noted a trend of decreasing tensile strength with increased reaction time, for both temperatures (70 and 80ºC). The observed effect of the crosslinking reaction under the film resistance was the opposite of the one obtained at 60ºC. This reveals that at higher temperatures other reactions occur that change the expected effect in the crosslinked films properties.

The highest resistance (TS = 4.62 MPa) was obtained for the non-crosslinked film with 20.5% glycerol. At 80ºC the elongation at break increased with increasing reaction time, with a maximum of 92.2% in 120 minutes of reaction. At 70ºC, only the film with 120 minutes of reaction was significantly more flexible than the control.

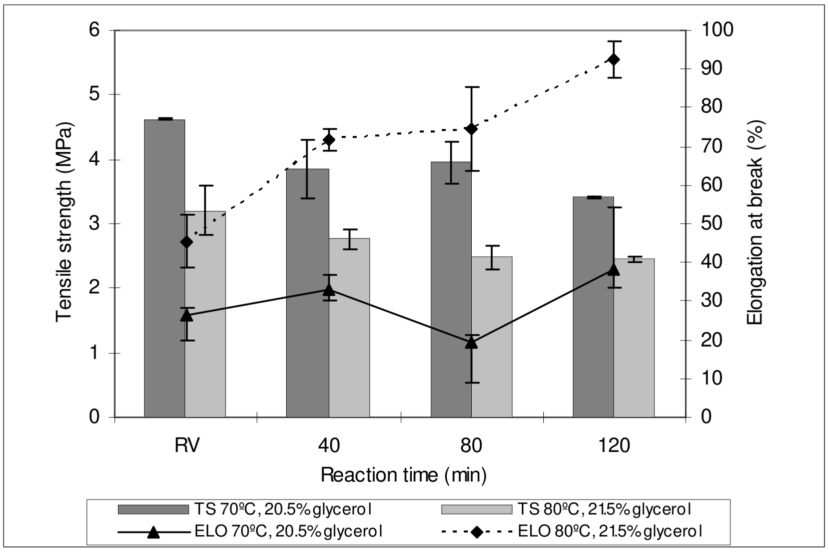

Figure 3.4 Tensile strength (MPa) and elongation at break (%) of *A.cruentus* flour films crosslinked with epichlorohydrin (5%) at 70 and 80ºC; 20.5 and 21.5 wt% glycerol respectively. Reference value (RV) without crosslinking (0% ECH).

Figure 3.5 shows the puncture test results. At 80 minutes of reaction, the films seemed to become more flexible and less resistant, for both temperatures. At 70ºC the puncture force was significantly lower at 80 minutes, but there was no difference between the puncture deformations. At 80ºC the PD was considerably higher at 80 minutes, but there was no difference between the film resistances (PF).

The most resistant film (PF = 5.05N) was produced at 70ºC for 40 minutes. Moreover, the most flexible (PD = 11.3%) at 80 minutes of reaction for the film crosslinked at 80ºC, with 21.5g/100g flour of glycerol.

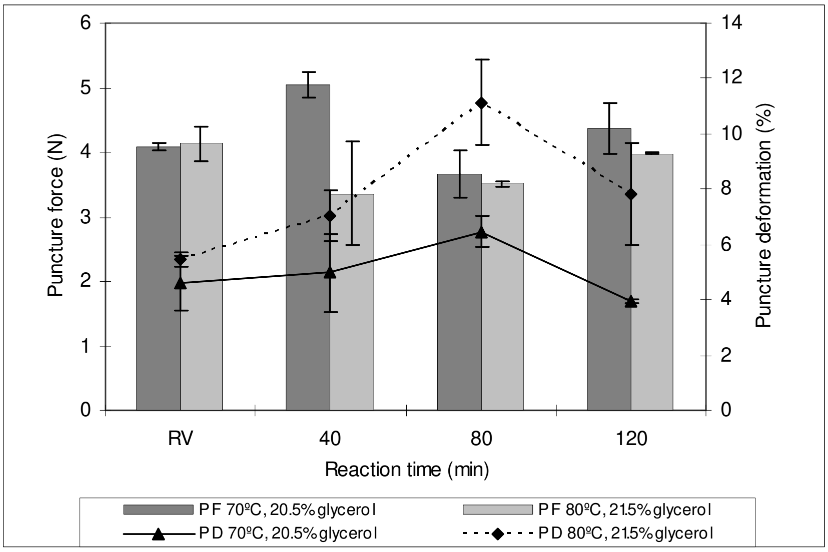

Figure 3.5 Puncture force (N) and deformation (%) of *A.cruentus* flour films crosslinked with epichlorohydrin (5%) at 70 and 80ºC; with 20.5 and 21.5 wt% glycerol respectively. Reference value (RV) without crosslinking (0% ECH).

It was observed from Figures 3.4 and 3.5 the impact that a small difference in the glycerol content (1g/100g flour) could have on the mechanical properties of the films, as the films prepared with 21.5% glycerol showed higher flexibilities (ELO and PD) and lower resistance (TS and PF).

3.3.1.3 Mechanical properties of *A.cruentus* flour films crosslinked with ECH (5 and 10 wt%) at 80ºC and different reaction time, with 20 wt% glycerol

Considering the great effect that the glycerol had on the mechanical properties of the crosslinked films, the glycerol concentration was fixed in 20g glycerol/100g flour. Films with higher resistance were obtained at this concentration. Once the glycerol content was fixed, a test to assess the effect of the crosslinked reaction at 80ºC (gelatinization temperature) for two epichlorohydrin concentrations (5 and 10g/100g flour), with the same reaction times considered previously (40, 80 and 120 min) was carried out.

Figure 3.6 shows the effect of two epichlorohydrin concentrations (5 and 10%) at 80°C with 20 wt% glycerol, as function of reaction time, on the mechanical properties of the films. It was observed from Figures 3.6 and 3.7 that the films produced with 5% ECH are more resistant (TS and PF) but less flexible (ELO and PD) than the ones crosslinked with 10% ECH at the same conditions.

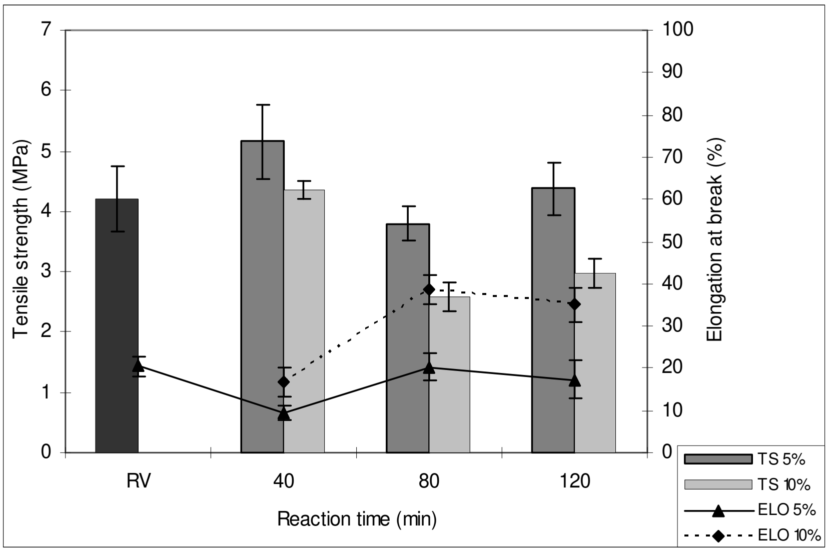

Figure 3.6 Tensile strength (MPa) and elongation at break (%) of *A.cruentus* flour films crosslinked with ECH (5 and 10%) at 80ºC and different reaction time, with 20% glycerol. Reference value (RV) without crosslinking (0% ECH).

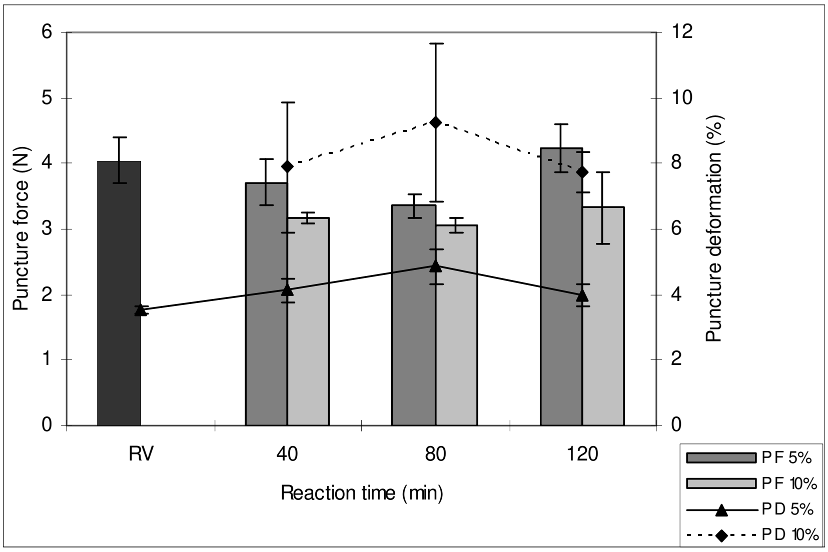

Figure 3.7 Puncture force (N) and deformation (%) at break of *A.cruentus* flour films crosslinked with ECH (5 and 10%) at 80ºC and different reaction time, with 20% glycerol. Reference value (RV) without crosslinking (0% ECH).

Only the film crosslinked with 5 wt% ECH for 40 min was more resistant than the non-crosslinked *A.cruentus* film (TS = 5.15 > 4.20 MPa).

A non-homogeneous behavior of the mechanical properties was detected with increasing reaction time, since the minimum resistance and maximum flexibility were achieved at intermediate time (80 min). This means that there was no real improvement of the mechanical performance of the crosslinked films, as one property was enhanced while the other was reduced.

Although no real improvement of the mechanical properties was observed, Figure 3.8 shows an outstanding reduction of the solubility in water of the crosslinked films compared to the non-treated *A.cruentus* flour film, from 67.5% to an average of 25% of soluble mater.

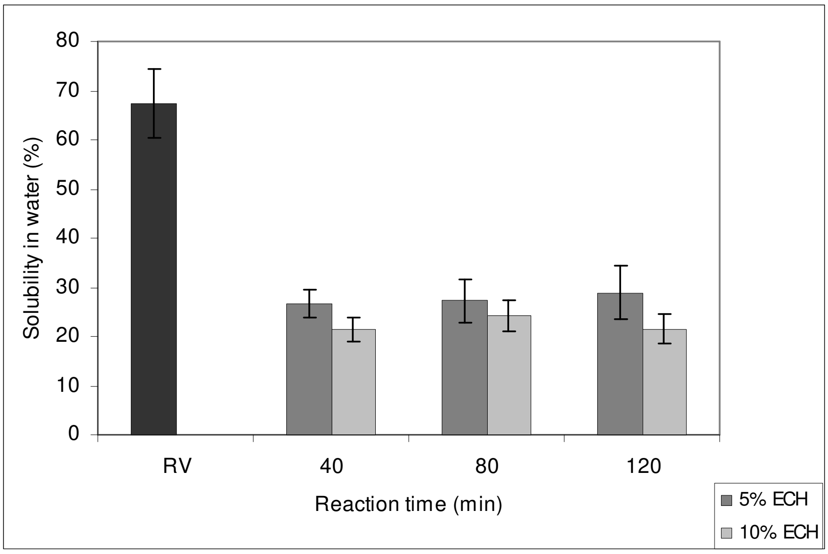

Figure 3.8 Solubility in water (%) of *A.cruentus* flour films crosslinked with epichlorohydrin (5 and 10%) at 80ºC and different reaction time, with 20g/100g flour glycerol. Reference value (RV) without crosslinking (0% ECH).

For the solubility in water of the crosslinked films, there was no significant difference between the films produced at the same concentration, but it was noted a lower solubility for the ones crosslinked with 10% ECH, with an average of 22.3%. It is important to remark that the crosslinked films maintained their integrity during the solubility test, which was not accomplished in the control film.

3.3.2 Response surface methodology (RSM)

After preliminary essays, it was decided to study more deeply the crosslinking reaction, as it has a significant effect in the solubility of the *A.cruentus* flour film. The chosen parameters were epichlorohydrin concentration from 1.6 to 18.4g ECH/100g flour, reaction temperature from 39.8 to 90.2ºC and reaction time from 12.8 to 147.2 minutes.

The developed films were homogeneous, slightly opaque and yellowish with an average thickness of 80 ± 2µm, and average moisture content of 13.5 ± 1.7%. In Table 3.3 are shown the mechanical properties and the solubility in water of the *Amaranthus cruentus* flour crosslinked films developed according to the 2^3 central composite design, for homogeneous reactions. In addition, the pH of the filmogenic solution determined at the end of the crosslinking reaction is shown in Table 3.3.

Table 3.3 Central composite design matrix with coded and real values of the variables and responses to mechanical properties (TS, ELO, PF and PD), solubility and pH of the solution

Assay	ECH (x_1) (g/100gflour)	Temperature (x_2) (ºC)	Time (x_3) (min)	TS (MPa)	ELO (%)	PF (N)	PD (%)	S (%)	pH
1	5 (-1)	50 (-1)	40 (-1)	2.01	65.03	3.29	8.98	19.83	10.20
2	15 (+1)	50 (-1)	40 (-1)	3.92	23.17	4.58	2.21	30.19	9.68
3	5 (-1)	80 (+1)	40 (-1)	2.92	31.78	3.52	6.50	23.36	7.71
4	15 (+1)	80 (+1)	40 (-1)	2.45	70.96	3.07	10.65	33.32	7.11
5	5 (-1)	50 (-1)	120 (+1)	3.68	40.69	4.37	3.28	30.10	9.77
6	15 (+1)	50 (-1)	120 (+1)	3.00	41.48	3.70	6.59	25.21	8.57
7	5 (-1)	80 (+1)	120 (+1)	2.94	63.09	3.52	7.62	33.66	7.01
8	15 (+1)	80 (+1)	120 (+1)	2.12	49.14	1.99	12.95	30.90	6.11
9	1.6 (-1.68)	65 (0)	80 (0)	3.69	31.95	4.76	4.23	30.31	9.50
10	18.4 (+1.68)	65 (0)	80 (0)	2.66	50.81	3.27	8.69	26.53	7.53
11	10 (0)	39.8 (-1.68)	80 (0)	2.17	75.40	3.55	9.37	22.80	10.09
12	10 (0)	90.2 (1.68)	80 (0)	2.19	25.65	3.19	8.92	39.46	6.31
13	10 (0)	65 (0)	12.8 (-1.68)	2.29	29.17	4.70	4.86	31.14	9.25
14	10 (0)	65 (0)	147.2 (+1.68)	3.60	28.44	3.65	5.28	33.26	7.44
15	10 (0)	65 (0)	80 (0)	2.53	57.48	3.12	11.87	30.38	7.76
16	10 (0)	65 (0)	80 (0)	3.23	61.18	2.85	12.91	30.36	7.88
17	10 (0)	65 (0)	80 (0)	2.25	64.72	2.59	13.94	32.31	8.03

TS, tensile strength; ELO, elongation at break; PF puncture force; PD puncture deformation; S, solubility and pH, pH of the solution.

3.3.2.1 Tensile strength and elongation at break

A small variation of the tensile strength (2-4 MPa) for the *A.cruentus* flour films crosslinked with epichlorohydrin was observed for all controlled conditions (Table 3.3), this difference is comparable to the one obtained with the central point essays (2.3 – 3.2 MPa). That explained why it was impossible to get a predictive model for this response.

For the elongation at break (ELO), the results were more variable (23-75%), but a lack of significance was obtained for its model, since the variation of this mechanical property could not be explained by the regression (R^2 = 37%). Therefore, it was not possible to get a predictive model for the responses either.

However, it was feasible to analyze the results obtained for the seventeen essays, the control (amaranth flour film without epichlorohydrin, numbered as 0) and the *A.cruentus* flour film developed by Tapia-Blácido at the same conditions of the control film (named as TB) (*21*), with a direct comparison between the tensile strength and elongation at break (Figure 3.9). It was observed that all the essays are located in the same diagonal area, descending from stiff and brittle (high TS and low ELO) to soft and ductile (low TS and high ELO) films, a typical behavior of the plasticizer effect. In this way, for the crosslinked films, when the elongation was improved the respective resistance was decreased.

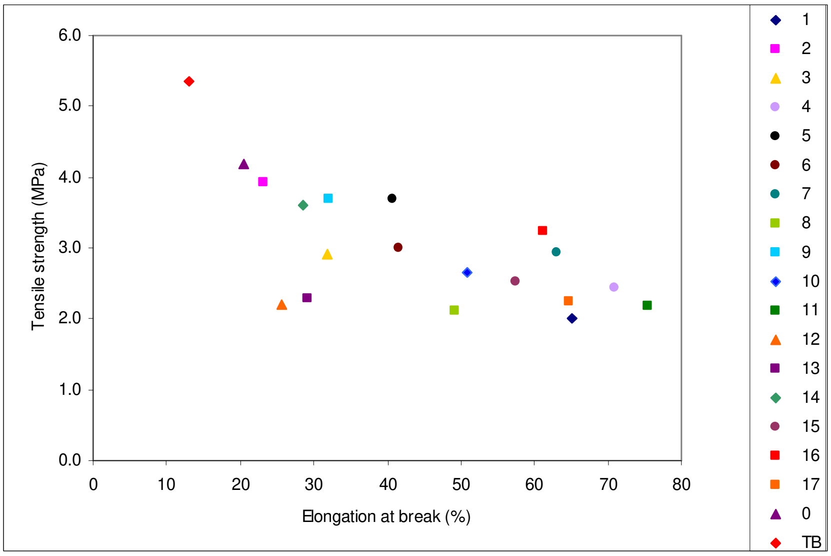

Figure 3.9 Tensile strength and elongation at break of the different crosslinking reactions performed in the experimental design (2^3)

The film developed by Tapia-Blácido (TB) was the stiffest (TS = 5.35 MPa and ELO = 12.9%), as well as the control film (numbered as 0) with TS = 4.20 MPa and ELO = 20.4%; therefore the reaction with ECH provided a plasticizing effect on the crosslinked films. The less desirable properties (low TS and ELO), film soft and ductile, was obtained from the 12th essay (TS = 2.2 MPa and ELO = 25.6%) developed with the higher reaction temperature (90.2ºC) and intermediate ECH concentration (10%) and reaction time (80 min).

The best tensile properties among the crosslinked films were achieved by the 5th essay (5% ECH, 50ºC, 120 min), which combined intermediate values of both responses: resistance (TS = 3.7 MPa) and flexibility (ELO = 40.7%). The most rigid film (high TS) among the crosslinked was from the 2nd essay (15% ECH, 50ºC, 40 min), whose mechanical properties (TS = 3.9 MPa and ELO = 23.2%) are near to those of the control, both with much lower solubility (S = 30.2%). The most flexible film (high ELO) was from the 11th essay (15% ECH, 50ºC, 40 min), with 75.4% of elongation at break. It is suitable to note that the desired characteristics (high TS and ELO) were obtained at low reaction temperature (50ºC).

The tensile strengths obtained from the crosslinked films are superior to those reported for the *A.cruentus* flour films with stearic acid addition, which varied from 0.8 to 3.0 MPa (*2*), but with a higher elongation at break (74 - 620%).

Although the mechanical properties of the *A.cruentus* flour films were not improved by the crosslinking reaction with epichlorohydrin, the solubility in water was significantly reduced. It could be considered that the covalent linkages introduced by the epichlorohydrin may obstruct the physical bonding of the polymer chains, making the matrix less resistant, but at the same time, this new covalent linkages limited the polymer disintegration due to hydration.

3.3.2.2 Puncture force and deformation

From Figure 3.10, it could be seen that the puncture force of the crosslinked films was generally lower to the one of the control film (numbered as 0) or the *A.cruentus* flour film developed by Tapia-Blácido (TB) (*21*) both with PF = 4.05N; only four samples (essays 2, 5, 9 and 13) were more resistant (PF > 4.0N). The highest puncture force was obtained from the 9th essay (PF = 4.76N) performed at the lowest epichlorohydrin concentration (1.6g/100g flour). The crosslinked films presented values slightly superior to those reported for the *A.cruentus* flour films with stearic acid addition, which varied from 1.0 to 2.0 N (*2*).

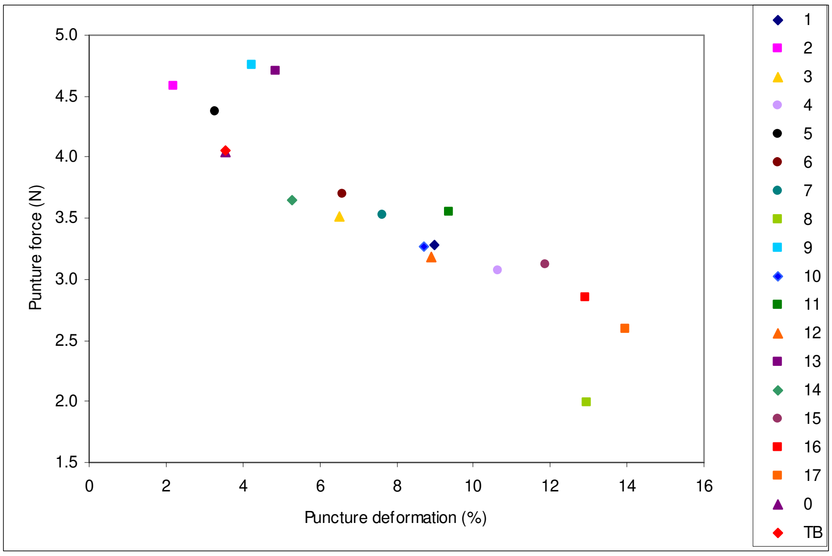

Figure 3.10 Puncture force and deformation of the different crosslinking reactions performed in the experimental design (2^3)

For the puncture deformation the inverse effect was observed, with higher PD for almost all the crosslinked films compared to the control sample (PD = 3.55%). The highest puncture deformation value was achieved by the central point of the experimental design (PD = 13 ± 1%) with 10g ECH /100g flour, 65ºC and 80 minutes of reaction. The optimized amaranth flour film developed by Tapia-Blácido (*3*), for another variety of amaranth (*A.caudatus*) with glycerol addition, presented superior flexibility (PD = 15.6%) than the *A.cruentus* flour crosslinked films, but with lower resistance (PF = 2.34N).

Models for puncture force and deformation

A regression analysis ($p < 0.05$) was performed to obtain a second order model equation for the puncture force (equation 5) and puncture deformation (equation 6) as function of epichlorohydrin concentration (x_1), reaction temperature (x_2) and time (x_3).

$$PF = 3.0 - 0.2\,x_1 + 0.3\,x_1^2 - 0.3\,x_2 + 0.3\,x_3^2 - 0.3\,x_1\,x_2 - 0.4\,x_1\,x_3 \qquad \text{Eq.5}$$

$$PD = 12.6 + 1.0\,x_1 - 2.0\,x_1^2 + 1.2\,x_2 - 1.1\,x_2^2 - 2.5\,x_3^2 + 1.6\,x_1\,x_2 + 1.4\,x_1\,x_3 \qquad \text{Eq.6}$$

The regression parameters reveal the importance of each independent variable (x_1, x_2, x_3) under the response (PF or PD), as well as the effect that it has increasing (+) or decreasing (-) the specific mechanical property. The inverse effect between the PF and PD is evident, as the regression signs for each variable of the PF equation generally had the opposite sign in the PD model. For example, the reaction temperature (x_2) has a decreasing effect under the PF (-0.3), although it has an increasing effect in the puncture deformation (+1.2). Regression coefficients are shown in Table A.2.

The statistical significance of these coded models was evaluated with an F test (ANOVA) and the results are given in Table 3.4.

Table 3.4 Analysis of variance for the quadratic model of puncture force and deformation

Source of	Puncture force				Puncture deformation			
variation	SS[a]	DF[b]	MS[c]	F ratio	SS[a]	DF[b]	MS[c]	F ratio
Regression	6.57	6	1.10	4.56	160.34	7	22.91	5.86
Residual	2.40	10	0.24		35.19	9	3.91	
Total	8.97	16			195.53	16		
		$R^2 = 0.73$				$R^2 = 0.82$		
		$F_{5\%,6,10} = 3.22$				$F_{5\%,7,9} = 3.29$		

[a] Sum of squares; [b] degrees of freedom; [c] Mean square

Both models are significant since each F ratio (MS regression / MS residual) is higher than the F critical value (4.56 > 3.22 for PF, and 5.86 > 3.29 for PD) (the absolute and relative deviations for each model are shown in Table A.4).

Since the models were significant ($p<0.05$) they were used to plot the response surfaces at three different reaction temperatures (39.8, 65 and 90.2ºC) for PF (Figure 3.11) and PD (Figure 3.12).

Figure 3.11 (a, b and c) shows that as the reaction temperature increases, the conditions to obtain more resistant films (high PF), varied from high ECH concentration and short reaction time at 39.8ºC, to low ECH concentration and long reaction time at 90.2ºC.

At low reaction temperatures (Figure 3.11 a) and short time, the film became more resistant as the ECH concentration increases. This reinforcing effect could be explained by the transformation of the hydrogen bonds to covalent linkages in the polymeric matrix (*10*). The inverse effect was noted at high reaction temperature (Figure 3.11 c) and long reaction time, where the film became less resistant as the ECH concentration increases. This behavior may be due to the formation of side reaction products, such as glycerol, whose production is favored under extreme reaction conditions (high ECH concentration, high temperature and long reaction time) and diluted solutions. Kartha and Srivastava (*10*) noted that in reactions under homogeneous conditions, the increasing amount of water in the system promoted the hydrolysis of the ECH, resulting in the formation of chlorohydrin and glycerol. Generally, the highest PF values were obtained for crosslinking reactions at low ECH concentration and long reaction time.

Figure 3.12 shows that the highest flexibilities (PD) for the crosslinked films are achieved at intermediate reaction time (~80 min), and the PD maximum goes from 5-10% ECH at 39.8ºC; to 10% ECH at 65ºC, and finally 10-15% ECH at 90.2ºC. The optimized region was dislocated to higher epichlorohydrin concentrations as the reaction temperature increases.

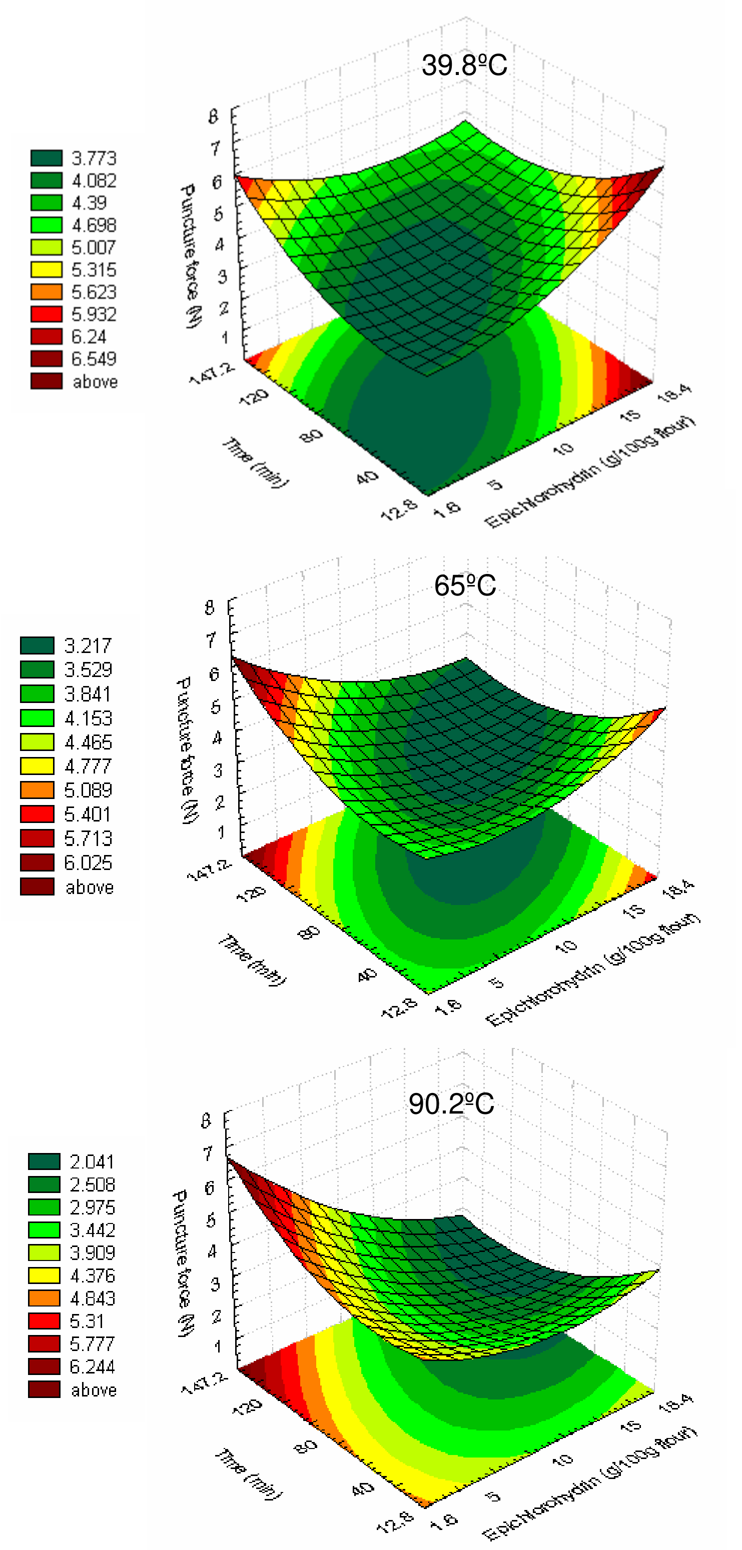

Figure 3.11 Response surfaces for puncture force (PF) as a function of epichlorohydrin concentration and reaction time, at different reaction temperature (39.8, 65 and 90.2ºC)

At low temperature (Figure 3.12 a) and short reaction time, the film became less flexible as the ECH concentration increased, denoting a strengthening of the film matrix due to the crosslinking of the polymeric chains. However, at high reaction temperature (Figure 3.12 c) the opposite effect was observed, since the film became more flexible as the ECH concentration increased, indicating the presence of side reaction products that act like plasticizers in the polymeric matrix. The maximum film flexibility was obtained at high reaction temperature (90.2ºC) and ECH concentration (10-15%), noting the strong effect that high temperature has on the mechanical properties.

These results (PF and PD) show the same plasticizing effect analyzed for the tensile strength and elongation at break, where the crosslinking reaction with ECH make the *A.cruentus* flour films more resistant but less flexible, or more flexible with lower resistance, depending of the reaction conditions. This phenomenon indicates that there was no real improvement on the mechanical properties of the films due to the crosslinking reaction.

A reinforcing effect from the crosslinking reaction was expected as it changed the hydrogen bonds of the polymeric matrix for covalent linkages, but this effect was just seen at low reaction temperature (39.8ºC). At medium and high temperatures was observed a plasticizing phenomenon. This observation could be explained by two factors. (a) the promotion of undesired side reactions: the glycerol formation from the hydrolysis of ECH in presence of water excess (*10*), as well as the monoglycerol ether starch produced by the competitive reaction of hydroxyl ions to open the oxirane during the crosslink reaction (*12*); and (b) the covalent linkages introduced by the ECH obstructed the physical bonding of the polymer chains, reducing the matrix resistance.

As indicated in the methodology, the films were prepared from a 4% amaranth flour solution, this fact could affect the crosslinking reaction, as the small epichlorohydrin molecule could be unable to reach easily the polymer chains to make the covalent linkages between them, and so reinforce the film matrix. Instead, the side reactions mentioned before were favored, making the films less resistant and more flexible.

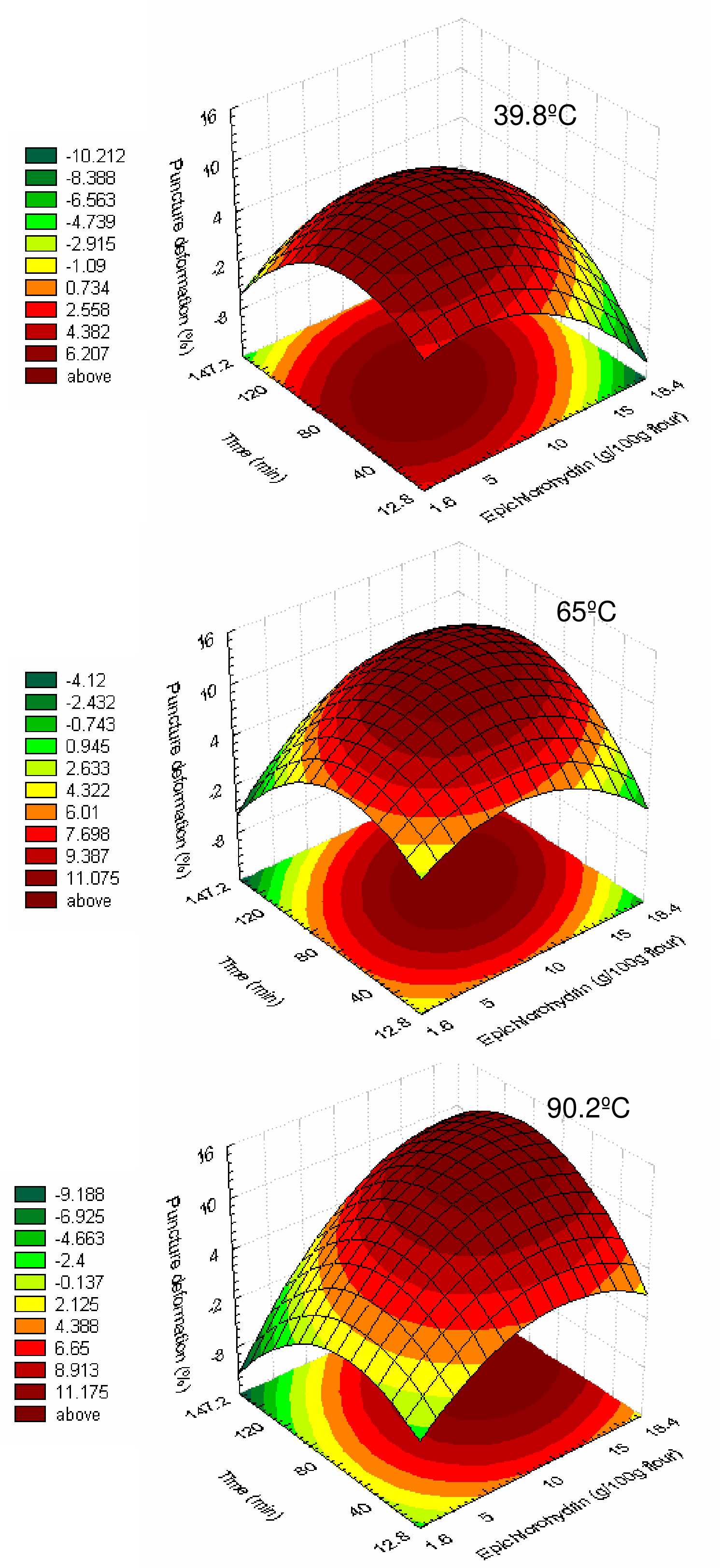

Figure 3.12 Response surface for puncture deformation (PD) as a function of ECH concentration and reaction time at different temperatures (39.8, 65 and 90.2ºC)

3.3.2.3 Solubility in water and pH of the solution

Since the integrity is one of the most important aspects in packaging materials, the solubility in water (S) of the films should be as low as possible to guarantee the food protection at high water activities (*17*). Although the mechanical properties of the modified films were not improved, the solubility in water was considerably reduced by the crosslinking reaction.

Figure 3.13 shows a direct comparision between solubility in water (S) of the films and the pH solution at the end of the crosslinking reaction. All reactions started at pH 10.4, and at the end, the pH was measured before being adjusted to pH 10.7. It was noticed that all crosslinked films presented an inferior solubility ($S_{average}$= 29.6 ± 4.8%) when compared to the non-crosslinked *A.cruentus* flour film (S = 67.5%), and that almost all maintain their integrity under the test conditions. Reduced values of solubility were expected as the crosslinked polymer became more hydrophobic due to the substitution of the hydrogen bonding by covalent linkages (*9*), but the major effect may be due to the network formation by the covalent links.

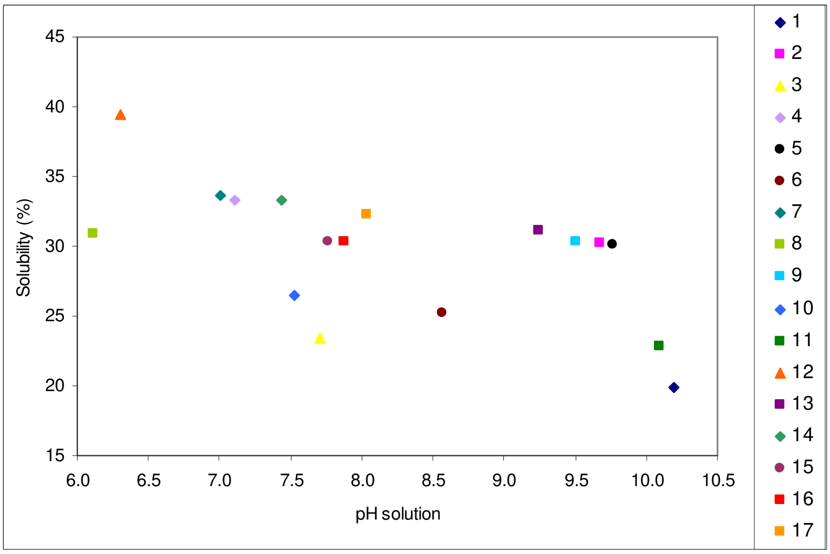

Figure 3.13 Solubility in water and pH of the solution of the different crosslinking reactions performed in the experimental design (2^3)

The lowest value of solubility observed was 19.8% for the 1st essay (5% ECH, 50ºC and 40 min), at the lowest conditions combination. The higher value (S = 39.5%) was obtained for the film developed at the higher reaction temperature from the 12th essay (10% ECH, 90.2ºC and 80 min). In general the *A.cruentus* flour films crosslinked with epichlorohydrin presented a lower solubility compared to the *A.caudatus* flour films with S = 42.2% (*3*), but higher than those of *A.cruentus* flour with stearic acid addition whose solubility was 15.2% (*2*).

It was observed that the pH adjustment of the filmogenic solution (to pH 10.7) after the crosslinked reaction, affected the solubility of the film, as the sodium hydroxide added is soluble in water. For the 12th essay conditions (10% ECH, 90.2ºC and 80 min), a film without pH regulation was formulated and it presented a decreased solubility (S = 31.6%), this difference could be explained by the NaOH addition needed to increase the pH from 6.3 to 10.7. However, this pH adjustment was required to make amaranth protein soluble in the film forming solution.

The pH solution was measured after the crosslinked reaction for each essay (Table 3.3); it was observed a decrease of the pH from the non-crosslinked *A.cruentus* flour solution (pH 10.4) to the crosslinked film forming solutions with an average pH 8.2 ± 1.3. The pH reduction is due to the consumption of OH^- along the crosslinked reaction as explained by Kartha and Srivastava (*10*) in the reaction mechanism of starch crosslinked with ECH.

The lowest pH was achieved by the 8th essay (pH 6.1) with the highest condition combination, 15% ECH, 80ºC and 120 min of reaction. On the contrary, the higher pH solution was from the 1st essay (pH 10.2) with the lowest conditions, 5% ECH, 50ºC and 40 minutes, which presented also the lowest solubility in water. The pH solution seems to be an indicator of the reaction intensity, but not precisely of the crosslinking degree, since side reactions are also involved.

Models for solubility in water and pH of the solution

A regression analysis ($p<0.05$) was performed to get a second order model equation for the solubility in water (equation 7) and the pH of the solution (equation 8) as function of epichlorohydrin concentration (x_1), reaction temperature (x_2) and time (x_3).

$$S = 30.63 - 1.29\, x_1^2 + 3.22\, x_2 + 1.23\, x_3 - 3.50\, x_1 x_3 \qquad \text{Eq.7}$$

$$pH = 8.12 - 0.48\, x_1 + 0.15\, x_1^2 - 1.22\, x_2 - 0.46\, x_3 \qquad \text{Eq.8}$$

Both, the reaction temperature (x_2) and time (x_3) enhancement produced an increase in solubility but a decrease in pH solution (Equations 7 and 8). Besides, an ECH concentration (x_1) enhancement caused a pH solution reduction, as more OH^- was consumed in the reaction. The factor with more effect under both responses (S and pH) was the reaction temperature (x_2). Regression coefficients are shown in Table A.3.

The statistical significance of these models was evaluated with a F test and the results are given in Table 3.5.

Table 3.5 Analysis of variance (ANOVA) for the quadratic model description of solubility in water and pH of the solution.

Source of	Solubility				Solution pH			
variation	SS[a]	df[b]	MS[c]	F ratio	SS[a]	df[b]	MS[c]	F ratio
Regression	281.3	4	70.32	10.20	26.56	4	6.64	111.8
Residual	82.7	12	6.89		0.71	12	0.06	
Total	364.0	16			27.28	16		
		$R^2 = 0.77$				$R^2 = 0.97$		
		$F_{5\%,4,12} = 3.26$				$F_{5\%,4,12} = 3.26$		

[a] Sum of squares; [b] degrees of freedom; [c] Mean square

Both models are significant since each F ratio (MS regression / MS residual) is higher than the F critical value (10.20 > 3.26 for S, and 111.8 > 3.26 for pH). The correlation coefficients (R^2) show that 77% of the solubility values variation and 97% of the pH solution variation could be explained by the regressions, equations 7 and 8 respectively. The absolute and relative deviations for each model are shown in Table A.5.

Since the models are significant ($p<0.05$) they were used to plot the response surface at three different reaction times for S (Figure 3.15) and pH (Figure 3.16). The response surfaces for solubility (S) as a function of epichlorohydrin concentration and reaction time (39.8ºC), is presented in Figure 3.14.

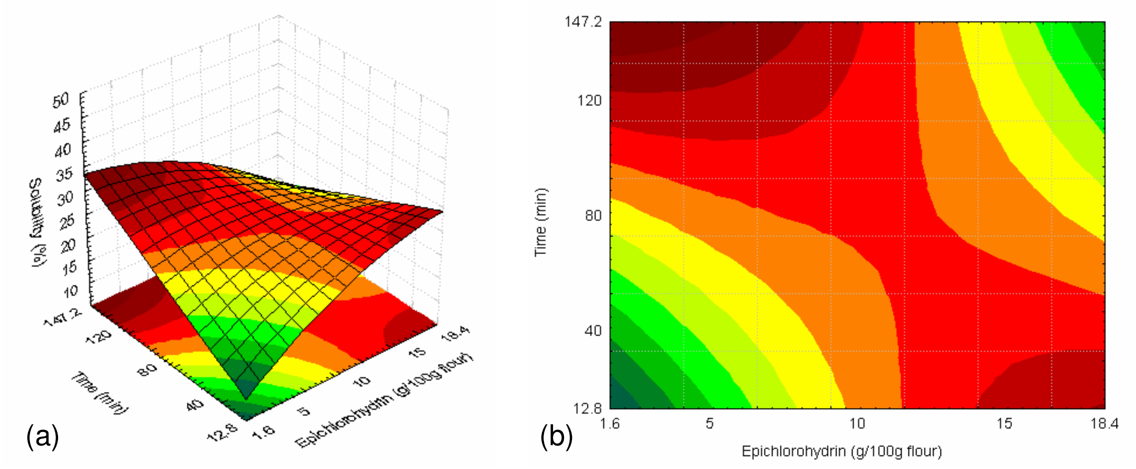

Figure 3.14 Response surface (a) and contour diagram (b) for solubility as a function of epichlorohydrin concentration and reaction time, at 39.8ºC

In accordance with the analysis made for the mechanical properties, the Figure 3.14 shows the effect of the epichlorohydrin concentration and the reaction time at 39.8ºC, where the lowest solubility was achieved. At short reaction time (12.8 - 40 min), the film turns to be more soluble as the ECH concentration increased, although it became more resistant. In contrast, at long reaction time (120 – 147.2 min) the film solubility decreased as the epichlorohydrin concentration increased.

This inverse effect is more clear in Figure 3.15 analysis (a, b and c). The lowest solubilities (S) for the crosslinked films were reached at 39.8 - 50ºC with 1.6% ECH for 12.8 minutes, and at 39.8 - 50ºC with 15 – 18.4% ECH for 147.2 min. The minimum region (low S) was dislocated from low to high ECH concentrations as the reaction time increased, always at low reaction temperatures (39.8 - 50ºC).

The higher reaction temperatures made the crosslinked films more soluble in water for all the ECH concentrations, since side reactions may be favored under these conditions. Moreover, at intermediate conditions of reaction time (~80ºC) and ECH concentration (~10 wt %) the films became more hydrophilic (Figure 3.15 b), same reaction conditions that make the crosslinked films more flexible (high PD) but less resistant (low PF).

The lowest solubilities were obtained at low reaction temperature (~39.8ºC) combined with: (a) low ECH concentration and short reaction time, or (b) high ECH concentration and long reaction time. In the first case, with the lowest condition combination, for example the 1st essay, the films were softer and ductile, with low resistance (PF) and relatively high flexibility (PD), and the low solubility may be favored by the small amount of NaOH that had to be added in order to adjust the pH from 10.2 to 10.7. In the second case, for example the 6th essay (15 g/100g flour ECH, 50ºC, 120 min), the films presented intermediate mechanical performance (PF and PD) and the pH of the solution had to be adjusted from 8.57 to 10.7, where the amount of NaOH added was significantly higher than the first case.

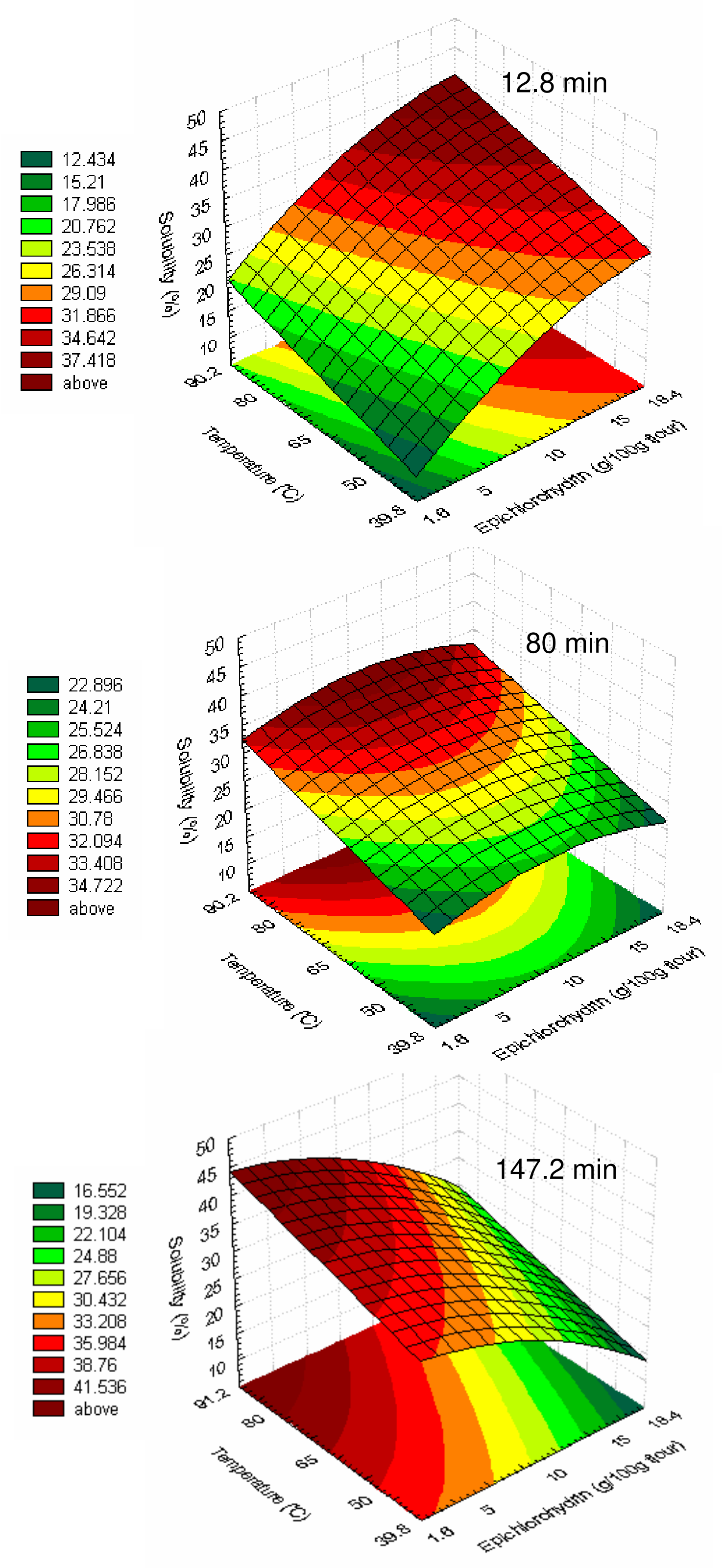

Figure 3.15 Response surface for solubility as a function of epichlorohydrin concentration and reaction temperature, at different reaction time (12.8, 80 and 147.2 min)

Figure 3.16 (a, b and c) shows the response surfaces for the pH solution. Evidently, the reaction temperature had the major effect under the pH solution, as the lowest pH values were obtained at higher temperatures. Besides, the ECH concentration and reaction time had a negative impact in the pH solution, as it decreased with the increase of both variables. That is why the pH solution appears like an indicator of the reaction intensity. In addition, it is important to consider that at more intense reaction conditions (low pH) it was necessary to add more NaOH to adjust the pH of the solution, and this fact affected the film solubility.

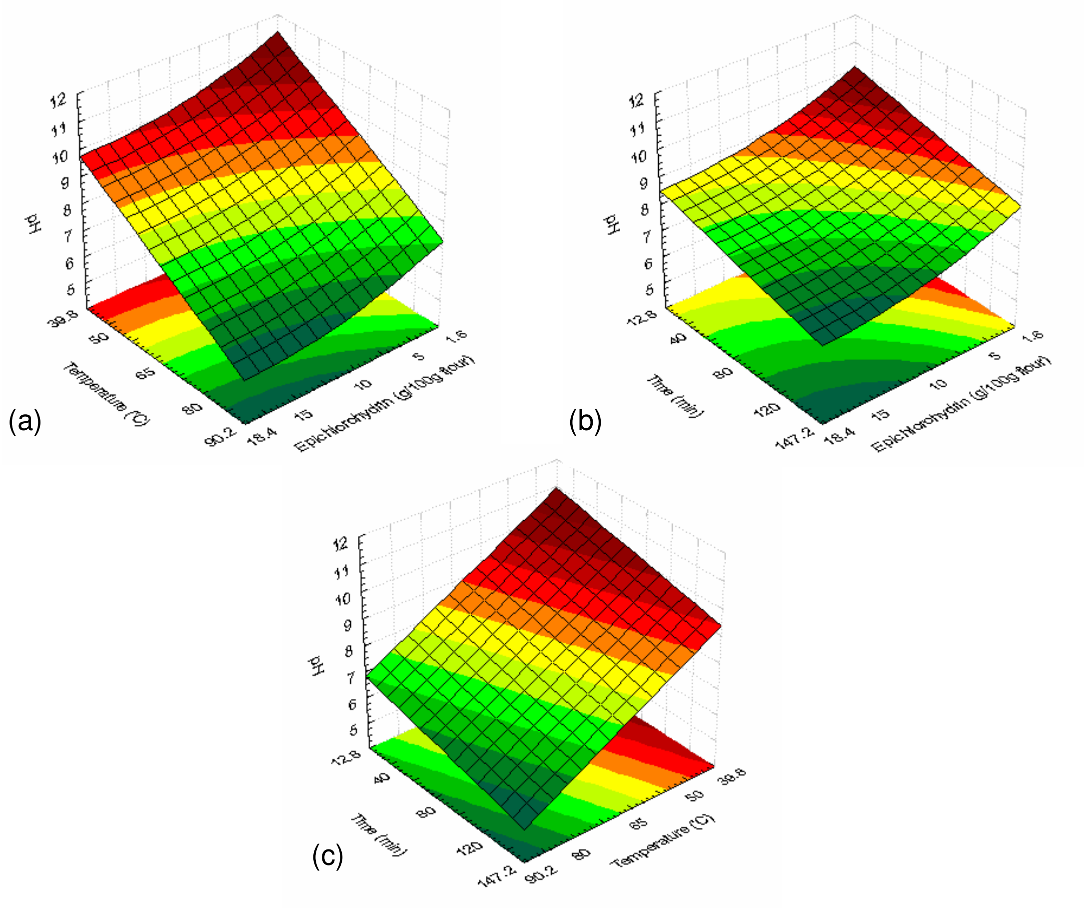

Figure 3.16 Response surface for pH of the solution as a function of (a) epichlorohydrin concentration and reaction temperature (reaction time, 80 min), (a) epichlorohydrin concentration and reaction time (reaction temperature, 65ºC), and (c) reaction temperature and time (ECH concentration, 10%)

The response surfaces analysis confirm that (a) the crosslinking reaction provided a plasticizing trend in the *A.cruentus* flour film as it enhanced one mechanical property (resistance or flexibility) with the consequent reduction of the other characteristics. (b) The reaction temperature had a strong impact on the film solubility and the pH solution, therefore is desired to perform the crosslinking reaction at low temperatures to ensure the reduction of the film solubility, as well as to decrease the pH variation along the reaction. And, (c) the pH adjustment affected the film solubility as the NaOH added increased this characteristic.

3.3.3 Effect of the epichlorohydrin concentration without pH adjustment

Based on the previous observations, it was decided to perform a series of crosslinking reactions with fixed temperature and time, and varying the epichlorohydrin concentration, without pH adjustment at the end of the reaction. The temperature was fixed at the lowest condition of the experimental design (39.8ºC), and the reaction time was set at 120 min. These conditions provided the best film characteristics (intermediate PF and PD, low S) in the experimental design. The epichlorohydrin concentration varied from 1.6 to 18.4g/100g of A.cruentus flour, at the same five levels used before. This was an attempt to study the effect of the ECH concentration on the mechanical and barrier properties of the crosslinked A.cruentus flour films without the pH adjustment effect.

3.3.3.1 Mechanical properties

In conformity with the observations made before, the crosslinked *A.cruentus* flour films became more resistant and less flexible with increasing epichlorohydrin concentration. The resistance of the films (TS and PF) increased and the flexibility (ELO and PD) decreased, as the epichlorohydrin concentration was enhanced (Figures 3.17 and 3.18). This behavior could be explained by the combination of three factors: (a) a reinforcing effect from the crosslinking reaction as it changed the hydrogen bonds of the polymeric matrix for covalent linkages (*10*), (b) the reduction of side reactions and (c) the decrease of the pH of the solution variation at low reaction temperature.

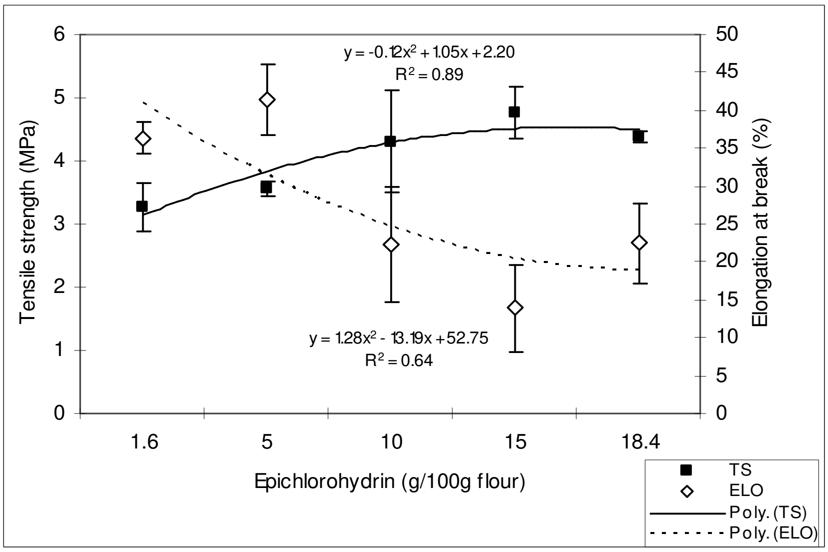

Figure 3.17 Tensile strength (MPa) and elongation (%) at break of *A.cruentus* flour films crosslinked with epichlorohydrin at different concentrations (39.8ºC, 120 min)

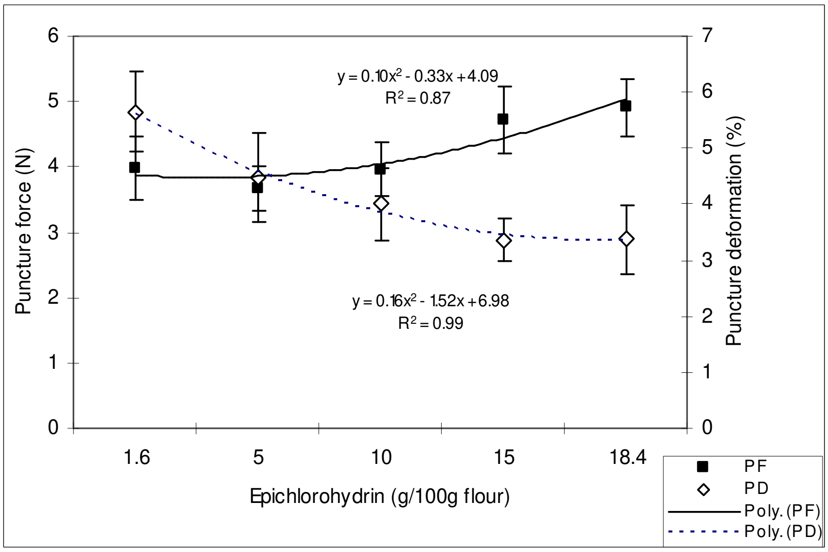

Figure 3.18 Puncture force (N) and deformation (%) at break of *A.cruentus* flour films crosslinked with epichlorohydrin at different concentrations (39.8ºC, 120 min)

Although the crosslinking reaction produced a reinforcement of the polymeric matrix due to the covalent bonding formation, it did not provide a real improvement of the mechanical properties when compared to the non-treated *A.cruentus* flour film. There was no significant difference ($p<0.05$) in the resistance responses (TS and PF) and the puncture deformation (PD) between the crosslinked and non-crosslinked films. Only the elongation at break (ELO) from the film crosslinked with 5 wt% ECH obtained a higher elongation at break (ELO = 41.3%) than the non-modified film (ELO = 20.4%) (Appendix 1, Table A.6). This observable fact could be explained as the covalent linkages introduced by the epichlorohydrin obstructed the natural bonding of the polymer chains, making the matrix less resistant.

In general, the crosslinked films with ECH presented a higher resistance than the *A.cruentus* flour– stearic acid films (TS, 2.6 MPa; PF, 2.5N), but a lower elongation at break (ELO, 148%) (*2*).

It is suitable to note that in the tensile test (Figures 3.17), the film crosslinked with 5g ECH/100g flour, showed the best mechanical performance among the other crosslinked films under the same conditions; since it obtained a favorable combination of both characteristics, resistance (TS) and flexibility (ELO). The mechanical properties are given in Table 3.6, the crosslinked films (5 wt% ECH) presented slightly lower resistance (TS and PF) but improved flexibility (ELO and PD) when compared to the non-crosslinked *Amaranthus cruentus* flour film. However, just the elongation at break presented a significant difference from the control film ($p<0.05$).

Table 3.6 Mechanical properties of crosslinked and non-crosslinked *A.cruentus* flour film

A. cruentus flour film with 20 wt.% of glycerol	Tensile test		Puncture test	
	TS (MPa)	ELO (%)	PF (N)	PD (%)
Crosslinked (5 wt.% ECH, 39.8ºC and 120 min)	3.56	41.35	3.67	4.47
Non crosslinked	4.20	20.41	4.05	3.55

TS, tensile strength; ELO, elongation at break; PF puncture force; PD puncture deformation

Rioux *et al.* (*1*) reported that the maximum values for the mechanical properties of the high amylose starch films crosslinked with epichlorohydrin (1-10g/100g polymer) were reached for the moderate crosslinking degree (CLD) at 6 g/100g flour. The researchers explained that at these CLD the polymer is less crystalline and the low crosslinking allows enough flexibility of chains to be stabilized by hydrogen bonding in the polymer network.

Nevertheless, the mechanical performance of the crosslinked films were poor as contrasted with other films developed, for example the starch-filled low-density polyethylene film (with 5% of crosslinked starch), which presented 15.5 MPa of tensile strength and 253% of elongation at break (*8*).

3.3.3.2 Solubility and water vapor permeability

Although the covalent bonds formed by epichlorohydrin in the crosslinking reaction were insufficient to enhance the mechanical properties of the *A.cruentus* flour films, they were able to improve their barrier properties compared to the non-modified film.

Figure 3.19 shows a significant reduction of the barrier properties (S and WVP) due to the crosslinking reaction with ECH. The solubility was decreased from 67.5% (non-crosslinked film) to 29.3 ± 2.2% of soluble mater for the crosslinked films. However, there was no significant difference between the films crosslinked with 1.6 – 15 wt% ECH, only the film crosslinked with 18.4g ECH/100g flour showed a significant lower solubility (S = 25.8%). The values and significant differences between the samples are shown in the Table A.7.

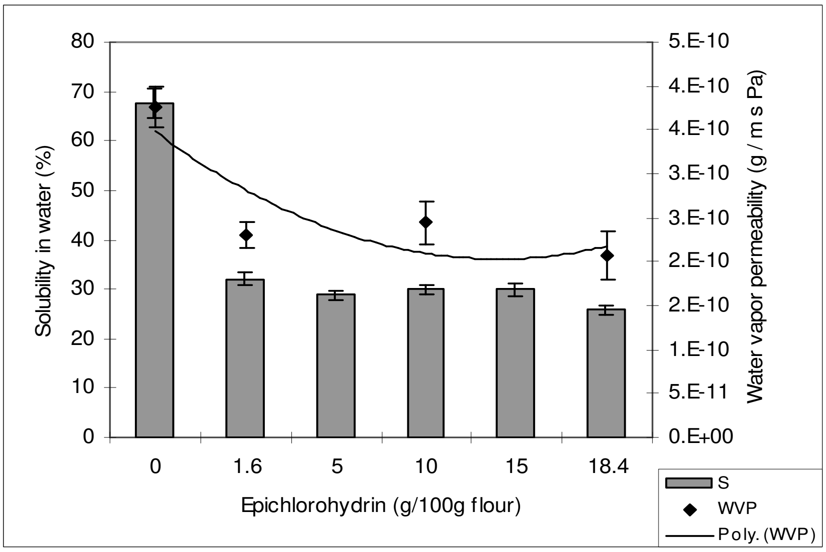

Figure 3.19 Solubility in water (%) and water vapor permeability (g / m s Pa) of *A.cruentus* flour films crosslinked with epichlorohydrin at different concentrations (39.8ºC, 120 min)

As mentioned before this improvement in the solubility was expected as the crosslinked polymer became more hydrophobic due to the covalent linkages that substituted the hydrogen bonding (*9*) and the network formation, therefore these new linkages limited the polymer disintegration and solubilization.

Figure 3.20 shows two types of *A.cruentus* flour films under the solubility test conditions, one crosslinked with epichlorohydrin (5 g/100g flour ECH, 39.8ºC, 120 min) and the other without modification. It was evident the effect that the crosslinking reaction had on the film solubility, as the modified film maintained its integrity when immersed in water for 24 hours at 25ºC with constant stirring (50 rpm).

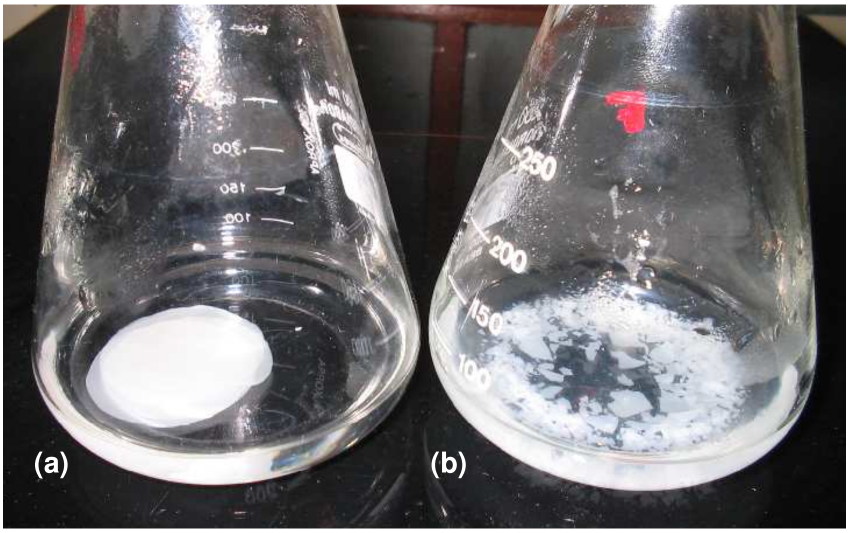

Figure 3.20 Image of the integrity of *A.cruentus* flour films in the solubility in water test, (a) film crosslinked with 5 g/100g flour ECH at 38.9C for 120 min, and (b) non-crosslinked film

The film solubility is related to the water vapor barrier property of the films. It is required an insoluble material or with low water solubility to ensure a low water vapor permeability at a wide range of relative humidity (*17*).

The water vapor permeability (WVP) of the crosslinked films with epichlorohydrin was in average 2.3 ± 0.2 x 10^{-10} g / m s Pa, value considerably lower to the non-crosslinked film, whose WVP was 3.8 x 10^{-10} g / m s Pa (Figure 3.19). However, there was no significant difference between the WVP of the crosslinked films as function of ECH concentration (39.8ºC, 120 min) see Appendix 1, Table A.7. However, these values were higher than the *Amaranthus cruentus* flour- stearic acid film WVP, reported as 8.9 x 10^{-11} g / m s Pa (*2*).

3.3.3.3 Fourier transformed infrared (FT-IR) spectroscopy

Figure 3.21 shows the FTIR spectra obtained for the *A.cruentus* flour films without modification and for the films crosslinked with three different epichlorohydrin concentrations (1.6, 10.0 and 18.4g/100g flour) at 39.8ºC for 120 minutes. Table 3.7 summarizes the main IR bands, their intensity and a tentative assignment of the functional groups that origin the peaks.

The amaranth flour films spectra displays the typical profile of a starch-based material (*4*). In FTIR analysis of polysaccharides, the region of 1300–900 cm^{-2} is the domain more sensitive to molecular conformation (*25*), so the discussion will be focused in this region. At 1150 cm^{-1} a medium peak from the C-O stretching glycosidic bond is characteristic of the amaranth starch and was not altered by the crosslinking reaction, as well as the peak at 1078 cm^{-1}. The peaks at 1078 and 1016 cm^{-1} are characteristic of the anhydroglucose ring (*4*).

The peak at 1016 cm^{-1}, associated to C-O / C-C stretching, which is characteristic of amorphous material (*24*), went through and enhancement compared to the 996 cm^{-1} peak for the 10 wt% ECH film. It was seen that this band became more prominent as the glycerol concentration increased in the films from 0 to 45% glycerol in cassava starch films (*22*). Van Soest et al. (*23*) observed that the peak at ~1022 cm^{-1} is characteristic of amorphous starch, changing the band shape and intensity as the starch goes from semi-crystalline to amorphous. The increase in this peak could corroborate to previous observation about the production of glycerol as a side product of the crosslinking reaction. This phenomenon was also observed for the spectra of the films from the essays 8 and 12 of the experimental design; these films were produced at the highest reaction conditions and were very plasticized and difficult to handle (Figure A.2).

The band at 996 cm^{-1}, which is mainly due to COH bending vibrations, is sensitive to the influence of water content on the intramolecular hydrogen bonding (*23*). Both 1016 and 996 cm^{-1} bands were increased in intensity for the crosslinked films with 1.6 and 18.4 wt% ECH, when compared to the non crosslinked amaranth flour film, suggesting additional C-O and C-C bonds present in the glyceryl bridge, characteristic of the crosslinking reaction with epichlorohydrin.

The IR spectra showed that chemical modifications were produced in the amaranth flour components by crosslinking and side reaction with epichlorohydrin, but unfortunately, could not provide information about the location of the bridges, the molecular mobility of the different polymers or the side reactions that were favored.

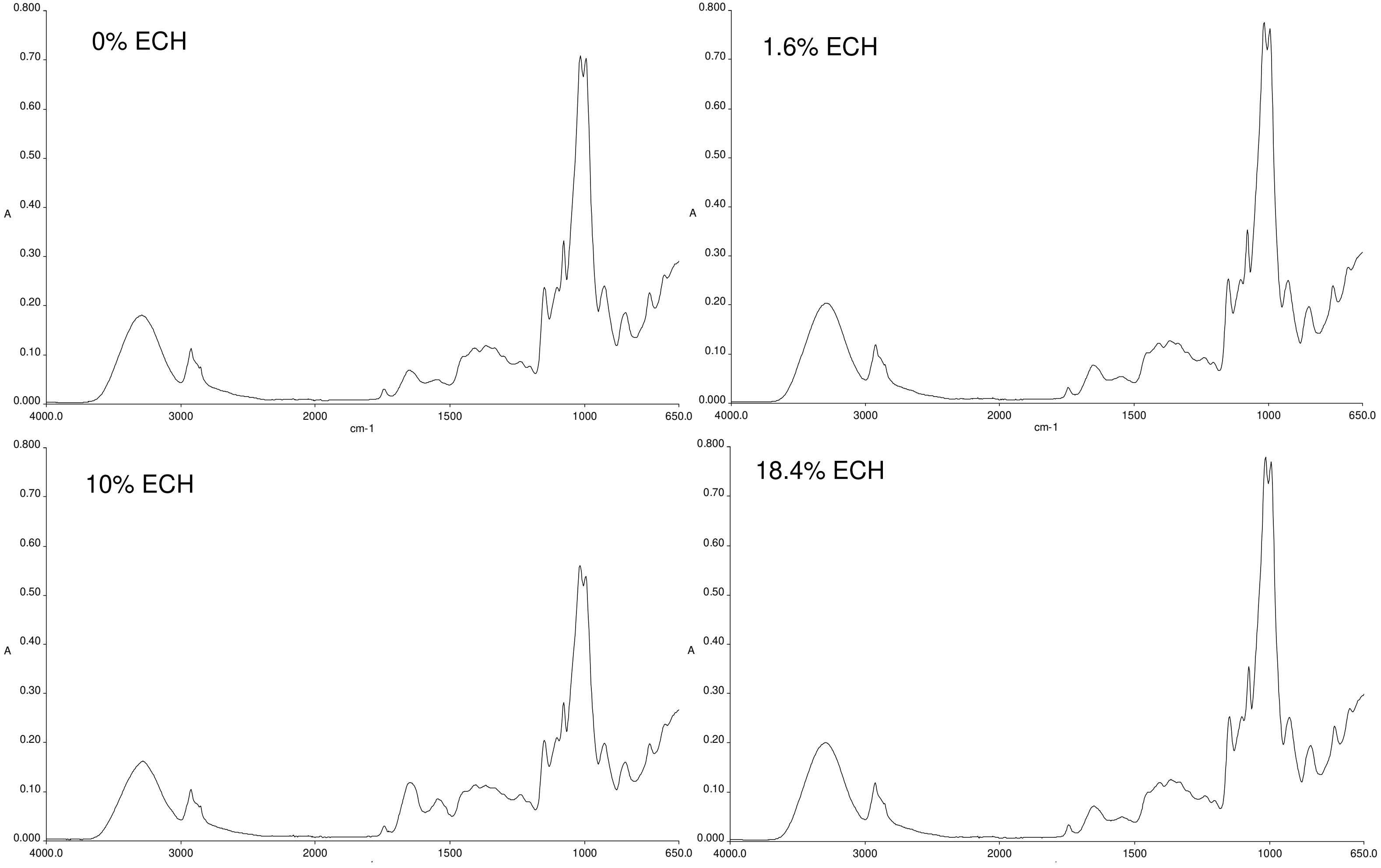

Figure 3.21 FT-IR spectra of *A.cruentus* flour films crosslinked with epichlorohydrin 0%, 1.6%, 10% and 18.4%, at 39.8°C for 120 min.

Table 3.7 Assignment of the main bands of the FT-IR spectra of the *A.cruentus* flour film

Non-crosslinked *A.cruentus* flour film			Crosslinked *A.cruentus* flour film	
Wavenumber (cm^{-1})	P.I.	Assignment	Wavenumber (cm^{-1})	Assignment
3293	M	OH stretching (H bonded)	3284-3292 [a]	OH stretching (H bonded)
2925	M	CH stretching (asym, sym)	2925	CH stretching (asym, sym)
1746	W		1745-1746	
1650	W	OH bending (water)	1649-1653 [b]	OH bending (water)
1549	W		1547-1549 [b, e]	
1409	M	CH bending (CH_2)	1405-1408 [e]	CH bending (CH_2)
1367	M	CH bending	1367-1368 [d]	CH bending
1239	W	CH_2-OH mode	1239 [e]	CH_2-OH mode
1150	M	C-O stretching glycosidic bond, C-O stretching / OH bending	1150 [d]	C-O stretching glycosidic bond, C-O stretching / OH bending
1103	M		1103	
1078	M	CH bending	1078 [d]	CH bending
1016	S	C-O / C-C stretching	1016-1017 [c, d]	C-O / C-C stretching
996	S	COH bending vibration	995-996 [c]	COH bending vibration
927	M	Asymmetrical ring mode	927-928	Asymmetrical ring mode
849		C-H deformation	849	C-H deformation
760		Symmetrical ring vibration	759 [d]	Symmetrical ring vibration
704			704	

P.I., Peak Intensity: W, weak; M, medium; S, strong.

[a] Shift to lower frequency, varying intensity (increase for 1.6 and 18.4%, decrease for 10%);

[b] Intensity increase for 10% ECH;

[c] Intensity increase for 1.6 and 18.4% and; decrease for 10% ECH;

[d] From *A.cruentus* starch;

[e] From *A.cruentus* protein

3.3.3.4 Scanning electron microscopy (SEM)

Microstructural images from the cross section and the surface of the non-crosslinked *A.cruentus* flour film are shown in Figures 3.22. It was observed a dense and rough appearance in the cross section, as well as a cracked surface, which could explain the increased water vapor permeability of the non-crosslinked film.

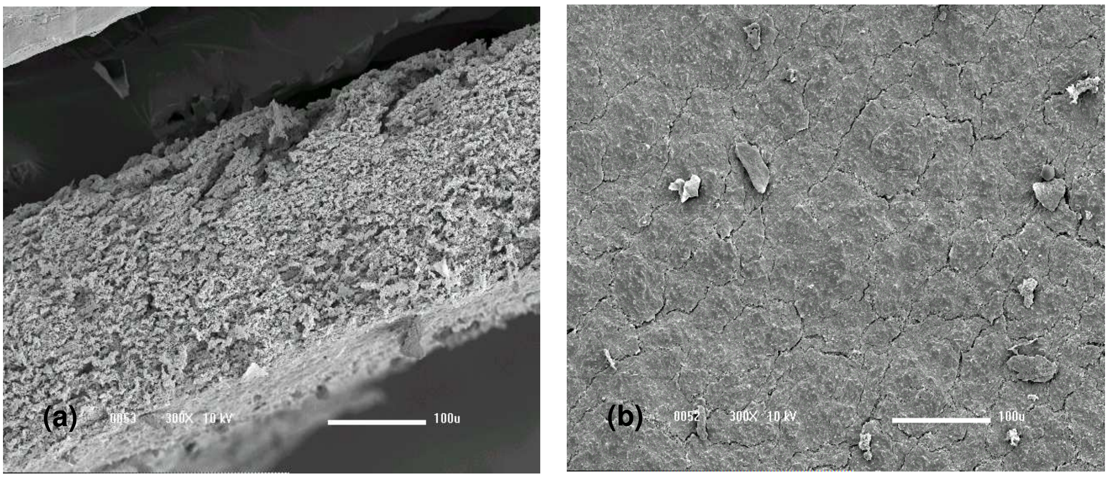

Figure 3.22 Scanning electron micrographs (300x) of *Amaranthus cruentus* flour film (a) Cross section, (b) Surface. (Donned by Tapia-Blácido)

Figures 3.23 and 3.24 show micrographs of the cross section (at two different magnifications) from the crosslinked films of the 12th essay (10% ECH, 90.2ºC, 80 min), and 8th essay (15% ECH, 80ºC, 120 min), respectively. The reaction conditions of these two essays provoke the lowest pH values among all the reactions performed in the experimental design, pH 6.1 in both cases.

The film from the 12th essay presented a similar appearance to the non-modified film. It showed a considerable amount of microporous, giving a spongy appearance, and a cracked surface. This spongy microstructure is characteristic of starch films with high amylopectin content (*2*). This film obtained the worst mechanical properties and solubility, with low resistance and flexibility (TS = 2.2, ELO = 26%), making the material soft and non-cohesive, and was partially disintegrated in water (S = 39%).

Moreover, Figure 3.24 shows the microstructure of the *A.cruentus* flour film from the 8th essay, crosslinked at the highest combination of conditions (15% ECH, 80ºC, 120 min).

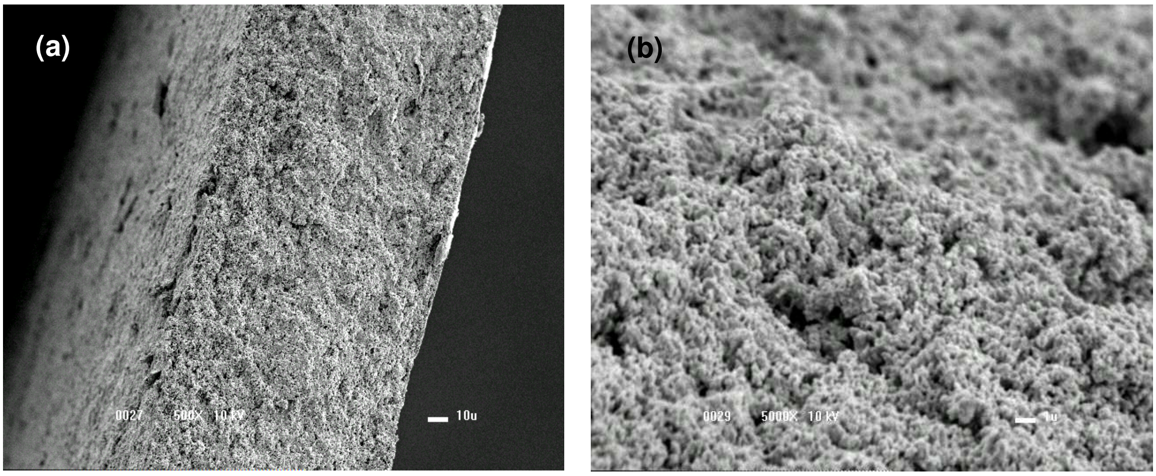

Figure 3.23 Scanning electron micrographs of *A.cruentus* flour films crosslinked with epichlorohydrin from assay 12 (10% ECH, 90.2ºC, 80 min), cross section at: (a) 500x and (b) 5000x

Under these conditions, the film was more flexible (ELO = 49%, PD = 13%), and with relatively low solubility (S = 31%). The film cross section presented the same dense structure with microporous, but in this case, with a smoother appearance. Although this film did not present outstanding mechanical properties, it gave evidence of the effect that the crosslinking reaction had under the microstructure of the *A.cruentus* flour film.

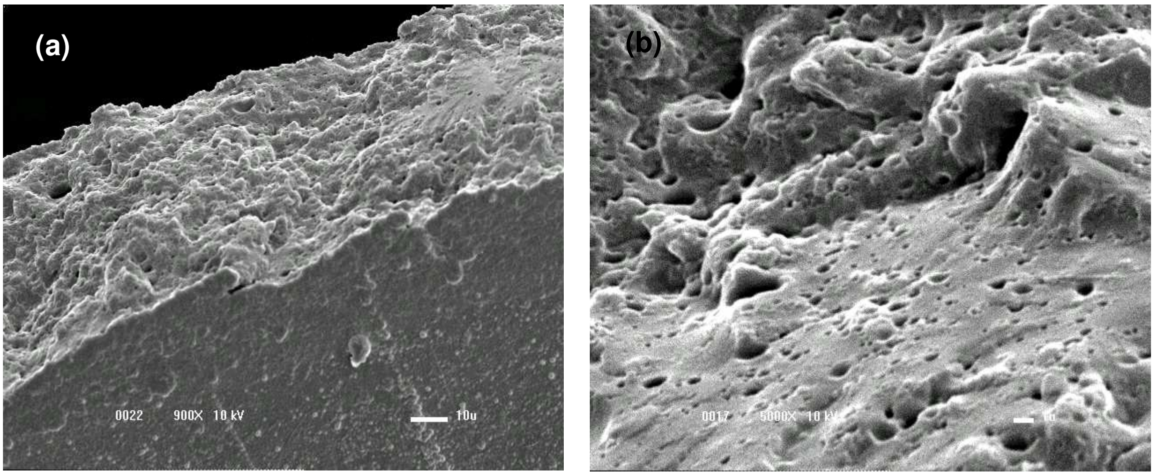

Figure 3.24 Scanning electron micrographs of *A.cruentus* flour films crosslinked with epichlorohydrin from assay 8 (15% ECH, 80ºC, 120 min), cross section at: (a) 900x and (b) 5000x

3.4 Conclusions

The crosslinking reaction provided a plasticizing effect in the *A.cruentus* flour film as it enhanced one mechanical property (resistance or flexibility) with the consequent reduction of the other characteristics. Besides, the reaction temperature had a strong impact on the film solubility and the pH solution, therefore is desired to perform the crosslinking reaction at low temperatures to ensure the reduction of the film solubility, as well as to decrease the pH variation along the reaction. In addition, the pH adjustment affected the film solubility as the NaOH added increased this characteristic.

Although the crosslinking reaction did not improve the mechanical performance of the *A.cruentus* flour films, it considerably reduced the solubility in water ($S_{average}$ = 29%) and the water vapor permeability ($WVP_{average}$ = 2.3 x 10^{-10} g / m s Pa) of the films crosslinked at 39.8ºC for 120 minutes. Moreover, the crosslinking reaction maintained the film integrity during the solubility test. FT-IR analysis showed evidence of chemical modification in the crosslinked *A.cruentus* flour films. SEM micrographs gave evidence of the effect that the crosslinking reaction had under the film microstructure.

3.5 Acknowledgement

We thank the CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and FAPESP (Fundação de Apoio para Pesquisa do Estado de São Paulo) for the financial support,.

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CAPÍTULO 2

Amarantus cruentus flour films: Effect of poly(vinyl alcohol) addition on the mechanical properties and solubility.

Amarantus cruentus flour films: Effect of poly(vinyl alcohol) addition on the mechanical properties and solubility

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Abstract

The aim of this work was to study the effect of poly (vinyl alcohol) (PVA) addition on the mechanical properties and solubility of *Amaranthus cruentus* flour films. First, different PVA grades were tested. Blends with PVA fully hydrolyzed (107, 325 and 350) were more resistant, with higher tensile strength (TS) and puncture force (PF). Moreover, the partially hydrolyzed PVA (523 and 540) films show higher elongation at break (ELO) and puncture deformation (PD), with exception of PVA 325. The latter was chosen due to it superior mechanical performance (TS = 10.2MPa, ELO = 89.8%, PF = 9.4N and PD = 16.3%). When blended films of amaranth flour / PVA (10-50%) were evaluated, all mechanical properties (TS, ELO, PF and PD) were enhanced as the PVA 325 content was increased. Values were at least doubled. The solubility in water (S) of the blended films decreased with increasing PVA content; reaching 44% of soluble mater for equal proportion of blended film. The formation of hydrogen bonds between the blend components was confirmed by the FT-IR spectra analysis.

Keywords: biofilms, *Amaranthus cruentus* flour, poly (vinyl alcohol), mechanical properties, solubility

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4.1 Introduction

The use of biodegradable polymers for packaging offers an alternative and partial solution to the problem of accumulation of solid waste composed of synthetic inert polymers (*1*). Recently, interest in using biodegradable materials for food packaging has increased because of the consumer's awareness of environmental damage caused by non-biodegradable packaging (*2*). The major ingredients that can be used for biodegradable films fabrication include carbohydrates, proteins and lipids. The interest in combining these ingredients in films production is due to the advantages and disadvantages of these components. The use of natural blends of protein, polysaccharides, and lipids directly obtained from agricultural sources takes advantage of each component in the original system and appears to be a new opportunity for material in the area of biofilms (*3*).

Amaranth flour is an interesting source of raw material for biofilm production, following the actual trends in the use of natural mixtures of starch, protein and lipids to reach the desired properties for biofilms. Amaranth is a pseudo cereal of rapid growth, with a high tolerance to arid conditions and poor soils where traditional cereals cannot be grown. The main cultivars used are *Amaranthus hipochondriacus, A. cruentus* and *A. caudatus*. Amaranth seeds contain a significant proportion of starch (62%), protein (14-17%) and fat (5-9%) which make the flour a promising material for film formation (*3,4*).

A. caudatus flour films has been developed, producing films slightly yellowish with moderate opacity, good flexibility and barrier properties, although mechanical resistance was relatively low (4). Besides, *A. cruentus* flour films with stearic acid addition where produced. The incorporation of stearic acid into the polymeric matrix provided an additional water vapor barrier to the film (*3*).

The attempts to improve the properties of biopolymer films are made in several ways. One strategy is the preparation of blend films through combined use of compatible polymers to enhance mechanical properties of biopolymers. Quite often, starch based polymers are blended with high-performance polymers (ex. aliphatic polyesters) to achieve the necessary performance properties for various applications (*5*). Nevertheless, these materials remain non-biodegradable. Thus, the better alternative may be the use of a biodegradable synthetic polymer, such as the poly (vinyl alcohol) (*1, 5,6*).

Poly (vinyl alcohol) (PVA) is water-soluble crystalline polymer with technological potential as a biodegradable material (*6*). Starch-PVA blend films are one of the most popular biodegradable plastics, and are widely used in packaging and agricultural sector (*5*).

The incorporation of PVA into starch changes the thermomechanical properties of the material and thus modifies the polymer structure at both the molecular and morphological levels; allowing its flexibility and functional properties (*7*).

Several researchers (*1, 5, 7-17*) have reported the characterization of starch-PVA blended films. The majority studied the properties of the films formulated with equal parts of starch-PVA (*7-11*), and many have used chemical or physical crosslinking to increase the compatibility of the two polymers (*5, 7, 14-17*).

The aim of this work was to improve the mechanical properties of the *Amaranths cruentus* flour films by the incorporation of poly(vinyl alcohol). Several PVA grades were tested to choose the best that enhance the most the mechanical properties of the blended film and then a series of flour-PVA blends were prepared by variations in the PVA content of 10-50% (w/w) and those films were characterized by mechanical properties and solubility. All films were plasticized by glycerol.

4.2 Materials and methods

4.2.1 Materials

Amaranth flour was prepared from *Amaranthus cruentus* seeds (cultivar BRS Alegria provided by Embrapa Cerrados, Brazilian Company of Agricultural Research, Federal District, Brazil) by the alkaline wet milling method described elsewhere (*3*), with a yield of 57%. Samples were analyzed following the standard methods of the AOAC (1997) and results reported on dry basis. Amylose content was determined using a colorimetric method (*18*) with modifications proposed by Martínez and Cuevas (*19*). The *A. cruentus* flour contained 72.5% starch (7.8 ± 0.2% amylose), 11.9 ± 0.3% protein, 7.7 ± 0.8% lipids, 2.2 ± 0.6% ash and 5.6 ± 0.2% moisture. Poly(vinyl alcohol) polymers having different molecular weights (viscosities) and acetyl group hydrolysis rates (Table 4.1) were from Celanese Chemicals and donated by Dermet Agekem (São Paulo, Brazil). Glycerol, NaOH, and other chemicals were analytical reagent grade, purchased from Synth (São Paulo, Brazil), and used without further purification.

Table 4.1 Characterization of selected poly(vinyl alcohol)

PVA Grade	Hydrolysis degree (%)	Molecular weight range
Celvol 107	98.0-98.8	13000-23000
Celvol 325	98.0-98.8	85000-124000
Celvol 350	98.0-98.8	146000-186000
Celvol 205	87.0-89.0	13000-23000
Celvol 523	87.0-89.0	85000-124000
Celvol 540	87.0-89.0	146000-186000

4.2.2 Film preparation and conditioning

Amaranth flour/PVA blended films were made by the casting technique (Figure 4.1). Firstly, the two solutions were prepared separately. The amaranth flour solution was made as established by Tapia-Blácido (*4*) from amaranth flour (4g/100g solution) dispersed in deionized water. The solution was mixed for at least 2 hour with a magnetic stirrer with adjusted pH (10.7) for protein dissolution. The solution was heated up to 70°C and glycerol (20 wt.%) was added, then it was gelatinized at 80°C for 15 minutes with constant stirring in a jacketed beaker connected to a water bath (Tecnalise, Brazil). The poly(vinyl alcohol) solution was prepared from PVA (4g/100g solution) with deionized water. It was dispersed by stirring for at least 4 hours at room temperature. The product was dissolved by heating, at 85°C for partially hydrolyzed PVA (87-89%) and at 90°C for fully hydrolyzed PVA (98-99%) for 30 minutes or until PVA was completely dissolved, and glycerol (20%) was added.

Blends of amaranth flour and PVA solutions were prepared for different poly(vinyl alcohol) content from 10 to 50%, and blends were homogenized using an Ultra-Turrax emulsifier (model T18-Basic, IKA Works Brazil) for 1 or 2 min at 6000 rpm, depending on the blend proportion, because PVA and starch are not compatible when each is added in high concentration (8). The blend was heated at 75°C for 30 minutes for increase the interaction between the components, and then it was kept under vacuum (Tecnal, TE-058) for defoaming, for 30-60 minutes, depending of the air incorporation during the homogenization. Afterward, the film forming solution was poured and spread evenly over a Teflon surface (18x21 cm). The weight of the film forming solution was controlled to obtain a constant thickness (80±2µm).

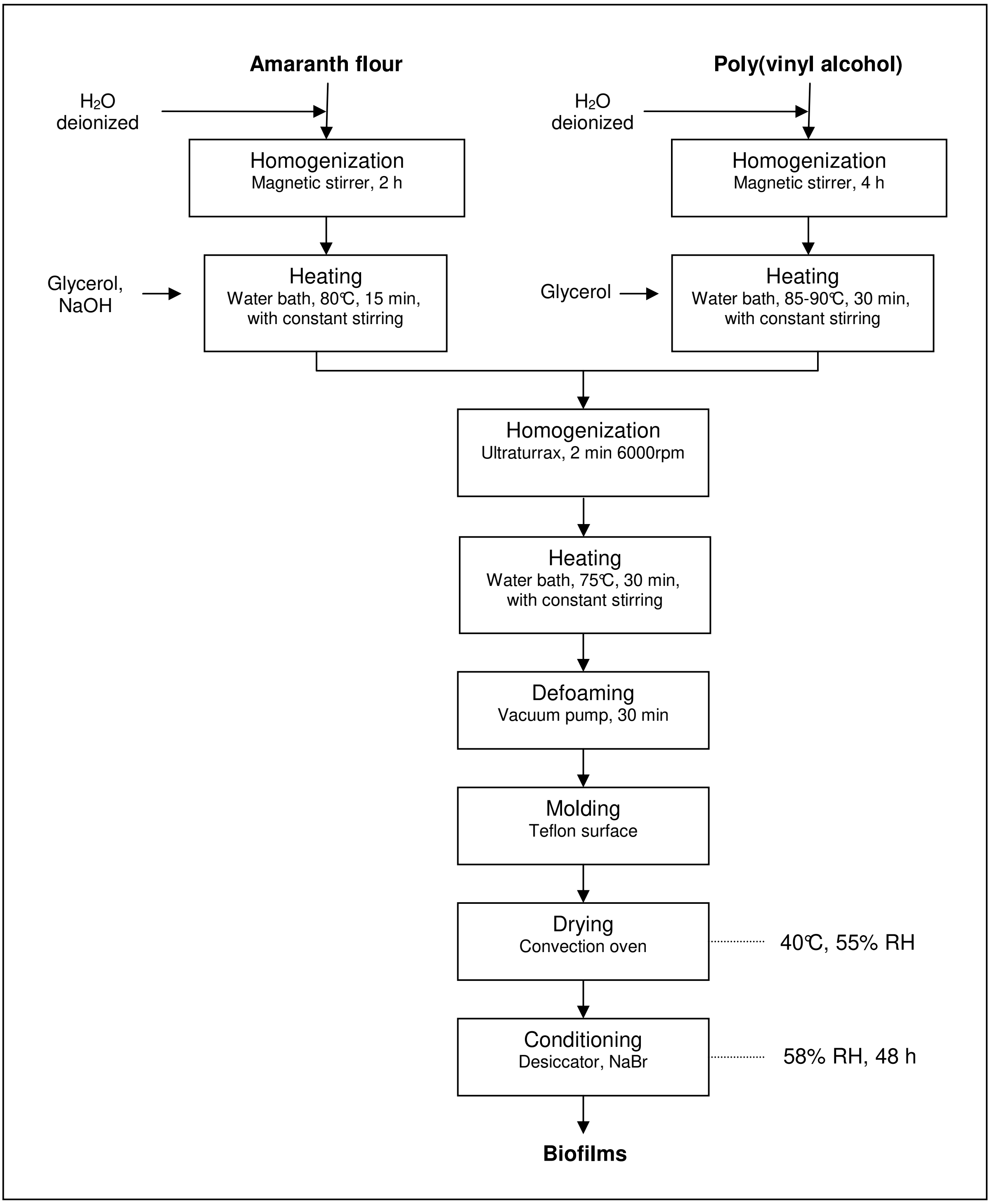

Figure 4.1 Flowchart for the elaboration of the A.cruentus flour – poly(vinyl alcohol) (PVA) blended films.

The solutions were dried in a controlled temperature and relative humidity oven (model MA 415UR, Marconi, Brazil) at 37°C and 55% relative humidity (RH) for 12-14h, when the equilibrium moisture was reached (11±2%). Dried films were peeled off the casting surface, cut into adequate samples. The films were conditioned at 25°C and 58% RH in desiccators with a saturated solution of NaBr for 72h prior to mechanical tests, and at 0% RH in desiccators with silica gel for solubility (48h) and FT-IR analysis (1 week). Films of *A.cruentus* flour without PVA and films of pure PVA were also prepared and analyzed.

4.2.3 Film characterizations

Thickness was measured using a micrometer (model FOW72-229-001, Fowler) with a range of 0-1in and an accuracy of 0.0001in. The mean thicknesses of the films were determined from the average of 15 measurements made at five different locations.

Moisture content of films was determinate by method of AOAC (1997) drying the sample (0.200±0.001g) at 105°C for 24h, in an air oven, in triplicate.

Mechanical properties were determined by puncture test (puncture force, PF and puncture deformation PD) and tensile test (tensile strength, TS, and elongation at break, ELO) using a TA-XT2i Stable Micro Systems texture analyzer (SMS). For puncture test, circular sample films were fixed in a 34mm diameter cell and perforated by a 3mm diameter cylindrical probe, moving at 1mm/s (*20*), in triplicate.

The puncture deformation was calculated as follows, equation 1:

$$PD = \Delta l/l_o = [(D^2 + l_o^2)^{1/2} - l_o] / l_o \qquad \text{Eq.1}$$

were l_o is the initial disc radius (17 mm) and D the distance penetrated at the break point.

Tensile test was performed according to the ASTM standard method D882-97 (*21*). Dumbbell shaped samples were cut for each film with 115mm length and 6mm wide in the middle. The initial grip separation was set at 80 mm and the crosshead speed at 1.0 mm/s. At least four samples from each film were evaluated. The elongation at break was obtained with equation 2:

$$ELO = (\Delta L/L_o)\ 100 \qquad \text{Eq.2}$$

were ΔL is the elongated distance at break and L_o the initial distance between the grips (80mm). Care was taken to remove the samples just before they were tested to minimize the chance of samples drying out or picking up moisture due to the change in humidity from the desiccator to the testing room. The TS (MPa), ELO (%), PF (N) and PD (%) were calculated using Texture Expert Analyzer version 1.15 (SMS).

Solubility in water was determinate according to the method described by Gontard et al. (*22*) with modifications. Three dry film discs (2cm diameter) were weighed (w_i) and immersed in 50mL deionized water (with sodium azide, 0.02%) at 25℃ for 24h, with constant agitation (50 rpm) in a shaker (Tecnal, TE-420). Then the films were recovered by vacuum filtration on filter paper with known weight, dried at 105℃ for 24h and weighed (w_f). The solubility (S) was calculated as the percentage of dissolved film, equation 3:

$S = [(w_i-w_f) / w_i]\ 100$ Eq.3

Fourier transformed IR spectroscopy (FT-IR). Infrared spectra of dry films were recorded between 4000 and 600 cm^{-1} at 4 cm^{-1} resolution, with a Spectrum One (Perkin Elmer) spectrometer, supplied with a universal attenuated total reflectance (UATR) accessory (*23*). For each spectrum, 16 scans were co-added.

Scanning electron microscopy (SEM) analyses were performed using a Leica (Cambridge, England) model LEO440i scanning electron microscope operating at 10 kV. Film samples were maintained in a desiccator with silica gel for 7 days and then randomly broken to investigate the cross section of the samples. A cylindrical aluminum stub cut like a straight chair, upon which the film was fixed using a double-sided cupper tape, was used in a specific way to observe the morphology of the cross section and the surfaces. Further, the stubs with films were coated with gold in a VG Microtech (Cambridge, England) model SC7620 sputter coater for 180 s at 4 mA.

For statistical analysis, mean values of the mechanical properties and solubility were analyzed by Tukey honest significant difference (HSD) test, performed using Statistica 5.0 software (Statsoft Inc.).

4.3 Results and discussion

4.3.1 Poly(vinyl alcohol) (PVA) grade selection

Different grades of poly(vinyl alcohol) were tested in the formulation of blended films with equal proportion of amaranth flour – PVA. Blended films made with PVA 205 were not included in the analysis, because phase separation occurred during drying due to the lack of compatibility between this partially hydrolyzed and low molecular weight PVA and amaranth flour.

Mechanical properties of blended films such as puncture force and deformation, tensile strength and elongation at break, were analyzed. Figure 4.2 shows the variation in the mechanical properties due to poly(vinyl alcohol) addition with different hydrolysis degrees and molecular weights (see Table 4.1). Significant difference between films ($p<0.05$) are shown in Table A.8.

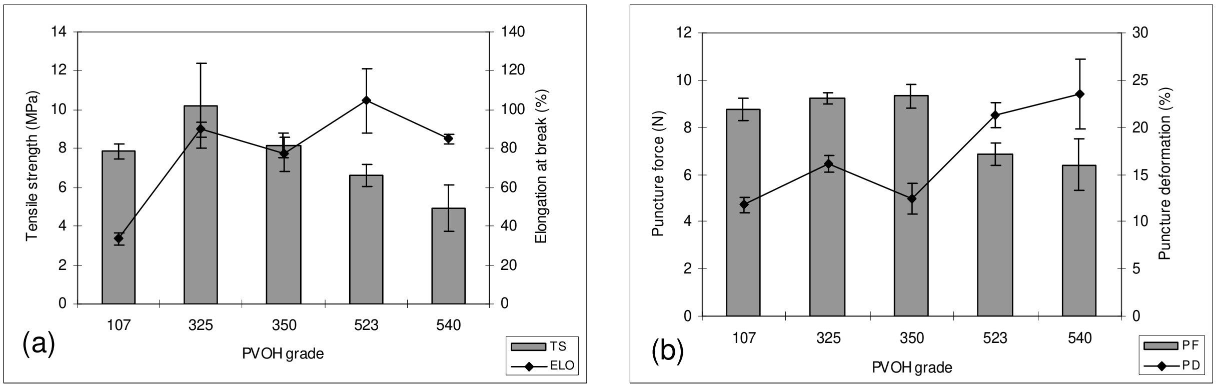

Figure 4.2 Mechanical properties of *A.cruentus* flour – poly(vinyl alcohol) blended films with different grades of PVA. (a) Tensile strength (MPa) and elongation (%) at break from tensile test, (b) Puncture force (N) and deformation (%) at break from puncture test

PVA fully hydrolyzed (107, 325 and 350) blended films were more resistant, with higher tensile strength and puncture force, than partially hydrolyzed PVA (523 and 540). It was expected because the higher hydrolysis degree increased the amount of hydroxyl groups present in the PVA molecule that allowed the formation of hydrogen bonds or chemical interactions between the –OH groups of PVA and –OH groups of amaranth flour, reinforcing the polymer structure.

The tensile strength, which is a measure of the resistance to direct pull, is important in machinability and packaging applications (*13*). Abd El-Kader, et al. (*24*) reported that the strength at break for PVA films decreased as the molecular weight is increased, but this effect was not evident in the present study, where no significant difference ($p>0.05$) was observed between tensile strength of samples with the same degree of hydrolysis and increased molecular weights. Elongation at break and puncture deformation reveal the flexibility of the polymer structure, and generally increase as the resistance decrease (*25*). Films with partially hydrolyzed PVA showed higher ELO and PD, except in the case of PVA 325 which was not different ($p>0.05$) from PVA 523 and 540.

PVA 325, which is a fully hydrolyzed (98%) and medium molecular weight poly(vinyl alcohol), presented the best mechanical characteristics between the other grades evaluated, with TS = 10.21MPa, ELO = 89.76%, PF = 9.40N and PD = 16.3%, and was chosen to improve the mechanical performance of *A.cruentus* flour-PVA blended films. These values are close to those reported by some researchers for starch-poly(vinyl alcohol) blended films in equal proportion (Table 4.2).

Table 4.2 Mechanical properties of starch-poly(vinyl alcohol) blended films reported in literature

Formulation and conditions	TS (MPa)	ELO (%)	Reference
Starch (21% amylose) – PVA (NH-17R), 15% glycerol	12.1	59.2	(*7*)
Waxy cornstarch (41%), PVA 325 (41%), Glycerol (15%) and EAA (3%) at 50% RH	~16	144	(*8*)
Cornstarch (5%), PVA (5%) and glycerol (20 wt%), mixed at 1500 rpm for 40 min. At 22ºC and 52% RH	~10	~75	(*9*)
Starch-g-PAN (HSPAN) – PVA, without plasticizer	~27	~10	(*17*)
Amaranth flour (4%), PVA 325 (4%) and glycerol (20wt.%), homogenized at 6000rpm for 2 min, at 58% RH	10.2	89.8	This work

4.3.2 *A.cruentus* flour - poly(vinyl alcohol) (PVA 325) blended films

4.3.2.1 Mechanical properties

Figures 4.3 and 4.4 show the mechanical properties of blended films, and significant difference between the films ($p<0.05$) are shown in Table A.9. Both the tensile strength and elongation at break of *A.cruentus* flour - PVA 325 blended films were higher than the *A.cruentus* flour without PVA films (TS 5.82MPa and ELO 13.81%), but lower than those of pure PVA films (TS 44.84MPa and ELO 84.35%). There was a tendency of increasing both tensile strength and elongation at break with increased PVA 325 content. However, at high PVA content, it was necessary to increase the homogenization time to overcome the phase separation during drying due to their incompatibility of both polymers at high concentrations (*8*).

This improvement in the tensile properties was also reported for absorbent hydrolyzed starch-g-PAN (HSPAN) and PVA blended films (*17*).

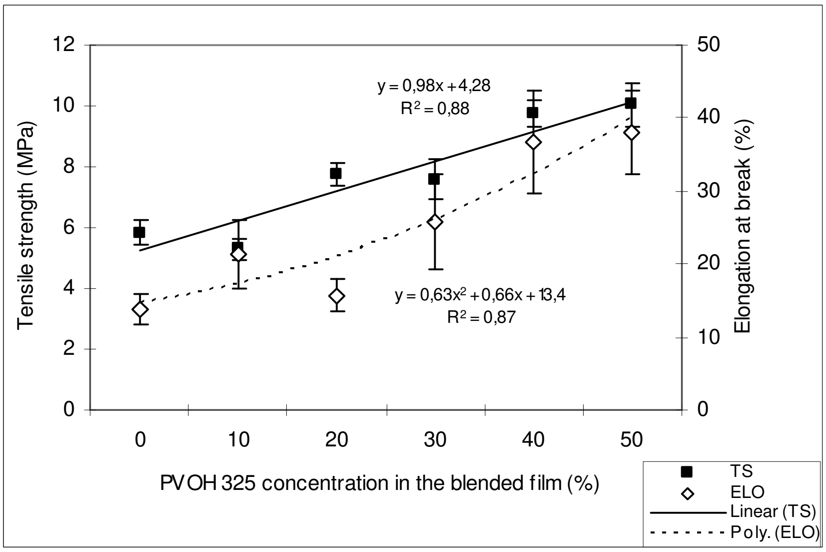

Figure 4.3 Tensile strength (MPa) and elongation (%) at break of poly(vinyl alcohol) (PVA 325) - *A.cruentus* flour blended films in different proportions.

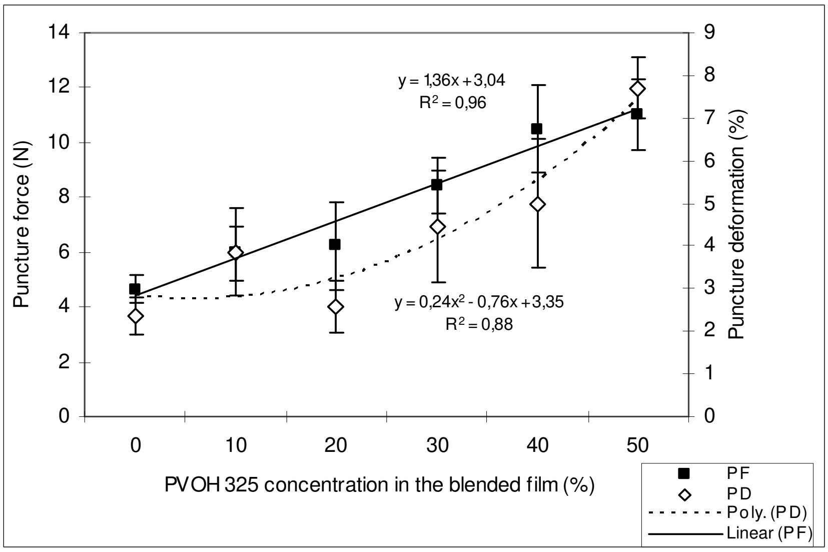

Figure 4.4 Puncture force (N) and deformation (%) at break of poly(vinyl alcohol) (PVA 325) -*A.cruentus* flour blended films in different proportions.

Both the puncture force and deformation of *A.cruentus* flour - PVA 325 blended films were also above those of *A.cruentus* flour films (PF 4.65N and PD 2.36%), but below to those of pure PVA films (PF 43.42N and PD 23.89%). Besides, there was a trend of increasing puncture force and deformation with higher PVA 325 content. These results demonstrate that it was possible to overcome the *A.cruentus* flour – PVA incompatibility by homogenization and heating of the blends. The enhancement in the mechanical properties could be due to the interaction of the components by hydrogen bonds formation between –OH groups of the PVA, amaranth starch and protein. The formation of hydrogen bonds between the components of the blend was corroborated by the FT-IR spectra analysis, as seen in section 4.3.2.3. This conformational modification induced by the incorporation of PVA in the blend achieved a real improvement in the mechanical performance of the blended films, as it enhanced both the resistance and flexibility of the polymeric matrix (*25*).

4.3.2.2 Solubility and moisture content

In Figure 4.5 are shown the solubility and moisture content of *A.cruentus* flour - PVA 325 blended films in different proportions.

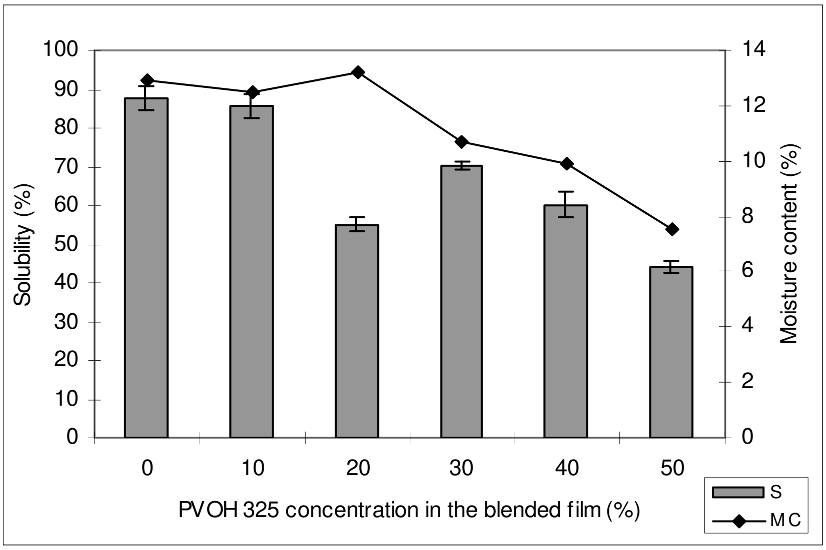

Figure 4.5 Solubility in water (%) and moisture content (%) of poly(vinyl alcohol) - *A.cruentus* flour blended films in different proportions.

It was noticed that as PVA content increased, both the solubility and moisture content decreased, the films became less hydrophilic, with the exception of the 20% PVA blended film which presented 55% of soluble mater, value lower to those of 30-70 and 40-60 blends. The diminution in the solubility in water and the moisture content of the blended films could be attributed to the formation of hydrogen bonds between the two polymers, which reduce its capacity to absorb water (*14*); this observation was confirmed by the FT-IR spectra analysis.

Another factor that could improve the solubility of the films was the homogenization (1-2 min at 6000 rpm) of the blends. It was observed for corn starch – PVA (99% hydrolyzed and MW: 89000-98000) blended films that the mixing time decreased the solubility until it was equilibrated at 40 min (1500 rpm), this phenomenon was explained by the free volume decrease in combination with hydrogen bonding formation between starch and poly(vinyl alcohol) (*9*).

4.3.2.3 Fourier Transformed Infrared (FT-IR) spectroscopy

Figure 4.6 shows the FTIR spectra obtained for the *A.cruentus* flour – PVA blended films at different proportions, it was observed that as the PVA concentration increases, occurred a decrease in the spectra intensity. This trend was explained as the crystalline films, such as the PVA films, generally provoke loss of radiation intensity due to an excessive dispersion of light (*26*).

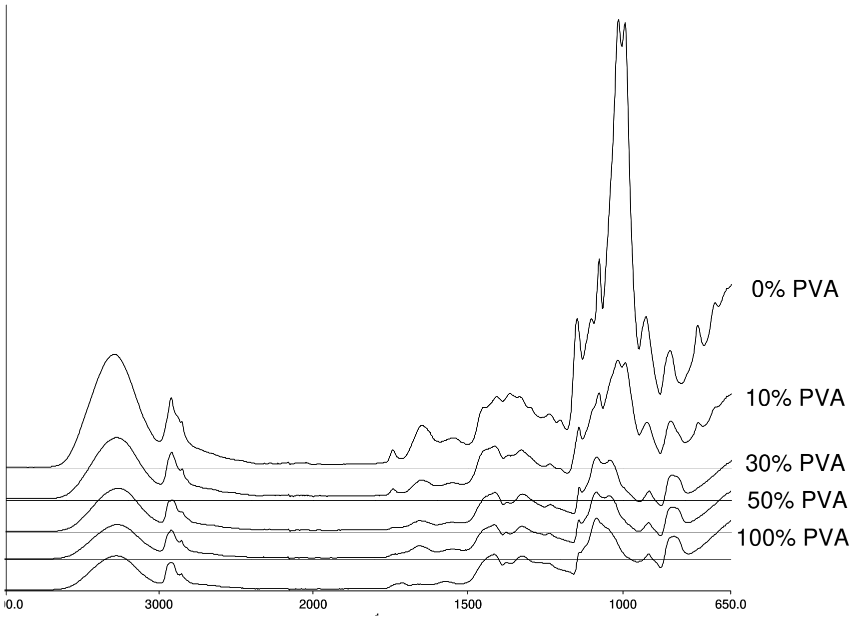

Figure 4.6 FT-IR spectra of *A.cruentus* flour films with poly(vinyl alcohol) addition 0%, 10%, 30% and 50%, and 100% PVA film.

The main IR bands and their assignments are summarized in Table 4.3. The *A.cruentus* flour film (0% PVA) spectrum displays the typical profile of a polysaccharide in the 1200–930-cm^{-1} range (characteristic peaks attributed to COC bond stretching), since the amaranth flour has a high content of starch (72.5%). The strong peaks at 996, 1016 and 1078 cm^{-1} are characteristic of the anhydroglucose ring, and shift to higher frequencies as the PVA concentration increase, only the 10% PVA blended film presented a peak at 995 and 1019 cm^{-1}. The peak at 1650 cm^{-1} is due to the water present in the amaranth flour, as it did not appear in the 100% PVA film. The band at 2925 cm^{-1} is characteristic of CH stretching (*27*).

Table 4.3 Assignment of the main bands of the FT-IR spectra of the *A.cruentus* flour – poly(vinyl alcohol) blended film

A.cruentus flour film			*A.cruentus* flour – PVA blended film	
Wavenumber (cm^{-1})	PI	Assignment	Wavenumber (cm^{-1})	Assignment
3293	M	OH stretching (H bonded)	3272-3278 [c]	OH stretching (H bonded)
2925	M	CH stretching (asym, sym)	2919-2921 [c]	CH stretching (asym, sym)
1746	W		1745 [a]	
1650	W	OH bending (water)	1650-1657 [b]	OH bending (water
1549	W			
1409	M	CH bending (CH_2)	1417 [d]	CH bending (CH_2)
1367	M	CH bending		
			1325-1331 [e]	
1239	W	CH_2-OH mode	1237 [a]	CH_2-OH mode
1150	M	C-O stretching glycosidic bond, C-O stretching / OH bending	1142-1144 [c]	C-O stretching glycosidic bond, C-O stretching / OH bending
1103	M			
1078	M	CH bending	1079-1086 [d]	CH bending
1016	S	C-O / C-C stretching	1019 [a, d]	C-O / C-C stretching
996	S	COH bending vibration	995 [a]	COH bending vibration
927	M	Asymmetrical ring mode	916-924 [c]	Asymmetrical ring mode
849		C-H deformation	835-847 [c]	C-H deformation
760		Symmetrical ring vibration	758 [a]	Symmetrical ring vibration

PI, peak intensity; W, weak; M, medium; S, strong peak intensity

[a] Only present in 10% PVA

[b] Not present in 100% PVA

[c] Shift to lower frequency

[d] Shift to higher frequency

[e] Not present in 0% PVA

The broad band at 3293 cm^{-1} is due to hydrogen-bonded hydroxyl groups (O-H). This band is of great importance as it indicates the presence of hydrogen bonding in the polymer. There was a shift to lower frequencies as the PVA concentration increases (3272-3278 cm^{-1}), this could mean an increase in the hydrogen bonds between the PVA and *A.cruentus* flour hydroxyl groups, which provoke an improvement in the mechanical properties and solubility in water of the blended films.

This fact was explained by SILVERSTEIN & WEBSTER (2000): the formation of hydrogen bonds modifies the force constant of both the groups involved in it, and thus, with this, the frequencies of axial and angular deformation are altered. The “free” hydroxyl groups absorbs strongly between 3650 and 3584 cm-1, but when the intermolecular hydrogen bonding becomes important, the bands start to appear at lower frequencies, typically 3550-3200 cm-1, observing themselves a wide absorption the 3333 cm-1 for polymeric structures (*26*).

4.3.2.4 Scanning electron microscopy (SEM)

Amaranth flour – PVA 325 blended film microstructure images are presented in Figure 4.7. Figure 4.8 shows the micrographs of the *A.cruentus* flour film without modification. The surface of the blended film (Figure 4.7a) was dense and with agglomerations distributed along the surface. Figure 4.8a shows the same dense structure of the *A.cruentus* flour film at lower magnification (300x).

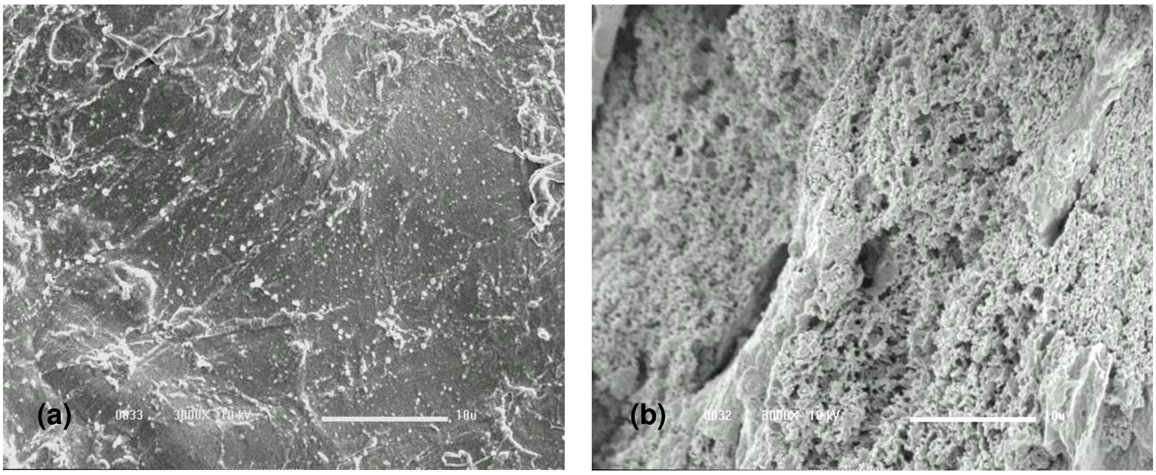

Figure 4.7 Scanning electron micrographs (3000x) of *A.cruentus* flour – poly(vinyl alcohol) (PVA 325) blended film in equal proportion (a) Surface, (b) Cross section.

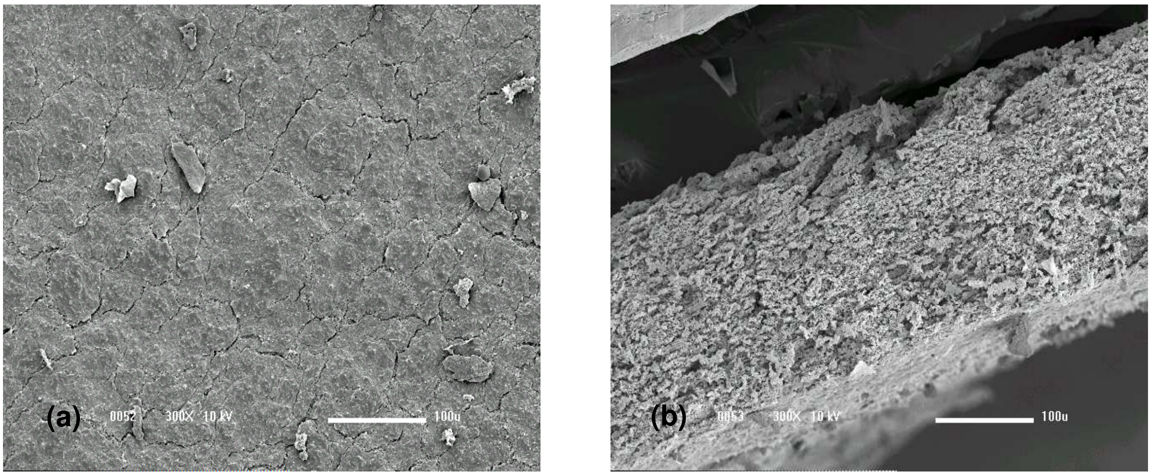

Figure 4.8 Scanning electron micrographs (300x) of *Amaranthus cruentus* flour film (a) Surface, (b) Cross section. (Donned by Tapia-Blácido)

Moreover, Figure 4.7b shows that the cross section of the blended film presented a considerable amount of microporous, giving a spongy appearance, this structure is characteristic of starch films with high amylopectin content, as seen in the Figure 4.8b for the film without PVA. The type of structure was reported for *A.cruentus* flour – stearic acid emulsified films (*3*).

4.4 Conclusions

Mechanical properties of amaranth (*A.cruentus*) flour films have been improved with the addition of poly(vinyl alcohol) (PVA). From blends with different grades of poly(vinyl alcohol), the PVA fully hydrolyzed (107, 325 and 350) blended films were more resistant, with higher tensile strength and puncture force, than the partially hydrolyzed PVA (523 and 540). Besides, the films with partially hydrolyzed PVA show higher elongation at break and puncture deformation, except in the case of PVA 325 which was not different ($p<0.05$) from PVA 523 and 540. PVA 325, which is a fully hydrolyzed (98%) and medium molecular weight poly(vinyl alcohol), was chosen because it presented the best mechanical performance between the other grades evaluated, with TS 10.21MPa, ELO 89.76%, PF 9.40N and PD 16.3%, for *A.cruentus* flour- PVA 325 blended films in equal proportions.

All mechanical properties (TS, ELO, PF and PD) were enhanced as the PVA 325 content was increased (up to 50%) in the blended film, values were at least doubled. The conformational modification induced by the incorporation of PVA in the blend achieved a real improvement in the mechanical performance of the blended films, because it enhanced both the resistance and flexibility of the polymeric matrix. The solubility in water of the blended films was decreased as the PVA content increased; reaching 44% of soluble mater for 50% amaranth flour- 50% PVA blended film. The formation of hydrogen bonding between the components of the blend was confirmed by the FT-IR analysis.

4.5 Acknowledgements

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CONCLUSÕES GERAIS

A aplicação das duas estratégias melhoraram algumas das características dos filmes de farinha de amaranto. A reticulação com epicloridrina reduz significativamente a solubilidade em água dos filmes; entretanto a adição de PVA melhorou o desempenho mecânico dos filmes.

5.1 Filmes de *A. cruentus* modificados com epicloridrina

A reação de reticulação com epicloridrina teve um efeito plasticizante sobre os filmes de farinha de amaranto (*A.cruentus*), ao diminuir a resistência mecânica enquanto aumenta a flexibilidade dos biofilmes reticulados, ou aumentando a resistência dos filmes com a conseqüente diminuição da flexibilidade.

A temperatura de reação (39,8 – 90,2ºC) foi a variável que teve mais impacto sobre a solubilidade em água dos filmes e o pH da solução filmogênica após a reação. O incremento da temperatura diminuiu o pH da solução e aumentou a solubilidade do filme, provavelmente devido à base adicionada para manter o pH em torno de 10,7.

Os filmes reticulados com temperatura baixa (39,8ºC) e tempo longo (120 min) não apresentaram diferença nas propriedades mecânicas em relação ao filme não reticulado.

A reação de reticulação dos filmes de amaranto (*A.cruentus*) com epicloridrina (1,6–18,4% ECH, 39,8ºC por 120 min) provocou uma melhora significativa das propriedades de barreira. A solubilidade em água ($S_{\text{média}}$ = 29,6 ± 4,8%) diminuiu mais da metade do valor em relação aos filmes não reticulados (S_{NR} = 67,5%). Assim como a permeabilidade ao vapor de água passou de $WVP_{NR} = 3,8 \times 10^{-10}$ g / m s Pa, no filme não reticulado, a $WVP_{\text{média}} = 2,3 \times 10^{-10}$ g / m s Pa, para os filmes modificados. Além disso, a reticulação manteve a integridade dos filmes durante o teste de solubilidade.

A análise do FT-IR evidenciou a presença de modificações químicas nos filmes de farinha de amaranto reticulados com epicloridrina.

As imagens de microscopia eletrônica de varredura (MEV) deram indícios do efeito que a reação de reticulação com epicloridrina teve sobre a microestrutura dos filmes de farinha de amaranto.

5.2 Filmes de *A.cruentus* misturados com poli(vinil álcool) (PVA)

O tipo de poli (vinil álcool), PVA 325, com alto grau de hidrólise (98%) e peso molecular intermediário (85000-124000g/mol) proporcionou filmes com melhores propriedades mecânicas (TS 10,21MPa, ELO 89,76%, PF 9,40N e PD 16,3%) em termos de resistência, mas sem efeito na flexibilidade, quando comparada com os filmes contendo PVA com hidrólise parcial.

As propriedades mecânicas dos filmes de farinha de amaranto (*A.cruentus*), tanto a resistência quanto a flexibilidade, aumentaram com o aumento da concentração do poli(vinil álcool) de 10 até 50%, sendo que estes valores foram ao menos dobrados. Dessa forma, pode-se concluir que houve uma melhora real das características mecânicas dos biofilmes.

Nas blendas de farinha de amaranto com poli (vinil álcool), a solubilidade em água diminuiu com o aumento da proporção de PVA na mistura, até atingir 44% de matéria solúvel para o filme com iguais proporções.

A análise do FT-IR confirmou a formação de pontes de hidrogênio entre os componentes dos filmes a partir de blendas de farinha de amaranto e poli(vinil álcool).

SUGESTÕES PARA TRABALHOS FUTUROS

Baseado nos resultados obtidos neste projeto é recomendável direcionar os futuros trabalhos com o objetivo de desenvolver materiais com propriedades mecânicas e de barreira que permitam a sua aplicação na área alimentar, bem como aprofundar na compreensão das alternativas escolhidas, a reação de reticulação com epicloridrina e a adição do poli(vinil álcool).

6.1 Reação de reticulação com epicloridrina

Avaliar o efeito de maiores concentrações da farinha de amaranto na solução filmogênica como alternativa de aumento do rendimento da reação de reticulação. Para assim favorer a formação de ligações entre as cadeias do polímero, e diminuir as reações secundárias.

Diminuir a concentração de plasticizante (glicerol), já que o mesmo é produzido nas reações secundárias da epicloridrina.

Deter a reação de reticulação com epicloridrina ao ajustar o pH da solução a pH 6, no final da reação. Avaliar o efeito do ajuste do pH na solubilidade das proteínas da farinha de amaranto.

6.2 Adição de poli(vinil álcool)

Otimizar a concentração de PVA adicionada, sendo a mínima necessária para provocar uma melhora significativa das propriedades do filme de farinha de amaranto.

Desenvolver uma metodologia que envolva as duas alternativas, ao preparar uma blenda de farinha de amaranto com poli(vinil álcool), cujas cadeias possam ser reticuladas pela epicloridrina. Assim seria obtida uma melhora conjunta das propriedades mecânicas e de barreira dos filmes de farinha de amaranto.

APÊNDICE 1

Efeito da reticulação com epicloridrina nas propriedades mecânicas e de barreira dos filmes de farinha de amaranto

Table A.1 Mechanical properties and solubility of *A.cruentus* flour films crosslinked with epichlorohydrin under different conditions for the preliminary tests

	Puncture test						Tensile test						S (%)	
	T (mm)		PF (N)		PD (%)		T (mm)		TS (MPa)		ELO (%)			
							60℃, 22.5 wt.% glycerol							
RV 0% ECH	0.09±0.01	a	4.7±0.2	a	5.3±0.9	a	0.083±0.007	c	2.6±0.2	b	21±4	a	81±17	a
5%, 30 min	0.08±0.01	a	5.4±0.5	a	3.8±0.5	ab	0.106±0.002	ab	3.1±0.7	ab	26±4	a	28±7	bc
5%, 60 min	0.09±0.01	a	5.2±0.6	a	4.3±0.4	ab	0.079±0.004	c	5.0±0.8	ab	33±7	a	26±12	bc
5%, 90 min	0.09±0.01	a	5.7±0.7	a	3.8±0.5	ab	0.090±0.005	bc	5.2±0.9	a	27±8	a	16±16	c
10%, 30 min	0.08±0.01	a	5.0±0.1	a	3.3±0.4	b	0.083±0.009	c	4.1±0.5	ab	33±1	a	56±13	ab
10%, 60 min	0.09±0.01	a	4.8±0.2	a	4.5±0.9	ab	0.115±0.007	a	4.0±0.4	ab	39±8	a	56±9	ab
10%, 90 min	0.09±0.01	a	5.2±0.1	a	4.6±0.8	ab	0.084±0.003	c	4.9±0.8	ab	29±7	a	50±7	abc
							5% ECH, 70℃, 20.5 wt.% glycerol							
RV 0% ECH	0.08±0.01	a	4.1±0.1	ab	4.6±0.9	a	0.093±0.008	a	4.6±0.1	a	26±2	a		
40 min	0.11±0.01	a	5.0±0.2	a	5.0±0.9	a	0.088±0.009	a	3.9±0.5	ab	33±4	a		
80 min	0.08±0.01	a	3.7±0.4	b	6.5±0.5	a	0.082±0.003	a	4.0±0.3	ab	19±2	a		
120 min	0.08±0.01	a	4.4±0.4	ab	4.0±0.1	a	0.094±0.002	a	3.4±0.1	b	38±16	a		
							5% ECH, 80℃, 21.5 wt.% glycerol							
RV 0% ECH	0.09±0.01	a	4.1±0.3	a	5.5±0.3	b	0.080±0.003	b	3.2±0.4	a	45±7	b		
40 min	0.09±0.01	a	3.4±0.8	a	7.0±0.9	ab	0.107±0.001	a	2.8±0.2	ab	72±3	ab		
80 min	0.09±0.01	a	3.5±0.1	a	11±2	a	0.087±0.002	b	2.5±0.2	ab	75±11	a		
120 min	0.10±0.01	a	4.0±0.1	a	8±2	ab	0.086±0.005	b	2.5±0.1	b	92±5	a		

Table A.1 Mechanical properties and solubility of A.cruentus flour films crosslinked with epichlorohydrin under different conditions for the preliminary tests.

	Puncture test						Tensile test						S (%)	
	T (mm)		PF (N)		PD (%)		T (mm)		TS (MPa)		ELO (%)			
80℃, 20 wt.% glycerol														
RV 0% ECH	0.08±0.01	a	4.1±0.4	a	3.6±0.1	b	0.072±0.005	d	4.2±0.5	ab	20±2	b	68±7	a
5%, 40 min	0.08±0.01	a	3.7±0.4	a	4.1±0.3	b	0.092±0.003	abc	5.2±0.6	a	9±2	b	27±3	b
5%, 80 min	0.08±0.01	a	3.4±0.2	a	4.9±0.6	ab	0.097±0.009	ab	3.8±0.3	bc	20±3	b	27±4	b
5%, 120 min	0.09±0.01	a	4.2±0.4	a	4.0±0.3	b	0.088±0.001	abc	4.4±0.4	ab	17±5	b	29±5	b
10%, 40 min	0.09±0.01	a	3.2±0.1	a	8±2	ab	0.077±0.004	cd	4.4±0.1	ab	17±3	b	21±2	b
10%, 80 min	0.10±0.01	a	3.1±0.1	a	9±2	a	0.102±0.002	a	2.6±0.2	d	39±3	a	24±3	b
10%, 120 min	0.08±0.01	a	3.3±0.6	a	7.8±0.6	ab	0.092±0.003	abc	3.0±0.2	cd	35±24	a	22±3	b

T, thickness; PF, puncture force; PD, puncture deformation; TS, tensile strength; ELO, elongation at break; S, solubility in water. Significant difference at $p<0.05$

Table A.2 Regression coeficients (MS Residual) for the puncture force and deformation

Factor	Puncture force			Puncture deformation		
	RC[a]	SE[b]	p value	RC[a]	SE[b]	p value
ECH (x_1)	-0.28	0.13	0.07[d]	0.99	0.58	0.13
ECH (x_1^2)	0.31	0.14	0.07[d]	-2.03	0.64	0.02[c]
Temp (x_2)	-0.33	0.13	0.04[c]	1.17	0.58	0.08[d]
Temp (x_2^2)	0.08	0.14	0.58	-1.08	0.64	0.13
Time (x_3)	-0.19	0.13	0.18	0.21	0.58	0.73
Time (x_3^2)	0.37	0.14	0.04[c]	-2.52	0.64	0.01[c]
x_1*x_2	-0.33	0.17	0.10[d]	1.62	0.75	0.07[d]
x_1*x_3	-0.38	0.17	0.06[d]	1.41	0.75	0.10[d]
x_2*x_3	-0.16	0.17	0.38	0.59	0.75	0.46

[a] Regression coefficient, [b] Standard error, Significant factor at [c] $p<0.05$ or [d] $p<0.1$

Table A.3 Regression coeficients (MS Residual) for the solubility in water and the pH of the solution

Factor	Solubility			Solution pH		
	RC[a]	SE[b]	p value	RC[a]	SE[b]	p value
ECH (x_1)	0.46	0.89	0.62	-0.48	0.06	0.00[c]
ECH (x_1^2)	-1.41	0.98	0.19	0.20	0.07	0.02[c]
Temp (x_2)	3.22	0.89	0.01[c]	-1.22	0.06	0.00[c]
Temp (x_2^2)	-0.46	0.98	0.66	0.09	0.07	0.24
Time (x_3)	1.23	0.89	0.21	-0.46	0.06	0.00[c]
Time (x_3^2)	-0.08	0.98	0.94	0.14	0.07	0.08[d]
x_1*x_2	0.22	1.17	0.86	0.03	0.08	0.74
x_1*x_3	-3.50	1.17	0.02[c]	-0.12	0.08	0.17
x_2*x_3	0.33	1.17	0.79	-0.02	0.08	0.81

[a] Regression coefficient, [b] Standard error, Significant factor at [c] $p<0.05$ or [d] $p<0.1$

Table A.4 Experimental and predicted values, absolute and relative deviations for the puncture force (PF) and deformation (PD)

Assay	Puncture force				Puncture deformation			
	PF exp	PF pred	AD[a]	RD[b]	PD exp	PD pred	AD[a]	RD[b]
1	3.286	3.541	-0.26	-7.76	8.982	7.896	1.09	12.09
2	4.582	4.392	0.19	4.14	2.211	3.828	-1.62	-73.11
3	3.518	3.542	-0.02	-0.69	6.502	6.991	-0.49	-7.52
4	3.067	3.086	-0.02	-0.62	10.652	9.392	1.26	11.83
5	4.371	4.304	0.07	1.53	3.279	5.082	-1.80	-55.01
6	3.702	3.629	0.07	1.95	6.587	6.642	-0.05	-0.83
7	3.525	4.305	-0.78	-22.14	7.620	4.177	3.44	45.19
8	1.988	2.324	-0.34	-16.88	12.948	12.207	0.74	5.72
9	4.757	4.295	0.46	9.70	4.229	5.249	-1.02	-24.13
10	3.271	3.344	-0.07	-2.25	8.694	8.581	0.11	1.30
11	3.552	4.530	-0.98	-27.52	9.373	7.643	1.73	18.46
12	3.188	3.432	-0.24	-7.66	8.924	11.561	-2.64	-29.55
13	4.701	3.014	1.69	35.90	4.863	5.527	-0.66	-13.65
14	3.648	3.014	0.63	17.40	5.284	5.527	-0.24	-4.60
15	3.115	3.014	0.10	3.27	11.870	12.642	-0.77	-6.50
16	2.853	3.014	-0.16	-5.63	12.905	12.642	0.26	2.04
17	2.588	3.014	-0.43	-16.44	13.938	12.642	1.30	9.30

[a] Absolute deviation, [b] Relative deviation

Table A.5 Experimental and predicted values, absolute and relative deviations for the solubility in water (S) and pH of the solution

Assay	Solubility in water				Solution pH			
	S exp	S pred	AD[a]	RD[b]	pH exp	pH pred	AD[a]	RD[b]
1	19.83	21.40	-1.57	-7.92	10.20	10.42	-0.22	-2.14
2	30.19	28.39	1.80	5.96	9.68	9.46	0.22	2.26
3	23.36	27.84	-4.48	-19.18	7.71	7.98	-0.27	-3.52
4	33.32	34.83	-1.51	-4.53	7.11	7.02	0.09	1.20
5	30.10	30.85	-0.74	-2.47	9.77	9.50	0.27	2.79
6	25.21	23.85	1.36	5.38	8.57	8.54	0.03	0.34
7	33.66	37.28	-3.62	-10.76	7.01	7.06	-0.05	-0.73
8	30.90	30.29	0.62	2.00	6.11	6.10	0.01	0.09
9	30.31	26.98	3.33	10.99	9.50	9.33	0.17	1.77
10	26.53	26.98	-0.45	-1.69	7.53	7.72	-0.19	-2.57
11	22.80	25.22	-2.43	-10.65	10.09	10.16	-0.07	-0.73
12	39.46	36.04	3.42	8.66	6.31	6.07	0.24	3.86
13	31.14	28.57	2.57	8.25	9.25	8.89	0.36	3.90
14	33.26	32.69	0.56	1.70	7.44	7.34	0.10	1.33
15	30.38	30.63	-0.26	-0.84	7.76	8.12	-0.36	-4.58
16	30.36	30.63	-0.27	-0.90	7.88	8.12	-0.24	-2.98
17	32.31	30.63	1.68	5.19	8.03	8.12	-0.09	-1.06

[a] Absolute deviation, [b] Relative deviation

Table A.6 Mechanical properties of *A.cruentus* flour crosslinked films with different concentrations of epichlorohydrin (ECH)

ECH (g / 100g flour)	Puncture test			Tensile test		
	T (mm)	PF (N)	PD (%)	T (mm)	TS (Mpa)	ELO (%)
0	0.083±0.004 a	4.7±0.5 ab	2.4±0.4 a	0.080±0.001 b	5.8±0.4 abc	14±2 bc
1.6 (-1.68)	0.089±0.003 a	4.0±0.3 ab	5.6±0.7 a	0.081±0.002 b	3.3±0.4 c	36±5 ab
5 (-1)	0.076±0.002 a	3.7±0.4 b	4.5±0.8 a	0.081±0.001 b	3.6±0.1 bc	41±8 a
10 (0)	0.079±0.003 a	4.0±0.5 ab	4.0±0.6 a	0.083±0.001 ab	4.3±0.8 abc	22±6 bc
15 (1)	0.083±0.003 a	4.7±0.4 ab	3.4±0.4 a	0.084±0.001 a	4.8±0.4 a	14±5 c
18.4 (1.68)	0.088±0.009 a	4.9±0.5 a	3.4±0.6 a	0.081±0.001 b	4.4±0.1 ab	23±8 bc

T, thickness; PF, puncture force; PD, puncture deformation; TS, tensile strength; ELO, elongation at break. Significant difference at p<0.05

Table A.7 Barrier properties of *A.cruentus* flour crosslinked films with different concentrations of epichlorohydrin (ECH)

ECH (g/100g flour)	Solubility in water (%)	WVP (g / m s Pa)
0	68±2	3.8±0.2E-10 a
1.6 (-1.68)	32±1 a	2.3±0.1E-10 b
5.0 (-1)	29±1 b	
10.0 (0)	30±1 ab	2.4±0.2E-10 b
15.0 (+1)	30±1 ab	
18.4 (+1.68)	26±1 c	2.1±0.3E-10 b

WVP: water vapor permeability. Significant difference at p<0.05

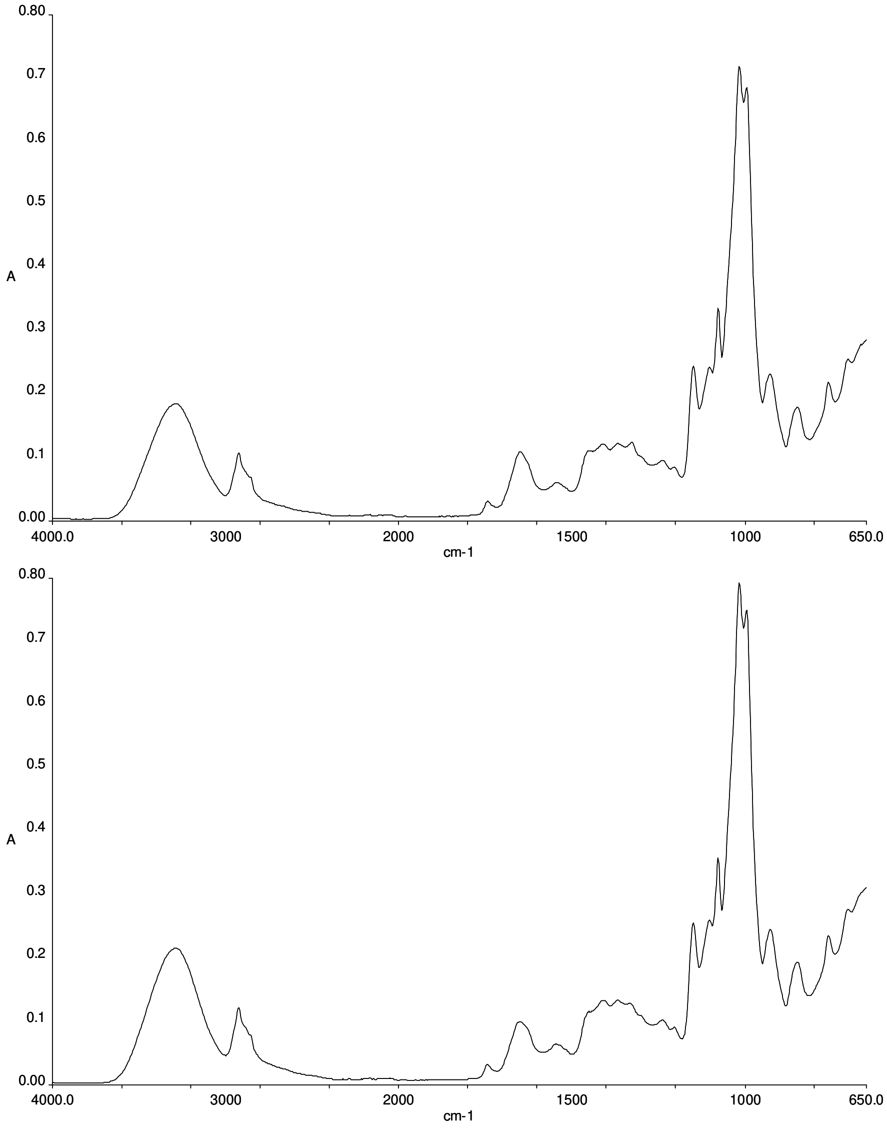

Figure A.1 FT-IR spectra of *A.cruentus* flour films crosslinked with epichlorohydrin from (a) assay 8 (15% ECH, 80ºC, 120 min) and (b) assay 12 (10% ECH, 90.2ºC, 80 min) of the experimental design

APÊNDICE 2

Efeito da adição de poli (vinil álcool) nas propriedades mecânicas e solubilidade dos filmes de farinha de amaranto

Table A.8 Mechanical properties of *A.cruentus* flour – poly(vinyl alcohol) blended films with different grades of PVA.

PVA	Puncture test						Tensile test					
Grade	T (mm)		PF (N)		PD (%)		T (mm)		TS (Mpa)		ELO (%)	
107	0.101±0.004	b	8.7±0.4	a	12±1	d	0.10±0.01	b	7.9±0.4	bc	33±3	c
325	0.098±0.006	b	9.2±0.2	a	16±1	c	0.11±0.01	a	10.2±0.9	a	90±3	ab
350	0.113±0.007	a	9.3±0.5	a	12±2	cd	0.11±0.01	a	8.2±0.6	ab	77±9	b
523	0.102±0.009	b	6.8±0.5	b	21±1	b	0.11±0.01	a	6.6±0.6	bc	105±16	a
540	0.104±0.004	b	6.4±0.9	b	24±4	a	0.10±0.01	b	4.9±0.9	c	85±2	ab

PF, puncture force; PD, puncture deformation; TS, tensile strength; ELO, elongation at break. Significative difference at $p<0.05$

Table A.9 Mechanical properties and solubility of poly(vinyl alcohol) - *A.cruentus* flour blended films in different proportions

PVA/	Puncture test						Tensile test							
ACF (%)	T (mm)		PF (N)		PD (%)		T (mm)		TS (Mpa)		ELO (%)		S (%)	
0-100	0.083±0.004	a	5±1	b	2.4±0.4	b	0.080±0.001	a	5.8±0.4	c	14±2	b	88±3	a
10-90	0.096±0.009	a	6±1	b	3.9±0.9	b	0.079±0.001	a	5.3±0.3	c	21±5	ab	86±3	a
20-80	0.085±0.009	a	6±2	b	2.6±0.6	b	0.081±0.002	a	7.8±0.3	b	16±2	b	55±2	c
30-70	0.087±0.001	a	8±1	b	4.5±0.9	b	0.079±0.002	a	7.6±0.7	b	26±7	ab	70±1	b
40-60	0.086±0.009	a	10±3	a	5.0±0.9	ab	0.079±0.002	a	9.7±0.4	a	37±7	a	60±3	c
50-50	0.082±0.001	a	11±1	a	7.7±0.7	a	0.081±0.002	a	10.0±0.7	a	38±6	a	44±2	b
100-0	0.083±0.004		43±2		23.9±0.5		0.080±0.001		44.8±0.6		84±5			

T, thickness; PF, puncture force; PD, puncture deformation; TS, tensile strength; ELO, elongation at break; S, solubility. Significant difference at $p<0.05$