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ELABORAÇÃO, ADITIVAÇÃO E CARACTERIZAÇÃO DE BIOFILMES À BASE DE FÉCULA DE MANDIOCA

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DEDICO

Ao meu filhinho Arthur, que mesmo antes de nascer,
já deixou a minha vida mais colorida.

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Resumo Geral

Os filmes à base de fécula de mandioca obtidos por moldagem ou “casting” se caracterizam por pobres propriedades mecânicas e alta permeabilidade ao vapor d’água. Entretanto, seu baixo custo e sua disponibilidade no Brasil justificam os esforços para viabiliza-lo como matéria-prima para a produção de filmes biodegradáveis. Na tentativa de reduzir as desvantagens apresentadas pelos filmes obtidos a partir de fécula de mandioca, foram analisados os efeitos dos parâmetros de processamento e aditivos nas suas propriedades físicas, químicas e estruturais. Ensaios preliminares destacaram a sacarose, propileno glicol, fosfato de sódio e óleo de soja como aditivos gerais. Como aditivos principais foram selecionados a goma xantana e a gelatina. A pesquisa foi dividida em duas fases, uma de seleção e outra de otimização. Para a primeira fase, o estudo foi dividido em dois grupos de materiais: os à base de fécula de mandioca e goma xantana e os à base de fécula de mandioca e gelatina. Para o grupo fécula-xantana, dois blocos foram investigados (através de planejamento fatorial 2^{7-3} com três pontos centrais), para avaliar o efeito dos aditivos gerais, do pH e da desacetilação da goma xantana, nas características mecânicas (alongamento e tração), na hidrofilicidade, na microestrutura (microscopia e cristalinidade), na atividade de água e na cor do material. Embora a desacetilação da goma xantana tenha afetado as propriedades mecânicas e a hidrofilicidade do material, foi a adição de sacarose que resultou em alterações mais positivas e significativas nos filmes. Devido ao custo elevado da goma xantana e o pequeno efeito observado nas características estudadas, a utilização deste aditivo foi descartada para continuidade deste estudo. Para o grupo fécula-gelatina, foram estudados três blocos (também através de planejamento fatorial 2^{7-3} com três pontos centrais) para os mesmo parâmetros. No primeiro bloco, foi investigado o efeito dos aditivos gerais (menos o fosfato de sódio), da gelatina, do pH e da temperatura. A temperatura mais elevada (60°C) destruiu completamente os filmes, razão pela qual, na continuidade deste estudo, a temperatura de secagem dos filmes foi fixada em 30°C. Para o segundo e terceiro blocos deste grupo foram investigados o efeito dos aditivos gerais, da gelatina, do pH e o efeito da substituição da sacarose por açúcar invertido. Novamente a sacarose mostrou ser o aditivo que mais afetou as propriedades do material. Entretanto, assim como no grupo fécula-xantana, a utilização de sacarose resultou em cristalização dos filmes, o que não foi observado quando substituída por açúcar invertido. Tal aditivo, embora em menor grau quando comparado à sacarose, também teve impacto positivo significativo nas propriedades do material. Portanto, para o planejamento final de otimização dos filmes foram selecionados os aditivos sacarose e açúcar invertido, a fim de se obter uma faixa ótima para a utilização conjunta destes aditivos. Somente o fosfato de sódio foi selecionado entre os demais aditivos gerais. Como inicialmente este aditivo foi investigado em uma pequena concentração, para a etapa de otimização o fosfato foi investigado em uma concentração superior para avaliação do efeito de possíveis ligações com a fécula. O óleo de soja mostrou não ter sido incorporado pelo filme, resultando em separação de fase. O propileno glicol foi descartado por agir da mesma forma que os plastificantes já selecionados (sacarose e açúcar invertido) e por não ter apresentado um efeito marcante nas propriedades avaliadas, nem prevenido a cristalização da sacarose. O pH do meio também não mostrou ter afetado grandemente o filme quanto às características estudadas, não sendo controlado durante a fase de otimização. A gelatina não resultou em grande alteração nas propriedades dos filmes, sendo também descartada para estudos posteriores. Na segunda fase deste estudo foi realizada uma etapa de otimização, através de metodologia de superfície de resposta (planejamento fatorial em estrela, 2^3, com três pontos centrais). Nesta fase foram, portanto, investigados o efeito da concentração de sacarose (0,0 a 1,4%), açúcar invertido (0,0 a 1,4%) e fosfato de sódio monobásico (0,0 a 0,6%) na microestrutura (difração por raios-X, microscopia óptica e eletrônica), propriedades mecânicas (resistência à tração e % alongamento), permeabilidade (vapor d'água e O_2), propriedades térmicas (DSC e TGA), atividade de água, solubilidade em água e cor dos filmes à base de fécula de mandioca. Na concentração estudada e mesmo em conjunto com o açúcar invertido, a sacarose afetou a cristalinidade dos filmes quando utilizada em concentração superior a 1,116% (mesmo na presença de açúcar invertido até 1,116%) durante a estocagem (120 dias). A combinação de até 0,8% de sacarose com qualquer concentração de açúcar invertido, ou o máximo de 1,2% de sacarose com 0,4% de açúcar invertido, são as faixas de utilização recomendadas para se evitar a cristalização durante a estocagem. Embora os filmes contendo açúcar invertido não tenham resultado em cristalização observada por microscopia, a recristalização da fécula foi observada por difração com raios-X. A adição da mais alta concentração de fosfato utilizada (0,48%) resultou na presença de fécula residual não gelificada nos filmes, também detectada como cristalização da fécula de mandioca. Entretanto, tal recristalização não afetou as características gerais do filme. A utilização dos aditivos (sacarose, açúcar invertido e fosfato de sódio) resultou em aumento da solubilidade em água, atividade de água e diferença total de cor entre amostras e o controle (contendo somente fécula de mandioca). Foi também observado diminuição da permeabilidade ao vapor d’água, diminuição na resistência à tração e aumento na % de alongamento das amostras experimentais quando comparadas ao controle. Os aditivos diminuíram a temperatura de transição vítrea do material e o fosfato afetou significativamente a perda de massa durante o aquecimento. Durante a análise de DSC também foi observado um pico endotérmico, provavelmente relacionado à degradação devido à perda de água do sistema e, somente para os filmes com aditivos, um pico exotérmico, talvez relacionado à cristalização do material.

Summary

The cassava starch films obtained by the casting technique results in poor mechanical properties and high water vapor permeability. However, their low cost and availability in Brazil justifies the efforts to make starch a feasible raw material for biodegradable films production. The effect of additives and process parameters on physical, chemical and structural properties of cassava starch films were investigated in attempt to minimize disadvantages presented by such materials. Preliminary assays detach sucrose, propylene glycol, sodium phosphate and soybean oil as general additives. Xanthan gum and gelatin were selected as main additives. This research was divided in two separate phases, one been a selection step and another, an optimization step. The first step was divided in two material groups: the cassava starch-xanthan gum materials and the cassava starch-gelatin materials. For the starch-xanthan group, it was evaluated the effect of general additives, pH and xanthan gum deacetylation on samples mechanical properties (tensile strength and elongation at break), hydrophilicity, color, water activity and microstructure (crystallinity and microscopy). For that matter, two statistical blocks were investigated (by the 2^{7-3} factorial design experiment, with three central points). Although xanthan gum deacetylation had affected mechanical and hydrophilicity properties, sucrose was the additive that had the most pronounced effect on such characteristics. Due to xanthan gum elevated cost and small effect observed on the studied characteristics, such additive utilization was discarded. For the starch gelatin group, three statistical blocks were investigated for the same parameters, also by the 2^{7-3} factorial planning with three central points. For the first block it was investigated the effect of general additives (except sodium phosphate), gelatin, pH and temperature. Under the highest temperature investigated (60°C), the films were completely destroyed. For that reason, on the continuance of this study, the dehydration temperature was fixed in 30°C. For the second and third blocks of this group, the effect of the general additives, gelatin, pH and the substitution of sucrose for invert sugar were investigated. Again, sucrose was the additive that most affected the material properties. However, as happen on the starch-xanthan group, sucrose addition resulted on film crystallization during storage. Crystallization, although, was not observed when substituting sucrose for inverted sugar. Even so in lower proportions when comparing to sucrose, inverted sugar also had a positive impact on material characteristics. For the final optimization step, the additives sucrose and inverted sugar was selected in order to find and optimum zone for the simultaneous usage of such additives. Among the general additives only sodium phosphate was selected for the optimization step. As initially investigated in very small concentrations, for the final step such additive was investigated in higher concentrations in order to evaluate possible interactions with the starch. The soybean oil was not completely incorporated by the films, resulting in phase separation. The propylene glycol acted the same way that plasticizer already selected (sucrose and inverted sugar), had a lower impact on materials properties and did not prevent sucrose crystallization. Such additive was thus, discarded. pH did not show great effect on films studied characteristics. For the final optimization step, pH adjustments were discarted. Gelatin utilization did not imply in important effects on the cassava starch films, been also discarded for further studies. On the second step of this study, surface response methodology (2^3 factorial planning, with tree central points) was performed to investigate the effect of sucrose (0.0 a 1.4%), inverted sugar (0.0 a 1.4%) and monobasic sodium phosphate (0.0 a 0.6%) on cassava starch films microstructure (X-ray diffraction, light and SEM microscopy), mechanical properties (tensile strength and elongation at break), permeability (water vapor and O_2), thermal properties (DSC and TGA), water activity, solubility and color. At the studied concentration, sucrose affected crystallinity when added above 1.116% (even on the presence of inverted sugar on concentrations bellow 1.116%), during 120 days of storage. The combination of until 0.8% of sucrose with inverted sugar concentration, or the maximum of 1.2% sucrose with 0.4% inverted sugar is the optimum band to prevent crystallization. Although inverted sugar addition did not result in crystallization observed by microscopy analysis, X-ray diffraction crystallization was observed due to the presence of cassava starch peaks. The higher phosphate concentration studied (0.48%) resulted in residual not jellified starch on the films, also detected as cassava starch crystallization. However, such crystallization did not affected the general characteristics of the films, been considered a semi-crystallization. The simultaneous usage of sucrose, inverted sugar and sodium phosphate resulted in increased waster activity, solubility and total color difference when comparing to the control (containing only cassava starch). It was also observed decrease on water vapor permeability, lower tensile strength and increased elongation at break. The additives decreased the glass transition temperature and during scanning, and phosphate had a significant effect on the mass loss during heating. DSC analysis allowed to observed an endothermic peak, probably related to degradation due to a water loss from the system. Also was observed, only for the films with additives, an exothermic peak, probably related to the material crystallization.

INTRODUÇÃO GERAL

Devido aos problemas ambientais acarretados pela utilização de embalagens convencionais (ARVANITOYANNIS & BILIADERIS, 1998), há um crescente interesse no desenvolvimento de embalagens biodegradáveis e obtidas a partir de fontes renováveis (LOURDIN *et al.*, 1997). Filme processado a partir de materiais biológicos, como polissacarídeos e lipídios (GUILBERT *et al.*, 1996 e KROCHTA & MULDER-JOHNSTON, 1997), têm sido avaliados nestee nicho.

O maior desafio da utilização de filmes biodegradáveis é substituir embalagens convencionais mantendo, com a mesma eficácia, a qualidade do produto; garantindo sua vida de prateleira através do controle de características mecânicas e de permeabilidade (KESTER & FENNEMA, 1986; KROCHTA & MULDER-JOHNSTON, 1997).

Embora a utilização de amidos na produção de biofilmes tenha sido bastante estudada (ARVANITOYANNIS *et al.*, 1997; KUUTTI *et al.*, 1998; BILIADERIS *et al.*, 1999; ARVANITOYANNIS & BILIADERIS, 1999; FISHMAN *et al.*, 2000 e GAUDIN *et al.*, 2000), poucos artigos enfocam amidos de fontes tropicais como a mandioca, que é uma cultura mais adaptada às condições sul-americanas (CEREDA, 2004). A fécula de mandioca mostrou ser um material viável na formação de revestimentos, também chamados de “coatings” (HENRIQUE & CEREDA, 1999; VICENTINI & CEREDA, 1999; OLIVEIRA & CEREDA, 1999 e CEREDA *et al.,* 2000) e de filmes flexíveis (HENRIQUE, 2002). Observa-se para tais materiais, entretanto, pequena resistência mecânica e baixa barreira à umidade (VICENTINI *et al.*, 1999), o que inviabiliza seu uso.

Radicais de fosfato conferem ao amido ligações cruzadas entre cadeias de amilose (WHISTLER & DANIEL, 1985), o que aumenta a resistência mecânica e melhorar a resistência à umidade dos filmes (MEZGHENI *et al.*, 2000). Também pode resultar na esterificação do amido, o que resultaria em uma rede com menos ligações intermoleculares (BEMILLER & WHISTLER, 1996), com maior maleabilidade, aumentando a porcentagem ao alongamento do material.

Apesar da utilização de goma xantana como aditivo em biofilmes ter sido pouco estudada, sugere-se que sua adição possa levar à aumento na viscoelasticidade dos filmes de amido e ajudar a manter suas propriedades reológicas após o congelamento (NAVARRO *et al.*, 1997). Também oferece como vantagem para este tipo de aplicação, o fato de ser resistente a variações de pH (BAIRD & PETITT, 1991), podendo ser utilizada em contato com produtos ácidos e básicos. Por ser obtida a partir de microorganismos, pode ser produzida em condições controladas, sem problemas acarretados por entressafras, assegurando a qualidade e homogeneidade de seus lotes (SILVA & SCAMPARINI, 2000).

Grupos acetil, presentes na molécula de goma xantana, previnem sua agregação (MORRIS *et al.*, 1996), indicando que a goma desacetilada poderia resultar em maior interação, tanto entre suas cadeias, como entre os componentes do filme; possibilitando a melhoria de suas propriedades de barreira e de resistência mecânica.

A utilização de plastificantes pode melhorar propriedades mecânicas de filmes de amido. Embora o sorbitol e o glicerol (GAUDIN *et al.*, 2000) estejam entre os plastificantes mais utilizados, ARVANITOYANNIS *et al.* (1996) observaram que a sacarose confere grande plasticidade a filmes hidrofílicos. Embora VEIGA *et al.* (2002) tenham confirmado o efeito plastificante da sacarose em filmes de fécula de mandioca, tais filmes apresentaram início de cristalização após 10 dias de estocagem. A adição de um umectante ao filme, como o propileno glicol (ANVISA, 2002), poderia minimizar tais efeitos.

A adição de propileno glicol a filmes biodegradáveis pode resultar em maior resistência mecânica e flexibilidade (LACROIX *et al.*, 1998). Por ser um dos poliésteres mais hidrofóbicos (KIM *et al.*, 2000), sua utilização também pode diminuir a hidrofilicidade dos filmes de amido, proporcionando menor permeabilidade ao vapor d' água.

Aditivos lipídicos, como o óleo de soja, também podem ser adicionados à filmes biodegradáveis na tentativa de melhorar propriedades de barreira à umidade (YANG & PAULSON, 2000 e GARCIA *et al.*, 2000).

A utilização de aditivos, ou alterações de processamento, que melhorem características mecânicas ou de barreira de filmes biodegradáveis elaborados à base de fécula de mandioca, poderia viabilizar a utilização industrial de embalagens que, além de serem biodegradáveis e advindas de fontes renováveis, também são de baixo custo. Para o Brasil, a viabilização de tais biofilmes representaria, além da detenção da tecnologia de produção de um material de grande interesse industrial e ecológico, também uma forma de agregar valor a importantes matérias-primas produzidas no país, como a mandioca, a sacarose e o óleo de soja. Em 2003, o Brasil produziu 386 milhões de toneladas de cana de açúcar (24,8 milhões de toneladas de sacarose), 51,6 milhões de toneladas de soja (5,4 milhões de toneladas de óleo de soja) e 22,4 milhões de toneladas de mandioca (FAO, 2004) (0,5 milhões de toneladas de fécula de mandioca) (CEREDA, 2004). Embora a produção de mandioca seja inferior quando comparada à da soja e sacarose, o Brasil é o segundo maior produtor mundial de mandioca (FAO, 2004), o que justifica a tentativa de motivar o setor.

Os objetivos deste trabalho foram selecionar aditivos e parâmetros de processo que tivessem influência significativa ($p<0,05$) nas propriedades mecânicas (resistência à tração e % alongamento), hidrofilicidade e microestrutura, cor e atividade de água de filmes à base de FÉCULA DE MANDIOCA e GOMA XANTANA e de filme à base de FÉCULA DE MANDIOCA e GELATINA. Posteriormente, através de metodologia de superfície de resposta, estudou-se o efeito dos aditivos selecionados (sacarose, açúcar invertido e fosfato de sódio) na microestrutura (difração por raios-X, microscopia óptica e eletrônica), propriedades mecânicas (resistência à tração e % alongamento), permeabilidade (vapor d'água e O_2), propriedades térmicas (DSC e TGA), atividade de água, solubilidade em água e cor dos filmes à base de fécula de mandioca.

As Figuras 1, 2 e 3 apresentam fluxogramas básicos da elaboração, aditivação e caracterização dos filmes dos grupos fécula-goma xantana, fécula-gelatina e da etapa de otimização, respectivamente.

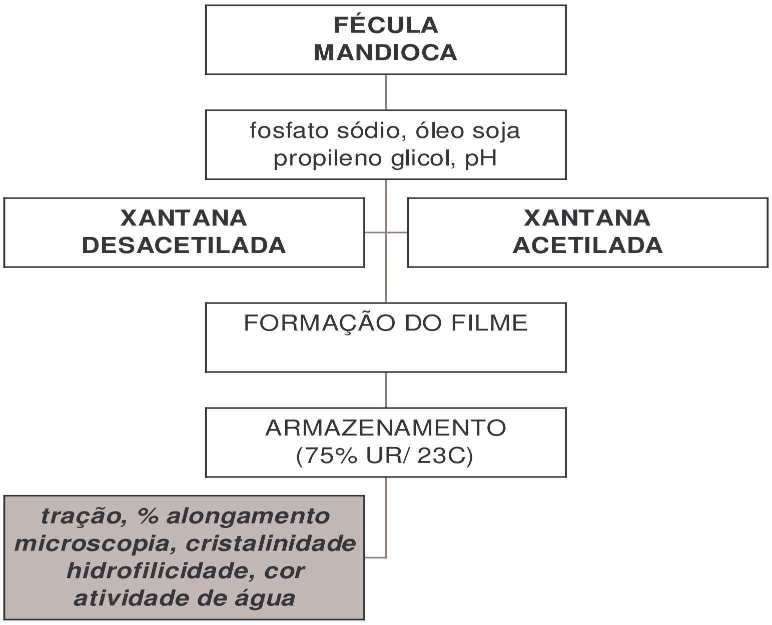

Figura 1. Fluxograma básico da elaboração, aditivação e caracterização dos filmes dos grupos fécula-goma xantana, da etapa de seleção.

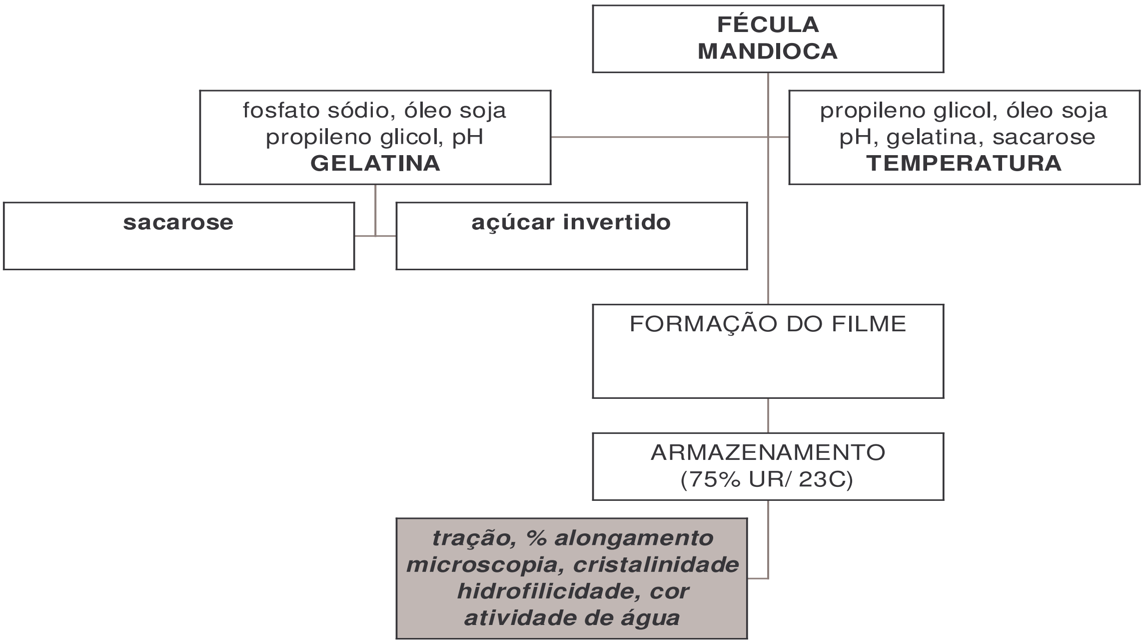

Figura 2. Fluxograma básico da elaboração, aditivação e caracterização dos filmes dos grupos fécula-gelatina, da etapa de seleção.

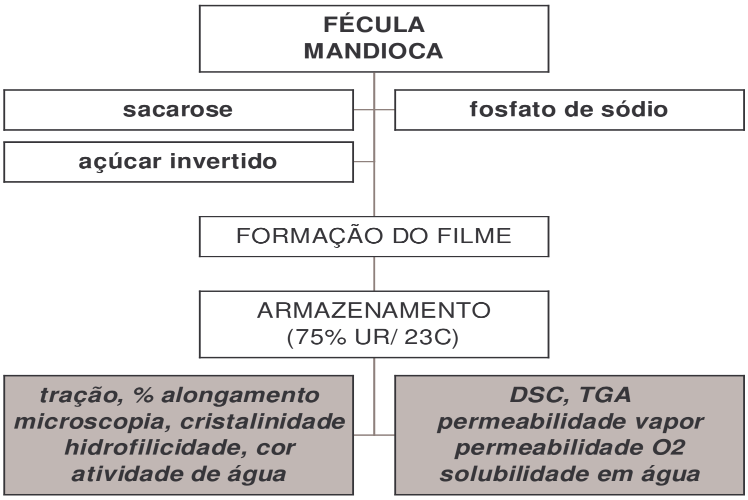

Figura 3. Fluxograma básico da elaboração, aditivação e caracterização dos filmes da etapa de otimização.

Este trabalho será apresentado na forma de capítulos, conforme descritos a seguir:

CAPÍTULO 1. Filmes flexíveis biodegradáveis à base de amido: aditivos e caracterização. Apresenta uma revisão bibliográfica sintética sobre filmes biodegradáveis. Aspectos relacionados à elaboração, aditivação e caracterização foram abordados. No início de cada um dos demais capítulos é apresentada uma revisão bibliográfica pertinente aos assuntos tratados em cada artigo. Este capítulo for redigido conforme normas da revista *Polímeros*.

CAPÍTULO 2. Mechanical properties, hydrophilicity and water activity of starch-gum films: Effect of additives and xanthan gum deacetylation. Part 1. Neste capítulo, o efeito da desacetilação da goma xantana, dos aditivos gerais (sacarose, propileno glicol, óleo de soja, fosfato de sódio) e do pH, nas características mecânicas, hidrofilicidade e atividade de água do material foram avaliados através de dois blocos estatísticos. Este capítulo foi redigido conforme normas da revista *Food Hydrocolloids*.

CAPÍTULO 3. Microstructure and color of starch-gum films: Effect additives and xanthan gum deacetylation. Part 2. Neste capítulo, o efeito da desacetilação da goma xantana, dos aditivos gerais (sacarose, propileno glicol, óleo de soja e fosfato de sódio) e do pH, na microestrutura e cor do material foram investigados através de dois blocos estatísticos. Este capítulo for redigido conforme normas da revista *Food Hydrocolloids*.

CAPÍTULO 4. Microstructure and color of biodegradable cassava starch-gelatin films: influence of additives and process parameters. Neste capítulo, o efeito de aditivos (gelatina, sacarose, propileno glicol, óleo de soja) e parâmetros de processamento (pH e temperatura) na microestrutura e cor do material foram investigados através de dois blocos estatísticos. Este capítulo for redigido conforme normas da revista *Journal of Food Chemistry*.

CAPÍTULO 5. Sucrose and inverted sugar as additives. Part 1: Effect on cassava starch-gelatin films mechanical properties, hydrophilicity and water activity. Neste capítulo, dois blocos estatísticos foram estudados para comparar o efeito da substituição da sacarose por açúcar invertido nas propriedades mecânicas, hidrofilicidade e atividade de água de filmes à base de fécula de mandioca e gelatina O efeito da utilização dos aditivos gerais (propileno glicol, óleo de soja e fosfato de sódio) e do pH também foram investigados. Este capítulo for redigido conforme normas da revista *Journal of Agriculture and Food Chemistry*.

CAPÍTULO 6. Sucrose and inverted sugar as additives. Part 2: Effect on biodegradable cassava starch-gelatin films microstructure and color.

Neste capítulo, dois blocos estatísticos foram estudados para comparar o efeito da substituição da sacarose por açúcar invertido na microestrutura e coloração de filmes à base de fécula de mandioca e gelatina O efeito da utilização dos aditivos gerais (propileno glicol, óleo de soja e fosfato de sódio) e do pH também foram investigados. Este capítulo for redigido conforme normas da revista *Journal of Agriculture and Food Chemistry*.

CAPÍTULO 7. Mechanical, barrier and thermal properties of cassava starch films: Effect of sucrose, inverted sugar and phosphate. Neste capítulo, foi investigado o efeito da adição de sacarose, açúcar invertido e fosfato de sódio, nas propriedades mecânicas, de barreira e na solubilidade de filmes à base de fécula de mandioca, através de metodologia de superfície de resposta. Este capítulo for redigido conforme normas da revista *Food Hydrocolloids*.

CAPÍTULO 8. Effect of sucrose, inverted sugar and phosphate on microstructure, color, water activity and solubility of cassava starch biodegradable films. Neste capítulo, foi investigado o efeito da adição de sacarose, açúcar invertido e fosfato de sódio, na microestrutura, coloração, atividade de água e solubilidade de filmes à base de fécula de mandioca, através de metodologia de superfície de resposta. Este capítulo for redigido conforme normas da revista *Food Hydrocolloids*.

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CAPÍTULO 1

Filmes flexíveis biodegradáveis à base de amido: Aditivos e caracterização.

Filmes flexíveis biodegradáveis à base de amido: aditivos e caracterização

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Resumo: Definição e processos de fabricação de filmes biodegradáveis, sendo ou não comestíveis, foram revisados. Ênfase especial foi dada para os filmes à base de amido, adicionados ou não de outros componentes principais como a gelatina e a goma xantana. Tentativas de melhoria das propriedades mecânicas e de barreira dos filmes através da utilização de aditivos como plastificantes e lipídios, foram brevemente discutidas. Métodos para caracterização dos filmes biodegradáveis quanto às suas propriedades mecânicas, de barreira, térmicas e microestrura, baseados em testes e experiências prévias dos autores, também foram considerados.

Palavras-chave: Filmes biodegradáveis, amido, aditivos, caracterização, revisão.

Starch biodegradable flexible films: additives and characterization

Summary: Definition and manufacture process of biodegradable films, edible or not, were reviewed. Special emphasis was given to starch films, with or without the addition of other main components as gelatin and xanthan gum. Attempts to improve films mechanical and barrier properties due to additives, such as plasticizers and lipids, utilization were briefly discussed. Methods for characterizing

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mechanical, barrier, thermal and microstructure properties of biodegradable films were also considered.

Keywords: Biodegradable films, starch, additives, characterization, review.

Introdução

Como alternativa aos problemas ambientais acarretados por embalagens poliméricas tradicionais estão as embalagens biodegradáveis, obtidas a partir de fontes renováveis[39]. Entre os materiais biológicos mais utilizados para tal finalidade estão os polissacarídeos[30, 36].

Entretanto, o maior desafio para a utilização industrial de filmes biodegradáveis obtidos de polissacarídeos é substituir as embalagens convencionais mantendo, com a mesma eficácia, a qualidade do produto e garantir sua vida de prateleira[34, 36].

Embora a utilização de amidos na produção de biofilmes tenha sido bastante estudada[3, 37, 15, 4, 24, 27], poucos artigos enfocam amidos de fontes tropicais como a mandioca, que é uma cultura mais adaptada às condições sul-americanas[18]. A fécula de mandioca mostrou ser um material viável na formação de revestimentos, também chamados de “coatings”[32, 57, 48]. Entretanto, possui pequena resistência mecânica e baixa barreira à umidade[57], o que inviabiliza seu uso.

A utilização de aditivos, ou alterações de processamento, que melhorem características mecânicas ou de barreira de filmes biodegradáveis elaborados à base de fécula de mandioca, poderia viabilizar a utilização industrial de embalagens que, além de serem biodegradáveis e advindas de fontes renováveis, também são de baixo custo. Para o Brasil, a viabilização de tais biofilmes representaria, além da detenção da tecnologia de produção de um material de grande interesse industrial e ecológico, também uma forma de agregar valor a importantes matérias-primas produzidas no país, como a mandioca, a sacarose e o óleo de soja. Em 2003, o Brasil produziu 22,4 milhões de toneladas de mandioca[23] (0,5 milhões de toneladas de fécula de mandioca)[18], sendo considerado segundo maior produtor mundial de mandioca[23]; o que justifica a tentativa de motivar o setor.

Este trabalho teve como objetivo apresentar uma sintética revisão bibliográfica sobre filmes biodegradáveis elaborados a partir de amido, com atenção especial à fécula de mandioca. Aspectos relacionados à elaboração de filmes de amido, possíveis aditivos e caracterização, foram também abordados.

Biofilmes

Filmes são estruturas utilizadas para envolver produtos. Diferentemente dos revestimentos, que são aplicados sobre o produto anteriormente à sua própria formação, of filmes primeiramente formam lâminas e só então são utilizados como revestimento[30]. Quando são completamente degradados por microorganismos são considerados biodegradáveis, sendo chamados de biofilmes[36]. Em alguns casos, também podem ser comestíveis.

Os filmes comestíveis devem ser reconhecidos como seguros para alimentação humana "GRAS - generally recognized as safe" e para tal, todos os seus componentes devem ser individualmente reconhecidos como "GRAS"[36].

Filmes são geralmente utilizados em alimentos com a finalidade de proteção, inibindo ou minimizando a permeação de umidade, oxigênio, dióxido de carbono, aromas e a migração de lipídios. Podem também carregar compostos antimicrobianos e antioxidantes, conferir proteção mecânica ou auxiliar no aumento da vida de prateleira após a abertura da embalagem, envolvendo individualmente cada produto contido no seu interior[36].

Filmes são formados principalmente por forças coesivas entre moléculas de polímeros. A desnaturação ou a incorporação de aditivos que promovam a formação de ligações cruzadas resultam em maior ordenação molecular, e consequentemente, maior coesão e rigidez dos filmes[30].

Os filmes feitos a partir de polímeros hidrofílicos são altamente impermeáveis a óleos e gorduras, e quando combinados com lipídios, podem apresentar melhor barreira à umidade[42].

Formação de Filmes

Para a formação de filmes, pode ser utilizado o método de secagem por moldagem, onde a suspensão formadora de filme é depositada sobre um molde ou superfície e, posteriormente, seca, geralmente em estufas ou secadores de bandeja[12, 59]. É forma mais recomendada para a produção de filmes flexíveis. Os filmes obtidos a partir de amido, secados por moldagem, resultam em um material com alta transparência. Entretanto, pela moldagem tradicional, os filmes devem ser secados a baixas temperaturas para que não quebrem durante a etapa de secagem. Equipamentos envolvendo uma primeira etapa de secagem por extrusão seguida de moldagem vêm sendo testados com sucesso, embora a transparência seja comprometida em muitos casos.

A extrusão é outro método bastante utilizado, por ser mais rápido e requerer menor energia na a remoção de água que o processo de moldagem, embora o equipamento seja de custo elevado. No caso de filmes à base de amido, que exigem ser pré-gelificados, os grânulos podem sofrer gelificação durante a extrusão, dispensando tratamentos térmicos anteriores[24]. Filmes feitos inteiramente de por extrusão resultam em materiais rígidos. Estes materiais, em geral, possuem boa resistência à permeabilidade de água.

Com a finalidade de agilizar a etapa de secagem do filme, métodos envolvendo equipamentos de microondas[33] e a utilização de irradiação[38, 45] vêm sendo também aplicados.

Tipos de biofilmes

Atualmente, o estudo de biofilmes comestíveis é mais voltado para a aplicação em alimentos, destacando-se o uso de polissacarídeos. No entanto, apesar de possuírem, em geral, boa barreira ao oxigênio, os polissacarídeos possuem resistência à umidade e barreira ao vapor d'água inferiores às apresentadas por filmes sintéticos[61].

Filmes de amido

Entre os primeiros filmes biodegradáveis e comestíveis estão os elaborados a partir do amido, por serem uma alternativa mais viável economicamente às resinas tradicionais[19] e por advirem de fontes renováveis. Tais filmes possuem moderada permeabilidade ao oxigênio, baixa barreira à umidade e baixa resistência mecânica. Adquirem propriedades termoplásticas quando plastificantes, como a água, são adicionados[36].

A obtenção de biofilmes a partir da fécula de mandioca é baseada na sua gelificação, que ocorre com aquecimento acima de 70°C, seguido de resfriamento. Ocorre então a retrogradação, com consequente formação de um filme transparente, com alto brilho, atóxico e de baixo custo[17]. No entanto, podem apresentar-se quebradiços[19].

A utilização de amido na produção de biofilmes é geralmente associada à interação com outros materiais, tanto sintéticos[50, 19], quanto orgânicos[24, 15, 4, 47], com e sem a adição de plastificantes e materiais lipídicos na tentativa de melhorar suas características mecânicas e de permeabilidade à água.

A aditivação de filmes de amido com fosfatos pode resultar em ligações cruzadas entre as cadeias de amilose, especialmente sob pH básico[60]. Tais ligações poderiam resultar em filmes com maior resistência mecânica e melhorar a resistência à umidade[45]. Radicais de fosfato também poderiam resultar na esterificação do amido, o que poderia estabelecer uma rede com menor número de ligações intermoleculares[14], com maior maleabilidade, podendo aumentar a porcentagem de alongamento do material.

Filmes de amido e goma xantana

Ao contrário da produção de filmes obtidos pela secagem do gel, resultando em colapso da rede estrutural deste gel, filmes obtidos da secagem de uma solução gelificante, como é o caso da goma xantana, são resultado da sedimentação de partículas em suspensão[59].

A goma xantana é compatível com amidos[11], influenciando positivamente suas propriedades viscoelásticas. Minimiza alterações estruturais em soluções de amido, mesmo a baixas temperaturas, prevenindo interações amilose-amilose[47]. Também pode ter efeito sinergístico com amidos, devido à formação de um complexo entre os polímeros do amido (amilose e amilopectina) e o hidrocolóide, quando submetidos a tratamento térmico[20].

A utilização de gomas (2%) em filmes pode resultar em materiais com propriedades mecânicas adequadas, e excelente barreira a aromas, gases e lipídios, mas baixa barreira à umidade[61]. O aumento da concentração de goma possibilita também uma diminuição na rugosidade da microestrutura da superfície e um aumento no seu brilho[59].

Grupos acetil, presentes na molécula de goma xantana, previnem sua agregação[45]. A formação de filmes a partir de goma xantana desacetilada poderia resultar em maior interação, tanto entre suas cadeias, como entre suas cadeias e os componentes do filme; o que poderia melhorar suas propriedades de barreira em comparação com a utilização de goma xantana não desacetilada. Também poderia melhorar as propriedades mecânicas, sendo que uma maior interação entre cadeias poderia aumentar a resistência à tração. Uma maior interação com os componentes do filme, também poderia aumentar a porcentagem de alongamento do material. Veiga *et al.* (2004)[54] observou melhoria nas propriedades de alongamento quando goma xantana desacetilada foi adicionada à filmes obtidos de fécula de mandioca.

Entretanto, para adicionar a goma xantana (desacetilada ou não) é recomendado sua completa dissolução antes de misturá-la aos demais componentes do material.

Filmes de amido e gelatina

A gelatina é obtida a partir do colágeno. De acordo com o tipo de pré-tratamento utilizado para retirar impurezas e iniciar a hidrólise da matéria-prima, a gelatina é classificada em tipo A (pré-tratamento com ácido, com ponto isoelétrico entre 7,0-9,4) e tipo B (pré-tratamento com base com ponto isoelétrico entre 4,5-5,3)[49]. A gelatina do tipo B é a mais comumente encontrada por ser o processo industrial mais utilizado[56].

Proteínas, como a gelatina, podem ser boas formadoras de filmes. Representam boa barreira a gases[16] e, quando utilizada em filmes de amido, podem melhor sua resistência mecânica[3].

Arvanitoyannis *et al.* (1997)[3] observaram que filmes de amido com adição de gelatina, mesmo estocados em diferentes condições de umidade, apresentaram melhoria nas propriedades mecânicas quando baixas temperaturas de secagem (20°C) foram utilizadas. Quando temperaturas de 60°C foram utilizadas, a gelatina e as condições de estocagem afetaram negativamente as propriedades mecânicas, indicando que quando secas a menores temperaturas, podem resultar em filmes com melhores propriedades mecânicas, caso gelatina esteja presente na suspensão filmogênica.

Para incorporar a gelatina aos filmes biodegradáveis, deve-se observar a forma com que será feita a sua dissolução já que ela exercerá efeito diferenciado conforme a forma de aquecimento, lentamente ou rapidamente.

Aditivos

Plastificantes

Plastificantes são utilizados em filmes para reduzirem as forças de ligação entre moléculas em sistemas de baixa umidade, aumentando a flexibilidade do filme. No entanto, seu aumento pode resultar em redução nas propriedades mecânicas de tração dos filmes, e em alguns casos, também na redução da barreira ao vapor d'água[21].

A permeabilidade mínima de filmes de amido ocorre quando cerca de 10% de plastificantes são adicionados, o que está relacionado à forte interação entre o plastificante e o polímero, provavelmente devido a ligações de hidrogênio[39]. Acima de 15% de plastificante, observa-se uma diminuição na temperatura de transição vítrea, no módulo de elasticidade e na resistência à tração. No entanto, pode ser observado um aumento nas propriedades de alongamento[3, 4].

Embora o sorbitol e o glicerol estejam entre os plastificantes mais utilizados[27], Aranitoyannis *et al.* (1996)[2] observaram que a sacarose também possui alta eficácia em filmes hidrofílicos.

Estudos preliminares indicaram que a sacarose exerce efeito plastificante em filmes de fécula de mandioca, entretanto, sua adição resultou em cristalização dos filmes após 10 dias de estocagem[52]. Um umectante, como o propileno glicol[1], poderia ser adicionado na expectativa de retardar a cristalização de filmes de fécula de mandioca e sacarose.

Caso seja utilizada a sacarose, deve-se garantir que todos os seus grânulos sejam completamente dissolvidos na suspensão filmogênica para que não ajam como núcleos de cristalização, afetando a estrutura final dos materiais ao longo da estocagem.

A utilização de propileno glicol em biofilmes também pode resultar em maior resistência mecânica e flexibilidade[38], e por ser mais hidrofóbico que outros poliésteres[34], sua utilização também pode diminuir a hidrofilicidade dos filmes de amido, proporcionando menor permeabilidade ao vapor d' água.

Água também é considerada um plastificante em filmes hidrofílicos, resultando em produtos com alto brilho[37, 21].

Em geral, sistemas plastificante-água-amido apresentam maior sensibilidade à umidade ambiente quando o plastificante está presente em altas concentrações (>27%), permitindo a formação de ligações de hidrogênio entre o plastificante e a água[39], sendo, portanto, recomendável a utilização de baixas concentrações.

Aditivos lipídicos

Triglicerídeos também são aditivos que vêm sendo avaliados para aplicação em biofilmes, uma vez que possibilitam um aumento na hidrofobicidade dos filmes[61].

A adição de lipídios, como o óleo de soja em filmes de amido, resultam em diminuição da permeabilidade à umidade[26], desde que o teor de lipídios não ultrapasse 20%[25]. Entretanto, podem causar redução nas propriedades ópticas e mecânicas dos filmes[61].

Métodos de Caracterização

Espessura:

Devido ao processo de fabricação utilizado, defeitos podem ocorrer nos filmes, o que, consequentemente, pode afetar o desempenho da embalagem confeccionada com este material[51].

Um dos mais graves defeitos de fabricação dos filmes biodegradáveis por moldagem é a heterogeneidade de espessura. Variações na espessura de um material podem afetar suas propriedades mecânicas e de barreira, comprometendo o desempenho da embalagem[51].

Para a análise de espessura, recomenda-se que as medidas sejam realizadas em corpos de prova sem irregularidade, a pelo menos 6 mm da borda. Principalmente no caso de biofilmes que tendem a trocar umidade com o meio, as amostras devem ser previamente acondicionadas em ambiente com umidade e temperatura controladas. As superfícies de medição do micrômetro devem ser limpas com óleo anticorrosivo, como éter de petróleo e anteriormente à análise, o micrômetro deve ser sempre zerado. A espessura é mais comumente expressa em μm[51].

Na falta de um micrômetro também pode-se avaliar a espessura através da gramatura, pesando-se uma área padronizada do material. Pode ser expressa em g/cm$^{2.}$

Propriedades Mecânicas

Entre as maiores desvantagens dos filmes biodegradáveis quando comparados a embalagens convencionais são suas características mecânicas, que em geral, são inferiores[34, 36].

As propriedades de tração expressam a resistência do material à deformação por alongamento quando submetido à tração. Observando uma curva característica de tensão de tração versus deformação de filmes flexíveis, inicialmente o material oferece resistência crescente à solicitação de tração, a qual provoca seu alongamento. A partir de certo ponto, o aumento de resistência passa a ser menor para um mesmo aumento de deformação até o ponto de escoamento, a partir do qual é possível alongar o filme sem que este responda com um aumento de resistência. Continuando o alongamento, o material resiste até que ocorra sua ruptura[51].

A resistência máxima à tração, como o próprio nome já diz, é a resistência máxima oferecida pelo material quando submetido à tração. A resistência à tração é a relação entre a força, medida pela área transversal inicial do corpo de prova. É comumente expressa em MPa, N/m^2 ou kgf/cm^2. Já o fator de ruptura, é a relação entre a força máxima de tração por unidade de largura inicial do corpo de prova. É um parâmetro alternativo para caracterizar a resistência à tração de filmes cuja seção transversal não é homogênea, como em filmes cuja espessura com grande desvio de espessura. É também expresso em MPa, N/m^2 ou kgf/cm^2 [51].

A porcentagem ao alongamento é a relação entre o alongamento do corpo de prova e seu comprimento inicial, podendo ser determinada para a situação de ruptura[51].

A metodologia padrão para os ensaios de resistência mecânica segue as especificações ASTM D882-00 (2001)[8], onde corpos de prova retangulares (8 x 2,5 cm) obtidos de filmes pré-condicionados (23°C; 75% UR) são fixados entre as garras de tensão do equipamento INSTRON. A separação inicial entre as garras e a velocidade de tração devem seguir recomendações, conforme apresentadas na Tabela 1.

Tabela 1. Separação inicial entre garras e velocidade de tração recomendadas para o ensaios de resistência mecânica[2].

Alongamento na ruptura (%)	**Taxa de alongamento inicial (mm/mm.min)**	**Separação inicial entre garras (mm)**	**Velocidade de tração (mm/min)**
< 20	0,1	125	12,5
20 a 100	0,5	100	50,0
> 100	10,0	50	500,0
*Módulos**	0,1	250	25,0

* módulos de elasticidade secante.

No caso de biofilmes, muitas vezes, o corpo de prova não possui tamanho suficiente para se adequar às condições de separação inicial entre as garras especificadas na Tabela 1. Neste caso, uma alternativa é a utilização de uma distância entre as garras menor que a recomendada. Entretanto, os outros parâmetros devem ser mantidos conforme recomendação inicial apresentada na Tabela 1 e as alterações feitas devem ser reportadas na metodologia da análise. Comparações entre amostras só devem ser feitas entre corpos de provas submetidos às mesmas condições de análise.

Deve-s lembrar a importância de se condicionar os filmes a uma umidade e temperatura controladas, não somente no momento da análise. Alguns filmes biodegradáveis podem levar dias para um acondicionamento uniforme. As análises também devem ser feitas sempre com o mesmo período de fabricação para que eventuais diferenças de degradação do material não alterem o resultado final de avaliação.

Microestrutura

Microscopia:

A análise de microscopia mais utilizada para avaliar a microestrutura de filmes biodegradáveis é a microscopia eletrônica de varredura. Entretanto, se o filme é diretamente submetido ao feixe de luz, ele será degradado. Uma alternativa é utilizar um feixe de luz de intensidade bem baixa, 5 a 10 kV. Para 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.

Embora a microscopia seja extremamente eficaz para avaliar a superfície dos filmes, para investigar a estrutura interna do material é necessário fazer um corte na amostra e olhar o perfil do corte no microscópio. Entretanto, para que ocorra o mínimo de alteração na estrutura do material, recomenda-se um corte crioscópico, geralmente utilizando-se nitrogênio líquido. O corte feito simplesmente com um material cortante como tesoura ou estilete pode mascarar eventuais falhas/defeitos na estrutura do filme.

Veiga-Santos *et al.*, 2004[55], investigaram a microestrutura de filmes biodegradáveis por observação direta em microscópio óptico de lente polarizada, sem preparo prévio, através de transmitância. Foi possível observar heterogeneidade de fase e cristalização. Também foi possível, através de luz polarizada, identificar grânulos de amido não gelificados, através da observação da "cruz de malta", que é somente encontrada em amidos não gelificados.

A utilização da microscopia óptica para observar materiais biodegradáveis é uma ferramenta bastante útil, rápida e de relativo baixo custo.

Cristalinidade:

A cristalinidade de filmes biodegradáveis pode ser facilmente determinada através de difração por raios-X, que pode revelar detalhes de estrutura interna do material da ordem de 10^{-8} cm de tamanho. Pela difração, pode-se determinar se um material é amorfo, semi-amorfo ou cristalino. Também permite avaliar a cristalização durante a estocagem do biofilme. Para os filmes semi-cristalinos ou cristalinos, a difração permite identificar qual ou quais elementos são responsáveis pela cristalinidade do material[22].

Embora existam bases de dados com o padrão de cristalinidade de muitos materiais, no caso de filmes biodegradáveis, especialmente os elaborados a partir de matérias-primas industriais que podem sofrer pequenas variações de um lote para outro, recomenda-se que também as matérias-primas sejam separadamente submetidas á análise de cristalinidade, fornecendo padrões para uma comparação mais eficaz com o padrão de cristalinidade obtido pela análise do filme.

Para a análise dos filmes, basta fixá-los em porta amostra de alumínio ou vidro, de maneira com que não apresente ondulações, mas também sem distendê-los, para que não haja possíveis distorções nos espaços intersticiais do material.

Propriedades de Barreira:

Embora a permeabilidade a lipídios, aromas e luz seja de enorme importância para o armazenamento de produtos alimentícios, a permeabilidade ao oxigênio e ao vapor d'água são os parâmetros mais estudadas para filmes biodegradáveis.

Em geral, filmes obtidos de polissacarídeos, como o amido, apresentam boa barreira ao oxigênio, mas baixa barreira ao vapor d'água[61].

Considera-se que o processo de permeação de gases e vapores através dos espaços intermoleculares do material acontece em 3 etapas: a) sorção e solubilização do permeante na superfície do material; b) difusão do permeante através do material devido à ação de um gradiente de concentração; c) dessorção e evaporação de permeante na outra face do material[51].

Permeabilidade ao Vapor D'água:

A taxa de permeabilidade ao vapor d'água é comumente expressa em $g_{\text{água}}/m^2$.dia e a permeabilidade ao vapor d'água é comumente expressa em $g_{\text{água}}.\mu m/m^2$.dia.mmHg, e não devem ser utilizadas para caracterizar um filme não heterogêneo[51].

Uma das formas mais utilizadas avaliar a taxa de permeabilidade ao vapor d'água dos filmes biodegradáveis é através de método gravimétrico. Tal método consiste em pesagens sucessivas de uma cápsula hermeticamente fechada, contendo o filme na sua superfície e substância dessecante (cloreto de cálcio ou sílica gel) no seu interior; colocadas em ambientes com umidade controlada. Caso o filme tenha grande afinidade pela água, é necessária a utilização de corpos de prova adicionais, preparados sem dessecante (branco) para que possa ser descontada ou acrescida a variação de peso do material. Alternativamente, a solução com umidade controlada também pode ser colocada no interior da cápsula, e no seu exterior, o material dessecante[51]. Em ambos os casos, as umidades e temperaturas para o teste de permeabilidade ao vapor recomendadas pela norma ASTM E104-85 (1996)[7] são: a) 23°C e 75% UR, utilizando cloreto de sódio; b) 30°C e 80% UR, utilizando sulfato de amônia, ou c) 38°C e 90% UR, utilizando cloreto de bário.

O coeficiente angular é determinado plotando-se o tempo de ensaio *versus* o ganho de peso. A taxa de permeação ao vapor d'água e a permeabilidade ao vapor d'água são calculadas de acordo com as equações 1 e 2, respectivamente[5].

$$TVPA = \text{inclinação}/\text{área do filme} \qquad \textbf{Equação 1}$$

Onde:

- TVPA: taxa de permeabilidade ao vapor d'água (g/hm^2)
- inclinação: perda de peso x tempo (g/h)
- área do filme: π x (raio da boca da cápsula)^2 (m^2)

. *Permeabilidade ao vapor d´água = TVPA.e/ps(UR₁ – UR₂)* **Equação 2**

$$\text{Permeabilidade ao vapor d´água} = TVPA.e/ps(UR_1 - UR_2)$$

Onde:

- e = espessura média do corpo de prova (μm)
- ps = pressão de saturação do vapor à temperatura do ensaio ($mmHg$)
- R = Constante de gases ($82{,}1x10^{-6}\ m^3\ atm/g\ mol\ K$)
- UR_1 = umidade relativa no interior da câmara (%)
- UR_2 = umidade relativa no interior da cápsula (%)

Taxas de correções para os valores de permeabilidade ao vapor d'água são propostas por McHugh *et al.* (1993)[44] e Gennadios *et al.* (1994)[29], entre outros.

Permeabilidade ao Oxigênio:

Uma das metodologias mais utilizadas para avaliar a taxa de permeabilidade ao oxigênio é definida pela norma ASTM D3985-95 (2001)[6]. Um corpo de prova, na forma de filme, é fixado como divisória no meio de uma célula de difusão, formando duas câmaras, ambas à pressão atmosférica ambiente. Numa das câmaras há um fluxo contínuo de nitrogênio (gás de arraste), enquanto na outra tem-se um fluxo de oxigênio (gás permeante), na forma pura ou combinado com outros gases. À medida que o oxigênio permeia pelo filme, ele é carregado pelo gás de arraste para um detector coulométrico[51].

No caso de filmes biodegradáveis sensíveis à umidade, recomenda-se a utilização de equipamentos que permitam controlar a umidade durante a análise.

Também é muito comum para biofilmes, o uso de máscaras de alumínio, que diminuem a área de troca de gases, geralmente para 5 cm^2 [29]. Muitos filmes biodegradáveis ressecam durante a análise, formando micro fissuras no local onde prendem-se as amostras. Nestes casos, faz-se necessário a utilização das máscaras.

As condições de análise recomendadas pela norma ASTM D3985-95 (2001)[6] são: gradiente de pressão parcial de oxigênio de 1 atm, à 25°C e 75% UR.

Ângulo de Contato

A análise do ângulo de contato pode ser utilizada em filmes biodegradáveis para avaliar sua polaridade e o seu caráter hidrofílico através do ângulo de contato inicial e da cinética de absorção da gota de água em função do tempo[10].

As medidas de ângulo de contato baseiam-se na deposição de uma gota de água sobre o filme. O ângulo formado entre o filme e a gota é chamado de ângulo de contato. Em geral, para filmes convencionais obtidos de petróleo, o ângulo de contato é constante, não variando com o tempo. Entretanto, para filmes biodegradáveis, o ângulo de contato pode variar grandemente, chegando a ser completamente absorvido pelo material[53]. A cinética de absorção de água pelo filme é um indicativo de sua hidrofilicidade[9].

Filmes com alta hidrofilicidade tendem a ser atraídos pela gota de água. Dependendo do tipo de equipamento utilizado, é necessário fixar as extremidades do corpo de prova com, por exemplo, fita adesiva dupla face, para que não ocorra distorção nas leituras. Entre a amostra e o suporte metálico sobre o qual é feita a leitura, deve ser colocado um papel absorvente para garantir que a superfície abaixo do filme não retenha a gota de água, alterando o real ângulo formado ao longo do tempo, evitando a interpretação errônea de que a gota pouco interagiu com a superfície do material. A evolução do perfil da gota deve ser gravada e a análise de imagem utilizada para determinar a evolução do ângulo de contato da gota ao longo do tempo[53].

Entretanto, alguns equipamentos já possuem dispositivos para que a amostra fique fixada durante a análise, não sendo necessário os passos propostos na etapa anterior.

Análises Térmicas:

Análises térmicas são um conjunto de técnicas que permitem medir as mudanças de uma propriedade física em função da temperatura.

DSC:

A calorimetria diferencial de varredura (DSC) é a técnica na qual se mede a diferença de energia fornecida à amostra em relação a um material de referência, enquanto ambos são submetidos a uma programação controlada de temperatura. A medida de temperatura é feita por meio de termopares fixados à base do suporte da amostra e da referência. Alterações de temperatura da amostra são devidas a variações de entalpia endotérmicas ou exotérmicas, decorrentes de transições físicas ou de reações químicas (Tabela 2). A área contida sob o pico é representativa da variação de entalpia (ΔH) sofrida pela amostra. As variações de entalpia são chamadas transições de primeira ordem (fusão, cristalização, vaporização, solidificação e adsorção). As transições térmicas ditas de segunda ordem são acompanhadas de variação da capacidade calorífica da amostra, juntamente com variações dimensionais e viscoelásticas (como, por exemplo, a transição vítrea Tg), mas não apresentam variações de entalpia, não gerando picos nas curvas de DSC e sim, alterações na linha de base (Tabela 2)[41, 40].

Tabela 2. Transições relacionadas aos processos físicos e químicos (DSC)[40].

	Processos	**Transição** **Endotérmica**	 **Exotérmica**
Transições físicas	Absorção	x	
	Adsorção		x
	Cristalização		x
	Dessorção	x	
	Fusão	x	
	Sublimação	x	
	Transição cristalina	x	
	Transição líquido-cristalina	x	
	Transição de ponto de Currie	x	
	Transição de capacidade calorífica	Alteração de linha de base	
	Transição vítrea	Alteração de linha de base	
	Vaporização	x	
Reações químicas	Combustão		x
	Decomposição	x	x
	Degradação oxidativa		x
	Desidratação	x	
	Oxidação em atmosfera gasosa		x
	Polimerização		x
	Pré-cura		x
	Reação catalítica		x
	Reação no estado sólido	x	x
	Reação redox	x	x
	Redução em atmosfera gasosa		x
	Sorção química		x

Embora sejam comuns duas varreduras para a análise de DSC, para filmes biodegradáveis obtidos de amido, ao final da primeira varredura, o amido presente no material se expande, impossibilitando uma segunda varredura. Também quando se utilizam plastificantes como a sacarose ou outro açúcar, após o final da primeira varredura já ocorreu uma acentuada deterioração deste componente, inviabilizando uma segunda varredura.

DTG:

Na Termogravimetria Derivativa (DTG), mede-se a variação de massa em função do tempo[31].

Se utilizada em conjunto com a análise de DSC, permite observar se houve deterioração significativa da massa do material após a primeira varredura, para o analista avaliar se deve ou não ser feita uma segunda varredura. Também possibilita acompanhar a degradação no material durante picos do DSC, o que ajuda a explicar o que está ocorrendo com o material.

No caso de embalagens biodegradáveis, que muitas vezes são elaboradas a partir de compostos mais sensíveis à temperatura, a termogravimetria mostra-se uma ferramente bastante poderosa na avaliação térmica geral do material.

DTMA:

A análise termodinâmico-mecânica (DTMA) é capaz de fornecer informações a respeito do comportamento viscoelástico do sistema, desmembrado o módulo em duas componentes: a contribuição elástica e a viscosa. Neste tipo de experimento, a tensão ou a deformação é uma função oscilatória, normalmente senoidal. Supondo que o equipamento aplique uma deformação senoidal e meça a tensão resposta como um função da variação de temperatura ou da freqüência. Essa tensão resposta irá depender do comportamento do material. Dividindo-se cada componente da tensão resposta pela deformação senoidal aplicada, obtém-se duas componentes: o módulo oriundo da componente da tensão resposta em fase com a deformação (E') e o módulo oriundo da componente de resposta 90° fora de fase com a deformação (E''). E' é denominado módulo de armazenamento e E'', módulo de perda. Assim, um material viscoelásticos será caracterizado por dois valores de módulo, o E' e o E''. A razão entre estes dois módulos define uma grandeza denominada tangente de perda (tan δ), muito útil na caracterização de sistemas poliméricos. Materiais mais rígidos irão apresentar valores de tan δ menores, enquanto os mais flexíveis apresentarão valores maiores [39].

De um modo resumido, as transições que ocorrem nas moléculas podem ser divididas em transições que ocorrem na fase amorfa e transições que ocorrem na fase cristalina, conforme descritas na Tabela 3[40].

Tabela 3. Relaxações que podem ocorrer em polímeros durante análises de DTMA (LUCAS *et al.*, 2001).

	Tipo de transição	**Movimento molecular**
Transições na parte cristalina	Primária	Movimento das cadeias que fazem parte do cristal. Transição observada na fusão.
	Secundária	Movimento de cadeias dos cristais que constituem fases metaestáveis, com posterior recristalização e fusão definitiva.
Transições na parte amorfa	Primária	Movimento molecular de um segmento de cadeia, em fase amorfa, como um todo. Transição vítrea.
	Secundária	Movimento de grupos ou parte de grupos laterais da cadeia polimérica, quando a cadeia principal como um segmento contínuo não apresenta movimento.

Cor

A cor de um filme pode ser avaliada tanto por transmitância como por absorbância, dependendo do tipo do material. Uma forma de avaliar por transmitância é através da transmitância total, com ou sem brilho. Entre os vários tipos de equipamentos para avaliar a cor está o espectrofotômetro de cor COLOR QUEST II, marca Hunter Lab, com área de visão de uma polegada quadrada. Para leitura da cor utiliza-se o sistema CIELAB Ttan D65, ângulo 10°. Embora seja usual a prática de se fazer três leituras por amostra, também podem ser realizadas 4 medições em quadrantes diferentes do material. A leitura de cor segue o sistema Hunter, onde “L” (±) é a luminosidade, “b” varia de vermelho (+) a verde (-) e “a” varia de amarelo (+) a azul (-).

Se a cor for avaliada através de transmitância, pode-se também avaliar o *haze*. Um objeto observado através de um filme com *haze* elevado possui sua visibilidade e distinção de cores comprometidas porque a dispersão de luz transmitida provoca uma perda de contraste da imagem[51].

Pode-se também calcular a diferença total de cor em relação à amostra avaliada e um controle (ΔE %), que geralmente é o ar ou um outro filme com o qual deseja-se comparar a amostra, conforme indicado na equação a seguir.

$$\Delta E = \left[(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2\right]^{\frac{1}{2}}$$ **Equação 3**

A opacidade dos filmes também pode ser determinada, embora para tal deva ser utilizada luz refletida.

Para a avaliação dos parâmetros de cor também é recomendo que todas as amostras sejam avaliadas com a mesma data de fabricação para que possíveis degradações do material não interfiram nos resultados.

Atividade de água

A atividade de água de um produto está intrinsecamente relacionada a sua vida de prateleira. Produtos com atividade de água mais baixa geralmente estão menos sujeitos à degradação por microorganismos e por reações químicas[13, 43].

Para a determinação da atividade de água os métodos mais utilizados são através de isotermas de sorção e de equipamentos como o equipamento DECAGON modelo CX-2, marca AQUALAB. No caso da utilização de equipamentos recomenda-se a padronização do tamanho das amostras, o local de onde serão recortadas.

No caso dos filmes biodegradáveis, é importante também que todas as amostras sejam previamente acondicionadas em uma condição de temperatura e umidade constantes.

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.

Para determinar a solubilidade de materiais biodegradáveis, uma das metodologias mais utilizadas é a proposta por Gontard, Guilbert & Cuq (1992)[28]. A solubilidade em água é avaliada pela porcentagem de peso seco não solubilizado, após a imersão da amostra (discos de 2cm de diâmetro) em água por 24 horas. Pela diferença entre o peso seco inicial da amostra e peso seco após a etapa de solubilização, calcula-se a porção solúvel da amostra. Embora a metodologia original sugira a “agitação esporádica da amostra”, Veiga-Santos (2004)[55] sugeriram a padronização da agitação utilizando-se agitação constante (100 rpm) e temperatura controlada (30°C), através da utilização de shaker.

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CAPÍTULO 2

Mechanical properties, hydrophilicity and water activity of starch-gum films: Effect of additives and deacetylated xanthan gum. Part 1.

Mechanical properties, hydrophilicity and water activity of starch-gum films: Effect of additives and deacetylated xanthan gum. Part 1.

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Abstract

The effect of deacetylated xanthan gum, additives (sucrose, soybean oil, sodium phosphate and propylene glycol) and pH modifications on mechanical properties, hydrophilicity and water activity of cassava starch-xanthan gum films has been studied. Sucrose addition resulted in the highest effect observed on cassava starch films elongation at break. The deacetylated xanthan gum had higher effect on elongation at break when comparing to the acetylated gum, although both gums presented an inferior effect in relation to the obtained with sucrose. However, lower tensile strength resistance values were observed when adding sucrose, propylene glycol and soybean oil. Increased water activity was observed for films added with sucrose, thus, increasing the material biodegradation. Sucrose and deacetylated xanthan gum addition resulted in a slight hydrophilicity increase.

Keywords: Biofilms, Mechanical properties, Water activity, Hydrophilicity.

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1. Introduction

Starch is considered one of the most promising natural polymers for packaging application (Guilbert, Gontard & Gossis, 1996 and Krochta & Mulder-Johnston, 1997) because of their low cost, renewability and biodegradability

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(Avérous, Fringant & Moro, 2001). Unfortunately, there are some strong limitations for developing starch-based products, due to its poor mechanical properties and high moisture sensitivity (Martin, Schwach, Avérous. & Couturier, 2001). To overcome these weaknesses, association with additives (Arvanitoyannis & Biliaderis, 1998; Coupland, Shaw, Monahan, O'Riordan & O'Sullivan 2000, and Garcia, Martino & Zaritzki, 2000) and process parameters modifications (Gontard, Guilbert & Cuq, 1992) are recommended.

Hydrocolloid increases starch systems viscosity capacity (Baird & Pettitt, 1991) and decreases retrogradation rate (Yoshimura, Takaya & Nishinari, 1999). Starch and hydrocolloids (such as xanthan gum) simultaneous usage in films can modify the material mechanical resistance. Xanthan gum is the most important commercial microbial hydrocolloid and exhibits synergism interaction with starch (Katzbauer, 1997). Is largely used as thickening, stabilizing (Katzbauer, 1997) and structuring agent in different technology areas (Capron, Brigand & Muller, 1997). Its primary structure consists of a linear β-1,4 linked D-glucose chain substituted on every second glucose residues by charged trisaccharide side chain containing a glucuronic acid residue between two mannose residues. The inner mannose residue is normally acetylated at C(6) (Pettitt, 1982), located on the periphery of the helix, preventing aggregation. Removal of these groups, allows the polymer chain to associate (Morris, Gothard, Hember, Manning & Robinson, 1996). The utilization of deacetylated xanthan gum as additive could, therefore, affect biofilms structure.

Chen & Nusinovitch (2000, 2001) have already introduced xanthan gum into wax-based coatings, however, xanthan gum application on hydrophilic films have not been investigated. Neither the potential of deacetylated xanthan gum has been explored as additive for biodegradable materials.

Sucrose has shown a higher plasticizing efficacy when compared with sorbitol and glycerol (Arvanitoyannis; Psomiadou & Nakayama, 1996). Thus, demonstrating that sucrose, which has a low coast when comparing to polyols, can also be a good alternative for increasing mechanical resistance of biodegradable films. Mechanical properties can also be improved by phosphate (Mezgheni, Vachon & Lacroix, 2000) and propylene glycol (Lacroix, Jobin, Mezgheni, Srour & Boileau, 1998) addition.

Moisture sensitivity of hydrophilic films may be reduced by addition of hydrophobic materials, such as soybean oil (Yang & Paulson, 2000; Garcia, Martino & Zaritzki, 2000).

With the aim to evaluate the simultaneous and isolate effect of xanthan gum (deacetylated or not), additives (sucrose, propylene glycol, sodium phosphate and soybean oil) and pH modifications on cassava starch films; tensile strength, elongation at break, contact angle measurement and water activity were investigated.

2. Material and Methods

2.1. Materials

Commercial cassava starch (Flor de Lotus, Brazil), acetylated xanthan gum-rodigel (Rhodia, Brazil), deacetylated xanthan gum (CP Kelco Brazil S.A.) and soybean oil (Cargill Agricola S.A., Brazil). Analytically pure sucrose, propylene glycol and monobasic sodium phosphate (Synth, Brazil).

2.2. Sample Preparation

Cassava starch (3-5% w/w) was blended with water and the additives: acetylated or deacetylated xanthan gum (0-1% w/w), sucrose (0-2% w/w), propylene glycol (0-1% w/w), sodium phosphate (0-0.2% w/w) and soybean oil (0-0.06% w/w) considering the total weight of the film forming suspension. pH was adjusted (4-8) with 50% citric acid solution or 5% sodium hydroxide solution, heated to 75°C with constant stirring and placed under ultrasonic bath. The films were prepared according to the casting technique, by dehydration under renewable circulated air (30°± 2°C) over Petri plastic dishes. Films containing 5% cassava starch (no additives or pH adjustment) served as the control. Samples were storage (23°C, 75% RH) for at least 4 days prior to testing.

2.3. Mechanical Properties

Films tensile strength and elongation at break were measured using an Instron Universal Testing Instrument (model 5500R) operated according to ASTM standard method D882-00 (ASTM, 2001). Tested filmstrips (8 x 2.5cm) were cut from each preconditioned (75% RH, 23°C) sample and mounted between the grips of the machine. The thickness of each specimen was measured in four points along its length with a flat and parallel surfaces Mitutoyo digital micrometer. Initial grip separation and crosshead speed were set to 50mm and 12.5 mm/min, respectively. The maximum load (kgf) and extension (mm) were recorded and used to calculate the tensile strength (kgf/cm^2) and elongation at break. Ten specimens were tested for each sample. The experimental design samples tensile strength (ΔT%) and elongation at break (ΔE%) increase or decrease size in relation to the control were calculated by equations 1 and 2, respectively.

$$\Delta T = \left[\left(\frac{sample\ tensile\ strength}{control\ tensile\ strength}\right)\times 100\right] - 100$$

Equation 1

$$\Delta E = \left[\left(\frac{sample\ elongation}{control\ elongation}\right)\times 100\right] - 100$$

Equation 2

2.4. Water Activity

Water activity measurements were performed with a DECAGON CX-2T, AQUALAB apparatus. Pure water (a_w=1.000 ± 0.003%) and KCl (a_w=0.843± 0.003 %) were used as standard for equipment calibration. Preconditioned (75% RH, 23°C) samples (4cm^2) were cut from the center of the films and evaluated in triplicate. The experimental design samples water activity (Δa_w%) increase or decrease size in relation to the control was calculated according to equation 3.

$$\Delta a_w = \left[\left(\frac{sample\ a_w}{control\ a_w}\right)\times 100\right] - 100$$

Equation 3

2.5. Contact Angle Measurement

The contact angle measurements were determined using a VCA Optima Equipment AST products, USA, to evaluate samples hydrophilic feature (Avérous & Fringant, 2001). The evolution of the water drop (3µL) shape on the surface of a preconditioned (75% RH, 23°C) 4cm^2 sample was recorded (in duplicate) to determine the initial contact angle and the material absorption kinetic (during 5 min) (Veiga, Alves, Sinézio, Scamparini & Cereda, 2003). The initial contact angle values just after deposition indicate the material hydrophilicity behavior. In order to illustrate the kinetic of absorption, the slope at the origin (º/min) was also calculated by linear regression from the points of the contact angle versus time (Avérous, Moro, Dole & Fringant, 2000).

2.6. Statistical analysis

Two blocks of a 2^{7-3} experimental factorial design were performed in order to select additives and process parameters that significativelly ($p<0.05$) influenced cassava starch films mechanical properties, hydrophilicity and water activity. The blocks were designed with three central points, totalizing 19 experiments per block, differing only for one variable (xanthan gum, deacetylated or not). The other variables were cassava starch, sucrose, soybean oil, sodium phosphate, propylene glycol and pH (Table 1).

Table 1.
Independent variables, coded and real values for the acetylated (X) and deacetylated (DX) gum factorial experiment blocks, considering the total weight of the film forming suspension.

Symbols	**Variables**		***Levels***		
	Block-X	**Block-DX**	***-1***	***0***	***+1***
A	Sodium phosphate (%)	Sodium phosphate (%)	*0.00*	*0.35*	*0.70*
B	pH	pH	*4.00*	*6.00*	*8.00*
C	Cassava starch (%)	Cassava starch (%)	*3.00*	*4.00*	*5.00*
D	Sucrose (%)	Sucrose (%)	*0.00*	*1.00*	*2.00*
E	Propylene Glycol (%)	Propylene Glycol (%)	*0.00*	*0.25*	*0.50*
F	Soybean oil (%)	Soybean oil (%)	*0.00*	*0.03*	*0.06*
G	Acetylated gum (%)	Deacetylated gum (%)	*0.00*	*0.05*	*0.10*

The independent variables significant ($p<0.05$) influence was evaluated by the Pareto chart of standardized effect, considering the ANOVA pure error. The experimental data were generated and analysed using the software Statistica for Windows, version 5.0.

The experimental designs demonstrated in Table 2 were used to estimate the main effects of independent variables.

Table 2.
2^{7-1} factorial design with coded values for the acetylated (X) and deacetylated (DX) xanthan gum factorial experiment blocks.

Assays		**Variables**						
Acetylated gum Block	**Deacetylated gum Block**	***A***	***B***	***C***	***D***	***E***	***F***	***G***
X-1	DX-1	*-1* (0.00)	*-1* (4.0)	*-1* (3.0)	*-1* (0.0)	*-1* (0.00)	*-1* (0.00)	*-1* (0.00)
X-2	DX-2	*+1* (0.70)	*-1* (4.0)	*-1* (3.0)	*-1* (0.0)	*+1* (0.50)	*-1* (0.00)	*+1* (0.10)
X-3	DX-3	*-1* (0.00)	*+1* (8.0)	*-1* (3.0)	*-1* (0.0)	*+1* (0.50)	*+1* (0.06)	*-1* (0.00)
X-4	DX-4	*+1* (0.70)	*+1* (8.0)	*-1* (3.0)	*-1* (0.0)	*-1* (0.00)	*+1* (0.06)	*+1* (0.10)
X-5	DX-5	*-1* (0.00)	*-1* (4.0)	*+1* (5.0)	*-1* (0.0)	*+1* (0.50)	*+1* (0.06)	*+1* (0.10)
X-6	DX-6	*+1* (0.70)	*-1* (4.0)	*+1* (5.0)	*-1* (0.0)	*-1* (0.00)	*+1* (0.06)	*-1* (0.00)
X-7	DX-7	*-1* (0.00)	*+1* (8.0)	*+1* (5.0)	*-1* (0.0)	*-1* (0.00)	*-1* (0.00)	*+1* (0.10)
X-8	DX-8	*+1* (0.70)	*+1* (8.0)	*+1* (5.0)	*-1* (0.0)	*+1* (0.50)	*-1* (0.00)	*-1* (0.00)
X-9	DX-9	*-1* (0.00)	*-1* (4.0)	*-1* (3.0)	*+1* (2.0)	*-1* (0.00)	*+1* (0.06)	*+1* (0.10)
X-10	DX-10	*+1* (0.70)	*-1* (4.0)	*-1* (3.0)	*+1* (2.0)	*+1* (0.50)	*+1* (0.06)	*-1* (0.00)
X-11	DX-11	*-1 (0.00)*	*+1* (8.0)	*-1* (3.0)	*+1* (2.0)	*+1* (0.50)	*-1* (0.00)	*+1* (0.10)
X-12	DX-12	*+1* (0.70)	*+1* (8.0)	*-1* (3.0)	*+1* (2.0)	*-1* (0.00)	*-1* (0.00)	*-1* (0.00)
X-13	DX-13	*-1* (0.00)	*-1* (4.0)	*+1* (5.0)	*+1* (2.0)	*+1* (0.50)	*-1* (0.00)	*-1* (0.00)
X-14	DX-14	*+1* (0.70)	*-1* (4.0)	*+1* (5.0)	*+1* (2.0)	*-1* (0.00)	*-1* (0.00)	*+1* (0.10)
X-15	DX-15	*-1* (0.00)	*+1* (8.0)	*+1* (5.0)	*+1* (2.0)	*-1* (0.00)	*+1* (0.06)	*-1* (0.00)
X-16	DX-16	*+1* (0.70)	*+1* (8.0)	*+1* (5.0)	*+1* (2.0)	*+1* (0.50)	*+1* (0.06)	*+1* (0.10)
X-17(c)*	DX-17(c)*	*0* (0.35)	*0* (6.0)	*0* (4.0)	*0* (1.0)	*0* (0.25)	*0* (0.03)	*0* (0.05)
X-18(c)*	DX-18(c)*	*0* (0.35)	*0* (6.0)	*0* (4.0)	*0* (1.0)	*0* (0.25)	*0* (0.03)	*0* (0.05)
X-19(c)*	DX-19(c)*	*0* (0.35)	*0* (6.0)	*0* (4.0)	*0* (1.0)	*0* (0.25)	*0* (0.03)	*0* (0.05)

(c): central points.*

3. Results and discussion

3.1. Mechanical Properties

It is well known that the selection of additives and process parameters is important since it can strongly affect film tensile strength and elongation at break. Therefore, few additives have been investigated to improve mechanical performance of cassava starch films.

The additives and pH tested in this study have affected cassava starch films tensile strength and elongation at break (Table 3 and Fig. 1 and 2, respectively).

Table 3.
Cassava starch films tensile strength (T), elongation at break (E) and increase or decrease size of tensile strength (ΔT) and elongation at break (ΔE) measurement results.

	Acetylated gum Block (X)				**Deacetylated gum Block (DX)**			
assays	**T** (kgf/cm^2)**	**ΔT (%)**	**E (%)**	**ΔE (%)**	**T** (kgf/cm^2)**	**ΔT (%)**	**E (%)**	**ΔE (%)**
control	379.96	na***	3.99	na***				
1	344.40	-9.36	3.82	-4.48	349.70	-7.96	3.90	-2.33
2	321.60	-15.36	4.65	16.42	300.80	-20.83	6.12	53.25
3	226.90	-40.28	3.88	-2.95	214.20	-43.63	3.80	-4.88
4	218.80	-42.41	3.22	-19.45	242.10	-36.28	5.26	31.77
5	248.20	-34.68	3.90	-2.43	199.00	-47.63	4.59	14.85
6	259.80	-31.62	3.98	-0.28	262.80	-30.83	4.08	2.15
7	354.50	-6.70	5.19	29.84	337.80	-11.10	4.47	11.94
8	259.90	-31.60	4.82	20.56	248.20	-34.68	4.88	22.28
9	58.10	-84.71	54.92	1275.09	57.40	-84.89	54.23	1257.66
10	26.30	-93.08	119.26	2886.03	20.60	-94.58	120.62	2920.08
11	35.90	-90.55	77.93	1851.05	28.30	-92.55	69.52	1640.69
12	47.70	-87.45	82.38	1962.47	51.10	-86.55	82.28	1960.02
13	40.20	-89.42	70.16	1656.69	45.00	-88.16	71.10	1680.17
14	77.70	-79.55	54.31	1259.89	86.00	-77.37	56.82	1322.73
15	54.70	-85.60	73.30	1735.35	59.90	-84.24	73.29	1734.88
16	31.10	-91.81	69.29	1634.88	25.20	-93.37	68.14	1606.16
17 (c)*	77.90	-79.50	32.74	719.75	49.30	-87.02	44.40	1011.74
18 (c)*	74.40	-80.42	32.19	706.01	57.80	-84.79	39.44	887.53
19 (c)*	62.80	-83.47	40.36	910.49	68.20	-82.05	46.59	1066.37
R^2****	0.889	na***	0.925	na***	0.870	na***	0.925	na***

(c)*: central points. T**: tensile strength as maximum resistance area. na***: not applicable. **R^2****: coefficient of determination.**

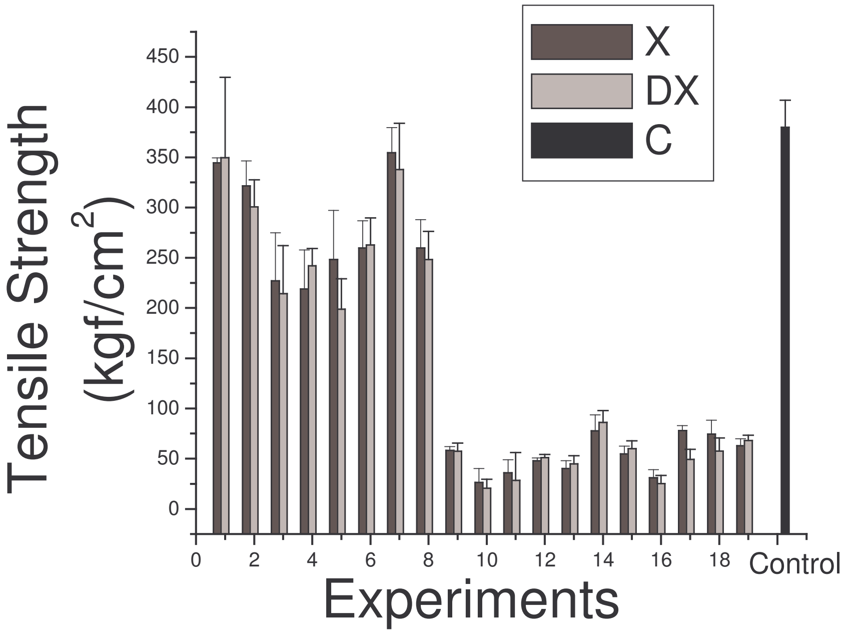

Fig. 1. Tensile strength measurements and standard deviation obtained for the control and acetylated (X) and deacetylated (DX) xanthan gum statistical experiments.

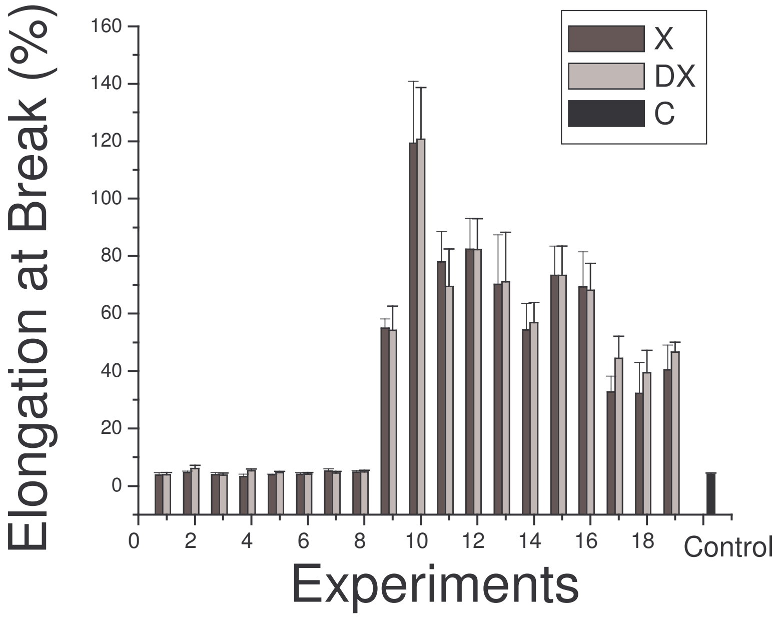

Fig. 2. Elongation at break measurements and standard deviation obtained for the control and acetylated (X) and deacetylated (DX) xanthan gum statistical experiments.

As can be clearly observed by Table 3 and visualized by Fig. 1 and 2, the experimental samples added with sucrose (X9 to X19 and DX9 to DX19) presented a very different behavior from the other films, independent of xanthan gum deacetylation. Results shown on Table 3 have also indicated that films with 2% sucrose addition (X9 to X16 and DX9 to DX16) presented higher elongation at break when comparing to films with 1% sucrose addition (X17 to X19 and DX17 to DX19). Therefore, indicating that increasing sucrose addition increases cassava starch film elongation at break. Sucrose addition can increase elongation at break up to 2900% when comparing to the control. The Pareto chart of standardized effect, confirmed that cassava starch films elongation at break is positively ($p<0.05$) affected by sucrose addition for both studied blocks.

The preferential hydration of sugars molecules (Stansell, 1995) could limit plasticizing effect of sucrose on starch films. However, at the concentration added in this study, sucrose have demonstrated efficacy as plasticizing agent. Arvanitoyannis, Psomiadou & Nakayama (1996) have also observed plasticizing effect for sucrose, mentioning an even more pronounced influence that observed when utilizing polyols. Plasticizer agents reduce intermolecular forces and increase polymer chains mobility, thereby improving films flexibility and extensibility (Coupland, Shaw, Monahan, O'Riordan & O'Sullivan, 2000).

However, when adding sucrose, the Pareto chart of standardized effect indicated also a significant ($p<0.05$) decrease in films tensile strength (90%) in relation to the control (Table 3). According to Coupland, Shaw, Monahan, O'Riordan & O'Sullivan (2000), an increase in plasticizer content can result in lower tensile strength resistance. Although the film forming sucrose concentration is low (< 2%), after the casting step, the final sucrose concentration increases significantly with the water removing, what could explain the lower tensile strength results observed for samples X9 to X19 and DX9 to DX19.

Although xanthan gum presented little influence on elongation at break measurements (Fig. 2 and Table 3), the Pareto chart of standardized effect, have indicated a significant ($p<0.05$) negative effect when using both gums, deacetylated or not. The xanthan gum effect on film elongation at break may be explained by a synergism interaction between the gum and the cassava starch (Katzbauer, 1997), preventing amylose-amylose interactions (Navarro, Martino & Zaritzky*,* 1997). The more unfolded network could present a weaker interaction forces, resulting in films with lower elongation at break performance. Allonce & Doublier (1991) and Annabel, Fitton, Harris, Phillips & Williams (1994), also observed weak gel network when adding gums to starch dispersion due to a binary hydrocolloid phase separation, which could also be an explanation to the lower elongation at break observed in this study.

The deacetylated xanthan gum effect can be observed comparing both blocks central point (X17 to X19 and DX17 to DX19) measurement results. Once the only difference between them is the xanthan gum deacetylation, observing their elongation at break measurement results (Table 3), it can be noticed an increase in relation to the control (ΔE) for the deacetylated gum (DX) block when comparing to the acetylated gum (X) block (~988% and ~778%, respectively). The removal of the acetylated groups allows the xanthan gum chain to associate, presenting increased viscoelastic properties (Morris, Gothard, Hember, Manning, & Robinson, 1996), which when interacting with the starch network, may result in higher elongation behavior compared to the starch-acetylated gum films.

The Pareto chart of standardized effect has indicated significant ($p<0.05$) negative influence of sucrose, soybean oil and propylene glycol on tensile strength for both blocks. The negative effect on tensile strength observed when adding propylene glycol can possibly be explained by its plasticizing character (Kim, Ko & Park, 2002). The great amount of plasticizer, caused by the simultaneous usage of propylene glycol and sucrose, could result in excessive interaction among the plasticizers and the film network (Arvanitoyannis, Psomiadou & Nakayama, 1996). Therefore, leading to a weak network, with low tensile strength resistance. The same reason can explain the soybean oil negative effect on tensile strength, which also can be considered a plasticizer agent (Garcia, Martino & Zaritzki, 2000). Another explanation could be due to the fact that lipids form inclusion compounds with amylose (Belitz & Grosh, 1999b), which could result in an opened network, lowering the films tensile strength resistance. Lower mechanical resistance when using lipid additives was also reported by Yang & Paulson (2000).

Although the decrease size of tensile strength (∆T) (Table 3) has indicated that all the additives and process parameters studied resulted in films with lower tensile strength resistance, the enormous increase size observed on elongation at break (∆E) when using sucrose (Table 3) justify the continuance of this study.

3.2. Water Activity

Perishable products shelf life extension consists in preventing its degradation by biochemical reactions and/or microbial growth. Such degradation mechanisms can generally be inhibited by low water activity (a_w) contents (Mathlouthi, 2001).

Samples have presented a_w content between 0.53 and 0.89 (Table 4), thus being considered intermediate moisture products, which are susceptible to microorganism growth and chemical reactions (Belitz & Grosch, 1999a).

Table 4
Water activity (a_w) measurement values, increase size of water activity in relation to the control (Δa_w%), initial contact angle (CA) and slope (°/min) for the acetylated (X) and deacetylated (DX) xanthan gum blocks.

	Acetylated gum Block (X)				**Deacetylated gum Block (DX**			
assays	**a_w**	**Δa_w**	**CA (°)**	**Slope**	**a_w**	**Δa_w**	**CA (°)**	**Slope**
control	0.507	*na***	70.10	-4.79				
1	0.576	*13.54*	45.90	-9.56	0.590	*16.37*	69.80	-8.20
2	0.722	*42.47*	71.60	-7.86	0.667	*31.56*	47.60	-8.67
3	0.886	*74.69*	71.20	-8.55	0.859	*69.43*	71.20	-12.82
4	0.614	*21.10*	75.80	-10.97	0.566	*11.64*	68.90	-11.09
5	0.621	*22.49*	73.30	-11.20	0.772	*52.27*	57.00	-11.66
6	0.599	*18.15*	70.70	-11.75	0.605	*19.33*	70.70	-11.75
7	0.632	*24.65*	65.80	-12.56	0.563	*11.05*	61.40	-9.43
8	0.535	*5.52*	76.10	-12.85	0.529	*4.34*	76.10	-12.85
9	0.703	*38.72*	77.70	-5.57	0.702	*38.46*	58.70	-4.40
10	0.751	*48.13*	57.70	-3.33	0.751	*48.13*	57.70	-3.33
11	0.721	*42.27*	76.70	-7.81	0.726	*43.20*	50.20	-2.52
12	0.759	*49.70*	80.10	-9.72	0.748	*47.53*	80.10	-5.72
13	0.806	*58.97*	64.00	-5.97	0.811	*59.96*	64.00	-5.97
14	0.745	*46.94*	38.20	-3.84	0.758	*49.51*	43.40	-3.47
15	0.739	*45.83*	61.30	-9.13	0.733	*44.58*	61.30	-5.13
16	0.748	*47.53*	64.40	-9.02	0.773	*52.47*	64.50	-2.20
17 (c)*	0.761	*50.10*	50.60	-7.11	0.708	*39.64*	60.10	-8.07
18 (c)*	0.742	*46.35*	70.00	-9.72	0.669	*31.95*	45.30	-8.69
19 (c)*	0.701	*38.26*	59.10	-9.00	0.749	*47.73*	58.30	-8.45
R^{2***}	0.82	*na***	0.29	0.99	0.94	*na***	0.31	0.80

(c)*: central points. *na***: not applicable. R^{2***}: coefficient of determination

Considering the biodegradable nature of the material and the soybean oil addition, microorganism growth and lipid oxidation may have the most concerning effect on material shelf life. The insaturation on soybean oil molecules combined with the films a_w contents (above 0.6%), indicates that the material is susceptible to a fast rate of lipid oxidation (Nawar, 1996; Belitz & Grosch, 1999a). Microbial growth can be expected, however molds growth is more likely to be observed. Optimum a_w range growth of most molds is between 0.80 and 0.87% (Fennema & Tannenbaum, 1996). Thus, in general, the sucrose content films (samples X9 to X19 and DX9 to DX19) are more susceptible for microbial, especially molds, growth during storage.

Only sucrose had significant ($p<0.05$) effect on films water activity for both studied blocks. Although sucrose is known as a water activity depressor (Mathlouthi, 2001), the opposite was observed in this study. Sucrose increased the material a_w (Table 4). A possible explanation is the plasticizing effect of sucrose on the film network structure (Mitchell, 1998), mainly related to the creation of highly mobile regions, which allow a even more pronounced water uptake (Cheryan, Gennadios, Woller & Chinachoti, 1995).

3.3. Contact Angle Measurement

The films hydrophilicity was quantitatively illustrated by measurements of the water droplet absorption kinetics given by the contact angle slope at the origin (º/min) (Table 4).

All samples presented a quickly absorption, probably due to the hydrophilic nature of the cassava starch film and additives (with exception of soybean oil) (Avérous, Moro, Dole & Fringant, 2000). Soybean oil, which has a hydrophobic character, did not influenced ($p<0.05$) water absorption during time. Such result can be explained by the low soybean oil concentration (< 0.06%) used in attempt to completely incorporate the lipid additive to the film network. Thus, indicating that soybean oil concentrations lower than 0.06% is not enough to affect cassava starch films hydrophilicity.

The Pareto chart of standardized effect has indicated that no additive or pH modifications affected ($p<0.05$) the material initial contact angle. However, the R^2 (coefficient of determination) for both analysed blocks were very low (0.29 and 0.31) to predict trustfully the main effects of the independent variables (Table 4). Thus, the results should be analysed with caution.

The Pareto chart of standardized effect has also indicated that, for the acetylated gum block (X), increasing sucrose concentration increases ($p<0.05$) the water absorption kinetic. For the deacetylated xanthan gum block (DX), water absorption kinetic increased ($p<0.05$) with sucrose and deacetylated xanthan gum addition.

The sucrose addition effect can be observed when comparing the water absorption behavior of samples added with sucrose (X9 to X19 and DX9 to DX19) with the other studied films (Table 4).

Plasticizer addition reduces chain-to-chain interactions (Sothornvit & Krochta, 2001). Such reducing interactions could result in opened structures, which may be an explanation for the hydrophilicity increase effect when using sucrose.

Deacetylated xanthan gum resulted on films with a slight higher water absorption behavior when comparing to the acetylated gum, as can be observed comparing the slope values of the acetylated block central point films (X17 to X19) to the deacetylated block samples (DX17 to DX19) (Table 4).

The associative effect of xanthan gum deacetylation (Morris, Gothard, Hember, Manning & Robinson; 1996) could increased the water absorption capacity of the xanthan gum molecule, which could explain the positive effect of the deacetylated xanthan gum on the water absorption kinetic of the film.

4. Conclusions

Additives and xanthan gum deacetylation influence on cassava starch films characteristics were clearly demonstrated. Sucrose addition improved cassava starch films elongation at break (up to 2900%). However, a decrease on elongation at break was observed with xanthan gum addition, with lower values obtained for the acetylated xanthan gum. Also cassava starch films tensile strength decreased (up to 94%) with sucrose, soybean oil and propylene glycol addition. Cassava starch film water activity was only affected by sucrose concentration, resulting in materials more susceptible for microbial growth during storage, especially molds. The cassava starch films hydrophilicity, evaluated by the water absorption kinetic, was increased by sucrose addition and deacetylated xanthan gum. Soybean oil has no effect on the material hydrophilicity, probably due to the lower concentration added. pH modifications did not affect the parameters analysed in this study. Sucrose addition presented the most pronounced effect on all characteristics evaluated. Considering the low cost of sucrose and the possibility of producing more feasible cassava starch films, such additive is an interesting approach to increase the performance of friendly packaging materials.

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CAPÍTULO 3

Microstructure and color of starch-gum films: Effect of additives and deacetylated xanthan gum. Part 2

Microstructure and color of starch-gum films: Effect of additives and deacetylated xanthan gum. Part 2

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Abstract

The influence of xanthan gum deacetylation, additives (sucrose, soybean oil, sodium phosphate and propylene glycol) and pH modifications on cassava starch films microstructure and color has been studied. X-Ray diffraction and microscopic analysis have demonstrated that sucrose addition influenced ($p<0.05$) film crystallinity during storage. Although not enough to prevent sucrose crystallization, deacetylated xanthan gum addition delayed the crystallization process. Comparing to the control, only cassava starch concentration and the additives sucrose and sodium phosphate affected samples total color difference (ΔE). However, all samples presented high lightness and low color values for "a" redness and "b" yellowness, indicating that, independent of the additives or pH modifications, the materials were almost colorless, with a high brilliancy.

Keywords: Biofilms, Microstructure, Color, Starch, Xanthan gum

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1. Introduction

Despite of the importance of the material biodegradablility for the environment, starch films present, in general, low mechanical resistance and water vapor barrier when comparing to traditional petroleum plastic films (Kester & Fennema, 1986 & Krochta & Mulder-Johnston, 1997). However, protective characteristics can be

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improved through additives incorporation (Arvanitoyannis & Biliaderis, 1998; Coupland, Shaw, Monahan, O'Riordan & O'Sullivan, 2000; Garcia, Martino & Zaritzki, 2000) and modifications of process parameters (Gontard, Guilbert & Cuq, 1992).

On the first paper of this series (Veiga-Santos, Oliveira, Cereda, Alves & Scamparini, 2004), the effect of deacetylation on cassava starch-xanthan gum films mechanical properties, hydrophilicity and water activity was investigated. The effect of additives (sucrose, soybean oil, propylene glycol and monobasic sodium phosphate) and pH modifications were also investigated for the same parameters.

Xanthan gum deacetylation and additives, especially sucrose, have affected mechanical properties and hydrophilicity of the final materials. However, little is known about the effect of such additives and pH modifications on the material microstructure.

Removal of the acetylated groups allows the xanthan gum chain to associate (Morris, Gothard, Hember, Manning & Robinson, 1996). The utilization of deacetylated xanthan gum as additive could, therefore, affect biofilms network, changing also the material structure.

Sucrose crystallization is a known and well-studied phenomenon (Jackson & Howling, 1995), however the sucrose effect on biofilms crystallinity has not been investigated. Propylene glycol, which is a humectant agent, may also affect film microstructure, having a protective effect against sucrose crystallization (Jackson & Howling, 1995).

Soybean oil utilization as additives has been reported (Yang & Paulson, 2000; Garcia, Martino & Zaritzki, 2000), although the homogeneity patterns of starch-soybean oil films have not been extensively investigated.

Ortophosphate group with acidic function, such as monosodium phosphate, forms an ester linkage with one of the hydroxyls in the starch chain, producing starch phosphate monoesters. The introduction of such ionizing groups may cause a little separation between the molecules (Ellinger, 1972), which may alter the microstructure of starch films added with sodium phosphate.

With the aim to evaluate the influence of xanthan gum (deacetylated or not), additives (sucrose, propylene glycol, soybean oil and sodium phosphate) and pH modifications on cassava starch-xanthan gum films microstructure; crystallinity, microscopy and color were investigated.

2. Material and Methods

2.1. Materials

Commercial cassava starch (Flor de Lotus, Brazil), acetylated xanthan gum-rodigel (Rhodia, Brazil), deacetylated xanthan gum (CP Kelco S.A., Brazil) and soybean oil (Cargill Agricola S.A., Brazil), and analytically pure sucrose, propylene glycol and monobasic sodium phosphate (Synth, Brazil).

2.2. Sample Preparation

Cassava starch (3-5% w/w) was blended with water and the additives: acetylated or deacetylated xanthan gum (0-1% w/w), sucrose (0-2% w/w), propylene glycol (0-1% w/w), sodium phosphate (0-0.2% w/w) and soybean oil (0-0.06% w/w) considering the total weight of the film forming suspension. pH was adjusted (4-8) with 50% citric acid solution or 5% sodium hydroxide solution, heated to 75°C with constant stirring and placed under ultrasonic bath. The films were prepared according to the casting technique, by dehydration under renewable circulated air (30°± 2°C) over Petri plastic dishes. Films containing 5% cassava starch (no additives or pH adjustment), served as the control. Samples were storage (23°C, 75%RH given by a NaCl saturated solution) for at least 4 days prior to testing.

2.3. Microstructure Analyses

A. Crystallinity

The relative crystallinity of the films was investigated with wide-angle X-ray diffraction (WAXS). Measurements were carried out using a DMAX-2200 Rigaku International Corporation $\theta/2\theta$ diffractometer, operating with voltage 40 kV and amperage 20mA, with CuKα radiation. Samples were fixed at an aluminum sample holder, and analysed from 5 to 60° (2θ), with angular step of 0.1° (2θ), and sampling interval time of 3 seconds. The films were analysed at each 15 days, during a storage period of 60 days (75% RH, 23°C), in duplicate. Crystallinity was also investigated (in the same conditions) for the raw materials cassava starch and sucrose in order to obtain crystallinity standards.

B. Light Microscopy

Samples surface was observed by LEICA-DMLP light microscope, with no further preparation, by transmitted light. Images 200x-magnification were collected with a CCD camera.

C. Scanning Electron Microscopy (SEM)

SEM digital images of the samples surfaces and fractures were obtained by Jeol JSM-5900LV scanning electron microscope. Cross section images were obtained by cryogenic fracture of the films, using liquid nitrogen. Samples were sputtered with a 16µg gold layer. Images were taken at 5-10KV, spotsize 28-30, 1000x-magnification.

2.4. Color

The color of preconditioned (75% RH, 23°C) samples was analysed in duplicate by total transmittance, using Color Quest II-Hunter lab equipment, CIELAB Ttran D65, lecture angle 10°, measuring area 2.54cm^2 (1.0 square inch). Four measurements taken at the different quadrants of each sample were averaged and expressed as Hunter system “L” (lightness), “a” (redness) and “b” (yellowness) values. Total color difference (ΔE) was evaluated as the size of color difference between the control and the experimental design samples, was calculated by the following equation (Francis, 1983; cited by Hong & Park, 2000):

$$\Delta E = \left[(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2\right]^{\frac{1}{2}}$$

2.5. Statistical analysis

Two blocks of a 2^{7-3} experimental factorial design were performed in order to select additives and pH modifications that significativelly ($p<0.05$) influenced cassava starch films microstructure and color. The blocks were designed with three central points, totalizing 19 experiments per block, differing only for one variable (xanthan gum, deacetylated or not). The other variables were cassava starch, sucrose, soybean oil, sodium phosphate, propylene glycol and pH (Table 1). The independent variables significant ($p<0.05$) influence was evaluated by the Pareto chart of standardized effect, considering the ANOVA pure error. The experimental data were generated and analysed using the software Statistica for Windows, version 5.0.

Table 1.
Independent variables coded and real values for the acetylated (X) and deacetylated (DX) gum factorial experiment blocks.

Symbol	**Variables**		***Levels***		
	Block-X	**Block-DX**	***-1***	***0***	***+1***
A	Sodium phosphate (%)	Sodium phosphate (%)	*0.00*	*0.35*	*0.70*
B	pH	pH	*4.00*	*6.00*	*8.00*
C	Cassava starch (%)	Cassava starch (%)	*3.00*	*4.00*	*5.00*
D	Sucrose (%)	Sucrose (%)	*0.00*	*1.00*	*2.00*
E	Propylene Glycol (%)	Propylene Glycol (%)	*0.00*	*0.25*	*0.50*
F	Soybean oil (%)	Soybean oil (%)	*0.00*	*0.03*	*0.06*
G	Acetylated gum (%)	Deacetylated gum (%)	*0.00*	*0.05*	*0.10*

The experimental designs demonstrated in Table 2 were used to estimate the main effects of independent variables.

Table 2.
2^{7-1} factorial design with coded values for the acetylated (X) and deacetylated (DX) xanthan gum factorial experiment blocks.

Assays		**Variables**						
Block X	**Block DX**	***A***	***B***	***C***	***D***	***E***	***F***	***G***
X-1	DX-1	*-1* (0.00)	*-1* (4.0)	*-1* (3.0)	*-1* (0.0)	*-1* (0.00)	*-1* (0.00)	*-1* (0.00)
X-2	DX-2	*+1* (0.70)	*-1* (4.0)	*-1* (3.0)	*-1* (0.0)	*+1* (0.50)	*-1* (0.00)	*+1* (0.10)
X-3	DX-3	*-1* (0.00)	*+1* (8.0)	*-1* (3.0)	*-1* (0.0)	*+1* (0.50)	*+1* (0.06)	*-1* (0.00)
X-4	DX-4	*+1* (0.70)	*+1* (8.0)	*-1* (3.0)	*-1* (0.0)	*-1* (0.00)	*+1* (0.06)	*+1* (0.10)
X-5	DX-5	*-1* (0.00)	*-1* (4.0)	*+1* (5.0)	*-1* (0.0)	*+1* (0.50)	*+1* (0.06)	*+1* (0.10)
X-6	DX-6	*+1* (0.70)	*-1* (4.0)	*+1* (5.0)	*-1* (0.0)	*-1* (0.00)	*+1* (0.06)	*-1* (0.00)
X-7	DX-7	*-1* (0.00)	*+1* (8.0)	*+1* (5.0)	*-1* (0.0)	*-1* (0.00)	*-1* (0.00)	*+1* (0.10)
X-8	DX-8	*+1* (0.70)	*+1* (8.0)	*+1* (5.0)	*-1* (0.0)	*+1* (0.50)	*-1* (0.00)	*-1* (0.00)
X-9	DX-9	*-1* (0.00)	*-1* (4.0)	*-1* (3.0)	*+1* (2.0)	*-1* (0.00)	*+1* (0.06)	*+1* (0.10)
X-10	DX-10	*+1* (0.70)	*-1* (4.0)	*-1* (3.0)	*+1* (2.0)	*+1* (0.50)	*+1* (0.06)	*-1* (0.00)

X-11	DX-11	*-1 (0.00)*	*+1* (8.0)	*-1* (3.0)	*+1* (2.0)	*+1* (0.50)	*-1* (0.00)	*+1* (0.10)
X-12	DX-12	*+1* (0.70)	*+1* (8.0)	*-1* (3.0)	*+1* (2.0)	*-1* (0.00)	*-1* (0.00)	*-1* (0.00)
X-13	DX-13	*-1* (0.00)	*-1* (4.0)	*+1* (5.0)	*+1* (2.0)	*+1* (0.50)	*-1* (0.00)	*-1* (0.00)
X-14	DX-14	*+1* (0.70)	*-1* (4.0)	*+1* (5.0)	*+1* (2.0)	*-1* (0.00)	*-1* (0.00)	*+1* (0.10)
X-15	DX-15	*-1* (0.00)	*+1* (8.0)	*+1* (5.0)	*+1* (2.0)	*-1* (0.00)	*+1* (0.06)	*-1* (0.00)
X-16	DX-16	*+1* (0.70)	*+1* (8.0)	*+1* (5.0)	*+1* (2.0)	*+1* (0.50)	*+1* (0.06)	*+1* (0.10)
X-17(c)*	DX-17(c)*	*0* (0.35)	*0* (6.0)	*0* (4.0)	*0* (1.0)	*0* (0.25)	*0* (0.03)	*0* (0.05)
X-18(c)*	DX-18(c)*	*0* (0.35)	*0* (6.0)	*0* (4.0)	*0* (1.0)	*0* (0.25)	*0* (0.03)	*0* (0.05)
X-19(c)*	DX-19(c)*	*0* (0.35)	*0* (6.0)	*0* (4.0)	*0* (1.0)	*0* (0.25)	*0* (0.03)	*0* (0.05)

(c): central points.*

3. Results and discussion

3.1. Microstructure analyses

Crystallinity

WAXS crystallinity diffractograms, collected during 60 days of storage, demonstrated that at the first day of storage, all samples from both blocks, presented an amorphous behavior (Fig. 1).

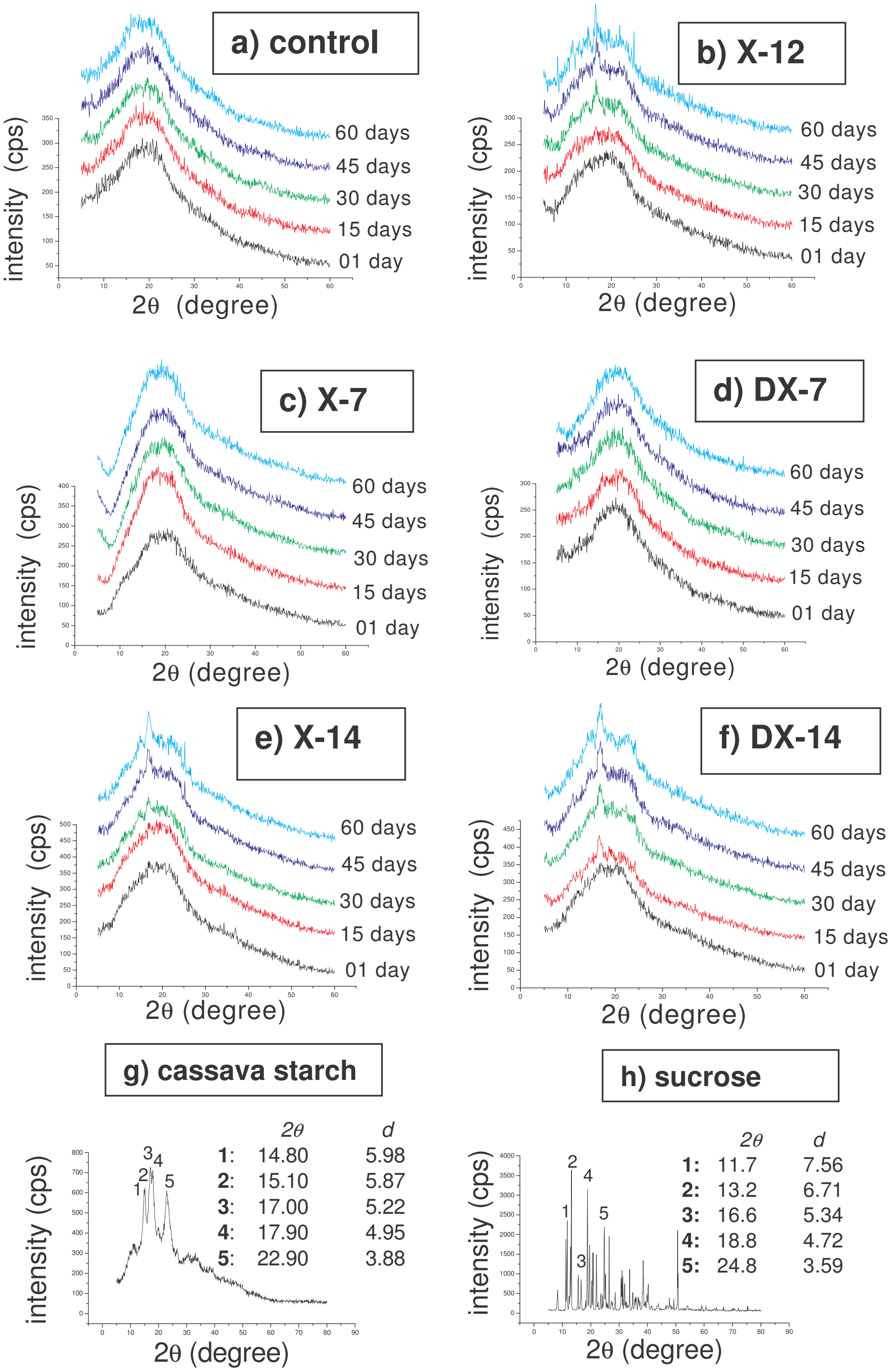

Fig. 1. WAXS crystallinity diffractograms of samples amorphous (a) control, (c) X-7, (d) DX-7 and crystalline (b) X-12, (e) X-14, (f) DX-14 behavior during 60 days of storage. Standards WAXS crystallinity diffractograms of (g) native cassava starch and (h) sucrose.

The native cassava starch contains both crystalline and noncrystalline regions in alternating layers (BeMiller & Wistler, 1996). It is considered to have a semi-crystalline structure, which is characterized by X-ray diffractograms of crystalline peaks (crystallites) superposed to an amorphous halo (Blennow, Bay-Smidt, Olsen & Moller, 2000), as can be observed in Fig. 1g. However, no semi-crystalline behavior was observed for the cassava starch films at the first day of storage (Fig. 1a, 1b, 1c, 1d, 1e). Although cassava starch is the main component on the studied films, the amorphous behavior observed for all films at the first day of storage can be explained by the crystallites instability near the jellification temperature. During jellification, the ordered crystalline regions undergo melting (Zobel & Stephen, 1995), which according to Jenkins & Donald (1998), could decrease the crystallization ratio until zero, resulting in an amorphous structure. Also the low dehydratation temperature utilized in this study (30°C) may be responsible for the amorphous behavior. According to Rindlav, Hulleman & Gatenholm (1997), dehydratation temperatures bellow 50°C result in starch films with amorphous structures.

For both (acetylated-X and deacetylated-DX gum) blocks, the films formulated without sucrose addition presented an amorphous behavior during storage. However, when sucrose was added to the films, the cassava starch films developed a crystalline structure during storage (Fig. 1b, 1e and 1f).

The crystalline samples behavior is represented by samples X-12 (added with sodium phosphate and sucrose, pH 8), X-14 (added with sodium phosphate, sucrose and acetylated xanthan gum, pH 4) and DX-14 (added with sodium phosphate, sucrose and deacetylated xanthan gum, pH 4). The crystallization behavior observed during storage of samples X-12, X-14 and DX-14 (Fig. 1b, 1e and 1f, respectively) was observed for all other samples containing sucrose, independent of additives or pH modifications. These results indicate that probable sucrose addition is the responsible for film crystallization during storage.

Such theory is reinforced when comparing the X-ray diffractograms of crystalline samples with sucrose standard (Fig. 1h). The 2θ angle and *d*-spacing (distance between lattice planes) of the crystalline peaks, observed on the cassava starch-sucrose films, matches with sucrose standard peaks, as can be observed in Table 3 and Fig. 1h.

Table 3. 2θ angle and *d*-spacing (*d*) matches for main peaks presented by crystalline samples (X-9 to X-19 and DX-9 to DX-19) WAXS diffractograms for the acetylated (X) and deacetylated (DX) xanthan gum blocks.

Sample / *[sucrose]*	**2θ**	***d* (Å)**	**2θ**	***d* (Å)**	**2θ**	***d* (Å)**	**2θ**	***d* (Å)**	**dc****
Sucrose Standard/ *99.7%*	11.7	*(7.56)*	16.6	*(5.34)*	18.8	*(4.72)*	24.8	*(3.59)*	*na****
Acetylated Block X									
X-9 / *2.0%*	11.6	*(7.62)*	16.7	*(5.31)*	18.8	*(4.72)*	25.2	*(3.53)*	30
X-10 / *2.0%*	11.6	*(7.62)*	16.7	*(5.31)*	18.8	*(4.72)*	24.8	*(3.59)*	15
X-11 / *2.0%*	11.6	*(7.62)*	16.7	*(5.31)*	18.8	*(4.72)*	25.2	*(3.53)*	30
X-12 / *2.0%*	----	----	----	----	18.8	*(4.72)*	24.7	*(3.60)*	30
X-13 / *2.0%*	----	----	16.7	*(5.31)*	----	----	----	----	45
X-14 / *2.0%*	----	----	16,7	*(5.31)*	----	----	25.2	*(3.53)*	30
X-15 / *2.0%*	----	----	16.6	*(5.34)*	----	----	----	----	30
X-16 / *2.0%*	----	----	16.7	*(5.31)*	18.8	*(4.72)*	----	----	45
X-17(c)* / *1.0%*	11.6	*(7.62)*	16.7	*(5.31)*	----	----	----	----	30
X-18(c)* / *1.0%*	----	----			18.8	*(4.72)*	----	----	30
X-19(c)* / *1.0%*	----	----	16.7	*(5.31)*	----	----	----	----	30
Deacetylated Block DX									
DX-9 / *2.0%*	11.5	*(7.69)*	16.7	*(5.31)*	18.7	*(4.75)*	24.6	*(3.62)*	15
DX-10 / *2.0%*	11.6	*(7.62)*	16.7	*(5.31)*	18.8	*(4.72)*	----	----	15
DX-11 / *2.0%*	----	----	16.7	*(5.37)*	----	----	25.0	*(3.53)*	15
DX-12 / *2.0%*	11.6	*(7.62)*	----	----	18.8	*(4.72)*	24.8	*(3.59)*	30
DX-13 / *2.0%*	----	----	16.7	*(5.31)*	----	----	----	----	45
DX-14 / *2.0%*	----	----	16.7	*(5.31)*	----	----	----	----	15
DX-15 / *2.0%*	----	----	16.7	*(5.31)*	----	----	----	----	30
DX-16 / *2.0%*	----	----	16.7	*(5.30)*	18.8	*(4.72)*	----	----	15
DX-17(c)* / *1.0%*	----	----	16.6	*(5.34)*	----	----	----	----	45
DX-18(c)* / *1.0%*	----	----	16.7	*(5.31)*	----	----	----	----	45
DX-19(c)* / *1.0%*	----	----	16.7	*(5.31)*	----	----	----	----	45

(c)*: central points. dc**: days that samples remained amorphous. *na****: not applicable. [sucrose]: sucrose concentration % (w/w film forming suspension).

Sucrose crystallization is a known phenomenon that starts with a crystallinity nucleus (Jackson & Howling, 1995). When in supersaturated conditions, spontaneous nucleation can occur. Nucleation and crystal formation could arise from rearrangement of randomly bound sugar molecules (Stansell, 1995).

Statistical Pareto analyses were performed to evaluate the additives and pH influence on the time required for crystallization beginning. For that matter, it was considered the total of days that the material remained amorphous (Table 3). In order to present values for the statistical calculations, for the films that did not present crystallinity during storage, the days that the material remained amorphous were considered the total storage period (60 days) evaluated in this study. The Pareto chart of standardized effect confirmed that sucrose positively affected ($p<0.05$) the crystallinity during storage of the acetylated (R^2: 0.92) and deacetylated (R^2: 0.99) xanthan gum blocks.

The crystallinity behavior of samples X-14 and DX-14, added only with sucrose and xanthan gum (acetylated-X and deacetylated-DX) (Fig. 1e and 1f, respectively) indicates that xanthan gum (deacetylated or not) did not prevent the sucrose crystallization. However, comparing the central point films of both blocks (X-17 to X-19 and DX-17 to DX-19) that differs only by the type of xanthan gum addition (acetylated or deacetylated), it can be observed that the deacetylated xanthan gum block (DX) presented a slight increase on the days that the samples remained amorphous. Such results indicate a possible protective effect retarding sucrose crystallization. The Pareto chart of standardized effect has indicated significant influence ($p<0.05$) on films crystallinity when adding deacetylated xanthan gum ($R^2=0.99$).

The removal of the acetylated groups allows the xanthan gum chain to associate (Morris, Gothard, Hember, Manning, & Robinson, 1996). Such association could result in a closer structure, which could reduce the sucrose crystals mobility, retarding the crystallization process (Stansell, 1995).

Scanning Electron Microscopy

Samples surface and cross section SEM micrographs were recorded at 5 and 60 days (± 4) of storage in order to examine the material surface characteristics during storage.

At the first week storage period, all samples presented a compact and smooth structure, for both surface and cross section, as exemplified by the control and samples X-14 and DX-14 (added only with sucrose and xanthan gum: acetylated-X and deacetylated-DX) (Fig. 2).

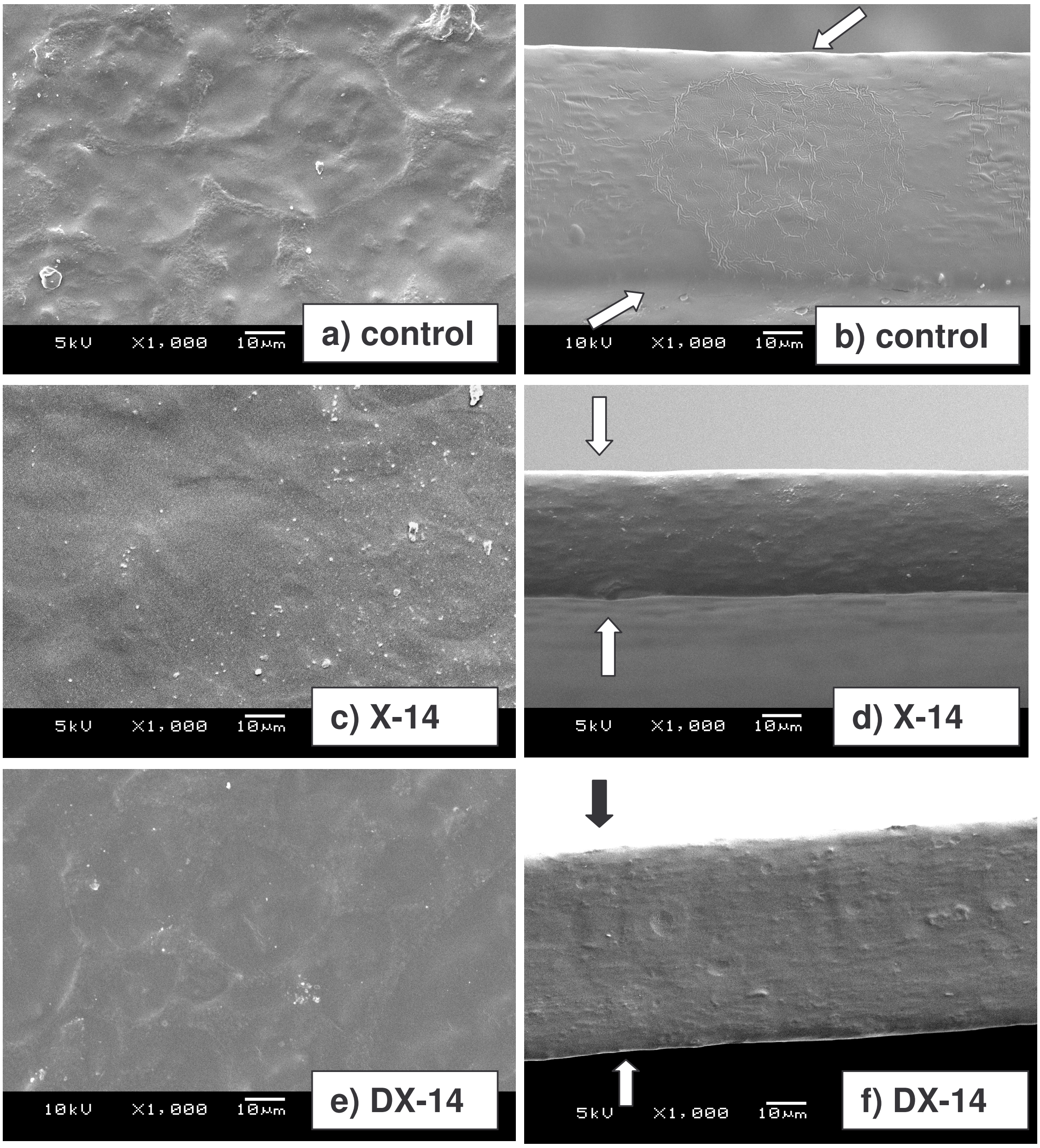

Fig. 2. Scanning electron micrographs of samples surfaces (a) control, (c) X-14, (e) DX-14 and cross sections (b) control, (d) X-14, (f) DX-14, at the first week of storage.

However, after 60 days of storage, samples containing sucrose as additive, presented a visible crystallinity, as exemplified by samples X-14 and DX-14 (added only with sucrose and xanthan gum: acetylated-X and deacetylated-DX) surfaces and cross-sections SEM micrographs (Fig. 3).

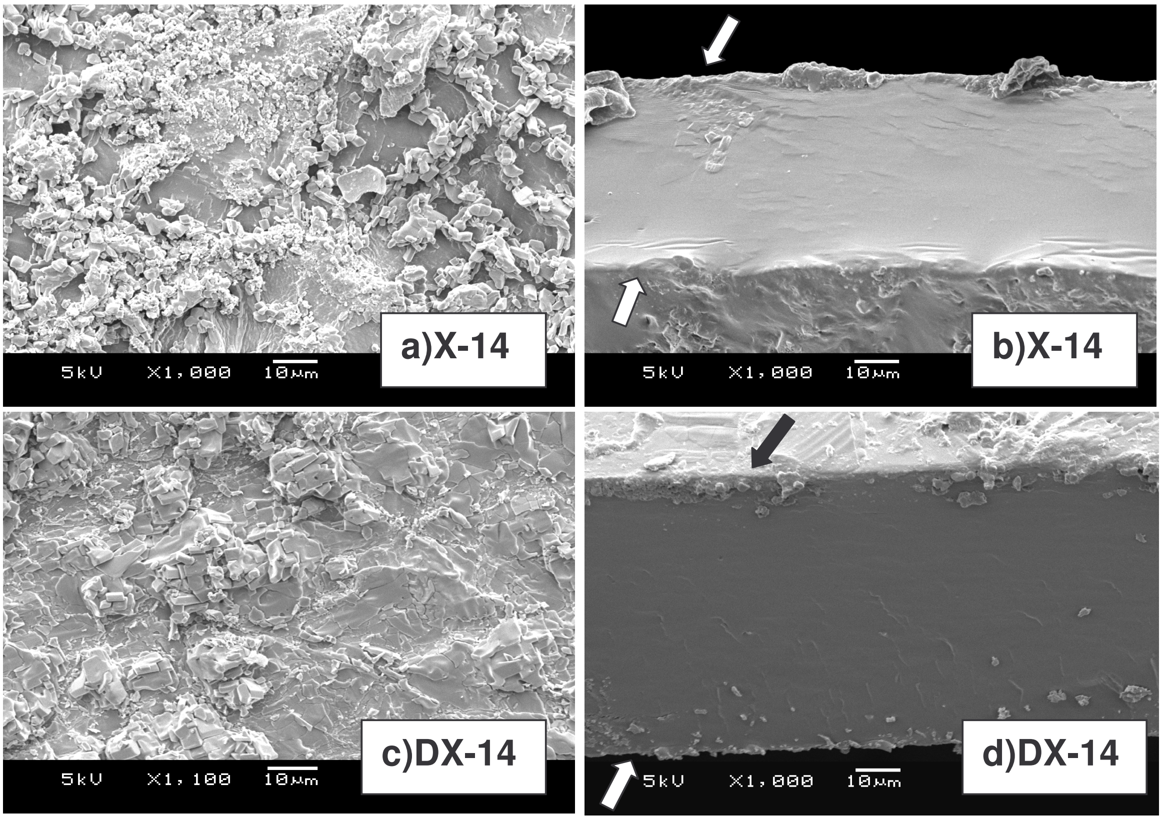

Fig. 3. Scanning electron micrographs of samples surfaces (a) x-14, (c) DX-14 and cross sections (b) X-14 and (d) DX-14, after 60 days of storage.

The crystal growth observed in Fig. 3 micrographs is in agreement with the crystalline behavior observed on X-ray diffractograms, confirming the crystalline behavior of the cassava starch films added with sucrose.

Cassava starch-sucrose cross-section micrographs (Fig. 3b and 3d) demonstrated also that the crystallinity began at the surface and not at the material interior. Such result indicates that the crystallization was not due to insoluble residual sucrose crystals.

Optical Microscopy

Although not often utilized for analyzing biodegradable structure, light microscopy demonstrated to be a very important tool in characterizing the component interaction on the samples, as exemplified by the central point samples

X-19, DX-19 (containing intermediate concentrations of all additives, pH 6.0) and the control, at 5 (5d) and 60 (60d) days (± 4) of storage (Fig. 4).

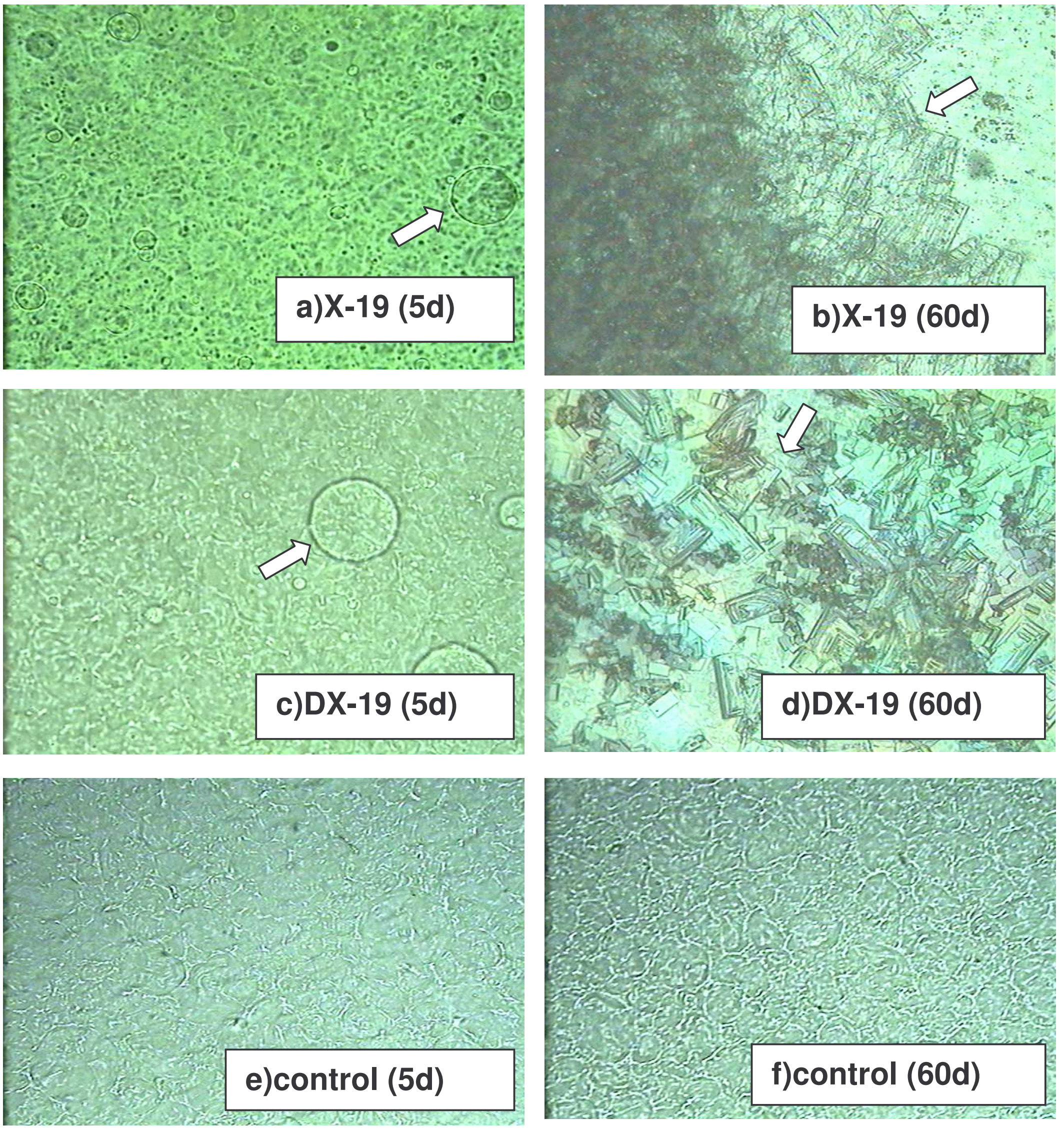

Fig. 4. Light optical micrographs of samples at 5 days of storage: a) X-14, c) DX-14, e) control, and at 60 days of storage: b) X-14, d) DX-14, f) control.

Light micrographs have demonstrated lack of homogeneity on films added with soybean oil, showing dispersed oil drops along the material structure (Fig. 4a, 4c). Such phenomenon was not possible to observe by SEM micrographs, indicating that light microscopy should be used in combination with SEM for characterizing the biofilms structure.

Fanta, Felker, Eskins & Baker (1999) also observed soybean oil separation for starch films produced by jet cooking technique. The lipophilic fraction was comprised of oil droplets surrounded by thin films of starch at the oil–water interface.

The heterogeneity observed indicates that although added in low concentration (0.06%), considering the sample preparation utilized in this study, the cassava starch film network structure was not able to embody the soybean oil, even in the presence of another hydrocolloid, as xanthan gum (deacetylated or not).

The material crystallization during storage was also clearly observed by light micrographs images (Fig 4b, 4d). The crystallinity results are in agreement with SEM images and X-ray diffractograms, demonstrating thus, that this analyse is a feasible, fast and low cost tool for observing sucrose content films crystallinity during storage.

3.2. Color

Samples color was expressed as Hunter system "L" (lightness), "a" (redness) and "b" (yellowness) values, as shown in Table 4.

Table 4.
Samples Hunter color parameters “L”, “a”, “b” and the total color difference between samples and the control (ΔE).

	Acetylated xanthan gum (X)				**Deacetylated xanthan gum (X)**			
assays	**Color “L”**	**Color “a”**	**Color “b”**	**ΔE (%)**	**Color “L”**	**Color “a”**	**Color “b”**	**ΔE (%)**
control	98.10	0.05	2.47	*na***				
1	98.36	0.06	2.55	0.32	98.21	0.07	2.48	0.16
2	98.85	-0.01	2.20	0.85	98.78	0.02	2.27	0.76
3	98.42	0.07	3.03	0.67	98.54	0.05	3.00	0.72
4	98.83	0.10	2.96	0.92	98.48	0.27	3.81	1.24
5	98.69	0.07	2.69	0.68	98.56	0.07	2.69	0.56
6	98.40	0.09	2.91	0.56	98.46	0.08	2.89	0.59
7	98.55	0.07	2.93	0.68	98.62	0.06	2.90	0.71
8	98.45	0.01	2.78	0.51	98.43	0.03	2.81	0.51
9	98.71	0.01	2.24	0.70	98.62	-0.01	2.22	0.63
10	98.39	0.11	3.13	0.74	98.46	0.08	3.05	0.71
11	98.72	-0.05	2.30	0.70	98.67	-0.01	2.11	0.72
12	98.30	0.00	2.45	0.26	98.45	0.02	2.49	0.40
13	98.55	0.02	2.46	0.50	98.46	0.03	2.44	0.41
14	98.52	-0.01	2.34	0.49	98.31	0.11	3.33	0.90
15	98.46	0.05	3.22	0.85	98.53	0.05	3.22	0.89
16	98.28	0.06	3.38	0.94	98.28	0.06	3.38	0.94
17 (c)*	98.46	0.21	3.49	1.11	98.43	0.22	3.67	1.27
18 (c)*	98.53	0.20	3.43	1.08	98.67	0.25	3.72	1.41
19 (c)*	98.52	0.19	3.41	1.06	98.70	0.30	3.91	1.60
R^2	0.68	0.28	0.60	0.40	0.74	0.21	0.58	0.50

(c): central points. na**: not applicable.* R^2****: coefficient of determination.*

For the acetylated xanthan gum block (X), the Pareto chart of standardized effect has indicated that no additives or pH modifications (R^2=0.68) have affected ($p<0.05$) the samples lightness (“L”). However, increasing sucrose addition, the lightness of films from the deacetylated xanthan block (DX) increased ($p<0.05$).

The Pareto chart of standardized effect has indicated that the material redness (“a”) also increased ($p<0.05$) with sucrose addition for the acetylated xanthan gum block (X) and with sucrose and deacetylated xanthan gum addition for the deacetylated xanthan gum block (DX). However, the analysis of variance (ANOVA) for both blocks, showed very low coefficient of determination (R^2) for the independent variable “color a” ($R^2 < 0.28$), indicating that these statistical analysis could not be adequately explained and the results are not trustworthy.

The yellowness (“b”) of films from the acetylated xanthan gum block (X) increased ($p<0.05$) with sucrose and cassava starch addition. For the deacetylated xanthan gum block (DX) samples, the yellowness also increased ($p<0.05$) with sucrose and cassava starch addition, but decreased ($p<0.05$) with soybean oil addition and acidic pH.

Among the samples evaluated, the effect of sucrose, cassava starch and soybean oil on Hunter “L”, “a” and “b” values can be attributed to the characteristic colors of such raw materials. Samples yellowness increased with alkaline pH. Since the most reactions with starch occur in acidic pH (Belitz & Grosch, 1999), probably the increasing in yellowness is due to the characteristic color of the NaOH solution, used to modify the alkaline pH (and not used for acidic adjustments).

The total color difference between samples and the control (ΔE) is a good indicative for evaluating which additives and pH modification alter the total color of the films when comparing to the control. The total color difference between the control and the samples (ΔE) increased ($p<0.05$) with sucrose and sodium phosphate addition for the acetylated xanthan gum block (X) and with sucrose and cassava starch addition for the deacetylated xanthan gum block (DX).

The increases observed on the total color difference (ΔE) of the material can probably be attributed to the characteristic color of the raw materials sucrose, cassava starch and sodium phosphate. However, the coefficient of determination (R^2) considering the “ΔE” as independent variable was not high enough ($R^2 < 0.50$) to adequately explain the effects and the results should be observed with caution.

All samples presented high lightness (> 98.21%) and low color values for “a” redness (< 0.30) and “b” yellowness (< 3.91) as can be observed in Table 4. When compared to the control (L: 98.10; a:0.05; b:2.47), samples presented similar results, indicating that the additives and pH modifications investigated in this study have little effect on the cassava starch films color parameters and that all materials were almost colorless, with a high brilliancy.

4. Conclusions

Sucrose utilization as additive resulted on cassava starch film crystallization during storage. Although not enough to prevent sucrose crystallinity, deacetylated xanthan gum addition delay in a few days the material crystallization, probably due to a closer structure, which could have reduced the sucrose crystals mobility, retarding the crystallization process. No other additive or pH modification affected the cassava starch films microstructure or the sucrose crystallization during the storage period. The additives sucrose, cassava starch, soybean oil and alkaline pH have affected the materials Hunter “L”, “a” and “b” values. However, in relation to cassava starch films formulated without additives, only sucrose and sodium phosphate affected samples total color difference (ΔE). The cassava starch concentration also changed the total color difference (ΔE). However, all experimental samples presented similar color parameters when comparing to the cassava starch control sample, maintaining high lightness and low color values for “a” redness and “b” yellowness, independent of the additives or pH modifications.

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CAPÍTULO 4

Microstructure and color of biodegradable cassava starch-gelatin films: Influence of additives and process parameters.

Microstructure and color of biodegradable cassava starch-gelatin films: Influence of additives and process parameters.

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Abstract

The influence of additives (gelatin, sucrose, propylene glycol and soybean oil) and process parameters (pH and temperature), singly and in association at different levels, had been evaluated by a 2^{7-3} factorial experiment design. X-ray diffractograms have indicated that only sucrose influenced film crystallinity during storage, which was also observed by scanning electron and light microscopy images. Although light microscope utilization for analyzing biofilms microstructure is unusual, light micrographs have allowobserving phase separation and sucrose crystallization. All biofilm samples presented a high lightness and low redness and yellowness, indicating to be almost colorless. Although temperature did not influenced film microstructure and color, dehydration temperature of 60°C had completely destroyed the films, not been recommended for further investigations.

Keywords: Biodegradable films, Additives, Crystallization, Microstructure, Color

1. Introduction

Despite of the biodegradable films importance for the environmental, starches materials present, in general, low mechanical resistance and water vapor barrier when comparing to traditional petroleum plastic films [11-12]. However, protective characteristics can be improved through additives incorporation [3-5] and process

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parameters [7] modifications. Nevertheless, few studies have reported the effect of additives and process parameters on the material microstructure.

Biodegradable films with increased water vapor barrier properties can be obtained by soybean oil addition [17,5] although the homogeneity patterns of starch-soybean oil films have not been extensively investigated.

Additivation with gelatin [2] and propylene glycol [13] can improve biofilms mechanical properties. Plasticizers are also recommended for increasing film elongation, being polyols the most frequently used [6]. Although not much investigated, sucrose, which has a low coast when comparing to polyols, can also be utilized [1].

Sucrose crystallization is a known and well-studied phenomenon [9], however the sucrose effect on biofilms crystallinity has not been studied yet. Propylene glycol is a humectant agent, and so, can also affect film microstructure, having a protective effect against sucrose crystallization [9].

With the aim to evaluate the isolate and simultaneous influence of additives (sucrose, propylene glycol, gelatin and soybean oil) and process parameters (pH and dehydration temperature) on cassava starch films microstructure; crystallinity, microscopy and color were investigated.

2. Materials and methods

2.1. Materials

Commercial cassava starch (Flor de Lotus, Brazil), gelatin type B (Liner Davis Gelatin, Brazil), soybean oil (Cargill Agricola S.A., Brazil), and analytically pure sucrose and propylene glycol (Sinth, Brazil) were used.

2.2. Sample Preparation

Cassava starch (3-5%, w/w) was blended with water and the additives: gelatin (0-0.7%, w/w), sucrose (0-3%, w/w), propylene glycol (0-1.0%, w/w) and soybean oil (0-0.06%, w/w) considering the total weight of the film forming suspension. The film forming suspension pH was adjusted (4-8) with 50% citric acid solution or 5% sodium hydroxide solution, heated to 75°C for starch jellification, with constant stirring. The films were prepared using the casting technique. 30g of the film forming suspension were dehydrated under renewable circulated air (30°- 60° ± 2°C), over Petri plastic dishes. Films containing only cassava starch (5% w/w; no additives or pH adjustment), casted at 30°C, served as the control. Samples were storage (23°, 75%RH given by a NaCl saturated solution) for at least 4 days prior to testing.

2.3. Microstructure Analyses

A. Crystallinity

The relative crystallinity of the films was investigated with wide-angle X-ray diffraction (WAXS). Measurements were carried out using a DMAX-2200 Rigaku International Corporation $\theta/2\theta$ diffractometer, operating with voltage 40 kV and amperage 20mA, with CuKα radiation. Samples were fixed at an aluminum sample holder, and analysed from 5 to 60° (2θ), with angular step of 0.1° (2θ), and sampling interval time of 3 seconds. The films were analysed at each 15 days, during a storage period of 60 days (75% RH, 23°C), in duplicate. Crystallinity was also investigated (in the same conditions) for the raw materials cassava starch and sucrose in order to obtain crystallinity standards.

B. Light Microscopy

Samples surface was observed by LEICA-DMLP light microscope, with no further preparation, by transmitted light. Images of 200x-magnification were collected with a CCD camera.

C. Scanning Electron Microscopy (SEM)

SEM digital images of the samples surface and fracture were obtained by Jeol JSM-5900LV scanning electron microscope. Cross section images were obtained by cryogenic fracture of the films, using liquid nitrogen. Samples were sputtered with a 16μg gold layer. Images were taken at 5-10KV, spotsize 28-30, 1000x-magnification.

2.4. Color

The color of preconditioned (75% RH, 23°C) samples was analysed in duplicate by total transmittance, using a Color Quest II-Hunter lab equipment, CIELAB Ttran D65, lecture angle 10°, measuring area 2.54cm^2 (1.0 square inch). Four measurements taken at the different quadrants of each sample were averaged and expressed as Hunter system "L" (lightness), "a" (redness) and "b" (yellowness) values. Total color difference (ΔE) was evaluated as the size of color difference between the control and the experimental design samples, and calculated by the following equation [8]:

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

2.5. Statistical analysis

A 2^{7-3} experimental factorial design was performed in order to select additives and process parameters that significativelly ($p<0.05$) influenced cassava starch films microstructure and color. The block was designed with three central points, totalizing 19 experiments. The variables were cassava starch, gelatin, sucrose, soybean oil, propylene glycol, temperature and pH (Table 1). The experimental data were analysed using the software Statistica for Windows, version 5.0.

Table 1.
Independent variables coded and real values for film preparation, considering the total weight of the film forming suspension.

Levels	A Temperature (°C)	B pH	C Cassava starch (%)	D Sucrose (%)	E Propylene glycol (%)	F Soybean oil (%)	G Gelatin (%)
-1	30	4	3	0	0.00	0.00	0.00
0	45	6	4	1	0.25	0.03	0.35
+1	60	8	5	2	0.50	0.06	0.70

The experimental design demonstrated in Table 2 was used to estimate the main effects of independent variables on films microstructure and color.

Table 2.
Factorial 2^{7-3} design with coded and real values of the experimental block.

Assays	**Variables**						
	A	***B***	***C***	***D***	***E***	***F***	***G***
1	*-1* (30)	*-1* (4.0)	*-1* (3.0)	*-1* (0.0)	*-1* (0.00)	*-1* (0.00)	*-1* (0.00)
2	*+1* (60)	*-1* (4.0)	*-1* (3.0)	*-1* (0.0)	*+1* (0.50)	*-1* (0.00)	*+1* (0.70)
3	*-1* (30)	*+1* (8.0)	*-1* (3.0)	*-1* (0.0)	*+1* (0.50)	*+1* (0.06)	*-1* (0.00)
4	*+1* (60)	*+1* (8.0)	*-1* (3.0)	*-1* (0.0)	*-1* (0.00)	*+1* (0.06)	*+1* (0.70)
5	*-1* (30)	*-1* (4.0)	*+1* (5.0)	*-1* (0.0)	*+1* (0.50)	*+1* (0.06)	*+1* (0.70)
6	*+1* (60)	*-1* (4.0)	*+1* (5.0)	*-1* (0.0)	*-1* (0.00)	*+1* (0.06)	*-1* (0.00)
7	*-1* (30)	*+1* (8.0)	*+1* (5.0)	*-1* (0.0)	*-1* (0.00)	*-1* (0.00)	*+1* (0.70)
8	*+1* (60)	*+1* (8.0)	*+1* (5.0)	*-1* (0.0)	*+1* (0.50)	*-1* (0.00)	*-1* (0.00)
9	*-1* (30)	*-1* (4.0)	*-1* (3.0)	*+1* (2.0)	*-1* (0.00)	*+1* (0.06)	*+1* (0.70)
10	*+1* (60)	*-1* (4.0)	*-1* (3.0)	*+1* (2.0)	***+1* (0.50)**	*+1* (0.06)	*-1* (0.00)
11	*-1* (30)	*+1* (8.0)	*-1* (3.0)	*+1* (2.0)	***+1* (0.50)**	*-1* (0.00)	*+1* (0.70)
12	*+1* (60)	*+1* (8.0)	*-1* (3.0)	*+1* (2.0)	*-1* (0.00)	*-1* (0.00)	*-1* (0.00)
13	*-1* (30)	*-1* (4.0)	*+1* (5.0)	*+1* (2.0)	***+1* (0.50)**	*-1* (0.00)	*-1* (0.00)
14	*+1* (60)	*-1* (4.0)	*+1* (5.0)	*+1* (2.0)	*-1* (0.00)	*-1* (0.00)	*+1* (0.70)
15	*-1* (30)	*+1* (8.0)	*+1* (5.0)	*+1* (2.0)	*-1* (0.00)	*+1* (0.06)	*-1* (0.00)
16	*+1* (60)	*+1* (8.0)	*+1* (5.0)	*+1* (2.0)	***+1* (0.50)**	*+1* (0.06)	*+1* (0.70)
17(c)*	*0* (45)	*0* (6.0)	*0* (4.0)	*0* (1.0)	*0* (0.25)	*0* (0.03)	*0* (0.35)
18(c)*	*0* (45)	*0* (6.0)	*0* (4.0)	*0* (1.0)	*0* (0.25)	*0* (0.03)	*0* (0.35)
19(c)*	*0* (45)	*0* (6.0)	*0* (4.0)	*0* (1.0)	*0* (0.25)	*0* (0.03)	*0* (0.35)

*(c): central point.s.

3. Results and Discussion

3.1. Microstructure analyses

Crystallinity

WAXS crystallinity diffractograms, collected during 60 days of storage, demonstrated an amorphous behavior for all samples at the first day of storage (Fig. 1).

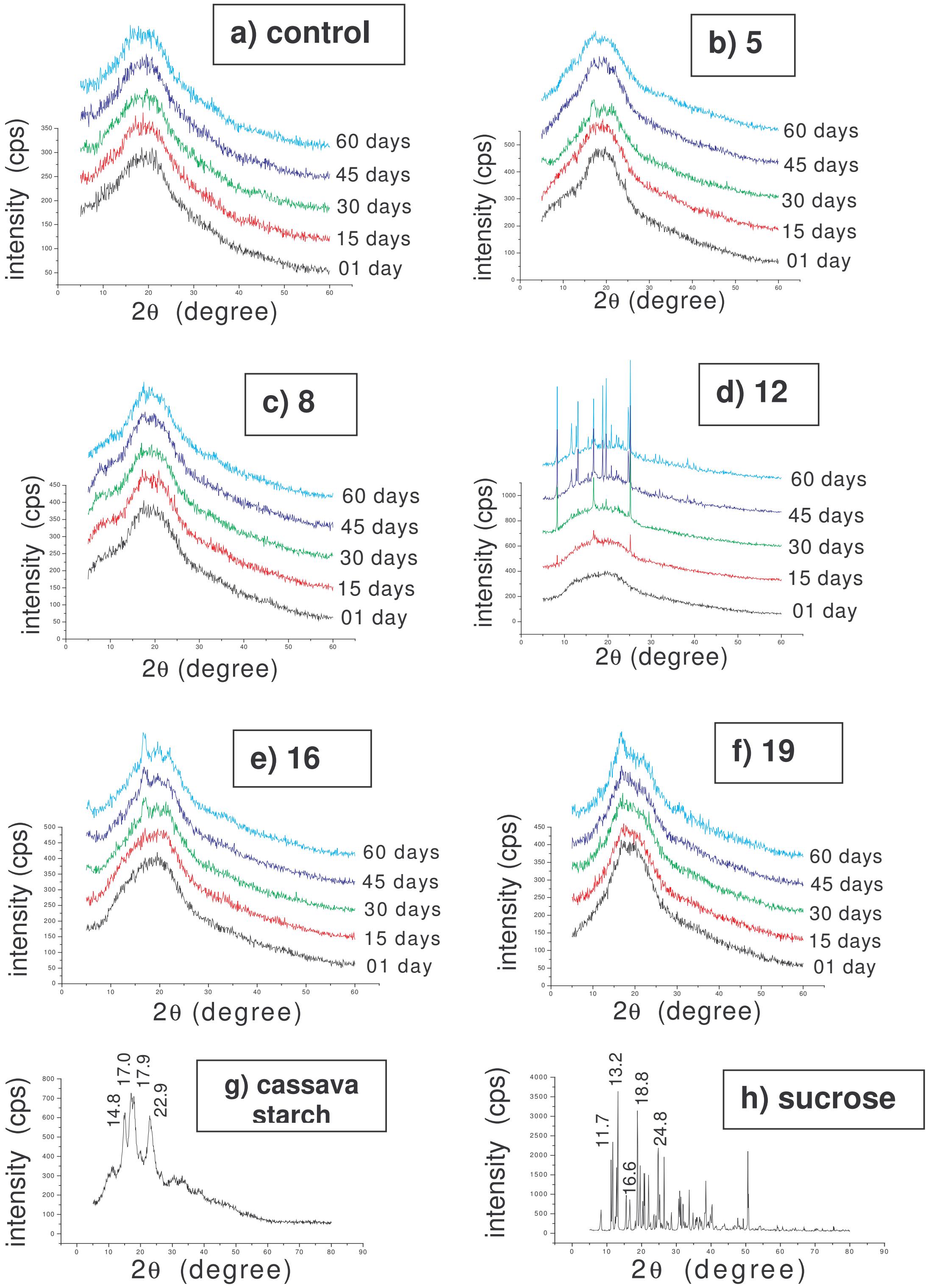

Fig. 1. WAXS crystallinity diffractograms of *amorphous*: (a) control, (b) 5, (c) 8, and *crystalline*: (d) 12, (e) 16, (f) 19 samples behavior during 60 days of storage. WAXS crystallinity diffractograms of (g) native cassava starch and (h) sucrose standards.

No crystallinity was observed during the control sample storage period (Fig. 1a), indicating that without additives or pH controlling (pH 5.78), the cassava starch film shows an amorphous behavior. The same amorphous behavior was also observed during storage of all samples formulated without sucrose, independent of the other additive, pH or dehydration temperature. Such results are exemplified by samples 5 (Fig. 1b, containing all additives except sucrose, pH 4.0, 30°C dehydration temperature and 3% cassava starch) and 7 (Fig. 1c, pH 8.0, 60°C dehydration temperature and 5% cassava starch). The X-ray diffractograms indicated that the additives gelatin, soybean oil and propylene glycol did not influenced the amorphous behavior of cassava starch films. Also no influence was observed when modifying pH, dehydration temperature and cassava starch concentration.

Cassava starch granules have a semi-crystalline structure, characterized by crystalline peaks (or crystallites) superposed to an amorphous halo [Katz, 1930, cited by 15]. Native root starch semi-crystalline behavior is designated as C-type [18].

Although cassava starch is the main component on the studied films, the amorphous behavior observed for all films at the first day of storage (Fig. 1a, 1b, 1c, 1d, 1e) can be explained by the jellification step used for film preparation. During jellification, the ordered crystalline regions undergo melting [18], which according to [10], could decrease the crystallization ratio until zero, resulting in an amorphous structure. Also the low dehydration temperature (30°C) can be responsible for the amorphous structure. According to Rindlav, Hulleman & Gatenholm (1997) [10], dehydration temperatures bellow 50°C result in amorphous behavior for starch films.

However, when sucrose was added (samples 9 to 19), with (Fig. 1e, 1f) or without (Fig. 1d) the presence of the other additives and process parameters, the cassava starch films developed a crystalline structure during storage. Such results indicate that sucrose addition is the responsible for film crystallization during storage.

Such theory is reinforced when comparing the X-ray diffractograms of crystalline samples with sucrose standard. The 2θ angle and *d*-spacing (distance between lattice planes) of the crystalline peaks observed on the cassava starch-sucrose films, matches with sucrose standard peaks (Fig. 1h), as can be observed in Table 3.

Table 3.
2θ angle and *d*-spacing (*d*) matches for main peaks presented by crystalline samples WAXS diffractograms.

Sample / [sucrose]	**2θ**	***d* (Å)**	**2θ**	***d* (Å)**	**2θ**	***d* (Å)**	**2θ**	***d* (Å)**	**dc****
Sucrose Standard/ (99.72%)	**11.7**	***(7.56)***	**16.6**	***(5.34)***	**18.8**	***(4.72)***	**24.8**	***(3.59)***	**na*****
Assays									
9 / (2.00%)	**11.6**	***(7.62)***	**16.6**	***(5.34)***	**18.8**	***(4.72)***	**24.7**	***(3.60)***	**15**
10 / (2.00%)	**11.6**	***(7.62)***	----	----	**18.8**	***(4.72)***	**24.8**	***(3.59)***	**15**
11 / (2.00%)	**11.6**	***(7.62)***	**16.7**	***(5.31)***	**18.8**	***(4.72)***	----	----	**30**
12 / (2.00%)	**11.6**	***(7.62)***	----	----	**18.8**	***(4.72)***	**24.7**	***(3.60)***	**15**
13 / (2.00%)	----	----	**16.6**	***(5.34)***	----	----	----	----	**45**
14 / (2.00%)	----	----	**16.6**	***(5.34)***	----	----	----	----	**45**
15 / (2.00%)	----	----	**16.6**	***(5.31)***	----	----	----	----	**30**
16 / (2.00%)	----	----	**16.7**	***(5.31)***	**18.8**	***(4.72)***	----	----	**30**
17(c)* / (1.00%)	----	----	**16.7**	***(5.31)***	----	----	----	----	**45**
18(c)* / (1.00%)	**11.6**	***(7.62)***	**16.7**	***(5.31)***	----	----	----	----	**45**
19(c)* / (1.00%)	----	----	**16.7**	***(5.31)***	----	----	**24.7**	***(3.60)***	**30**

(c)*: central points; dc**: days that samples remained amorphous; na***: not applicable.

Sucrose crystallization is a known phenomenon that starts with a crystallinity nucleus [9]. When in supersaturated conditions spontaneous nucleation can occur. Nucleation and crystal formation could arise from rearrangement of randomly bound sugar molecules [16].

Statistical Pareto analyses were performed to evaluate the additives and process pH influence on the time required for crystallization beginning. For that matter, it was considered the total of days that the material remained amorphous (Table 3). In order to present values for the statistical calculations, for the films that did not present crystallinity during storage, the time required for crystallinity beginning was considered the total storage period (60 days) evaluated in this study. Only sucrose has affected ($p<0,05$) (R^2:0.86) film crystallinity during the storage period evaluated, lowering the shelf life of the material.

The sucrose crystallization observed resulted in brittle materials. As mechanical resistance is essential to a packaging material, the sucrose crystallinity during storage is undesired and determines the product shelf life expiration.

Scanning Electron Microscopy

Samples surface and cross section SEM micrographs were recorded at 5 and 60 days (± 4) of storage. At the first week storage period, all samples presented a compact and smooth structure, both for surface and cross section, as exemplified by the control and samples 5 (containing all additives except sucrose) and 16 (containing the maximum level of all additives, pH and temperature) (Fig. 2).

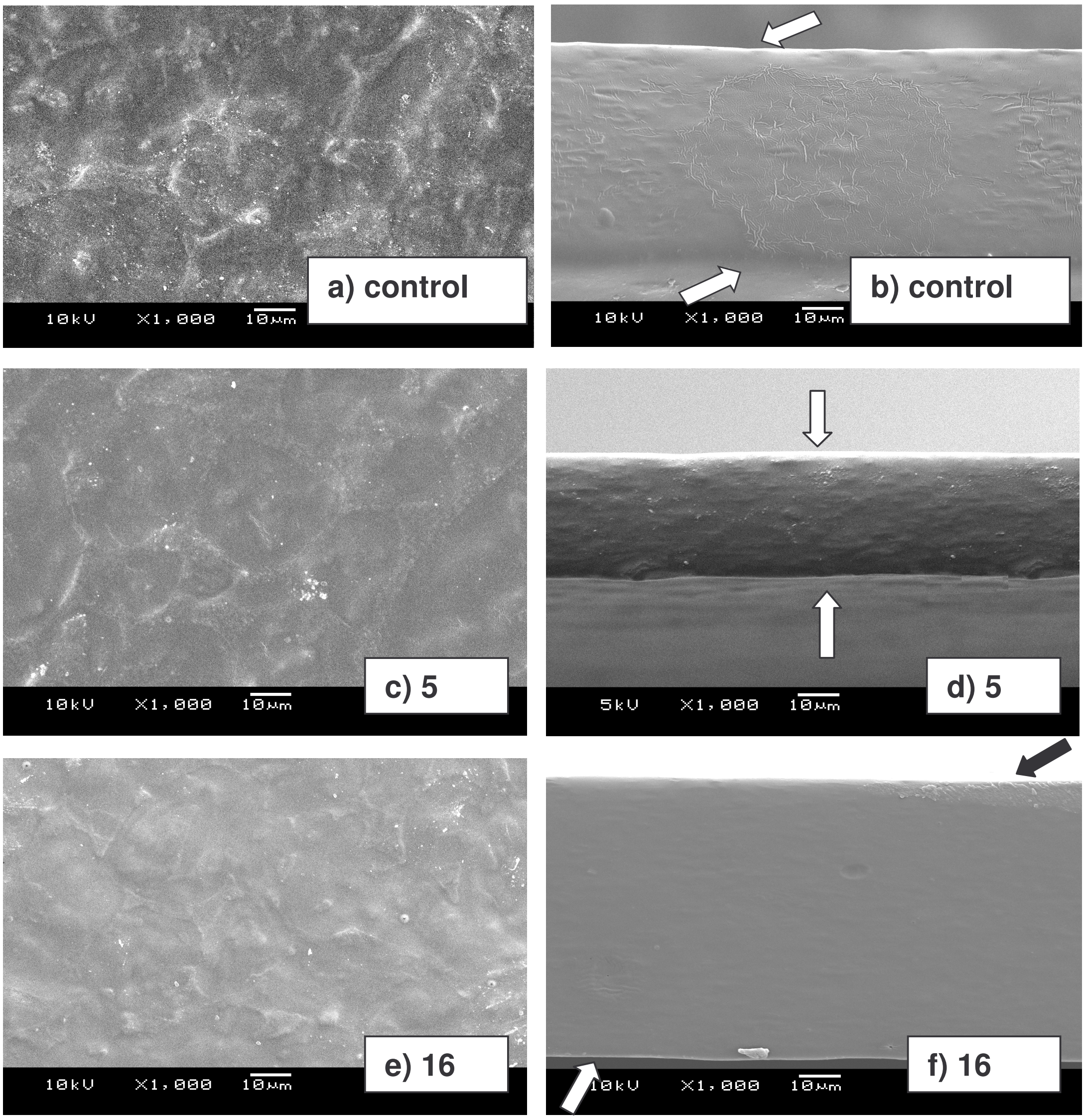

Fig. 2. Scanning electron micrographs of samples surfaces (a) control, (c) 5, (e) 16, and cross sections (b) control, (d) 5, (f) 16, at the first week of storage.

However, after 60 days of storage, a visible crystallinity pattern was observed on surface and cross-section SEM micrographs of samples containing sucrose (Fig. 3), as exemplified by samples 19 (containing intermediate values of all additives and process parameters) and 16 (containing the maximum level of all additives and process parameters).

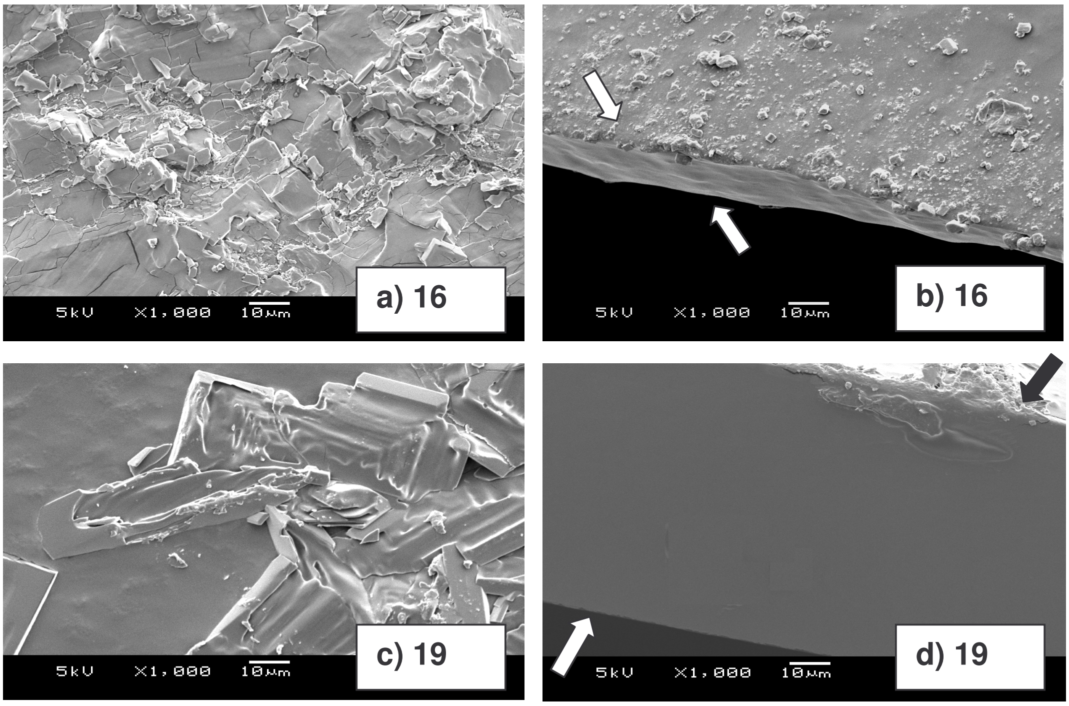

Fig. 3. Scanning electron micrographs of samples surfaces (a) 16, (c) 19, and cross sections (b) 16, (d) 19, after 60 days of storage.

The crystalline surfaces observed in Fig. 3 micrographs are in agreement with the crystallinity behavior observed by X-ray diffraction analyses, confirming the crystalline behavior presented by cassava starch films added with sucrose.

Cassava starch-sucrose films cross-section micrographs (Fig. 3b, 3d) demonstrate also that the crystallization began at the surface and not at the interior of the material. Such result indicates that the crystallization probably occurred by spontaneous nucleation of sucrose crystals and not due to residual non-dissolved sucrose crystals. If non-dissolvable sucrose grains were present along the interior of the film structure, they would have act as seeding agents. The other sucrose molecules would lean and stick to this nucleus beginning crystallization [9] all over the film, and not only at the surface.

The fact that the film surface area is a more susceptible to water vapor exchange with the environmental could be an explanation for the sucrose crystallization beginning at the surface. According to Lees [14], outside disturbance, as changes in the supersaturated level, can start the crystallization process.

Optical Microscopy

Although not often utilized for analyzing biodegradable films structure, light microscopy demonstrated to be a very important tool in characterizing the component interaction of the samples. Light microscopy images allowed observing film homogeneity and crystallization during storage (Fig. 4).

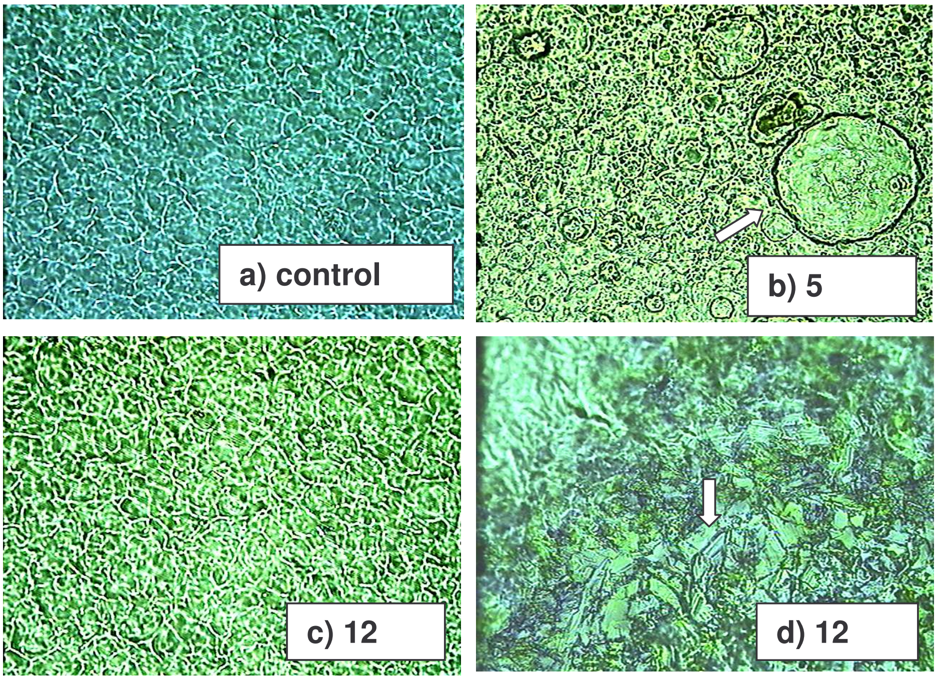

Fig. 4. Samples light micrographs (a) control, (c) 12, at the first week of storage; and at (b) 5, (d) 12, 60 days of storage.

Light micrographs have demonstrated phase separation for films added with soybean oil (0.06%), as demonstrated by sample 5, showing dispersed oil drops along the material structure (Fig. 4b), since the first day of storage. Such phenomenon was not possible to observe by SEM micrographs, indicating that light microscopy should be used in ensemble with SEM for characterizing biofilms microstructure.

The heterogeneity observed indicates that the cassava starch films network structure was unable to embody the soybean oil, although added in low concentration (0.06%), even in the presence of gelatin (Fig. 4b).

The sucrose crystallization was also clearly observed by light micrographs images, as exemplified by sample 12 (added with 2% sucrose) after 60 days of storage (Fig. 4d). The crystal growths observed are in agreement with SEM images and X-ray diffractograms.

Light micrographs demonstrated to be a feasible, fast and low cost tool for observing sucrose content film crystallinity during storage and phase separation in oil-starch systems.

3.2. Color

The Pareto chart of standardized effect, considering ANOVA pure error has indicated that no additives and process parameters affected ($p<0.05$) the films lightness (“L”), redness (“a”) and yellowness (“b”).

All samples presented high lightness (above 97.9%), and low color values for “a” redness (< 0.24) and “b” yellowness (< 4.14), as can be observed in Table 4. Such results indicate that, independent of the additives, pH or dehydration temperature investigated in this study, the materials were almost colorless, with a high brilliancy. When compared to the control (L=98.5; a=0.25; b=3.37), samples presented similar results, indicating that the additives and pH modifications investigated in this study have little effect on the cassava starch films color parameters and that all materials were almost colorless, with a high brilliancy.

Table 4.
Samples Hunter color parameters “L”, “a” and “b” and total color difference between samples and the control (ΔE %).

Assays	**“L”**	**“a”**	**“b”**	***ΔE (control)***
control	98.5	0.25	3.37	*na***
1	98.60	0.24	3.38	*0.11*
2	98.70	0.17	3.41	*0.23*
3	98.72	0.27	4.14	*0.80*
4	98.12	0.19	3.40	*0.38*
5	98.34	0.15	3.19	*0.25*
6	98.05	-0.01	2.29	*1.19*
7	97.97	0.22	4.05	*0.86*
8	97.98	-0.10	2.41	*1.14*
9	98.35	0.21	3.59	*0.26*
10	98.39	0.11	3.13	*0.30*
11	98.14	0.19	3.48	*0.37*
12	98.38	0.09	3.64	*0.33*
13	98.48	0.07	2.29	*1.09*
14	98.15	0.00	2.91	*0.62*
15	98.39	0.12	2.65	*0.74*
16	98.23	-0.01	3.59	*0.43*
17 (c)*	98.59	0.01	2.66	*0.81*
18 (c)*	98.56	-0.01	2.47	*0.94*
19 (c)*	98.61	0.03	2.59	*0.82*
R^{2***}	0.33	0.59	0.52	*0.28*

(c)*: central point.s; n*a**:* not applicable, R^{2***}: correlation coefficient.

The total color difference between samples and the control (ΔE) is a good indicative to measure how much the additives affected the total color of the films in relation to the control.

Although the Pareto chart of standardized effect has indicated that no additive, pH or temperature modification affected ($p<0.05$) the films total color difference (ΔE), the R^2 obtained was very low (0.26) indicating that effect results given by the Statistica program may not be trustfully.

Temperature has not influenced the films microstructure and color parameters. Although, when using process temperature of 60ºC, samples were completely destroyed during dehydration, not been recommended in future experiments (Fig. 5).

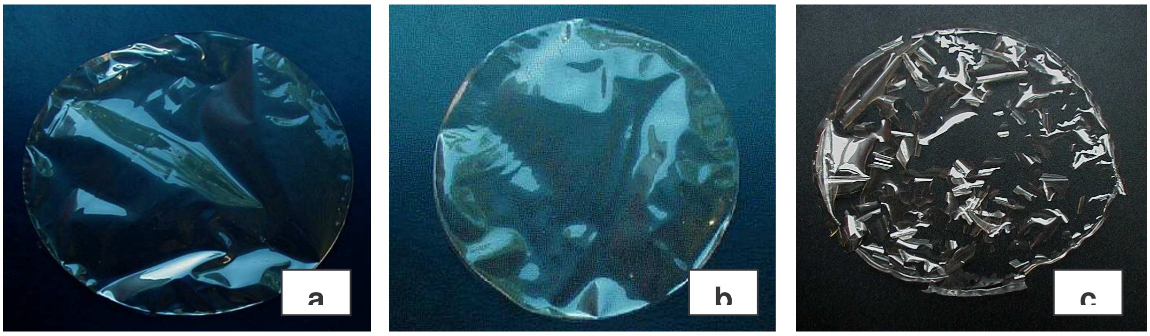

Fig. 5. Samples prepared at (a) 30°C, (b) 45°C and (c) 60°C drying temperatures.

4. Conclusions

Among the additives and process parameters investigated, cassava starch film microstructure can only be affected by sucrose and soybean oil addition. X-ray diffractograms, SEM and light microscopy indicated that sucrose addition resulted in crystallization during storage. Light micrographs also allowed observing phase separation when using soybean oil and also confirmed crystallization during storage. The investigated additives, pH and temperatures did not affect cassava starch color parameters. All films were almost colorless with a high brilliancy. Although temperature did not influence cassava starch film microstructure or color, dehydration temperature of 60°C has completely destroyed the films, not been recommended for further investigations.

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CAPÍTULO 5

Sucrose and inverted sugar as additives. Part 1: Effect on cassava starch-gelatin films mechanical properties, hydrophilicity and water activity

Sucrose and inverted sugar as additives. Part 1: Effect on cassava starch-gelatin films mechanical properties, hydrophilicity and water activity

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The effect of sucrose and inverted sugar as additives on mechanical properties, hydrophilicity and water activity of cassava starch-gelatin films have been studied. Other additives (soybean oil, sodium phosphate and propylene glycol) and pH effect have also been investigated by the same parameters. Sucrose addition resulted in the highest effect observed on cassava starch-gelatin films elongation at break, however inverted sugar also had a great effect on such property. Sucrose and inverted sugar also decreased tensile strength and increased water activity of the material. Plasticizer addition and acidic pH increased film water absorption kinetic, while cassava starch concentration had the opposite effect.

KEYWORDS: biofilms, mechanical properties, water activity, hydrophilicity.

INTRODUCTION

Starch is considered one of the most promising natural polymers for packaging application (Guilbert, Gontard & Gossis, 1996 and Krochta & Mulder-Johnston, 1997) because of their low cost, renewability and biodegradability (Avérous, Fringant & Moro, 2001). Unfortunately, there are some strong limitations to the development of

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starch-based products, as their poor mechanical properties when comparing to conventional petroleum films (Martin, Schwach, Avérous & Couturier, 2001). To overcome these weaknesses, association with additives (Arvanitoyannis & Biliaderis, 1998; Coupland, Shaw, Monahan, O'Riordan & O'Sullivan, 2000; Garcia, Martino, Zaritzki, 2000), such as sucrose is recommended (Arvanitoyannis, Psomiadou & Nakayama, 1996).

Previous work has indicated that sucrose addition to cassava starch films has greatly increased elongation at break of starch-xanthan gum films (Veiga-Santos, Oliveira, Cereda, Alves & Scamparini, 2004), although the materials presented crystallinity during storage, becaming britlle. Substituting sucrose for inverted sugar may prevent sucrose crystallinity; however, inverted sugar effect on biodegradable films mechanical properties and hydrophilicity has not yet been studied.

Sucrose, which is a disaccharide, can be broken down into a 1:1 mixture of two monosacharides, dextrose (glucose) and laevulose (fructose), known as inverted sugar (James, 1995), which presents a lower crystallinity tendency when comparing to sucrose (Lees, 1995). The inverted sugar can increase the film forming suspension viscosity, difficulting crystals approaching (Jackson, & Howling, 1995).

With the aim to evaluate the effect of substituting sucrose for inverted sugar as additive on cassava starch films characteristics; tensile strength, elongation at break, contact angle measurement and water activity were investigated. The effect of other additives (gelatin, propylene glycol, sodium phosphate and soybean oil) and pH modifications were also investigated for the same parameters.

MATERIAL AND METHODS

Materials.

Commercial cassava starch (Flor de Lotus, Brazil), inverted sugar cane (Copersucar União, Brazil), gelatin type B (Liner Davis Gelatin, Brazil), soybean oil (Cargill Agricola S.A., Brazil), and analytically pure sucrose, propylene glycol and monobasic sodium phosphate (Sinth, Brazil).

Sample Preparation.

Cassava starch (3-5%, w/w) was blended with the additives: sucrose or inverted sugar (0-2%, w/w), propylene glycol (0-1%, w/w), sodium phosphate (0-0.2%, w/w) and soybean oil (0-0.06%, w/w). Gelatin (0-1%, w/w) was dispersed in distilled water (100°C), cooled to 40°C and added to the previous blended ingredients. It was considered the total weight of the film forming suspension. The pH was adjusted (4-8) with 50% citric acid solution or 5% sodium hydroxide solution and heated to 75°C with constant stirring, to obtain starch jellification. The cassava starch-gelatin films were prepared according to the casting technique, by dehydration under renewable circulated air (30°± 2°C) over Petri plastic dishes. Films containing only cassava starch (no additives or pH adjustment) served as the control. Samples were storage (23°C, 75%RH) for at least 4 days prior to testing.

Mechanical Properties.

Films tensile strength and elongation at break were measured using an Instron Universal Testing Instrument (model 5500R) operated according to ASTM standard method D882-00 (ASTM, 2001). Tested filmstrips (8 x 2.5cm) were cut from each preconditioned (75% RH, 23°C) sample and mounted between the grips of the machine. The thickness of each specimen was measured in four points along its length with a flat and parallel surfaces Mitutoyo digital micrometer. Initial grip separation and crosshead speed were set to 50 mm and 12.5 mm/min, respectively. The maximum load (kgf) and extension (mm) were recorded and used to calculate the tensile strength (kgf/cm^2) and elongation at break. Ten specimens were tested for each sample. The experimental design sample tensile strength (*ΔT*%) and elongation at break (*ΔE*%) increase or decrease size in relation to the control were calculated by equations 1 and 2, respectively.

$$\Delta T = \left[\left(\frac{sample\ tensile\ strength}{control\ tensile\ strength} \right) \times 100 \right] - 100$$

Equation 1

$$\Delta E = \left[\left(\frac{sample\ elongation}{control\ elongation} \right) \times 100 \right] - 100$$ **Equation 2**

Water Activity.

Water activity measurements were performed with a DECAGON CX-2T, AQUALAB apparatus. Pure water (a_w=1.000 ± 0.003%) and KCl (a_w=0.843± 0.003 %) were used as standard for equipment calibration. Preconditioned (75% RH, 23°C) samples (4cm^2) were cut from the center of the films and evaluated in triplicate. The experimental design samples water activity (Δa_w%) increase or decrease size in relation to the control was calculated according to equation 3.

$$\Delta a_w = \left[\left(\frac{sample\ a_w}{control\ a_w} \right) \times 100 \right] - 100$$ **Equation 3**

Contact Angle Measurement.

The contact angle measurements were determined using a VCA Optima Equipment AST Products, to evaluate samples hydrophilic feature (Avérous & Fringant, 2001). The evolution of the water drop (3μL) shape on the surface of preconditioned (75% RH, 23°C) 4cm^2 samples was recorded (in duplicate) to determine the material absorption kinetic (during 5 min) (Veiga, Alves, Sinézio, Scamparini & Cereda, 2003). The initial contact angle values just after deposition indicate the material hydrophilicity behavior. In order to illustrate the kinetic of absorption, the slope at the origin (º/min) was also calculated by linear regression from the points of the contact angle versus time (Avérous, Moro, Dole & Fringant, 2000).

Statistical analysis.

Two blocks of a 2^{7-3} experimental factorial design were performed in order to select additives and process parameters that significativelly ($p<0.05$) influenced cassava starch films mechanical properties, hydrophilicity and water activity. The blocks were designed with three central points, totalizing 19 experiments per block, differing only for one variable (sucrose or inverted sugar concentration). The other variables were cassava starch, sucrose, soybean oil, sodium phosphate, propylene glycol and pH (Table 1).

Table 1. Independent variables coded and real values for the sucrose (S) and inverted sugar (I) factorial experiment blocks, considering the total weight of the film forming suspension.

symbol	**Variables**		***Levels***		
	Block-S	**Block-I**	***-1***	***0***	***+1***
A	Sodium phosphate (%)	Sodium phosphate (%)	*0.00*	*0.35*	*0.70*
B	pH	pH	*4.00*	*6.00*	*8.00*
C	Cassava starch (%)	Cassava starch (%)	*3.00*	*4.00*	*5.00*
D	Sucrose (%)	Inverted Sugar (%)	*0.00*	*1.00*	*2.00*
E	Propylene Glycol (%)	Propylene Glycol (%)	*0.00*	*0.25*	*0.50*
F	Soybean oil (%)	Soybean oil (%)	*0.00*	*0.03*	*0.06*
G	Gelatin (%)	Gelatin (%)	*0.00*	*0.35*	*0.70*

The independent variables significant ($p<0.05$) influence was evaluated by the Pareto chart of standardized effect, considering the ANOVA pure error. The experimental data were generated and analysed using the software Statistica for Windows, version 5.0.

The experimental design demonstrated in Table 2 was used to estimate the main effects of independent variables.

Table 2. 2^{7-3} factorial design with coded values for the sucrose (S) and inverted sugar (I) blocks.

Assays		**Variables**						
Block S	**Inverted I**	***A***	***B***	***C***	***D***	***E***	***F***	***G***
S-1	I-1	*-1* (0.00)	*-1* (4.0)	*-1* (3.0)	*-1* (0.0)	*-1* (0.00)	*-1* (0.00)	*-1* (0.00)
S-2	I-2	*+1* (0.70)	*-1* (4.0)	*-1* (3.0)	*-1* (0.0)	*+1* (0.50)	*-1* (0.00)	*+1* (0.70)
S-3	I-3	*-1* (0.00)	*+1* (8.0)	*-1* (3.0)	*-1* (0.0)	*+1* (0.50)	*+1* (0.06)	*-1* (0.00)
S-4	I-4	*+1* (0.70)	*+1* (8.0)	*-1* (3.0)	*-1* (0.0)	*-1* (0.00)	*+1* (0.06)	*+1* (0.70)
S-5	I-5	*-1* (0.00)	*-1* (4.0)	*+1* (5.0)	*-1* (0.0)	*+1* (0.50)	*+1* (0.06)	*+1* (0.70)
S-6	I-6	*+1* (0.70)	*-1* (4.0)	*+1* (5.0)	*-1* (0.0)	*-1* (0.00)	*+1* (0.06)	*-1* (0.00)
S-7	I-7	*-1* (0.00)	*+1* (8.0)	*+1* (5.0)	*-1* (0.0)	*-1* (0.00)	*-1* (0.00)	*+1* (0.70)
S-8	I-8	*+1* (0.70)	*+1* (8.0)	*+1* (5.0)	*-1* (0.0)	*+1* (0.50)	*-1* (0.00)	*-1* (0.00)
S-9	I-9	*-1* (0.00)	*-1* (4.0)	*-1* (3.0)	*+1* (2.0)	*-1* (0.00)	*+1* (0.06)	*+1* (0.70)
S-10	I-10	*+1* (0.70)	*-1* (4.0)	*-1* (3.0)	*+1* (2.0)	***+1* (0.50)**	*+1* (0.06)	*-1* (0.00)
S-11	I-11	*-1 (0.00)*	*+1* (8.0)	*-1* (3.0)	*+1* (2.0)	***+1* (0.50)**	*-1* (0.00)	*+1* (0.70)
S-12	I-12	*+1* (0.70)	*+1* (8.0)	*-1* (3.0)	*+1* (2.0)	*-1* (0.00)	*-1* (0.00)	*-1* (0.00)
S-13	I-13	*-1* (0.00)	*-1* (4.0)	*+1* (5.0)	*+1* (2.0)	***+1* (0.50)**	*-1* (0.00)	*-1* (0.00)
S-14	I-14	*+1* (0.70)	*-1* (4.0)	*+1* (5.0)	*+1* (2.0)	*-1* (0.00)	*-1* (0.00)	*+1* (0.70)
S-15	I-15	*-1* (0.00)	*+1* (8.0)	*+1* (5.0)	*+1* (2.0)	*-1* (0.00)	*+1* (0.06)	*-1* (0.00)
S-16	I-16	*+1* (0.70)	*+1* (8.0)	*+1* (5.0)	*+1* (2.0)	***+1* (0.50)**	*+1* (0.06)	*+1* (0.70)
S-17(c)*	I-17(c)*	*0* (0.35)	*0* (6.0)	*0* (4.0)	*0* (1.0)	*0* (0.25)	*0* (0.03)	*0* (0.35)
S-18(c)*	I-18(c)*	*0* (0.35)	*0* (6.0)	*0* (4.0)	*0* (1.0)	*0* (0.25)	*0* (0.03)	*0* (0.35)
S-19(c)*	I-19(c)*	*0* (0.35)	*0* (6.0)	*0* (4.0)	*0* (1.0)	*0* (0.25)	*0* (0.03)	*0* (0.35)

(c): central points.*

RESULTS AND DISCUSSION

*Mechanical Properties***.**

The additives and pH tested in this study have affected cassava starch-gelatin films tensile strength (maximum resistance area) and elongation at break (Table 3 and Figures 1 and 2, respectively).

Table 3. Measurement results of cassava starch films tensile strength (T), elongation at break (E) and increase or decrease size of tensile strength (ΔT) and elongation at break (ΔE) in relation to the control.

	Sucrose Block (S)				**Inverted sugar Block (I)**			
assays	**T** (kgf/cm^2)**	***ΔT (%)***	**E (%)**	***ΔE (%)***	**T** (kgf/cm^2)**	***ΔT (%)***	**E (%)**	***ΔE (%)***
control	379.96	*na****	3.99	*na****				
1	339.58	*-10.63*	3.74	*-6.36*	344.40	*-9.36*	3.65	*-8.61*
2	219.40	*-42.26*	3.80	*-4.86*	225.60	*-40.63*	3.46	*-13.37*
3	221.92	*-41.59*	3.95	*-1.10*	226.90	*-40.28*	3.42	*-14.37*
4	219.00	*-42.36*	3.33	*-16.62*	218.80	*-42.41*	3.17	*-20.63*
5	240.67	*-36.66*	4.09	*2.40*	236.00	*-37.89*	3.92	*-1.85*
6	252.22	*-33.62*	4.03	*0.90*	259.80	*-31.62*	4.03	*0.90*
7	342.59	*-9.84*	3.80	*-4.86*	350.10	*-7.86*	4.09	*2.40*
8	260.88	*-31.34*	4.74	*18.68*	259.90	*-31.60*	3.46	*-13.37*
9	56.00	*-85.26*	74.57	*1767.05*	55.99	*-85.26*	48.73	*1120.11*
10	26.30	*-93.08*	115.60	*2794.34*	37.33	*-90.17*	54.42	*1262.54*
11	53.53	*-85.91*	65.24	*1533.45*	43.93	*-88.44*	54.14	*1255.48*
12	47.70	*-87.45*	85.06	*2029.69*	50.64	*-86.67*	50.19	*1156.74*
13	40.20	*-89.42*	71.66	*1694.19*	91.07	*-76.03*	48.06	*1103.25*
14	42.70	*-88.76*	86.75	*2072.01*	47.70	*-87.45*	56.21	*1307.39*
15	54.70	*-85.60*	71.13	*1680.92*	60.60	*-84.05*	39.68	*893.57*
16	36.88	*-90.29*	70.25	*1658.89*	36.88	*-90.29*	40.64	*917.45*
17 (c)*	146.90	*-61.34*	4.90	*22.68*	91.17	*-76.01*	10.83	*171.23*
18 (c)*	136.40	*-64.10*	5.87	*46.97*	99.36	*-73.85*	12.66	*216.98*
19 (c)*	135.00	*-64.47*	5.85	*46.47*	100.08	*-73.66*	9.59	*140.16*
R^{2****}	0.96		0.85		0.92		0.92	

(c)*: central points. T**: tensile strength as maximum resistance area. na***: not applicable. R^{2****}: coefficient of determination.

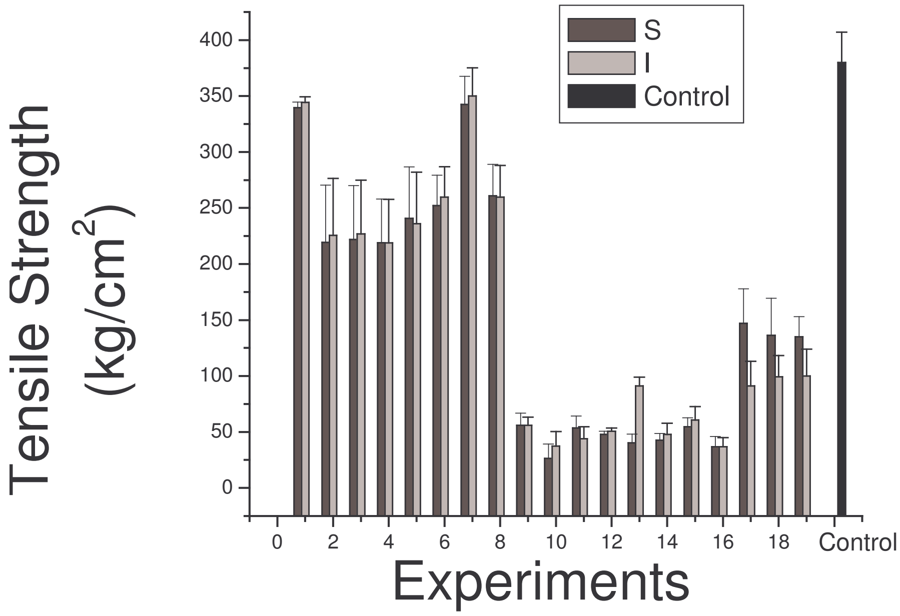

Figure 1. Tensile strength measurements and standard deviation obtained for the control and sucrose (S) and inverted sugar (I) statistical experiments.

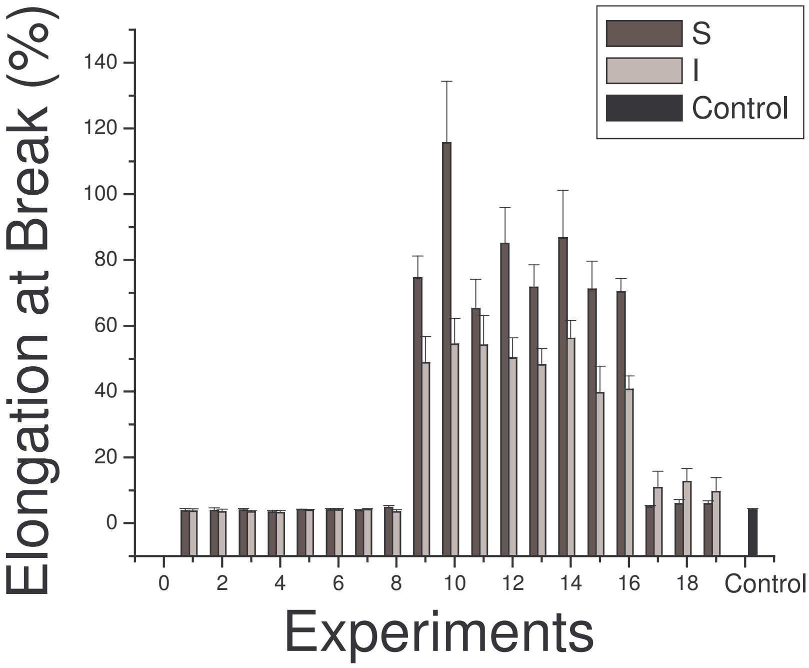

Figure 2. Elongation at break measurements and standard deviation obtained for the control and sucrose (S) and inverted sugar (I) statistical experiments.

As can clearly be observed by Table 3, and visualized by Figures 1 and 2, the experimental samples added with sucrose (S-9 to S-19) and inverted sugar (I-9 to I-19) presented a very different behavior from the other films.

Results shown on Table 3 have also indicated that films with 2% sucrose (S-9 to S-16) or inverted sugar addition (I-9 to I-16) presented higher elongation at break when comparing to films with 1% sucrose (S-17 to S-19) or inverted sugar addition (I-17 to I-19). Therefore, indicating that, increasing sucrose or inverted sugar addition, increases the cassava starch films elongation at break.

Sucrose addition can increase elongation at break up to 2970% when comparing to the control. The inverted sugar effect was lower, although significant. It has increased elongation at break up to 1300% when comparing to the control (Table 3). Such results indicate that sucrose has a higher efficacy in increasing elongation at break than inverted sugar.

The Pareto chart of standardized effect has confirmed that cassava starch films elongation at break is positively ($p<0.05$) affected by sucrose and inverted sugar addition.

Veiga-Santos, Oliveira, Cereda, Alves & Scamparini (2004) has observed a plasticizing effect when using sucrose as additive for cassava starch-xanthan gum films. It was observed an increase on materials elongation at break. Arvanitoyannis, Psomiadou & Nakayama (1996), have also observed plasticizing effect for sucrose, mentioning an even more pronounced influence that observed when utilizing polyols. According to Coupland, Shaw, Monahan, O'Riordan & O'Sullivan (2000), plasticizer agents reduce intermolecular forces and increase polymer chains mobility, thereby improving film flexibility and extensibility

The elongation increase observed when using inverted sugar, which is obtained form sucrose (James, 1995), could also be attributed to a plasticizing effect.

Although the sucrose has the most pronounced effect on elongation at break, sucrose block (S) samples were also positively affected ($p<0.05$) by sodium phosphate and soybean oil, and negatively affected ($p<0.05$) by pH, gelatin and cassava starch (R^2: 0.85).

The soybean oil increase effect on elongation at break can also be explained by its plasticizing effect (Garcia, Martino & Zaritzki, 2000). Also, the lipids ability to form inclusion compounds with amylose (Belitz & Grosh, 1999b) could result in more flexible network, increasing the material elongation at break.

Monobasic sodium phosphate can be added to starch to produce monoester phosphates, resulting in lower intermolecular association (BeMiller & Whistler, 1996), which could explain the increase on elongation at break observed.

Decrease in elongation at break with increasing gelatin concentration was also observed by Lee, Shim & Lee (2003). The authors attributed the reduction observed due to a closely packed state in gelatin, where extensive intermolecular bounding occurs, inhibiting further reorientation and better alignment of the gelatin and starch chain.

The negative effect of pH on films elongation at break can be attributed to the isoeletric point of the gelatin type B. The acidic conditions evaluated in this study (pH 4.0-6.0) were near the isoeletric point (pH 4.5-5.3) of gelatin type B. While at this pH the net charge of gelatin is zero (Poppe, 1997), no interaction between gelatin and starch will occur. However, increasing pH away form the isoeletric point (pH 6.0-8.0), starch-gelatin interaction may occur, which could result in lower elongation at break, as discussed previously.

The negative effect of cassava starch concentration on films elongation at break can possibly be attributed to the amylopectin fraction, which for the cassava starch utilized in this study, represents 84%. Amylopectin is a very branched molecule (BeMiller & Whistler, 1996), which could lower the flexibility of the polymer network, consequently reducing films elongation at break.

For the sucrose block, the Pareto chart of standardized effect also indicated significant ($p<0.05$) decrease in films tensile strength when adding sucrose, propylene glycol, sodium phosphate and soybean oil to the sucrose block (S) samples.

For the inverted sugar block (I) samples, the Pareto chart of standardized effect has indicated a significant ($p<0.05$) decrease in films tensile strength when adding the same additives (soybean oil, sodium phosphate, propylene glycol) and also inverted sugar and gelatin. Also indicated a significant ($p<0.05$) increase in films tensile strength when increasing cassava starch concentration.

According to Coupland, Shaw, Monahan, O'Riordan & O'Sullivan (2000), an increase in plasticizer content can result in lower tensile strength resistance, what could explain the decrease in tensile strength when adding sucrose (Aranitoyannis, Psomiadou & Nakayama, 1996), propylene glycol (Kim, Ko & Park, 2002) and soybean oil (Garcia, Martino & Zaritzki, 2000). Veiga-Santos, Oliveira, Cereda, Alves & Scamparini (2004) has already reported lower tensile strength cassava starch films when adding sucrose, propylene glycol and soybean oil.

The negative effect on tensile strength observed when adding inverted sugar, propylene glycol (Kim, Ko & Park, 2002), soybean oil (Garcia, Martino & Zaritzki, 2000) and gelatin (Arvanitoyannis, Nakayama, Aiba, 1998) can also be attributed to the plasticization effect of such additives. Propylene glycol and soybean oil negative effect on cassava-starch films tensile strength have already been reported (Veiga-Santos, Oliveira, Cereda, Alves & Scamparini, 2004).

Sodium phosphate negative effects on tensile strength can be attributed to interactions between sodium phosphate and starch that can produce monoester phosphates. The monoester starch presents lower intermolecular association when comparing to native starch (BeMiller & Whistler, 1996), which could result in networks with lower tensile strength. Henrique (2002) also observed lower tensile strength for esterifyed cassava starch films, when comparing to films formulated with native cassava starch.

Although the decrease size of tensile strength (ΔT) (Table 3) has indicated that all the additives and process parameters studied resulted in films with lower tensile strength resistance, the enormous increase size observed on elongation at break (ΔE) when using sucrose and inverted sugar (Table 3) justify the continuance of studying such additives.

Water Activity

Perishable product shelf life is dependent on preventing degradation by biochemical reactions and/or microbial growth. Such degradation mechanisms can generally be inhibited by low water activity (a_w) contents (Mathlouthi, 2001).

Samples have presented a_w content between 0.53 and 0.89 (Table 4), thus being considered intermediate moisture products, which are susceptible to microorganism growth and chemical reactions (Belitz & Grosch, 1999a).

Table 4. Water activity (a_w) measurement values, increase size on water activity in relation to the control (Δa_w%), initial contact angle (CA) and slope (°/min) for the sucrose (S) and inverted sugar (I) blocks.

	Sucrose Block (X)				**Inverted Sugar Block (DX)**			
assays	a_w	Δa_w	**CA (°)**	**Slope**	a_w	Δa_w	**CA (°)**	**Slope**
control	0.507	*na***	70.10	-4.79				
1	0.576	*13.54*	69.50	-5.18	0.576	*13.54*	70.90	-4.93
2	0.721	*42.21*	77.10	-1.81	0.694	*36.88*	77.10	-2.02
3	0.886	*74.69*	71.20	-8.55	0.680	*34.12*	71.20	-7,98
4	0.579	*14.20*	75.80	-10.97	0.579	*14.20*	75.80	-10.47
5	0.584	*15.19*	85.90	-7.40	0.584	*15.19*	85.90	-6,77
6	0.599	*18.15*	70.70	-11.75	0.599	*18.15*	70.70	-12.32
7	0.579	*14.20*	93.60	-19.03	0.579	*14.20*	93.60	-17.49
8	0.535	*5.52*	76.10	-12.85	0.535	*5.52*	76.10	-11.71
9	0.749	*47.73*	77.70	-5.57	0.749	*47.73*	73.30	-4.71
10	0.751	*48.13*	57.70	-3.33	0.753	*48.52*	42.30	-0.98
11	0.744	*46.75*	87.40	-7.03	0.761	*50.10*	57.40	-5.28
12	0.759	*49.70*	80.10	-10.72	0.699	*37.87*	86.30	-7.17
13	0.806	*58.97*	64.00	-5.97	0.755	*48.92*	56.50	0.98
14	0.757	*49.31*	82.20	-9.09	0.750	*47.93*	69.20	-10.28
15	0.739	*45.83*	61.30	-9.13	0.701	*38.26*	74.30	-2.52
16	0.773	*52.47*	78.80	-5.83	0.773	*52.47*	73.90	-15.65
17 (c)*	0.744	*46.75*	50.60	2.07	0.709	*39.84*	69.00	-3.65
18 (c)*	0.699	*37.87*	70.60	0.89	0.725	*43.00*	83.50	-3.40
19 (c)*	0.738	*45.56*	59.90	3.23	0.733	*44.58*	82.80	-6.20
R^2***	0.77	*na***	0.50	0.38	0.81	*na***	0.58	0.68

(c)*: central points. *na***: not applicable. R^2***: coefficient of determination.

Sucrose block (S) and inverted sugar block (I) samples water activity increased ($p<0.05$) with increasing sucrose addition. Inverted sugar block (I) samples water activity also increased ($p<0.05$) with increasing propylene glycol concentration.

Although sucrose is known as a water activity depressor (Mathlouthi, 2001), the opposite was observed in this study. Sucrose increased the material a_w (Table 4). A possible explanation is the plasticizing effect of sucrose on the film network structure (Mitchell, 1998), mainly related to the creation of highly mobile regions, which allow a even more pronounced water uptake (Cheryan, Gennadios, Woller & Chinachoti, 1995). The plasticizing character of propylene glycol (Kim, Ko & Park, 2002) and inverted sugar can also be the explanation for their significant effect on film a_w.

Observing the central point samples from both blocks (S-17 to S19 and I-17 to I-19), it can be noticed a slight decrease in a_w values when adding inverted sugar when comparing to sucrose (Table 4). A possible explanation is the humectancy capacity of such additive. Sucrose, that has humectancy (a_w depressor ability) equal to 1, may be less humectant than inverted sugar, which has greater depressor ability (1.4) (Mathlouthi, 2001).

Considering the biodegradable nature of the material and the presence of soybean oil, microorganism growth and lipid oxidation may have the most concerning effect on material shelf life. The soybean oil insaturation, combined with the film a_w contents (> 0.53%), indicates that the material is susceptible to a fast rate of lipid oxidation (Nawar, 1996; Belitz & Grosch, 1999a). Microbial growth can be expected, however mold growths are more likely to be observed (which have a optimum a_w range from 0.80 to 0.87%) (Fennema & Tannenbaum, 1996). Thus, films added with sucrose, inverted sugar or propylene glycol are susceptible for microbial, especially molds, growth during storage.

Contact Angle Measurement.

The films hydrophilicity was quantitatively illustrated by the initial contact angle and the water absorption kinetics were illustrated by measurements of the slope at the origin (º/min) (Table 4).

All samples presented a very quickly absorption, probably due to the hydrophilic nature of the cassava starch films (Avérous, Moro, Dole & Fringant, 2000) and additives (with exception of soybean oil). Soybean oil, which has a hydrophobic character, did not influence ($p<0.05$) water absorption during time. Such result can be explained by the low soybean oil concentration (< 0.06%) used in order to completely incorporate the lipid additive to the film network. Thus, indicating that higher soybean oil concentrations or an emulsifier agent should be used in attempt to affect cassava starch film hydrophilicity.

The Pareto chart of standardized effect, have indicated that no additive or pH modification affected ($p<0.05$) the material hydrophilicity (evaluated by the initial contact angel measurement).

However, the Pareto analyses also indicated that, for the inverted sugar block (I) samples, the water absorption kinetic (during 5 min) increased ($p<0.05$) with inverted sugar addition and acidic pH. Also indicated that the water absorption kinetic decreased with cassava starch concentration. For the sucrose block (S) samples, water absorption kinetic increased ($p<0.05$) with sucrose, propylene glycol and acidic pH and decreased ($p<0.05$) with cassava starch concentration. Such results indicate that inverted sugar, sucrose and propylene glycol increased the water absorption kinetic while cassava starch decreased the absorption kinetic.

Plasticizer addition, such as inverted sugar, sucrose and propylene glycol, reduces chain-to-chain interactions (Sothornvit & Krochta, 2001). Such interaction reducing could result in opened structures, which may be an explanation for the hydrophilicity increase effect when using such additives.

The acidic pH could have inverted sucrose in glucose and fructose. However, as both sucrose and inverted sugar block water absorption were affected by acidic conditions in similar manner, the observed increase was due to starch depolimerization. Hydrolysis of the glycosidic bounds may occur, resulting in smaller fragments (BeMiller & Whistler, 1996). Such hydrolysis could result in void spaces between the film network, which can explain water absorption increase in acidic pH.

Bialopiotrowicz (2003) also observed hydrophilicity decrease when increasing starch concentration. According to the author, starch gel surface attempted to maintain maximal hydrophobic character with polar domains formed by functional groups of D-glucose units. Increasing starch concentration may increase the films surface hydrophobicity, which could decrease the water absorption kinetic of the material.

The coefficient of determination for the water absorption kinetic of the sucrose block (S) samples was very low (R^2=0.38) and may not truthfully explain the main effects observed.

CONCLUSIONS

Additives and pH have affected cassava starch films characteristics. The greater effect was observed on elongation at break when adding sucrose and inverted sugar, however sucrose had an even more pronounced effect. Sucrose and inverted sugar also increased the material water activity. Plasticizer addition and acidic pH increased cassava starch film water absorption kinetic, while the increase on cassava starch concentration had the opposite effect.

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CAPÍTULO 6

Sucrose and inverted sugar Part 2: Effect on biodegradable cassava starch-gelatin films microstructure and color

Sucrose and inverted sugar Part 2: Effect on biodegradable cassava starch-gelatin films microstructure and color

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The influence of the additives sucrose and inverted sugar on microstructure and color of cassava starch-gelatin films has been studied. Other constituents (soybean oil, sodium phosphate and propylene glycol) and pH effect have also been investigated for the same parameters. X-ray diffraction and microscopic analysis have demonstrated that sucrose addition influenced ($p<0.05$) films crystallinity during storage. When substituting sucrose for inverted sugar, no crystallization was observed. However, the presence of inverted sugar resulted in semi-crystalline materials due to starch molecular re-organization. The only color parameter that was affected by additives and pH modifications was yellowness.

KEYWORDS: Biofilms, Microstructure, Starch, Additives, Color

INTRODUCTION

The repeating units of sucrose atoms and the rearrangement of such units makes up the crystal. Sucrose crystallization does not occur in the stable zone; however, it invariably occurs in the labile zone and is dependent on the rate of formation of the crystals nuclei and the subsequent speed of growth on these nuclei (Lees, 1995).

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Sucrose crystals have seven hydrogen bonds between each molecule. If each hydrogen is linked to a water molecule, than nucleation will not occur. However, when there is not enough water present to occupy all the hydrogen bonding sites, nucleation may occur (Stansell, 1995). The drying step of the casting technique utilized to produce the films can lower water into levels in which the resulting sucrose will have free hydrogen bounding sites, allowing crystallization.

Sucrose, which is a disaccharide, can be broken down into a 1:1 mixture of two monosaccharides, dextrose (glucose) and laevulose (fructose), known as inverted sugar (James, 1995). Inverted sugar has a lower crystallinity tendency when comparing to sucrose (Lees, 1995).

Previous study of this serie has indicated that sucrose, which has a low coast comparing to polyols, had a great efficacy in increasing cassava starch films elongation at break (Veiga-Santos, Oliveira, Cereda, Alves & Scamparini, 2004), although Veiga, Scamparini, Tomiyama, Suzuki & Cereda (2002) have reported that sucrose addition resulted in films crystallization during storage.

With the aim to evaluate the influence of sucrose and inverted sugar on cassava starch films crystallinity and color, X-ray diffraction; polarized light and scanning electron microscopy (SEM) and color were evaluated during the films storage. The effect of other additives and pH modifications was also investigated.

MATERIAL AND METHODS

Materials.

Commercial cassava starch (Flor de Lotus, Brazil), inverted sugar cane (Copersucar União, Brazil), gelatin type B (Liner Davis Gelatin, Brazil), soybean oil (Cargill Agricola S.A., Brazil) and analytically pure sucrose, propylene glycol and monobasic sodium phosphate (Sinth, Brazil).

Sample Preparation.

Cassava starch (3-5%) was blended with the additives: sucrose or inverted sugar (0-2%), propylene glycol (0-1%), sodium phosphate (0-0.2%) and soybean oil

(0-0.06%). Gelatin (0-1%) was dispersed in distilled water (100°C), cooled to 40°C and added to the previous blended ingredients. It was considered the total weight of the film forming suspension. pH was adjusted (4-8) with 50% citric acid solution or 5% sodium hydroxide solution and heated to 75°C for starch jellification with constant stirring. The cassava starch-gelatin films were prepared according to the casting technique, by dehydration under renewable circulated air (30°± 2°C) over Petri plastic dishes. Films containing only cassava starch (no additives or pH adjustment) served as the control. Samples were storage (23°C, 75%RH) for at least 4 days prior to testing.

Crystallinity:

The relative crystallinity of the films was investigated with wide-angle X-ray diffraction (WAXS). Measurements were carried out using a DMAX-2200 Rigaku International Corporation $\theta/2\theta$ diffractometer, operating with voltage 40 kV and amperage 20mA, with CuKα radiation. Samples were fixed at an aluminum sample holder, and analysed from 5 to 60° (2θ), with angular step of 0.1° (2θ), and sampling interval time of 3 seconds. The films were analysed at each 15 days, during a storage period of 60 days (75% RH, 23°C), in duplicate. Crystallinity was also investigated (in the same conditions) for the raw materials cassava starch and sucrose in order to obtain crystallinity standards.

Polarized Light Microscopy:

Samples surface was observed by LEICA-DMLP light microscope, with no further preparation, by transmitted polarized or ordinary (not polarized) light. Images of 200x-magnification were collected with a CCD camera.

Scanning Electron Microscopy (SEM).

SEM digital images of the samples surface and fracture were obtained by Jeol JSM-5900LV scanning electron microscope. Cross section images were obtained by cryogenic fracture of the films, using liquid nitrogen. Samples were sputtered with a 16μg gold layer. Images were taken at 5-10KV, spotsize 28-30, 1000x-magnification.

Color.

The color of preconditioned (75% RH, 23°C) samples was analysed in duplicate by total transmittance, using a Color Quest II-Hunter lab equipment, CIELAB Ttran D65, lecture angle 10°, measuring area 2.54cm^2 (1.0 square inch). Four measurements taken at the different quadrants of each sample were averaged and expressed as Hunter system "L" (lightness), "a" (redness) and "b" (yellowness) values. Total color difference (ΔE) was evaluated as the size of color difference between the control and the experimental design samples, and calculated by equation 1 (Francis, 1983, cited by Hong & Park, 2000):

$$\Delta E = \left[(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2\right]^{\frac{1}{2}} \qquad \textbf{Equation 1}$$

Statistical analysis.

Two blocks of a 2^{7-3} experimental factorial design were performed in order to select additives and process parameters that significativelly ($p<0.05$) influenced cassava starch film microstructure and color. The blocks were designed with three central points, totalizing 19 experiments per block, differing only for one variable (sucrose or inverted sugar concentration). The other variables were additives (gelatin, soybean oil, sodium phosphate and propylene glycol), cassava starch concentration, and pH (Table 1). The independent variables significant ($p<0.05$) influence was evaluated by the Pareto chart of standardized effect, considering the ANOVA pure error. The experimental data were generated and analysed using the software Statistica for Windows, version 5.0.

Table 1. Independent variables at different levels for the sucrose (S) and inverted sugar (I) factorial experiment blocks, considering the total weight of the film forming suspension.

Symbol	**Variables**		***Levels***		
	Block-S	**Block-I**	***-1***	***0***	***+1***
A	Sodium phosphate (%)	Sodium phosphate (%)	*0.00*	*0.35*	*0.70*
B	pH	pH	*4.00*	*6.00*	*8.00*
C	Cassava starch (%)	Cassava starch (%)	*3.00*	*4.00*	*5.00*
D	Sucrose (%)	Inverted Sugar (%)	*0.00*	*1.00*	*2.00*
E	Propylene Glycol (%)	Propylene Glycol (%)	*0.00*	*0.25*	*0.50*
F	Soybean oil (%)	Soybean oil (%)	*0.00*	*0.03*	*0.06*
G	Gelatin (%)	Gelatin (%)	*0.00*	*0.35*	*0.70*

The experimental design demonstrated in Table 2 was used to estimate the main effects of independent variables.

Table 2. Factorial 2^{7-3} design with coded and real values for the sucrose (S) and inverted sugar (I) blocks.

Assays		**Variables**						
Block S	**Block I**	***A***	***B***	***C***	***D***	***E***	***F***	***G***
S-1	I-1	*-1* (0.00)	*-1* (4.0)	*-1* (3.0)	*-1* (0.0)	*-1* (0.00)	*-1* (0.00)	*-1* (0.00)
S-2	I-2	*+1* (0.70)	*-1* (4.0)	*-1* (3.0)	*-1* (0.0)	*+1* (0.50)	*-1* (0.00)	*+1* (0.70)
S-3	I-3	*-1* (0.00)	*+1* (8.0)	*-1* (3.0)	*-1* (0.0)	*+1* (0.50)	*+1* (0.06)	*-1* (0.00)
S-4	I-4	*+1* (0.70)	*+1* (8.0)	*-1* (3.0)	*-1* (0.0)	*-1* (0.00)	*+1* (0.06)	*+1* (0.70)
S-5	I-5	*-1* (0.00)	*-1* (4.0)	*+1* (5.0)	*-1* (0.0)	*+1* (0.50)	*+1* (0.06)	*+1* (0.70)
S-6	I-6	*+1* (0.70)	*-1* (4.0)	*+1* (5.0)	*-1* (0.0)	*-1* (0.00)	*+1* (0.06)	*-1* (0.00)
S-7	I-7	*-1* (0.00)	*+1* (8.0)	*+1* (5.0)	*-1* (0.0)	*-1* (0.00)	*-1* (0.00)	*+1* (0.70)
S-8	I-8	*+1* (0.70)	*+1* (8.0)	*+1* (5.0)	*-1* (0.0)	*+1* (0.50)	*-1* (0.00)	*-1* (0.00)
S-9	I-9	*-1* (0.00)	*-1* (4.0)	*-1* (3.0)	*+1* (2.0)	*-1* (0.00)	*+1* (0.06)	*+1* (0.70)
S-10	I-10	*+1* (0.70)	*-1* (4.0)	*-1* (3.0)	*+1* (2.0)	***+1* (0.50)**	*+1* (0.06)	*-1* (0.00)
S-11	I-11	*-1 (0.00)*	*+1* (8.0)	*-1* (3.0)	*+1* (2.0)	***+1* (0.50)**	*-1* (0.00)	*+1* (0.70)
S-12	I-12	*+1* (0.70)	*+1* (8.0)	*-1* (3.0)	*+1* (2.0)	*-1* (0.00)	*-1* (0.00)	*-1* (0.00)
S-13	I-13	*-1* (0.00)	*-1* (4.0)	*+1* (5.0)	*+1* (2.0)	***+1* (0.50)**	*-1* (0.00)	*-1* (0.00)
S-14	I-14	*+1* (0.70)	*-1* (4.0)	*+1* (5.0)	*+1* (2.0)	*-1* (0.00)	*-1* (0.00)	*+1* (0.70)
S-15	I-15	*-1* (0.00)	*+1* (8.0)	*+1* (5.0)	*+1* (2.0)	*-1* (0.00)	*+1* (0.06)	*-1* (0.00)
S-16	I-16	*+1* (0.70)	*+1* (8.0)	*+1* (5.0)	*+1* (2.0)	***+1* (0.50)**	*+1* (0.06)	*+1* (0.70)
S-17(c)*	I-17(c)*	*0* (0.35)	*0* (6.0)	*0* (4.0)	*0* (1.0)	*0* (0.25)	*0* (0.03)	*0* (0.35)
S-18(c)*	I-18(c)*	*0* (0.35)	*0* (6.0)	*0* (4.0)	*0* (1.0)	*0* (0.25)	*0* (0.03)	*0* (0.35)
S-19(c)*	I-19(c)*	*0* (0.35)	*0* (6.0)	*0* (4.0)	*0* (1.0)	*0* (0.25)	*0* (0.03)	*0* (0.35)

(c)*: central points.

RESULTS AND DISCUSSION

Cassava starch-gelatin films WAXS crystallinity diffractograms were collected during 60 days of storage. At the first day of storage, all samples from the sucrose and the inverted sugar blocks presented an amorphous behavior (Figure 1a,1b, 1c, 1d ,1e and 1f).

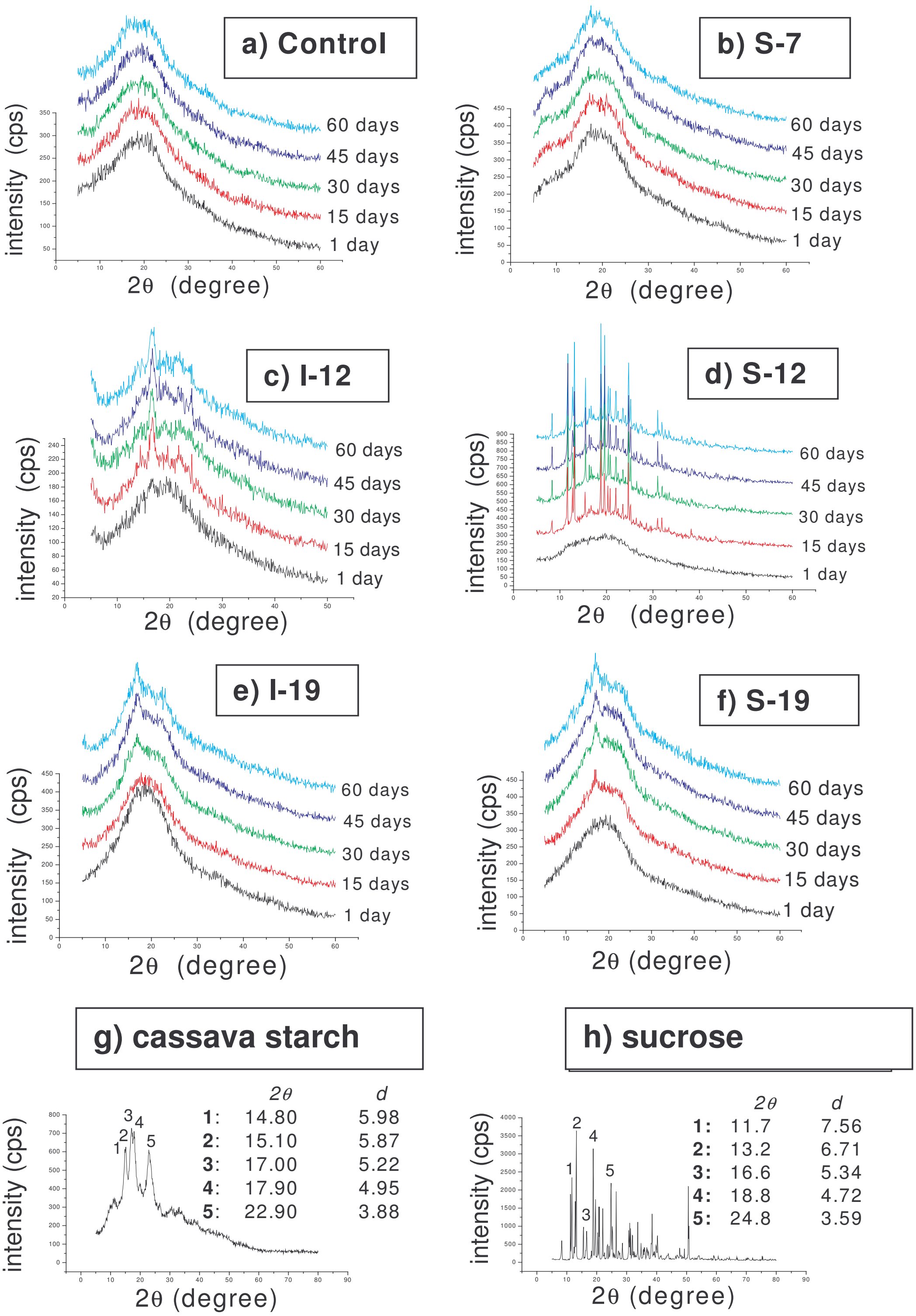

Figure 1. WAXS crystallinity diffractograms of samples *amorphous*: (a) control, (b) S-7; *semi-crystalline:* (c) I-12, (e) I-19, and *crystalline*: (d) S-12, (f) S-19 behavior during 60 days of storage. WAXS crystallinity diffractograms of (g) native cassava starch and (h) sucrose standards.

Amorphous behavior was also observed during storage of samples formulated without sucrose (S-1 to S-8) or inverted sugar (I-1 to I-8), as exemplified by the samples control (formulated only with cassava starch, no additives) and S-7 (added only with gelatin) (Figure 1a, 1b). Veiga, Scamparini, Tomiyama, Suzuki & Cereda (2002) have already observed an amorphous behavior during cassava starch film storage. Despite of the native cassava starch semi-crystalline structure, characterized for crystallites superposed to an amorphous halo (Blennow, Bay-Smidt, Olsen, & Moller, 2000) (Figure 1g), the amorphous behavior observed at the beginning of the film storage could be explained by crystallites instability due to the jellification step used for film preparation. According to Jenkins & Donald (1998), the jellification temperature could decrease the crystallization ratio until zero, resulting in an amorphous structure.

The low casting temperature (30°C) can also be responsible for the amorphous structure, once, according to Rindlav, Hulleman & Gatenholm (1997), casting temperatures bellow 50°C result in amorphous behavior for starch films.

However, during storage, samples added with sucrose (S-9 to S19) presented a crystalline behavior. The crystalline behavior was represented by samples S-12 (added with sodium phosphate and sucrose) and S-19 (added with intermediate concentration of all additives) (Figure 1d and 1e). Such phenomenon can be attributed to sucrose crystallization, which starts with a crystallinity nucleus (Jackson & Howling, 1995). When in supersaturated conditions, spontaneous nucleation can occur. Nucleation and crystal formation could arise from rearrangement of randomly bound sugar molecules (Stansell, 1995).

Sucrose crystallinity in films has already been reported (Veiga, Scamparini, Tomiyama, Suzuki & Cereda, 2002) and the peaks observed during the storage of samples containing sucrose (S-9 to S19) matches to sucrose standard diffractograms peaks (Figure 1h and Table 3).

Table 3. 2θ angle and *d*-spacing (*d*) matches for main peaks presented by crystalline (S-9 to S19) and semi-crystalline (I-9 to I-19) samples WAXS diffractograms for the sucrose (S) and inverted sugar (I) blocks.

	2θ angle (interplane distance- d)					**dc****
Cassava Starch Standard	*n.o.*****	17.0(5.22)	*n.o.*****	22.9(3.88)	*n.o.*****	*na****
Sucrose Standard	16.6(5.34)	*n.o.*****	18.8(4.72)	*n.o.*****	24.8(3.59)	*na****
Sucrose Block (S)						
S-9	16.7(5.30)	*n.o.*****	18.8(4.71)	*n.o.*****	*n.o.*****	15
S-10	16.7(5.31)	*n.o.*****	18.8(4.72)	*n.o.*****	24.7(3.60)	15
S-11	16.7(5.31)	*n.o.*****	*n.o.*****	*n.o.*****	*n.o.*****	30
S-12	16.6(5.34)	*n.o.*****	18.8(4.72)	*n.o.*****	24.7(3.60)	15
S-13	16.7(5.31)	*n.o.*****	*n.o.*****	*n.o.*****	*n.o.*****	45
S-14	16,7(5.31)	*n.o.*****	*n.o.*****	*n.o.*****	*n.o.*****	15
S-15	16.6(5.34)	*n.o.*****	*n.o.*****	*n.o.*****	*n.o.*****	30
S-16	16.7(5.31)	*n.o.*****	*n.o.*****	*n.o.*****	*n.o.*****	15
S-17	16.6(5.34)	*n.o.*****	*n.o.*****	*n.o.*****	*n.o.*****	15
S-18	16.7(5.31)	*n.o.*****	*n.o.*****	*n.o.*****	*n.o.*****	15
S-19	16.7(5.31)	*n.o.*****	*n.o.*****	*n.o.*****	*n.o.*****	30
Inverted Sugar Block (I)						
I-9	*n.o.*****	17.0(5.21)	*n.o.*****	*n.o.*****	*n.o.*****	15
I-10	*n.o.*****	16.9(5.24)	*n.o.*****	*n.o.*****	*n.o.*****	15
I-11	*n.o.*****	17.0(5.21)	*n.o.*****	22.2(3.99)	*n.o.*****	15
I-12	*n.o.*****	16.9(5.24)	*n.o.*****	*n.o.*****	*n.o.*****	15
I-13	*n.o.*****	16.9(5.24)	*n.o.*****	*n.o.*****	*n.o.*****	15
I-14	*n.o.*****	17.0(5.21)	*n.o.*****	*n.o.*****	*n.o.*****	15
I-15	*n.o.*****	16.9(5.24)	*n.o.*****	*n.o.*****	*n.o.*****	15
I-16	*n.o.*****	16.9(5.24)	*n.o.*****	*n.o.*****	*n.o.*****	15
I-17	*n.o.*****	17.1(5.19)	*n.o.*****	*n.o.*****	*n.o.*****	30
I-18	*n.o.*****	17.0(5.22)	*n.o.*****	*n.o.*****	*n.o.*****	30
I-19	*n.o.*****	17.1(5.19)	*n.o.*****	22.2(3.99)	*n.o.*****	15

(c): central points. dc**: days that samples remained amorphous. na***: not applicable. n.o.****: non observed.*

Statistical Pareto analyses were performed to evaluate the additives and pH influence on the time required for crystallization beginning. For that matter, it was considered the total of days that the material remained amorphous (Table 3). In order to present values for the statistical calculations, for the films that did not present crystallinity, the days that the material remained amorphous were considered the total storage period evaluated in this study (60 days).

The Pareto chart of standardized effect confirmed that sucrose positively affected ($p<0.05$) films crystallinity (R^2: 0.73) during storage of the sucrose block (S) samples.

Figures 1a, 1b, 1c, 1e, 1f and Table 3 have also indicated that when substituting sucrose for inverted sugar, no crystallization were developed on the material structure during the storage period, although a semi-crystalline structure was observed. Such result was expected due to the lower crystallinity tendency of inverted sugar when comparing to the sucrose crystallinity tendency (Lees, 1995). The inverted sugar can increase the film forming suspension viscosity, difficulting crystals approach (Jackson, & Howling, 1995). Semi crystalline behavior was represented by samples I-12 (added with sodium phosphate and inverted sugar) and I-19 (added with intermediate concentration of all additives) (Figure 1c and 1d).

The film semi-crystalline behavior can be attributed to a reorganization of the cassava starch molecules, once the peaks observed in the inverted sugar films match to the native cassava starch diffraction standard, as can be observed when comparing Figures 1c and 1d to Figure 1g and Table 3.

The degree of starch crystallinity depends on the water content of the material (Zobel & Stephen, 1995). Rindlav, Hulleman & Gatenholm (1997) have observed that crystallinity appear during higher water content film storage. If the equilibrium relative humidity (ERH) of a sugar is low, the product will attract humidity. As inverted sugar has a lower ERH than sucrose (180.2 and 342.3, respectively) (Jackson & Howling, 1995), its utilization as additive could result in networks with more free water, which could lead to cassava starch re-crystallization.

The Pareto chart of standardized effect confirmed that inverted sugar positively affected ($p<0.05$) the films crystallinity during storage for the inverted sugar block (I) (R^2: 0.94).

Scanning Electron Microscopy

Samples surface and cross section SEM micrographs were recorded at 5 and 60 (± 4) days of storage. At the first week storage period, all samples presented a compact and smooth structure, both for surface and cross section, as exemplified by the control and samples I-12 (added with sodium phosphate and inverted sugar) and S-12 (added with sodium phosphate and sucrose) (Figure 2).

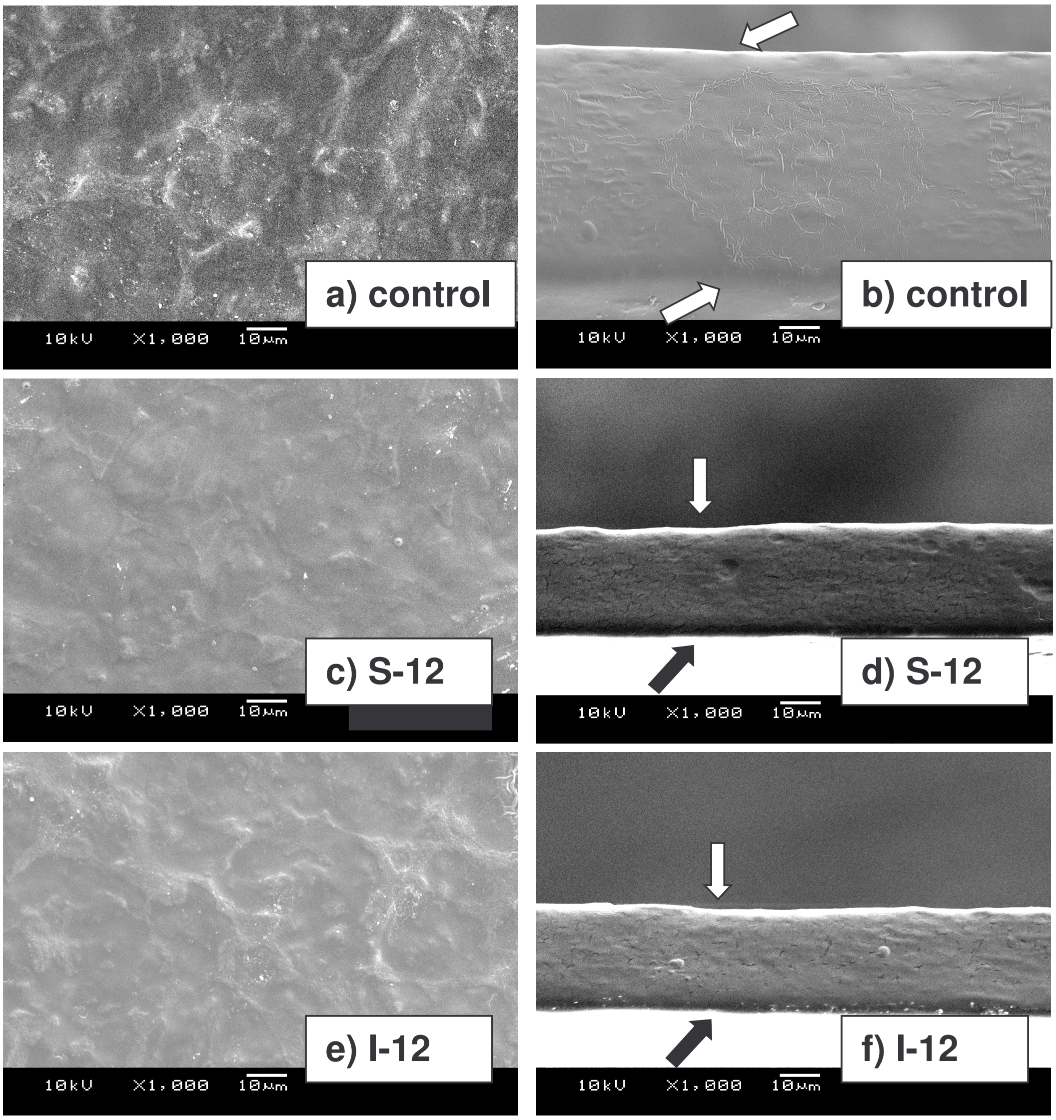

Figure 2. Scanning electron micrographs of samples *surface:* (a) control, (c) S-12, e) I-12 and samples *cross-section*: (b) control, (d) S-12, (f) I-12; at the first week of storage.

However, after 60 days of storage, samples containing sucrose as additive, presented a visible crystallinity, as exemplified by sample S-12 surface and cross-section SEM micrographs (Figure 3).

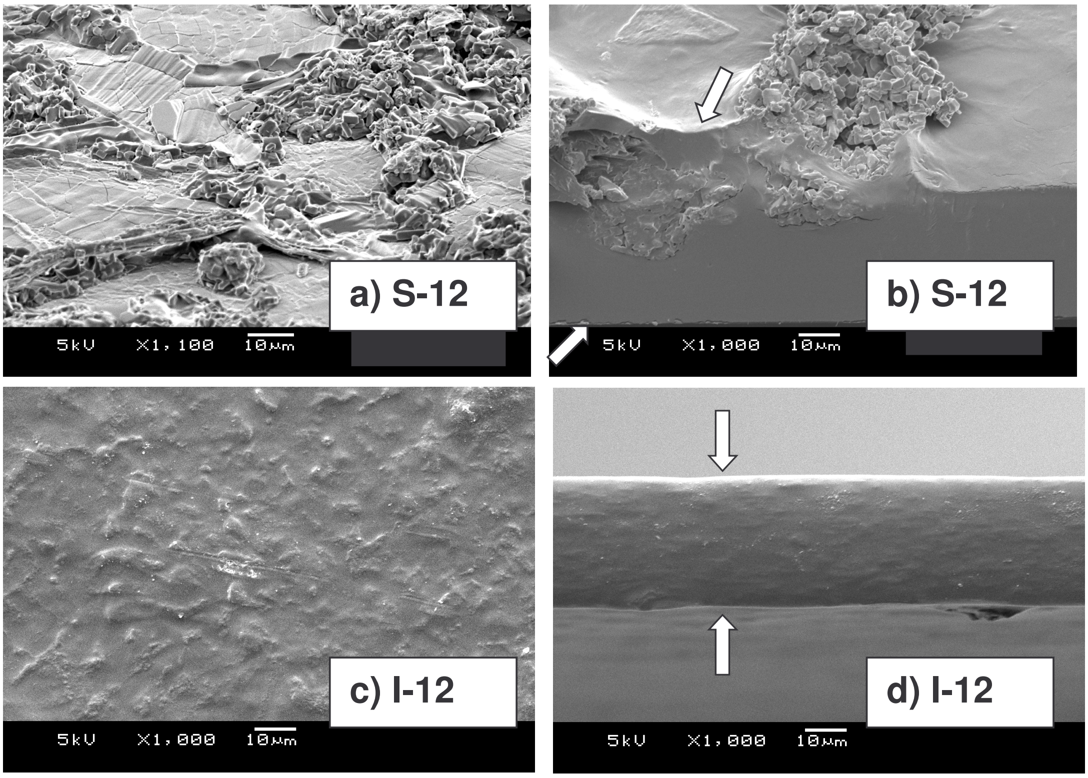

Figure 3. Scanning electron micrographs of samples *surface:* (a) S-12, c) I-12 and *cross-section*: (b) S-12, (f) I-12; after 60 days of storage.

By the micrographs presented in Figure 3, it can also be observed that the semi-crystalline samples did not present visible crystals. Such result reinforces the theory that the semi-crystalline behavior is due to cassava starch reorganization.

Polarized Light Microscopy

Although not often utilized for analyzing biodegradable structure, light microscopy had demonstrated to be a very important tool in characterizing sucrose crystallization on starch based films (Veiga-Santos, Suzuki, Cereda & Scamparini, 2004).

In this study, light micrograph was utilized for characterizing the material crystallization during 60 days of storage. Analyzing the light micrograph of sample S-12 (added only with sucrose and sodium phosphate) with ordinary (not polarized) light it can clearly be observed crystallization at the material surface (Figure 4a). It was also utilized polarized light to evaluate the material crystallinity patterns (Figure 4b).

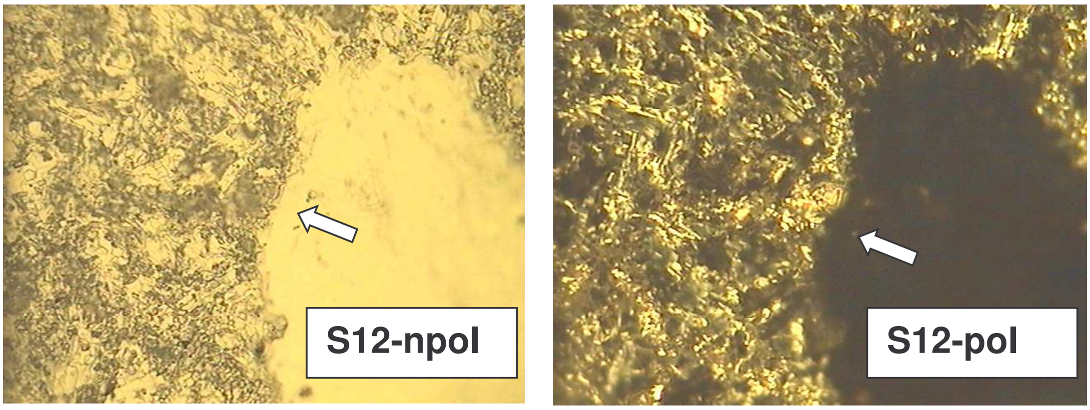

Figure 4. Samples light micrographs using ordinary light (not polarized – “npol”): (a) S-12 and polarized light (“pol”): (b) S-12; after 60 days of storage.

Sucrose crystallization is exemplified by sample S-12 (added with sodium phosphate and sucrose) and is clearly observed by ordinary light micrographs (Figure 4a) and confirmed by polarized light micrographs (Figure 4b). Crystalline materials have birefringence and are detected by polarized light. If the material had no crystalline structure, the field of view would have appeared completely black (Greaves & Saville, 1995).

Color

Samples color was expressed as Hunter system “L” (lightness), “a” (redness) and “b” (yellowness) values, as shown in Table 4.

Table 4. Samples Hunter color parameters “L”, “a”, “b” and the total color difference between samples and the control (ΔE).

assays	Sucrose Block (S)				Inverted Sugar Block (IS)			
	Color “L”	**Color “a”**	**Color “b”**	**ΔE (%)**	**Color “L”**	**Color “a”**	**Color “b”**	**ΔE (%)**
control	98.3	0.25	3.37	*na***				
1	98.15	0.19	3.25	0.17	98.26	0.24	3.38	0.02
2	98.34	0.26	3.55	0.20	98.34	0.26	3.55	0.20
3	98.72	0.27	3.78	0.62	98.72	0.27	3.78	0.62
4	98.12	0.19	3.40	0.15	98.12	0.19	3.40	0.15
5	98.45	0.35	4.16	0.82	98.43	0.35	4.16	0.82
6	98.40	0.09	2.91	0.51	98.40	0.09	2.91	0.51
7	97.99	0.22	4.05	0.73	98.01	0.22	4.05	0.72
8	98.45	0.01	2.78	0.67	98.45	0.01	2.78	0.67
9	98.35	0.20	3.59	0.25	98.31	0.23	3.59	0.23
10	98.39	0.11	3.13	0.31	98.43	0.06	2.47	0.94
11	98.14	0.19	3.48	0.17	98.87	0.38	4.39	1.20
12	98.30	0.00	2.45	0.95	98.69	0.35	4.80	1.50
13	98.48	0.07	2.29	1.12	98.14	0.11	3.07	0.35
14	97.96	0.23	3.88	0.59	98.00	0.35	3.26	0.29
15	98.39	0.12	2.65	0.74	98.55	0.38	3.73	0.49
16	98.23	-0.01	3.59	0.34	98.23	0.35	3.68	0.33
17 (c)*	98.46	0.21	3.42	0.22	98.42	0.22	3.40	0.18
18 (c)*	98.67	0.25	3.72	0.55	98.32	0.23	3.65	0.29
19 (c)*	98.70	0.30	3.91	0.70	98.56	0.29	3.69	0.45
*R^2****	*0.53*	*0.41*	*0.66*	*0.53*	*0.50*	*0.44*	*0.52*	*0.29*

(c)*: central points; na**: not applicable; R^2***: coefficient of determination.

All samples presented high lightness (> 98.15%) and low color values for “a” redness (< 0.38) and “b” yellowness (< 4.80), as can be observed in Table 4. When compared to the control (L=98.3; a=0.25; b=3.37), samples presented similar results, indicating that the additives and pH modifications investigated in this study have little effect on the cassava starch films color parameters and that all materials were almost colorless, with a high brilliancy.

The Pareto chart of standardized effect has indicated that samples lightness (“L”) and redness (“a”), for both blocks, were not affected ($p<0.05$) by any additive or pH modifications evaluated in this study. However, it was observed that yellowness (“b”) of the sucrose block (S) increased ($p<0.05$) with gelatin addition and alkaline pH, and decreased with sodium phosphate addition (R2=0.52). For the inverted sugar (I) block samples, yellowness increased ($p<0.05$) with increasing gelatin concentration ($R^2=0.66$).

The yellowness observed when adding gelatin can be attributed to the characteristic color of this raw material, which according to Poppe (1997), can vary form pale yellow to brown. Also the characteristic color of the NaOH solution, used to modify the alkaline pH, can explain the increase on samples yellowness. Gontard, Guilbert & Cuq (1992) also observed a slightly yellow color increase when adding sodium hydroxide in wheat gluten films.

The decrease in yellowness with increasing sodium phosphate may be due to a sodium phosphate-cassava starch interaction. Ester linkages can be form between sodium phosphate and the hydroxyl in the starch chain, resulting in starch phosphate monoesters (Ellinger, 1972). The jellified starch monoester has a higher clarity when compared to the gelatinized normal starch (Belitz & Grosch, 1999), what could affect other color parameters, such as yellowness. Also the characteristic color of the monobasic sodium phosphate may have reduced the materials yellowness.

The total color difference between samples and the control (ΔE), for both blocks (S and I), was not affected ($p<0.05$) by any additive or pH modifications. Such results indicate that in relation to the control, additives and pH (evaluated in this study) did not interfere on the material total color. However, the % variability explained (R^2) considering the "ΔE" as independent variable (0.53 for the sucrose block and 0.29 for the inverted sugar block), was not high enough to adequately explain the experimental data and the effects should be observed with caution.

CONCLUSIONS

Sucrose utilization as additive resulted on cassava starch film crystallization during storage. The substitution of sucrose with inverted sugar successfully avoid the material crystallization. However, a cassava starch molecular re-organization was observed, resulting in semi-crystalline structures. Although, the semi-crystallization did not affect films surface or cross-section structure observed by mycroscopic analyses. Between samples, the only color parameter that was affected by additives or pH modification was yellowness, although all samples presented high lightness and low color values for “a” redness and “b” yellowness, indicating that, independent of the additives or pH modifications, the materials were almost colorless, with a high brilliancy. Additives and pH modifications did not affect the total color difference between samples and the cassava starch films formulated without additives or gelatin.

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CAPÍTULO 7

Mechanical, barrier and thermal properties of cassava starch films: Effect of sucrose, inverted sugar and phosphate

Mechanical, barrier and thermal properties of cassava starch films: Effect of sucrose, inverted sugar and phosphate

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Abstract

The effect of additives (sucrose, inverted sugar and sodium phosphate) added singly and in association at different levels on cassava starch films protection performance were evaluated by response surface methodology. The additives reduced cassava starch films tensile strength and increased elongation at break. No effect was observed on the water vapor permeability. Oxygen permeability was evaluated for the films that presented a good relation between tensile strength and elongation at break. Two glass transition temperatures were observed for all experimental samples, probably indicating phase separation related to the additives. Interaction of the film network with phosphate diminished the total mass loss after an endothermic peak. The exothermic peak was not observed for the control and is probably a crystallization phenomenon related to the additives.

Keywords: *biofilms, response surface analysis, starch, additives.*

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1. Introduction

Starch is considered one of the most promising natural polymers for packaging application (Guilbert, Gontard & Gossis, 1996 and Krochta & Mulder-Johnston, 1997)

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because of their low cost, renewability and biodegradability (Avérous, Fringant & Moro, 2001). However, to such materials become feasible is necessary to overcome their poor mechanical properties and high moisture sensitivity (Martin, Schwach, Avérous, Couturier, 2001). For that matter, additive incorporation (Arvanitoyannis & Biliaderis, 1998; Coupland, Shaw, Monahan, 2000; Garcia, Martino, Zaritzki, 2000) is recommended.

In previous works of this series, when used singly, sucrose and inverted sugar has demonstrated to be excellent plasticizer. Sucrose addition increased elongation at break up to 2970% when comparing to the control (containing only cassava starch). The inverted sugar effect was lower, but also significant. It increased elongation at break up to 1300% when comparing to the control. Although sucrose addition allowed a higher elongation at break increase, both plasticizers decreased cassava starch films tensile strength (up to 90%) (Veiga-Santos, Oliveira, Cereda & Scamparini, 2004).

The simultaneous effect of sucrose and inverted sugar may lead to materials with a good relation between increased elongation at break, without much reduction in tensile strength properties.

It has also been reported the isolate effect of sodium phosphate addition on cassava starch film mechanical properties (Veiga-Santos, Oliveira, Cereda & Scamparini, 2004). Interactions between sodium phosphate and starch can produce monoester phosphates. The monoester starch presents lower intermolecular association when comparing to native starch (BeMiller & Whistler, 1996), which could result in networks with lower tensile strength and higher elongation at break properties.

A previous review indicates that the simultaneous effect of the additives sucrose, inverted sugar and sodium phosphate on mechanical, barrier and thermal properties of biodegradable films have not yet been investigated.

With the aim to evaluate the singly and asociated effect of sucrose, inverted sugar and sodium phosphate addition on cassava starch films, mechanical (tensile strength and elongation %), barrier (to water vapor and oxygen) and thermal (DSC, DMA and TGA) properties were investigated by surface response methodology.

2. Material and Methods

2.1. Materials

Commercial cassava starch (Flor de Lotus, Brazil), sucrose and inverted sugar (Copersucar, Brazil). Analytically pure monobasic sodium phosphate (Sinth, Brazil).

2.2. Sample Preparation

Cassava starch (5%, w/w) was blended with water and additives: sucrose (0.0-1.4%), inverted sugar (0.0-1.4%) and sodium phosphate (0.0-0.6%) considering the total weight of the film forming suspension. The mixture was heated to 75°C (for complete jellification of cassava starch) with constant stirring and placed under ultrasonic bath. The films were prepared according to the casting technique by dehydration under renewable circulated air (30°± 2°C) over Petri plastic dishes. Films containing 5% cassava starch (no additives or pH adjustment) served as the control. Samples were storage (23°C, 75%RH given by a NaCl saturated solution) for at least 4 days prior to testing.

2.2. Mechanical Properties

Films tensile strength and elongation at break were measured using an Instron Universal Testing Instrument (model 5500R) operated according to ASTM standard method D882-00 (ASTM, 2001). Tested filmstrips (8 x 2.5cm) were cut from each preconditioned (75% RH, 23°C) sample and mounted between the grips of the machine. The thickness of each specimen was measured in four points along its length with a flat and parallel surfaces Mitutoyo digital micrometer. Initial grip separation and crosshead speed were set to 50 mm and 12.5 mm/min, respectively. The maximum load (kgf) and extension (mm) were recorded and used to calculate the tensile strength (kgf/cm^2) and elongation at break. Ten specimens were tested for each sample. The experimental design samples tensile strength (ΔT%) and elongation at break (ΔE%) increase or decrease size in relation to the control were calculated by equations 1 and 2, respectively.

$$\Delta T = \left[\left(\frac{sample\ tensile\ strength}{control\ tensile\ strength} \right) \times 100 \right] - 100$$

Equation 1

$$\Delta E = \left[\left(\frac{sample\ elongation}{control\ elongation} \right) \times 100 \right] - 100$$

Equation 2

2.3. Water vapor permeability

The water vapor permeability (WVP) determination was based on ASTM E96-00 (2000). The films were fixed onto opening cells containing silica gel, inside desiccators containing NaCl saturated solution (75% RH). For all the tested samples, a cell containing just the films (no silica gel) were used as a control to estimate the weight modifications due to the humidity adsortion/desoption of the material (Sarantopóulos, Oliveira, Padula, Coltro, Alves & Garcia, 2002) for being a material with water affinity. The cells were weighed daily, during 5 days. The water vapor permeability rate (WVPR) and the water vapor permeability (WVP) were calculated according to equation 3 and 4, respectively, where *x* is the average thickness of the edible films (mm), *A* is the permeation area (m^2), *ps* is the vapor saturation pressure at 25°C, RH_1 is the relative humidity of the dissecator (75%), RH_2 is the relative humidity on the interior of the cells (0%), w/t was calculated by linear regression from the points of weight gain and time, in the constant period. All testes were made in triplicate. The experimental design samples increase or decrease water vapor permeability size (ΔW%) in relation to the control samples were calculated according to equation 5.

$$WVPR = \frac{w}{t}\frac{}{A}$$

Equation 3

$$WVP = \frac{WVPR\ .\ x}{ps\,(RH_1 - RH_2)}$$

Equation 4

$$\Delta W = \left[\left(\frac{sample\ water\ vapor\ permeability}{control\ water\ vapor\ permeability} \right) x\,100 \right] - 100$$

Equation 5

2.4. Oxygen permeability

Oxygen transmission rates through preconditioned (75% RH, 23°C) films were measured with an OX-TRAN 2/20 instrument (Mocon, Inc.) according to ASTM F1927-98 (2001). Testing was performed at oxygen partial pressure gradient of 1atm, 25°C, with controlled relative humidity of 75%. Oxygen transmission rate values (J) (cm^3/m^2.day) were obtained from the instrument. Oxygen permeability values were determined according to equation 6, where P is the oxygen permeability (cm^3/m.day.atm), x is the average thickness of the film (m), and Δp is the partial pressure difference of oxygen across the film (atm) (Felder and Huvard, 1980; cited by Gennadios, Weller & Testin, 1993). The thickness of individual film samples was determined as an average of 4 measurements taken on the testing area.

$$P = \frac{J.x}{\Delta p}$$ **Equation 6**

2.5. Thermal Properties

Differential scanning calorimetric (DSC) and thermal gravimetric analysis (TGA)

Differential scanning calorimetric (DSC) and thermal gravimetric analysis (TGA) measurements were determined with a Simultaneous Thermal Analysis STA 209 Instrument, Netzsch, equipped with a liquid N_2 cooling accessory. 15-20mg preconditioned (75% RH, 23°C) samples were hermetically sealed in aluminum pans to prevent water evaporation during scanning. An empty aluminum pan served as reference. The scan was performed between 20°C and 690ºC, at 10ºC/min rate, 60 mL/min flux with Helium as purge gas, to observe at the same time DSC and TGA (mass loss during heating) analyses. The endothermic and exothermic onset and peak temperatures and ΔH were given by the Netzsch program Proteus Analysis. A differential scanning calorimetric (DSC 2010, TA Instrument) analyse, was also performed between –150ºC and 100ºC, 10ºC/min rate, 10 mL N_2/min, for Tg determinations of 9-13 mg preconditioned (75% RH, 23°C) samples. The thermal effects were recorded during the scans and the glass transition (Tg) temperature was computed as the midpoint of the changes on the base line.

2.6. Statistical analysis

Independent variables (sucrose%, inverted sugar% and sodium phosphate%) effect and their interaction on cassava starch film water vapor permeability, mechanical and thermal properties was evaluated by Response Surface Methodology. A 2^3 experimental central composite design were performed, with 3 central points, totalizing 17 experiments. The experimental design coded and real values are presented in Table 1.

Table 1.
Independent variables coded and real values, considering the total weight of the film forming suspension.

Variables (%)	***Coded Values***				
	-1.68	***-1***	***0***	***1***	***1.68***
Sucrose (S)	0.000	0.284	0.700	1.116	1.400
Inverted sugar (I)	0.000	0.284	0.700	1.116	1.400
Sodium Phosphate (P)	0.000	0.122	0.300	0.478	0.600

The statistical model utilized for experimental adjustments is a second order polynomial equation, were b_n are the model parameters, Y_i are the dependent variables (water vapor permeability and mechanical, and thermal properties) and x_1, x_2 and x_3 are the independent variables (sucrose%, inverted sugar% and sodium phosphate%), as exemplified in Equation 1. The experimental data were analysed using the software Statistica for Windows, version 5.0.

$$Y_i = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_2^2 \quad \textbf{\textit{Equation 1}}$$

3. Results and discussion

The experimental designs presented in Table 2 were used to estimate the main effects of independent variables.

Table 2.
2^3 factorial response surface experimental design matrix and mechanical resistance permeability results.

Assay	**Coded values**			**Mechanical Resistance**				**Permeability**			
	S	**I**	**P**	**TS^b**	***ΔTS***	**E^c**	***ΔE***	**WVP^d x10^{-5}**	***ΔW***	**O$_2$ P^e**	***ΔO$_2$P***
control	*0*	*0*	*0*	368.85	*na^g*	4.06	*na^g*	58.11	*na^g*	8.70	*na^g*
1	*-1*	*-1*	*-1*	222.24	*-39.75*	4.45	*9.81*	9.72	*-83.27*	----	----
2	*-1*	*-1*	*1*	217.11	*-41.14*	4.72	*16.35*	5.72	*-90.15*	----	----
3	*-1*	*1*	*-1*	131.59	*-64.33*	6.15	*51.53*	7.24	*-87.54*	----	----
4	*-1*	*1*	*1*	71.38	*-80.65*	49.79	*1127.56*	6.56	*-88.71*	17.59	*102.18*
5	*1*	*-1*	*-1*	122.14	*-66.89*	6.61	*62.87*	7.32	*-87.41*	----	----
6	*1*	*-1*	*1*	67.94	*-81.58*	53.83	*1227.05*	6.68	*-88.51*	14.22	*63.45*
7	*1*	*1*	*-1*	32.99	*-91.06*	98.01	*2316.44*	5.82	*-89.98*	12.82	*47.36*
8	*1*	*1*	*1*	8.90	*-97.59*	93.61	*2207.86*	6.28	*-89.19*	----	----
9	*-1,68*	*0*	*0*	254.78	*-30.93*	4.07	*0.44*	9.63	*-83.44*	----	----
10	*1,68*	*0*	*0*	45.06	*-87.78*	96.27	*2273.42*	7.75	*-86.66*	11.39	*30.92*
11	*0*	*-1,68*	*0*	259.40	*-29.67*	3.51	*-13.41*	9.66	*-83.38*	----	----
12	*0*	*1,68*	*0*	62.31	*-83.11*	62.79	*1448.00*	7.63	*-86.87*	9.58	*10.11*
13	*0*	*0*	*-1,68*	200.00	*-45.78*	4.84	*19.30*	5.08	*-91.26*	----	----
14	*0*	*0*	*1,68*	50.08	*-86.42*	54.60	*1246.03*	7.66	*-86.81*	15.71	*80.57*
15^a	*0*	*0*	*0*	106.20	*-71.21*	7.17	*76.85*	7.74	*-86.67*	----	----
16^a	*0*	*0*	*0*	99.52	*-73.02*	8.58	*111.56*	8.52	*-85.34*	----	----
17^a	*0*	*0*	*0*	104.58	*-71.65*	8.67	*113.86*	10.41	*-82.09*	----	----
R$^{2\ f}$	----	----	----	0.92	----	0.94	----	0.49	----	na^g	----

^{a}: central points; TS^b: tensile strength(kgf/cm^2); E^c: elongation (%); WVP^d: water vapor permeability (g.mm/m^2.day.Pa); O$_2$P^e: oxygen permeablility (cm^3/m^2.day); R$^{2\ f}$: coefficient of determination; na^g: not applicable.

3.1. Mechanical Properties

Additives can strongly affect film tensile strength and elongation percentage. Veiga-Santos, Oliveira, Cereda & Scamparini (2004) has observed that cassava starch films elongation at break was greatly affected by both sucrose and inverted sugar.

All additives studied affected ($p<0.05$) cassava starch film tensile strength (evaluated as maximum resistance area) and elongation at break (Table 2 and Fig. 1). Such parameters were also affected ($p<0.05$) by the interaction between sucrose and inverted sugar.

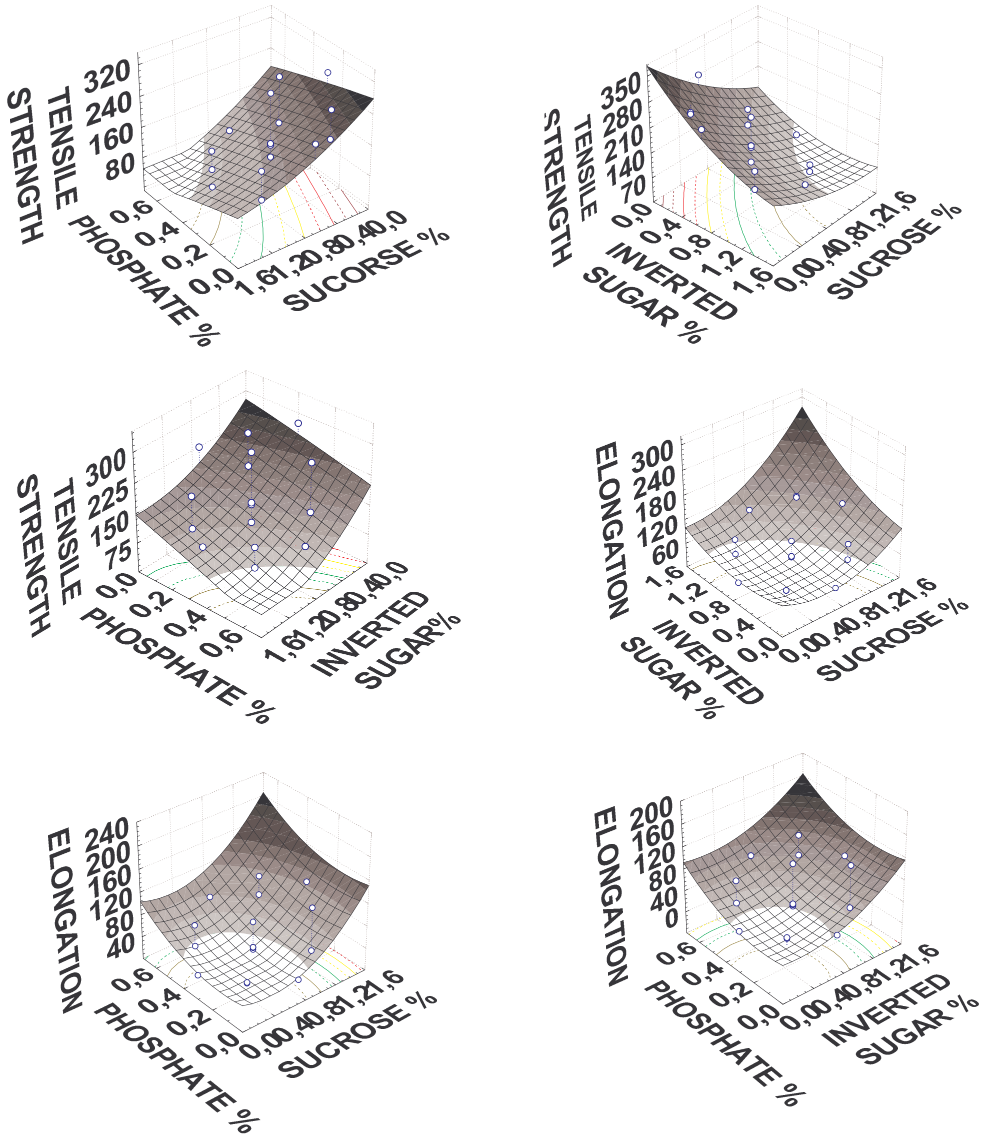

Fig 1. Response surfaces with the significant effects of sucrose, inverted sugar and sodium phosphate concentrations on cassava starch film tensile strength resistance and elongation at break.

As can be observed (Fig.1), increasing sucrose, inverted sugar and phosphate concentration, the material elongation can increase up to 2300 %, however, a decrease (up to 97%) in tensile strength was also observed.

In previous studies of this serie, the plasticizing effects of sucrose and inverted sugar have already been commented (Veiga-Santos, Oliveira, Cereda & Scamparini, 2004). The same increasing elongation at break and decreasing tensile strength behavior were observed with plasticizer addition. The effect on elongation was attributed to reducing intermolecular forces, resulting in a more flexible film network (Coupland, Shaw, Monahan, O'Riordan & O'Sullivan, 2000). A decrease in tensile strength may be observed in systems with high plasticizer content, resulting in excessive interaction among the plasticizer and the film network (Arvanitoyannis, Psomiadou & Nakayama, 1996). Therefore, leading to a weak network, with low tensile strength resistance.

A possible explanation for the interaction observed when using sucrose and inverted sugar is their plasticizing character, that when utilized together, increases the total plasticizer content of the films.

3.2. Water Vapor Permeability

Lower water vapor permeability values were observed with the combinations of the highest concentration of inverted sugar, sucrose and phosphate. Water vapor permeability comprises tree steps, sorption, diffusion and adsorption and is largely governed by the interactions between the polymer and the water vapor (Sangaj & Malshe, 2004). Films containing sucrose and inverted sugar may immobilize the absorbed water, reducing its diffusion trough the polymer chain and, consequently, lowering the water vapor permeability. Decrease on the water vapor permeability when adding sucrose has also been observed by Cherian, Gennadios, Weller & Chinachoti (1995).

The phosphate may act as the sucrose and inverted sugar molecules, interacting with the water, or altering the material polarity, diminishing permeation (Nivedita, Sangaj & Malshe, 2004).

However, none of the additives have significativelly ($p<0.05$) influenced film water vapor permeability.

Although, when comparing the experimental samples with the control, an increased of the water vapor barrier (up to 71%) can be observed when adding the studied additives.

3.3. Oxygen Permeability

The oxygen permeability was performed for samples 4, 6, 7, 10, 12 and 14; which presented elongation properties up to 1100% and a tensile strength up to 30kgf/cm^2 (Table 2 and Figure 2).

Such samples were chosen because they presented the best combination of improved elongation at break without much loose on tensile strength.

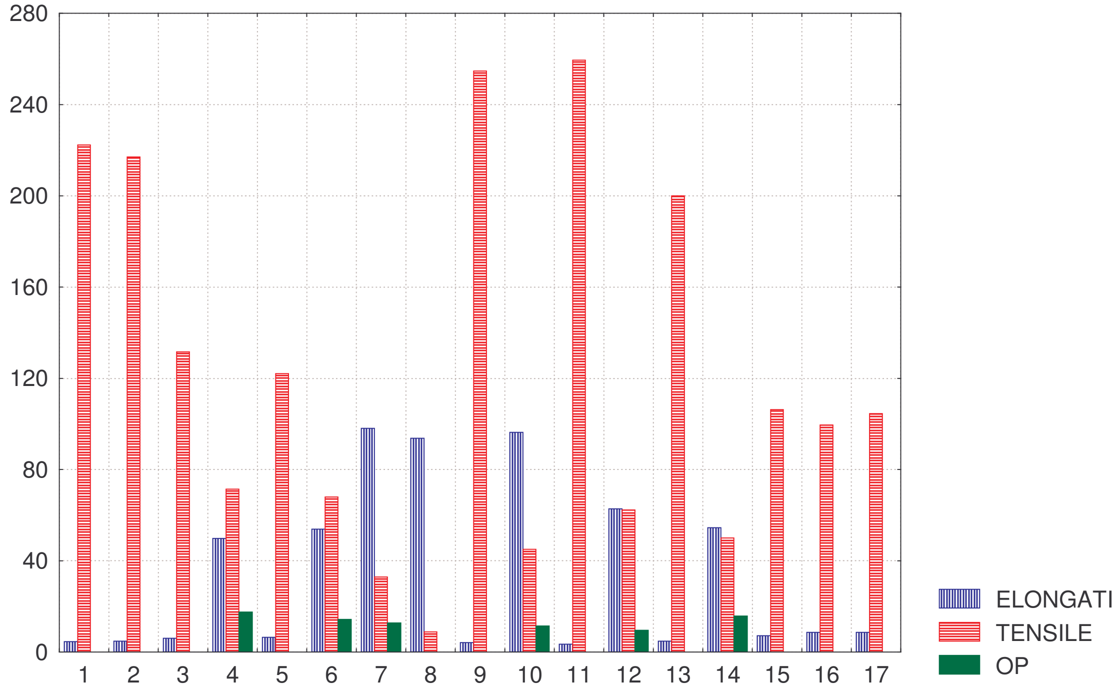

Fig. 2. Experimental samples (ELONGATI) elongation at break, (TENSILE) tensile strength and (OP) oxygen permeability behavior.

If lower oxygen permeability were requested, the samples 12, 10 and 7 would be more recommended (Table 2). Among the 6 studied films, the lowest oxygen permeability (9.58 x 10^{-5} g.mm/m^2.day.Pa) was observed for samples 12 (containing 0.7% of sucrose, 1.4% of inverted sugar and 0.3% of phosphate).

If, on the contrary, higher oxygen permeability was requested, the samples 4, 6 and 14 were be more recommended (Table 2). The highest oxygen permeability (17.59 x 10^{-5} g.mm/m^2.day.Pa) was observed for sample 4 (containing 0.28% of sucrose, 1.12% of inverted sugar and 0.48% of phosphate).

Although the control (containing just cassava starch, no additives) had presented the lowest oxygen permeability (8.70 x 10^{-5} g.mm/m^2.day.Pa) (Table 2), such material presented no elongation properties. As the elongation at break has an important characteristic for packaging, the control utilization as a film has a very low viability.

3.4. Thermal Analysis

Table 3 presents the surface response for the thermal analyses.

Table 3. Responses of the dependent variables obtained for the experimental samples.

Assays	**DTA**		**DSC**							
	ML_t^*	***ML_d****	**$T_{g,1}$**	**$T_{g,2}$**	**$T_{e,o}$**	**$T_{e,p}$**	**$T_{ex,o}$**	**$T_{ex,p}$**	**ΔH_e**	**ΔH_{ex}**
Control	79.94	71.91	nob*	nob*	289.7	306.6	nob*	nob*	-49.51	nob*
1	66.12	43.82	-8.72	57.29	193.4	211.8	281.9	288.5	-15.30	25.98
2	64.93	42.04	-30.89	22.39	185.2	201.8	261.6	275.5	-14.36	38.55
3	68.98	44.40	-16.43	28.15	183.1	209.8	279.0	287.5	-34.87	22.62
4	64.96	41.16	-22.89	15.46	201.3	198.7	260.7	273.9	-16.62	42.51
5	68.33	45.51	-2.21	49.72	187.1	210.6	279.3	287.2	-38.25	24.20
6	67.62	47.55	-18.04	31.88	191.7	200.0	261.6	274.2	-25.44	40.16
7	69.49	48.08	-16.35	20.38	197.4	208.4	262.9	281.6	-26.34	55.48
8	58.88	38.26	-23.0	44.48	182.2	199.4	255.0	273.6	-36.42	42.68
9	63.52	40.94	-32.24	nob*	186.6	208.5	276.7	282.7	-17.36	24.36
10	69.71	47.51	-19.11	9.41	187.6	202.1	260.6	277.6	-36.21	47.01
11	68.45	46.56	16.99	57.95	192.9	207.9	260.8	278.1	-13.08	47.97
12	68.20	45.62	-22.69	10.02	184.9	201.6	260.3	277.6	-29.19	48.89
13	77.92	61.98	-0.44	50.67	216.4	235.0	326.6	332.7	-28.94	17.78
14	65.33	42.90	-20.77	31.07	176.1	196.2	253.9	272.5	-30.01	49.77
15(c)*	69.58	46.18	-18.74	36.58	185.7	202.8	258.0	276.7	-29.45	73.12
16(c)*	65.75	42.28	-16.43	35.36	192.8	203.5	259.0	277.3	-17.49	60.26
17(c)*	65.63	44.43	-18.82	24.91	187.4	205.1	262.9	279.4	-17.98	62.51
R^2	0.47	0.38	0.48	0.72	0.25	0.55	0.40	0.37	0.68	0.44

(c): central points. nob*: not observed. $T_{g,1}$: negative glass transition temperature (°C). $T_{g,2}$: positive glass transition temperature(°C). ML_t: total mass loss (%). ML_d: mass loss (%) after exothermic degradation. e: endothermic. ex: exothermic. T: temperature (°C). ΔH: specific enthalpy (J/g). o: onset. p: peak. R^2: coefficient of determination.*

A depressor effect of plasticizer (sucrose and inverted sugar) on the glass transition temperature (T_g) of the materials can be observed in Table 3 and Fig. 3.

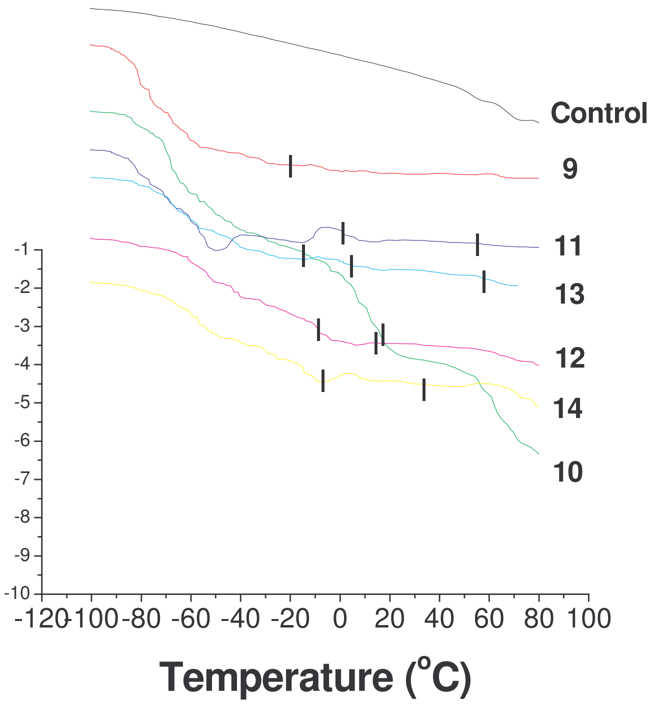

Fig. 3. DSC scanning for glass transition temperature (T_g) determination. Samples 9 (without sucrose addition), 10 (maximum sucrose concentration), 11 (without inverted sugar addition), 12 (maximum inverted sugar concentration), 13 (without sodium phosphate addition), 12 (maximum sodium phosphate concentration) and control (only cassava starch, no additives).

Such depressor effect was also observed (Forssell, Mikkilä, Moates & Parker, 1997; Arvanitoyannis, Psomiadou, Nakayama, Aiba & Yammoto, 1997). The plasticizer effect could possibly be due to changes in the polymer network, mainly related to the creation of highly mobile regions, which could allow an even more pronounced moisture uptake (Cherian, Gennadios, Woller & Chinachoti, 1995). Increasing water content decreases the material T_g due to enhanced starch-water hydrogen bounding interactions and less intra- and intermolecular hydrogen bounding between starch chains (Arvanitoyannis, Psomiadou, Nakayama, Aiba & Yamamoto; 1997).

DSC analysis have shown two different Tg, one negative ($T_{g,1}$) and one positive ($T_{g,2}$) (Table 3, Fig. 3), indicating phase separation. A miscible polymer mixture exhibits a single glass transition. With increasing immiscibility, there is a broadening of the transition, whereas an incompatible system would be marked by separate transitions of the polymer component blend (Biliaderis, Lazaridou & Arvanitoyannis, 1999).

The Pareto chart of standardized effect has indicated that all additives decreased ($p<0.05$) the material inferior (negative) glass transition temperatures ($T_{g,1}$). Also the interactions between sucrose and inverted sugar and between inverted sugar and phosphate affected ($p<0.05$) the film lower (negative) glass transition temperatures ($T_{g,1}$) (Fig. 4).

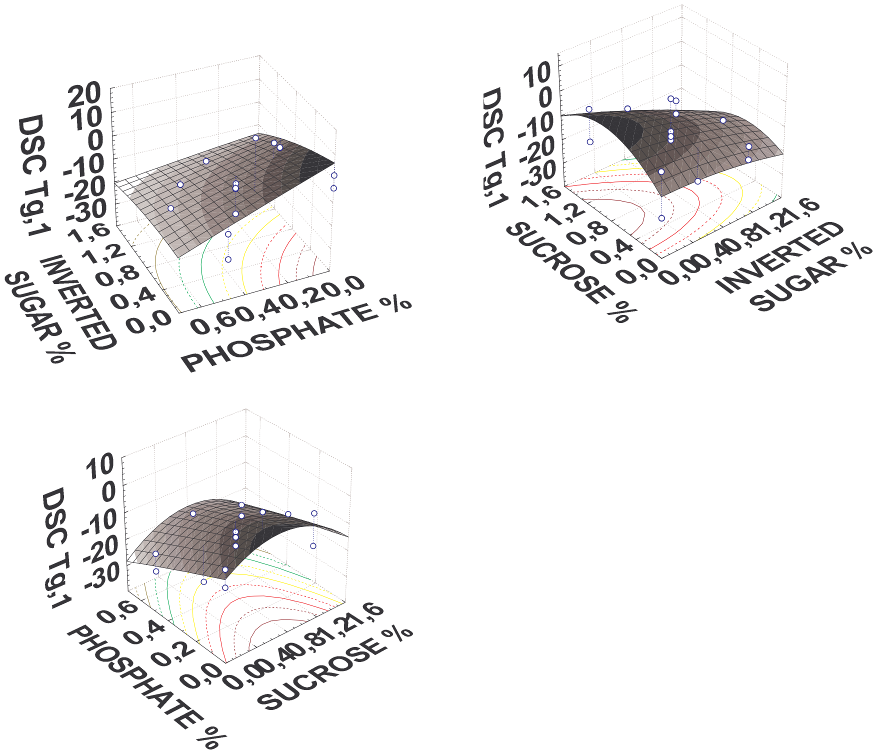

Fig. 4. Response surfaces with the significant effect of sucrose, inverted sugar and sodium phosphate concentrations on cassava starch film lower (negative) glass transition temperature ($T_{g,1}$).

Observing samples superior (positive) glass transition temperature (T_g) (Table 3, Fig. 5), it can be noticed that sample 9 (the only one not added with sucrose) did not present a positive T_g. Such result indicates that only inverted sugar (glucose and fructose) and/or phosphate affected the positive $T_{g,2}$.

The Pareto chart of standardized effect has also indicated that only inverted sugar decreased ($p<0.05$) the material superior (positive) glass transition temperatures ($T_{g,\ 2}$). Such results, analysed together with the two glass transition temperatures observed (Table 3, Fig. 4 and 5), reinforces the theory that have occurred phase separation.

The positive glass transition temperature response surfaces (Fig. 5) indicate that increasing inverted sugar addition decreases the material positive T_g. Such result was expected due to the plasticizer character of the inverted sugar (glucose and fructose).

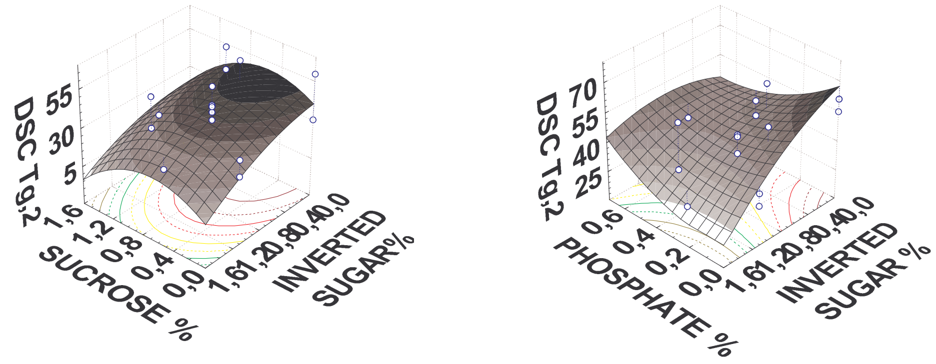

Fig. 5 Response surfaces with the significant effect of inverted sugar concentration on cassava starch films positive glass transition temperature ($T_{g,\ 2}$).

When observing the control (containing only cassava starch), no glass transition temperature was observed. A raw cassava starch DSC shows a T_g around 65 °C, which indicates the starch jellification temperature (Jenkins & Donald, 1998). However, the cassava starch of the studied films was already jellified and so, did not presented a T_g. Because no T_g was observed for the control, it is possible that the glass transition temperatures observed are related only to the additives (sucrose, inverted sugar and phosphate). Two glass transition temperatures (one positive and another negative) was also observed for cassava starch films plasticyzed with polyols. The author attributed such phenomenon to a possible excessive presence of additives (> 15%), resulting in fase separation between the polyol and the biopolymer (Vicentini, 2003)

All experimental samples presented an endothermic and an exothermic peak during the DSC analysis, with the exception of the control, which only presented an endothermic peak (Table 3). Observing the onset temperature of the endothermic peaks, it can be noticed that the endothermic peak of the experimental samples began at lower temperatures when comparing to the control.

Since the onset temperature is the temperature in which the material endothermic or exothermic modifications starts, the knowledge of the effect of additives on such parameter is extremely important. However, the R^2 of the Pareto chart of standardized effect of the endothermic onset temperatures was very low (0.25), indicating that the main effects observed may not be trustful. Thus, the effect of additives on the endothermic and exothermic peak was evaluated by their medium peak temperatures. The Pareto chart of standardized effect has indicated that phosphate, inverted sugar and sucrose affected ($p<0.05$) both endothermic and exothermic peak temperatures (Fig. 6).

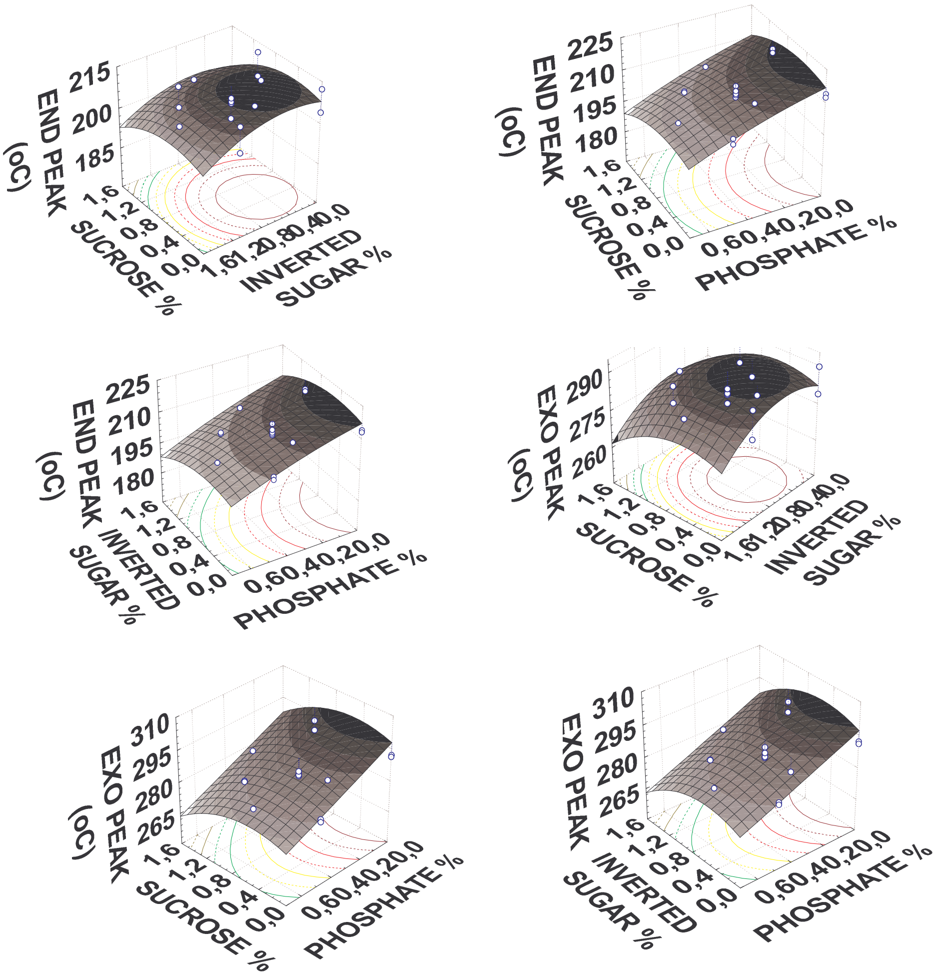

Fig. 6. Response surfaces with the significant effects of phosphate, sucrose and inverted sugar concentration on cassava starch film endothermic (ENDO) and exothermic (EXO) peak temperatures.

When comparing both endothermic and exothermic peaks of samples 9 (no sucrose addition), 11 (no inverted sugar addition) and 13 (no phosphate addition) peak temperatures (Table 3); it can be notice that the most distinct results were obtained when phosphate was not present (sample 13). The absence of phosphate increased sample endothermic and exothermic peaks. Such result indicates that, if the material would be submitted to high temperatures (> 200 °C), sodium phosphate addition should be avoided.

All samples, including the control, presented the endothermic peak. Observing the mass loss trough the TGA analyses, it can be observed that the major mass loss occurs just after the endothermic peak (Table 3). Thus, it is possible to believe that the endothermic peak is related to a degradation phenomenon.

The Pareto chart of standardized effect has indicated that no additive affected ($p<0.05$) the total mass loss. However, the mass loss just after the endothermic peak was negatively affected ($p<0.05$) by phosphate addition (Fig. 7). Such result indicates that phosphate addition reduce the mass loss just after the endothermic peak. Admitting that the endothermic peak is due to the material degradation, a possible explanation is that the association of the phosphate with starch in starch monoester (Belitz & Grosh, 1999) lowers the material degradability by heating.

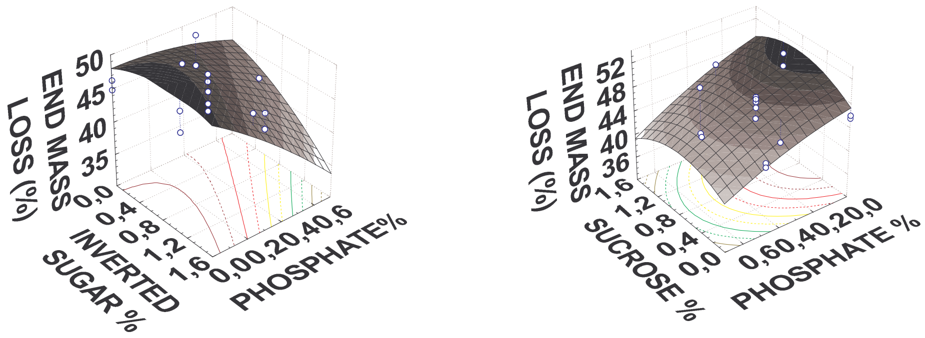

Fig. 7. Response surfaces with the significant effect of phosphate concentration on cassava starch film mass loss (%) just after the endothermic peak (END MASS LOSS).

The exothermic peak was only observed for the experimental samples (Table 3). The Pareto chart of standardized effect has indicated that all additives affected ($p<0.05$) the exothermic peak temperature and onset temperature (Fig. 6).

As the control (containing no additives) did not show an endothermic peak, such phenomenon may be explained by the crystallization of the material caused by the additives. After the DSC analyses the samples containing additives presented glassy and dark appearance, typical of the excessive high boiling of sugars (Cummings, 1995). The phosphate could have affected the sucrose and inverted sugar crystallization. Monobasic sodium phosphate can be added to starch to produce monoester phosphates, resulting in lower intermolecular association

(BeMiller & Whistler, 1996), which can facilitate the sucrose mobility allowing the molecules to lean and stick to the nucleus, accelerating the crystallization process (Jackson & Howling, 1995).

4. Conclusions

All additives studied affected cassava starch films tensile strength and elongation at break, but no effect was observed on the water vapor permeability. If lower oxygen permeability are requested, samples added with 0.7% sucrose, 1.4% inverted sugar and 0.3% sodium phosphate are the best alternative and present a good balance between tensile strength (>60 kgf/cm^2) and elongation at break (>60%). There were observed two glass transition temperatures, indicating phase separation. As the control (containing no additive) did no presented glass transition, the T_g is probably related to the additives. All additives affected the lower T_g, however only inverted sugar affected the higher T_g. Interaction of the film network with phosphate reduced the total mass loss after a endothermic peak. The exothermic peak was not observed for the control and is probably a crystallization phenomenon related to the additives.

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CAPÍTULO 8

Effect of sucrose, inverted sugar and phosphate on microstructure, color, water activity and solubility of cassava starch biodegradable films

Effect of sucrose, inverted sugar and phosphate on microstructure, color, water activity and solubility of cassava starch biodegradable films

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Abstract

Additives (sucrose, inverted sugar and sodium phosphate) added singly and in association at different levels effect on cassava starch films microstructure, color, water activity and solubility were evaluated by response surface methodology. None of the studied additives affected cassava starch water activity, solubility and color ("L", "a", "b" and haze). However, when comparing the experimental samples to the control, an increase on water activity, solubility and total color difference was observed when using the studied additives. X-ray diffractograms and SEM and light microscopy have indicated that inverted sugar can prevent sucrose crystallization if utilized the combination of until 0.8% sucrose with any concentration of inverted sugar, or the maximum of 1.2% sucrose with 0.4% inverted sugar. Increasing inverted sugar and phosphate concentration leads samples to a semi-crystalline behavior. Inverted sugar addition results in cassava starch re-organization. Phosphate addition (> 0.48%) affected the starch jellification temperature of the film forming suspension, with consequently not jellified starch molecules presented on the final material structure.

Keywords: *biofilms, microstructure, water activity, solubility, color.*

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1. Introduction

Starch is one of the most promising natural polymer because of its inhert biodegradability, its overwhelming abundance and its annual renewal (Arvanitoyannis, Kolokuris, Nakayama & Sei-Ichi, 1997).

Starch films presents low water vapor barrier and mechanical resistance when comparing to traditional petroleum plastic films (Kester & Fennema, 1986; Krochta & Mulder-Johnston, 1997). Although, protective characteristics can be improved through additives incorporation (Arvanitoyannis & Biliaderis, 1998; Coupland, Shaw, Monahan, O'Riordan & O'Sullivan, 2000; Garcia, Martino & Zaritzki, 2000).

Previous studies of this series had indicated that the isolate utilization of sucrose, inverted sugar and sodium phosphate have resulted in significant increase on materials elongation at break (Veiga-Santos, Oliveira, Cereda & Scamparini, 2004). However, sucrose utilization, which had the most pronounced effect on films elongation at break, also resulted on the crystallization of the material during storage (Veiga-Santos, Suzuki, Cereda & Scamparini, 2004).

Spontaneous nucleation of sucrose crystals can occur. Nucleation and crystal formation could arise from rearrangement of randomly bound sucrose molecules (Stansell, 1995).

The simultaneous effect of sucrose and inverted sugar may prevent or reduce the sucrose crystallization. The inverted sugar can increase the material viscosity, which would difficult sucrose molecules to bind each other beginning the crystallization process (Jackson, & Howling, 1995). Although the inverted sugar effect on sucrose crystallization has already been investigated (Stansell, 1995); none has been investigated about the inverted sugar effect on sucrose crystallization in biodegradable films.

The ester linkages between sodium phosphate and the starch chain hydroxyl, results in starch phosphate monoesters (Ellinger, 1972), which has lower intermolecular association than none modified starch (BeMiller & Whistler, 1996). Low concentrations of phosphate addition on cassava starch films have increased the material elongation at break (Veiga-Santos, Oliveira, Cereda & Scamparini, 2004), indicating that a phosphate concentration increase may lead to higher elongation at break values. Although, the phosphate effect on starch suspension has been already studied (Blennow, Bay-Smidt, Olsen & Moller, 2000; Nabeshima & Grossmann, 2001), little has been investigated about the phosphate effect on film microstructure, color, water activity and solubility.

With the aim to evaluate the ensemble and isolate effect of sucrose, inverted sugar and sodium phosphate addition on cassava starch films; microstructure, water activity, solubility and color were investigated by surface response methodology.

2. Material and Methods

2.1. Materials

Commercial cassava starch (Flor de Lotus, Brazil), sucrose and inverted sugar (Copersucar, Brazil) and analytically pure monobasic sodium phosphate (Sinth, Brazil).

2.2. Sample Preparation

Cassava starch (5%, w/w) was blended with water and additives: sucrose (0.0-1.4%), inverted sugar (0.0-1.4%) and sodium phosphate (0.0-0.6%) considering the total weight of the film forming suspension. The mixture was heated to 75°C (for complete jellification of cassava starch) with constant stirring and placed under ultrasonic bath. The films were prepared according to the casting technique, by dehydration under renewable circulated air (30°± 2°C) over Petri plastic dishes. Films containing 5% cassava starch (no additives) served as the control. Samples were storage (23°C, 75%RH given by a NaCl saturated solution) for at least 4 days prior to testing.

2.3. Color.

The color of preconditioned (75% RH, 23°C) samples was analysed in duplicate by total transmittance, using a Color Quest II-Hunter lab equipment, CIELAB Ttran D65, lecture angle 10°, measuring area 2.54cm^2 (1.0 square inch). Four measurements taken at the different quadrants of each sample were averaged and expressed as Hunter system “L” (lightness), “a” (redness), “b” (yellowness) and haze values. Total color difference (ΔE) was evaluated as the size of color difference between the control and the experimental design samples, was calculated by equation 1 (FRANCIS, 1983, cited by HONG & PARK, 2000):

$$\Delta E = \left[(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2\right]^{\frac{1}{2}}$$ **Equation 1**

2.4. Water activity

Water activity measurements were performed with a DECAGON CX-2T, AQUALAB apparatus. Pure water (a_w 1.000 ± 0.003%) and KCl (a_w 0.843± 0.003 %) were used as standard for equipment calibration. Preconditioned (75% RH, 23°C) samples (4cm^2) were cut from the center of the films and evaluated in triplicate. The experimental design samples water activity (Δa_w%) increase or decrease size in relation to the control was calculated according to equation 2.

$$\Delta a_w = \left[\left(\frac{sample\ a_w}{control\ a_w}\right) \times 100\right] - 100$$ **Equation 2**

2.5. Solubility

The solubility in water was evaluated by the percentage of dry matter of the film solubilized after 24 h immersion in water. The percentage of the initial dry matter of each samples was determined at 105℃, until constant weigh. Tree pre-conditioned (75RH, 25℃) discs (Ø 2 cm) were weighted, immersed in distilled water and constantly agitated (100 rpm) at 30℃. The remained insoluble parts of the disc were than dried (105℃, until constant weigh) in order to calculate the weight of the not solubilized dry matter. The solubility percentage was calculated by subtracting the weight of the not solubilized dry matter from the weight of the initial dry matter (Gontard, Guilbert & Cuq, 1992). The experimental samples solubility (ΔSol %) increase or decrease size in relation to the control was calculated according to equation 3.

$$\Delta Sol = \left[\left(\frac{sample\ so\mathrm{lub}ility}{control\ so\mathrm{lub}ility}\right) \times 100\right] - 100$$ **Equation 3**

2.6. Microstructure Analyses

Crystallinity*:*

The relative crystallinity of the films was investigated with wide-angle X-ray diffraction (WAXS). Measurements were carried out using a DMAX-2200 Rigaku International Corporation θ/2θ diffractometer, operating with voltage 40 kV and amperage 20mA, with CuKα radiation. Samples were fixed at an aluminum sample holder, and analysed from 5 to 60° (2θ), with angular step of 0.1° (2θ), and sampling interval time of 3 seconds. The films were analysed at each 15 days, during a storage period of 120 days (75%RH), in duplicate. Crystallinity was also investigated (in the same conditions) for the raw materials cassava starch and sucrose in order to obtain crystallinity standards.

Polarized Light Microscopy:

Samples surface was observed by LEICA-DMLP light microscope, with no further preparation, by transmitted polarized or ordinary (not polarized) light. Images of 200x-magnification were collected with a CCD camera.

Scanning Electron Microscopy (SEM):

SEM digital images of the samples surface and fracture were obtained by Jeol JSM-5900LV scanning electron microscope. Cross section images were obtained by cryogenic fracture of the films, using liquid nitrogen. Samples were sputtered with a 16μg gold layer. Images were taken at 5-10KV, spotsize 28-30, 1000x-magnification.

2.7. Statistical analyses

Independent variables (sucrose%, inverted sugar% and sodium phosphate%) effect on cassava starch film microstructure, color, water activity and solubility were evaluated trough Surface Response Methodology. A 2^3 experimental central composite design were performed, with 3 central points, totalizing 17 experiments. The experimental design coded and real values are presented in Table 1.

Table 1.
Independent variables coded and real values, considering the total weight of the film forming suspension.

Variables (%)	***Coded Values***				
	-1.68	***-1***	***0***	***1***	***1.68***
Sucrose (S)	0.000	0.284	0.700	1.116	1.400
Inverted sugar (I)	0.000	0.284	0.700	1.116	1.400
Sodium Phosphate (P)	0.000	0.122	0.300	0.478	0.600

The statistical model utilized for experimental adjustments is a second order polynomial equation, were b_n are the model parameters, Y_i are the dependent variables (color, water activity, solubility, crystallinity) and x_1, x_2 and x_3 are the independent variables (sucrose%, inverted sugar% and sodium phosphate%), as exemplified in Equation 4. The experimental data were analysed using the software Statistica for Windows, version 5.0.

$$Y_i = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^{\,2} + b_{22}x_2^{\,2} + b_{33}x_{2}^{\,2} \quad \textbf{\textit{Equation 4}}$$

3. Results and discussion

The experimental designs presented in Table 2 were used to estimate the main effects of independent variables.

Table 2.
2^3 factorial response surface experimental design and results for the independent variables sucrose (S), inverted sugar (I) and phosphate (P) concentration.

Assays	Coded values			Color					Water activity		Solubility	
	S	I	P	"L"	"a"	"b"	Haze	ΔE^d (%)	a_w^e	Δa_w^f	Sol^g	ΔSol^h
control	*-1*	*-1*	*-1*	97.10	0.75	3.77	16.55	*na^c*	0.51	*na^c*	9,60	***na^c***
T1	*-1*	*-1*	*-1*	97.11	0.95	3.62	12.98	*0.25*	0.70	*38.46*	26,32	*174.17*
T2	*-1*	*-1*	*1*	97.41	0.73	3.15	6.48	*0.71*	0.73	*44.77*	26,35	*174.48*
T3	*-1*	*1*	*-1*	96.97	0.72	3.56	13.38	*0.23*	0.70	*37.48*	18,54	*93.14*
T4	*-1*	*1*	*1*	97.09	0.69	3.45	9.29	*0.33*	0.71	*39.45*	22,78	*137.32*
T5	*1*	*-1*	*-1*	97.12	0.71	3.52	12.21	*0.26*	0.70	*38.66*	18,18	*89.41*
T6	*1*	*-1*	*1*	97.23	0.92	3.31	8.07	*0.52*	0.75	*47.53*	20,28	*111.23*
T7	*1*	*1*	*-1*	97.43	0.94	3.21	5.79	*0.69*	0.67	*32.35*	28,81	*200.08*
T8	*1*	*1*	*1*	97.01	0.69	3.38	8.67	*0.40*	0.73	*44.18*	19,49	*103.02*
T9	*-1,68*	*0*	*0*	97.25	0.94	3.91	16.92	*0.30*	0.75	*47.34*	24,19	*151.98*
T10	*1,68*	*0*	*0*	97.08	0.71	3.58	12.65	*0.19*	0.75	*47.53*	21,80	*127.08*
T11	*0*	*-1,68*	*0*	97.04	0.74	3.57	13.05	*0.20*	0.64	*25.84*	20,76	*116.23*
T12	*0*	*1,68*	*0*	97.33	0.95	3.60	10.75	*0.37*	0.71	*39.05*	17,36	*80.86*
T13	*0*	*0*	*-1,68*	97.10	0.95	3.48	9.40	*0.35*	0.73	*43.59*	22,29	*132.17*
T14	*0*	*0*	*1,68*	96.96	0.77	3.70	13.47	*0.13*	0.75	*47.93*	23,64	*146.28*
T15^a	*0*	*0*	*0*	97.19	0.93	3.77	13.73	*0.22*	0.72	*41.42*	19,70	*105.17*
T16^a	*0*	*0*	*0*	97.00	0.71	3.47	11.23	*0.31*	0.66	*30.37*	18,69	*94.70*
T17^a	*0*	*0*	*0*	97.32	0.94	3.66	12.16	*0.33*	0.70	*38.07*	22,76	*137.14*
R^{2b}				0.69	0.40	0.63	0.61	0.77	0.36	*na^c*	0.67	*na^c*

a: central points; R^{2b}: coefficient of determination; na^c: not applicable; ΔE^d: total color difference size between samples and the control (%); T^e: thickness (mm); a_w^f water activity; Δa_w^f: water activity size of increase in relation to the control (%); Sol^g: solubility in water (%); ΔSol^h: solubility in water size of increase in relation to the control (%);.Sol^g: solubility in water (%);

3.1. Color

Samples Hunter color parameters "L", "a", "b" and haze and the total color difference between samples and the control (ΔE) are presented in Table 2.

All samples presented high lightness (above 97.1%) and low color values for "a" redness (< 0.95) and "b" yellowness (< 3.91) as can be observed in Table 2. When compared to the control (L=97.10; a=0.75; b=3.77), samples presented similar results, indicating that the additives investigated in this study have little effect on the cassava starch films color parameters and that all materials were almost colorless, with a high brilliancy.

Sample haze evaluation provides some information of the homogeneity of the surface and internal defects of a material (Kaya & Kaya, 2000). Also a package with an elevated haze compromise the visibility and color distinction of the product due to a loss of image contrast caused by the dispersion of the transmitted light (Sarantopóulos, Oliveira, Padula, Coltro, Alves & Garcia, 2002).

All samples presented haze values below 13.73% (Table 2). Such result probably indicates that the utilization of sucrose, inverted sugar and phosphate as additives provides and homogeneous surface material with little internal defects. Also can indicate that a good color definition and visibility will be able observe trough the cassava starch films additivated with sucrose, inverted sugar and phosphate. When comparing to the control haze (16.55%), the experimental samples presented lower values (13,73%), indicating that the additives increased the color definition and visibility of the cassava starch film.

The Pareto chart of standardized effect has indicate that no additive affected ($p<0.05$) the material “L”, “a” and “b” and haze parameters. However, significant effect ($p<0.05$) on the total color difference between samples and the control (ΔE) was observed for the interaction between inverted sugar and phosphate.

The ΔE values are a good indicative to measure how much the additives increased or decreased samples total color difference in relation to the control.

The response surface for the effect of sodium phosphate and inverted sugar concentrations on the materials total color difference between samples and the control (ΔE) are represented on Fig. 1.

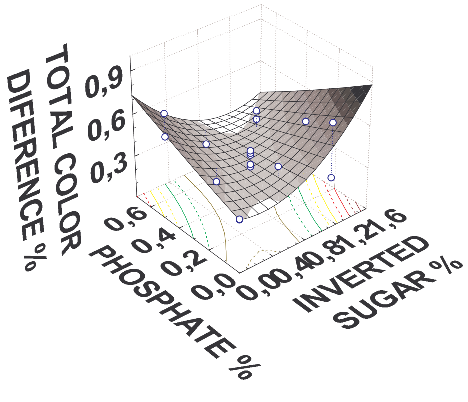

Fig. 1. Response surface for the significant effect of sodium phosphate and inverted sugar concentrations on the material total color difference between samples and the control (ΔE %).

The effect of inverted sugar on samples total color difference (ΔE %) can be explained by the characteristic yellowness presented by such raw material.

Ester linkages between sodium phosphate and the hydroxyl in the starch chain can be formed, resulting in starch phosphate monoesters (Ellinger, 1972). The jellified starch monoester has a higher clarity when comparing to the jellified normal starch (Belitz & Grosch, 1999b), what could affect the material total color difference (ΔE %). Also the characteristic color of the monobasic sodium phosphate could have affected the film total color difference (ΔE %).

3.2. Water activity

The shelf life of perishable products is generally extended on systems with low water activity (a_w) contents (Mathlouthi, 2001).

All samples have presented a_w content between 0.64 and 0.75 (Table 2), thus being considered intermediate moisture products, which are susceptible to microorganism growth, especially molds (Belitz & Grosch, 1999a). When comparing samples to the control (a_w: 0.51), it can be noticed that the studied additives increased cassava starch a_w. Such result indicates that when adding sucrose, inverted sugar and phosphate; cassava starch film shelf life may be decreased, been more susceptible to microorganism growth. However, considering the biodegradable application of such material, been more susceptible to microorganisms attach, in some cases, can be advantageous.

Among the experimental samples, however, the Pareto chart of standardized effect has indicated that no additive affected ($p<0.05$) the content of the films a_w.

3.3. Solubility

When the biofilm remains in contact with high water activity content products or when the material may be in contact with water, water solubility resistance is an important property (Guilbert & Biquet, 1989; cited by Gontard, Guilbert and Cuq, 1992).

As can be observed on Table 2, the studied additives increased the water solubility of the experimental samples (from 80 to 200%) when comparing to the control, which is probably due to the hydrophilic character of the additives.

Among the experimental samples, the Pareto chart of standardized effect, have indicated that no additive affected ($p<0.05$) the samples solubility in water.

3.4. Microstructure analyses

Crystallinity

WAXS crystallinity diffractograms, collected during 120 days of storage, demonstrated that, at the first day of storage, all samples from both blocks, presented an amorphous behavior (Fig. 2).

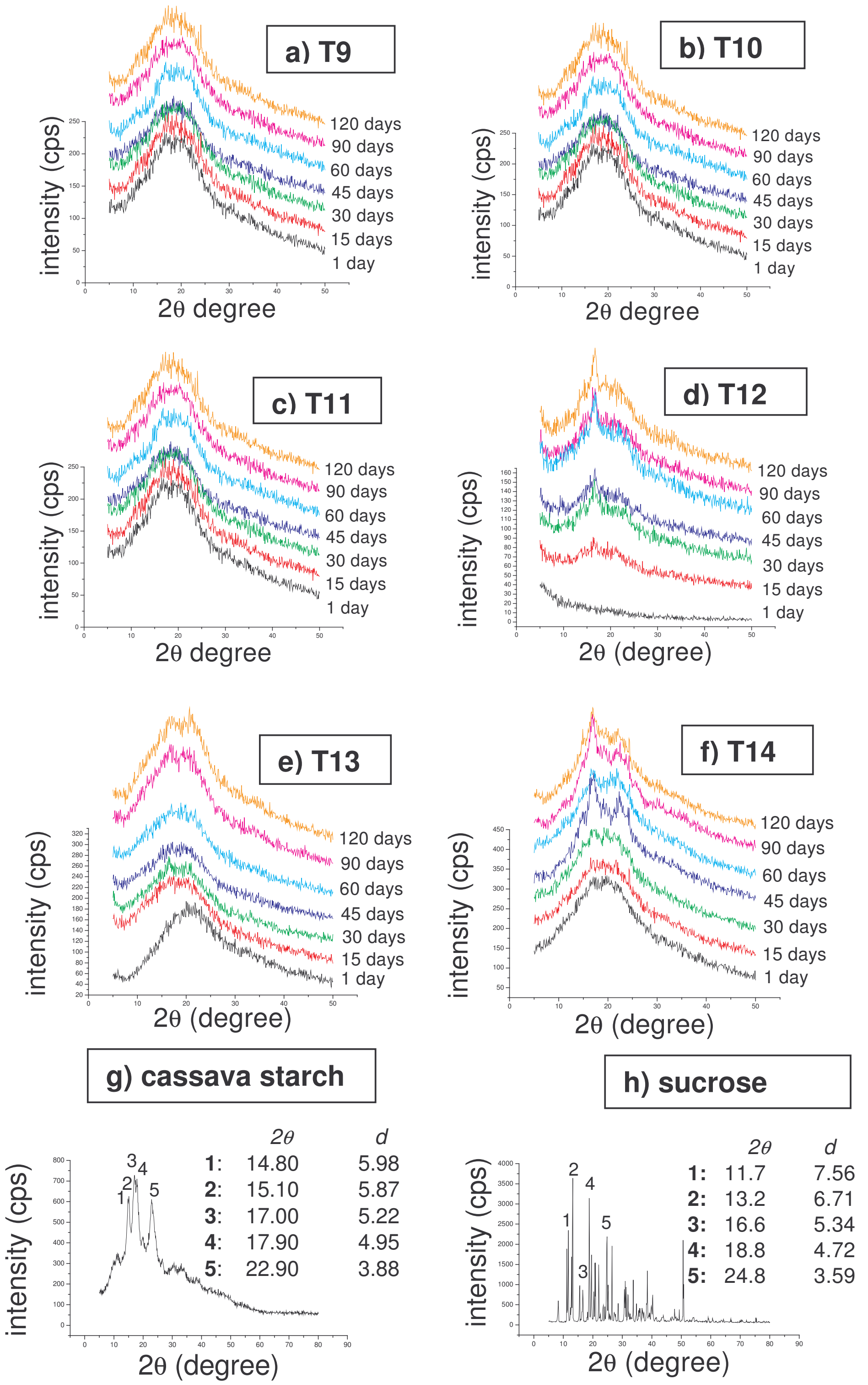

Fig. 2. WAXS crystallinity diffractograms of samples *amorphous*: (a) T9, (c) T11; *semi-crystalline*: (d) T12, (e) T13, (f) 14 and *crystalline:* (b) T10 behavior, during 120 days of storage. WAXS crystallinity diffractograms of (g) native cassava starch and (h) sucrose crystallinity standadrds.

The cassava starch films amorphous behavior at the first day of storage has already been reported by Veiga-Santos, Suzuki, Cereda & Scamparini (2004). Although cassava starch is the main component on the studied films, the amorphous behavior observed for all films at the first day of storage was explained by the crystallites instability near the jellification temperature. During jellification, the ordered crystalline regions undergo melting (Zobel & Stephen, 1995) which, according to Jenkins & Donald (1998), could decrease the crystallization ratio until zero, resulting in an amorphous structure. Also the low dehydratation temperature (30°C) can be responsible for the amorphous behavior. According Rindlav, Hulleman & Gatenholm (1997), dehydratation temperatures bellow 50°C can result in starch films with amorphous structures.

During the storage period (120 days), some samples remained amorphous, as exemplified by samples T9 (no sucrose addition), T11 (no inverted sugar addition) and T13 (no phosphate addition) (Fig. 2). A few other experimental samples also presented an amorphous behavior, as exemplified on Table 3.

Table 3.
2θ angle and *d*-spacing (*d*) matches for main peaks presented by semi-crystalline and crystalline samples WAXS diffractograms.

	2θ angle (interplane distance- d)				**Dgc**[b]	**Dsc**[c]
Cassava Starch Standard	nob[d]	*17.0(5.22)*	*22.9(3.88)*	nob[d]	na[e]	na[e]
Sucrose Standard	16.6(5.34)	nob[d]	nob[d]	24.8(3.59)	na[e]	na[e]
Samples						
T-1	nob[d]	nob[d]	nob[d]	nob[d]	120	120
T-2	nob[d]	*17.0(5.22)*	*22.7(3.90)*	nob[d]	15	120
T-3	nob[d]	nob[d]	nob[d]	nob[d]	120	120
T-4	nob[d]	*16.9(5.24)*	nob[d]	nob[d]	15	120
T-5	nob[d]	nob[d]	nob[d]	nob[d]	120	120
T-6	nob[d]	*16.9(5.24)*	nob[d]	nob[d]	30	120
T-7	16.7(5.31)	nob[d]	nob[d]	nob[d]	15	15
T-8	16.7(5.31)	nob[d]	nob[d]	nob[d]	15	15
T-9	nob[d]	nob[d]	nob[d]	nob[d]	120	120
T-10	16.6(5.34)	nob[d]	nob[d]	nob[d]	15	15
T-11	nob[d]	nob[d]	nob[d]	nob[d]	120	120
T-12	nob[d]	*16.9(5.24)*	nob[d]	nob[d]	30	120
T-13	nob[d]	nob[d]	nob[d]	nob[d]	120	120
T-14	nob[d]	*17.0(5.21)*	*22.2(3.99)*	nob[d]	45	120
T-15(c)[a]	nob[d]	*16.9(5.24)*	nob[d]	nob[d]	75	120
T-16(c)[a]	nob[d]	*16.9(5.24)*	nob[d]	nob[d]	75	120
T-17(c)[a]	nob[d]	*16.9(5.24)*	nob[d]	nob[d]	60	120
Control	nob[d]	nob[d]	nob[d]	nob[d]	120	120
R^2 [f]	na[e]	na[e]	na[e]	na[e]	0.89	0.94

(c)[a]: central points; Dgc[b]: days that samples remained amorphous for general crystallization; Dsc[c]: days that samples remained amorphous for sucrose crystallization; nob[d]: non observed; na[e]: not applicable; R^2 [f]: coefficient of determination.

The experimental X-ray diffractograms results (Table 3 and Fig. 2) indicate that no sucrose crystallization was observed for samples containing until 0.7% sucrose, if no inverted sugar was added. Also when adding 1.116% sucrose and 0.284% of inverted sugar, samples remained amorphous (sample T5, Table 3). The results indicate that inverted sugar can prevent sucrose crystallization depending on the concentration added. The protective effect of inverted sugar on sucrose crystallization was expected due to its lower crystallinity tendency when comparing to sucrose (Lees, 1995). The inverted sugar can increase the film forming suspension viscosity, difficulting crystals approaching (Jackson, & Howling, 1995).

However, inverted sugar addition was not enough to prevent sucrose crystallization in all samples. Samples T7 and T8 (containing the same concentration of sucrose and inverted sugar, 1.116%) and T10 (containing 1.400% sucrose and

0.700% inverted sugar) presented a crystalline behavior during storage (Table 3). Samples crystalline behavior was exemplified in Fig. 2b by sample T10.

In order to provide crystallization values for statistical analysis, it was considered the total days that samples remained amorphous, in relation to sucrose crystallization. For samples that did not present sucrose crystallization it was considered the total days that samples remained amorphous during storage (120 days). Semi-crystalline samples were considered amorphous for the evaluation of films crystallization due to sucrose addition. The Pareto chart of standardized effect has indicated that sucrose and inverted sugar have affected ($p<0.05$) the material crystallization by sucrose.

The response surfaces of sucrose crystallinity (Fig. 3) indicates that the best combination of sucrose and inverted sugar to avoid sucrose crystallization is until 0.8% sucrose with any concentration of inverted sugar, or the maximum of 1.2% sucrose with 0.4% of inverted sugar.

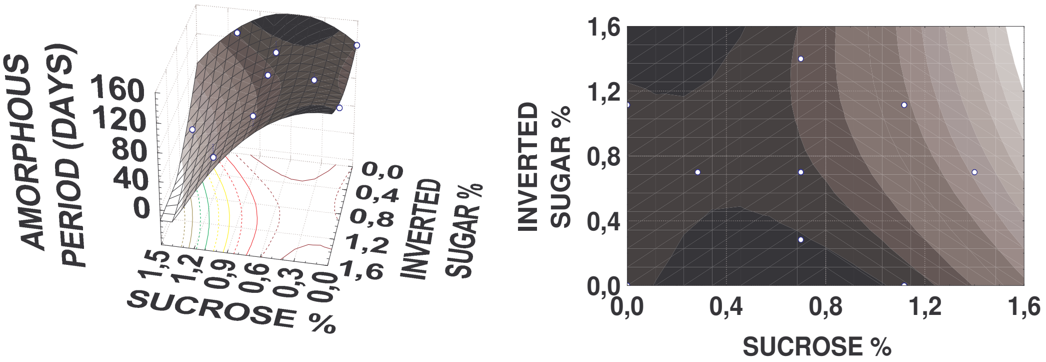

Fig. 3. Response surface for the significant effect of sucrose and inverted sugar on the sucrose crystallization during storage (120 days).

Sucrose crystallization starts with a crystallinity nucleus (Jackson & Howling, 1995) that could arise from rearrangement of randomly bound sugar molecules (Stansell, 1995). Sucrose crystallinity in cassava starch films has already been reported (Veiga-Santos, Suzuki, Cereda & Scamparini, 2004).

The 2θ angle and *d*-spacing (distance between lattice planes) of main peaks observed during storage of samples T7, T8 and T10, matches with sucrose standard main peaks 2θ angle and *d*-spacing (Fig. 2h and Table 3), reinforcing the theory that the observed crystallization was due to sucrose.

It was also noticed a cassava starch reorganization on some films (Table 3). Observing samples T11 (no inverted sugar addition) and T12 (maximum inverted sugar addition), it can be clearly noticed that increasing inverted sugar concentration leads samples to a semi-crystalline behavior. Comparing the main peaks 2θ angle and *d*-spacing of sample T12 to the cassava starch standard main peaks (Table 3, Fig. 2g), it can possibly be concluded that the crystallization observed was due to cassava starch reorganization. When crystallinity was due to cassavas starch peaks, the materials were considered semi-crystalline.

The degree of starch crystallinity depends on the water content of the material (Zobel & Stephen, 1995). Rindlav, Hulleman & Gatenholm (1997) have observed that crystallinity appear during higher water content films storage. If the equilibrium relative humidity (ERH) of a sugar is low, the product will attract moisture. As inverted sugar has a lower ERH than sucrose (180.2 and 342.3, respectively) (Jackson & Howling, 1995) and, inverted sugar is more humectant than sucrose (Mathlouthi, 2001), inverted sugar addition could result in films with higher water content, which consequently, could lead to cassava starch re-crystallization.

When comparing samples T13 and T14, which differed only by the phosphate content, it can be observed that sample T14 added with the maximum phosphate concentration resulted in semi-crystalline behavior. Comparing the main peaks 2θ angle and *d*-spacing of sample T14 to the cassava starch standard main peaks (Table 3, Fig. 2), it can be concluded that the crystallization observed was also due to cassava starch.

Phosphate addition increases the jellification temperature of starch. While native starch granules rupture gradually on heating above 70℃, the phosphorilated starch did not rupture until 100℃ (Morikawa & Nishinari, 2000). As all film forming suspensions were heated to the same temperature (75℃), samples added with phosphate content higher than 0.484%, could increased the jellification temperature of the suspension. Thus, some starch granules could remain not jellified on the film forming suspension, been detected by the X-ray analysis.

Statistical Pareto chart of standardized effect was also performed to evaluate the additives influence on the time required for general crystallization to begin, considering the sucrose crystallization and the cassava starch crystallization. For that matter, it was considered the total of days that the material remained amorphous (Table 3). The Pareto chart of standardized effect confirmed that all additives affected ($p<0.05$) the material crystallinity or semi-crystallinity behavior during storage.

Scanning Electron Microscopy

Samples surface and cross section SEM micrographs were recorded at 5 and 120 days (± 4) of storage. At the first week storage period, all samples presented a compact and smooth structure, both for surface and cross section, as exemplified by samples T10, T12 and T14 (Fig. 4).

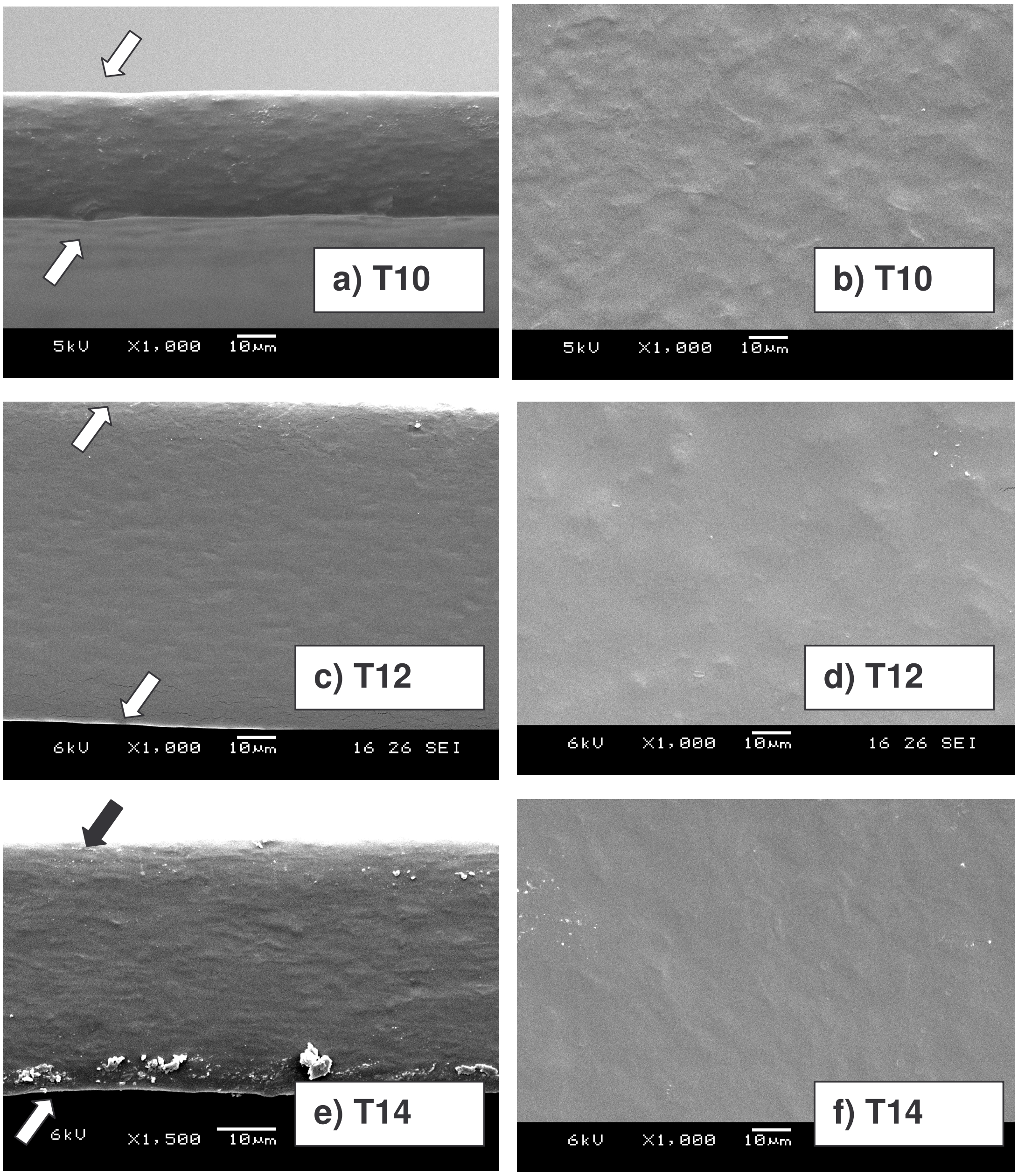

Fig. 4. Surface and cross section scanning electron micrographs of samples *surface*: (a) T10, (c) T12, (e) T14 and *cross-section*: (b) T10, (d) T12 and (f) T14, at the first week of storage.

However, after 120 days of storage, some samples containing sucrose as additive, presented a visible crystallinity, as exemplified by sample T10 (7% sucrose, 3.5% inverted sugar and 1.5% phosphate) surface and cross section scanning electron micrographs (Fig. 5).

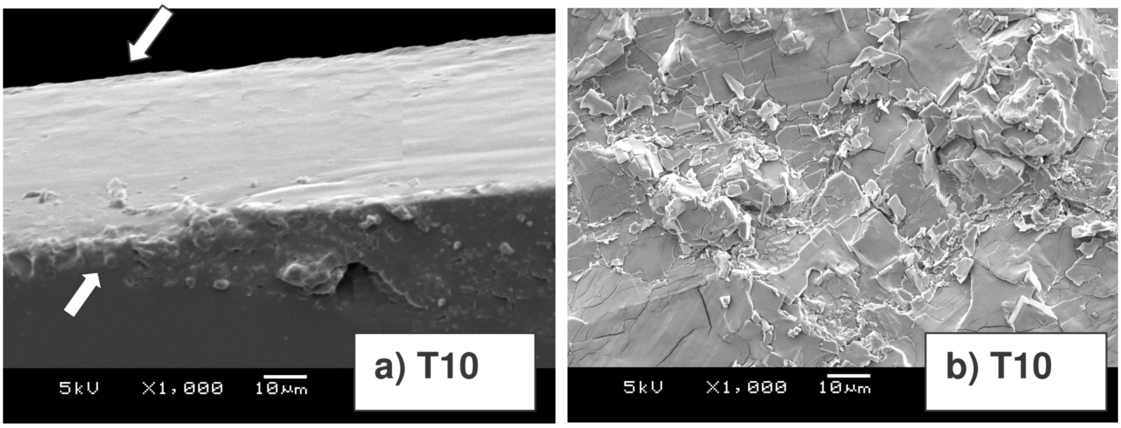

Fig. 5. Scanning electron micrographs of sample T10 (a) surface and (b) cross-section, after 120 days of storage.

The crystal growth observed in Fig. 5 micrographs is in agreement with the crystalline behavior observed on X-Ray diffractograms, confirming the crystalline behavior of the cassava starch films added with higher content of sucrose (1.4% in this case).

Crystals were also observed for samples T7 and T8, again, in agreement with the X-ray diffraction results.

Cassava starch-sucrose cross-section micrograph (Fig. 5b) demonstrated also that the crystallinity began at the surface and not at the material interior. Such result indicates that the crystallization was not due to residual not dissolved sucrose crystals (Veiga-Santos, Suzuki, Cereda & Scamparini, 2004).

For the amorphous samples and the semi-crystalline samples (samples tat present matched peaks with the cassava starch standard), no visible crystal was observed. Such results indicate that the semi-crystalline behavior did not affect the material microstructure.

Optical Microscopy

Although not often utilized for biodegradable films analyses, light microscopy demonstrated to be a very important tool in characterizing the film microstructure, as exemplified by samples T10 and T14 ordinary (not polarized “npol”) and polarized (“pol”) light micrographs (Fig. 6).

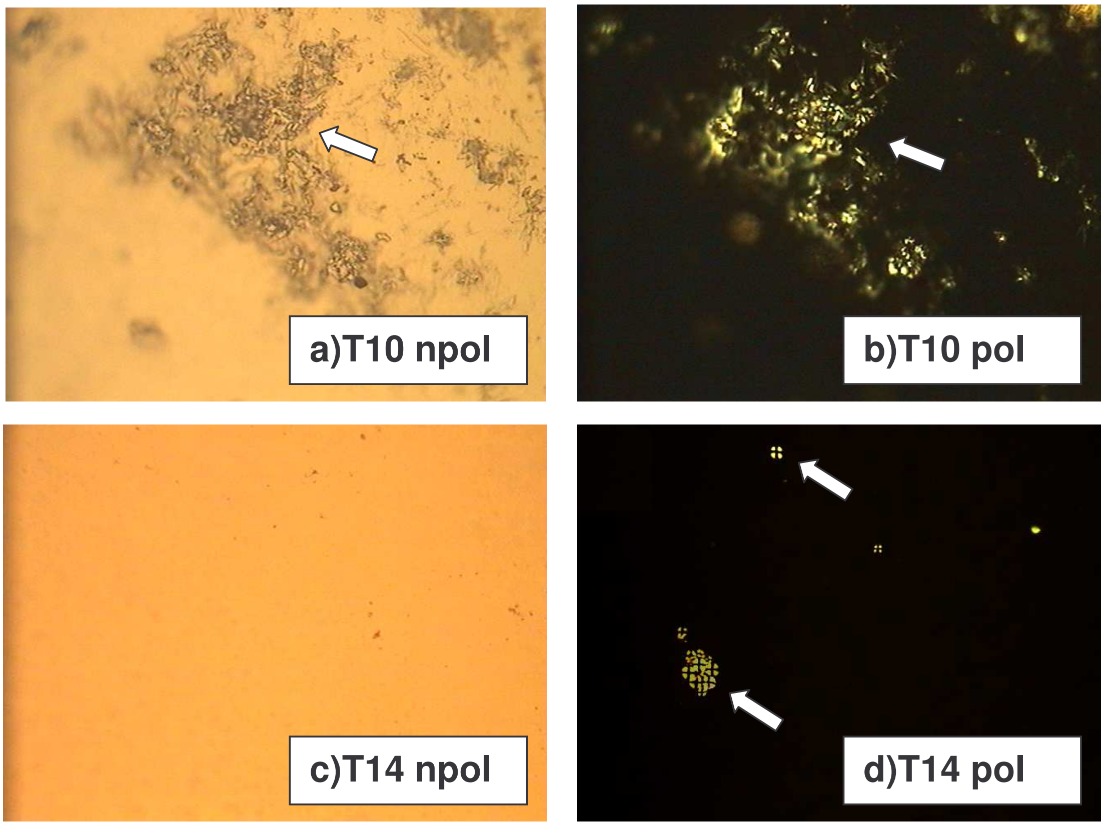

Fig. 6. Samples *ordinary* (not polarized: “npol”): (a) T10, (c) T14 and *polarized* (“pol”): (b) T10, (d) T14 light micrographs, after 120 days of storage.

In this study, light micrograph was utilized for characterizing the material crystallization during 120 days of storage. Analyzing the light micrograph of sample T10 (added with the maximum level of sucrose) it can be observed crystallization at the material surface (Fig. 6a). Also polarized light was used to confirm the material crystallinity patterns (Fig. 6b), since crystalline materials have birefringence detected by polarized light. The material areas that were not yet crystallized appeared completely black in the field of view (Greaves & Saville, 1995).

The crystallization of the material during storage was also clearly observed by light micrographs images. The crystallinity results observed by ordinary and polarized light microscopy are in agreement with SEM images and X-ray diffractograms, demonstrating thus, that this analysis is a feasible, fast and low cost tool for observing sucrose content films crystallinity during storage.

Polarized light microscopy also allowed to confirm that the semi-crystalline structure observed for samples added with sodium phosphate concentration up to 0.48% is probably due to non totally jellified cassava starch molecules (as exemplified by sample T14, added with the maximum phosphate concentration; Fig. 6d).

When polarized light is used on no jellified starch granules, a typical birefringence is evident from the polarization cross (white cross on a black background - the “Maltese cross”) seen in a polarizing microscope with the polarizes set 90º to each other, (Zobel & Stephen, 1995; BeMiller & Whistler, 1996). Loss of birefringence, and therefore molecular order, is observed during jellification (Zobel & Stephen, 1995). The “Maltese cross” observation by polarized light microscope could, therefore, indicate the presence of residual non jellified cassava starch on the film structure.

4. Conclusions

None of the studied additives had significant influence on materials water activity, solubility and the color (“L”, “a”, “b” and haze). However, when comparing to cassava starch films formulated without additives, an increase on water activity, solubility and total color difference was observed. X-ray diffractograms and SEM and light microscopy have indicated that inverted sugar can prevent sucrose crystallization if utilized the combination up to 0.8% sucrose with any concentration of inverted sugar, or the maximum of 1.2% sucrose with 0.4% of inverted sugar. Increasing inverted sugar and phosphate concentration leads samples to a semi-crystalline behavior. Inverted sugar addition probably resulted in films with higher water content, leading to cassava starch re-organization. Samples added with phosphate, probably increased the cassava starch jellification temperature, resulting in remained not-jellified starch granules on the film forming suspension.

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Conclusões Gerais

1) A goma xantana desacetilada, embora não tenha prevenido a cristalização da sacarose, retardou em alguns dias o início da cristalização. Também aumentou a cinética de absorção de água dos filmes obtidos de fécula de mandioca. Entretanto, os benefícios atribuídos ao filme foram pouco marcantes, não justificando sua utilização devido ao seu alto custo.
2) A adição de gelatina resultou em diminuição na % de alongamento e resistência à tração das amostras analisadas. Sua aplicação em filmes obtidos de fécula de mandioca, nas condições avaliadas, não é recomendada.
3) A variação na concentração de fécula de mandioca afetou a coloração e a diferença total de cor do material. Diminuiu a cinética de absorção de água e a % de alongamento, embora tenha aumentado ligeiramente a resistência à tração dos materiais. Como todos os filmes mantiveram um alto brilho e pouca coloração, sugere-se que a concentração de fécula seja mantida em 5%.
4) O óleo de soja afetou a coloração, aumentou a % alongamento e diminuiu a resistência à tração dos filmes. Não afetou a hidrofilicidade dos materiais, como era esperado e não foi totalmente incorporado à rede estrutural do filme, havendo separação de fases. Sua aplicação em filmes obtidos de fécula de mandioca, nas condições avaliadas, não é recomendada.
5) O propileno glicol diminui a resistência à tração dos filmes e não teve efeito sobre a cristalização da sacarose. Sua aplicação em filmes obtidos de fécula de mandioca, nas condições estudadas, não é, portanto, recomendada.
6) O fosfato de sódio afetou a diferença total de cor e a % de alongamento dos materiais. Também afetou a temperatura de gelificação da suspensão formadora do filme e diminuiu a perda de massa do filme após o pico endotérmico, provavelmente relacionado à degradação do material. Tais resultados indicam que houve ligações entre o fosfato e a fécula. A continuidade da avaliação da aplicação deste aditivo em filmes obtidos de fécula de mandioca é recomendada.

7) O pH alcalino afetou a coloração e o pH ácido aumentou a cinética de absorção de água de filmes. Em filmes contendo gelatina, o pH ácido, próximo ao ponto isoelétrico da proteína, diminuiu interações deste aditivo com a rede estrutural do filme. Entretanto, seu efeito nos filmes obtidos de fécula de mandioca foi pouco pronunciado. Pela facilidade da obtenção de um processo onde não é necessário controlar o pH e pelo fato da adição de gelatina ter sido descartada, sugere-se que o pH da suspensão formadora do filme não seja controlado.

8) A utilização da temperatura de 60℃ destruiu completamente os filmes, sendo recomendadas temperaturas mais baixas de secagem. Temperaturas de 30°C garantiram a integridade dos filmes, independentemente da adição de aditiovs.

9) A utilização da sacarose resultou em aumento da % de alongamento dos filmes (em até 2900%) quando comparados ao filmes obtido de amido de mandioca sem aditivos. Entretanto uma redução na resistência à tração (de até 100%) foi também observada. Sua utilização resultou em cristalização dos filmes durante a estocagem, tornando-os extremamente quebradiços. Afetou a diferença total de cor dos filmes e também aumentou a atividade de água de todos os grupos de filmes estudados, tornando-os mais susceptíveis à contaminação microbiológica, especialmente por fungos. Aumentou a cinética de absorção de água de todos os grupos das amostras, tornando-as mais hidrofílicas. O grande ganho que a sacarose confere a % de alongamento dos filmes obtidos de amido de mandioca justifica a continuidade de seu estudo em conjunto com outros aditivos que possam amenizar problemas causados pela cristalização e aumento na atividade de água, as maiores desvantagens de sua utilização.

10) O açúcar invertido aumentou a atividade de água e a hidrofilicidade dos materiais. Aumentou (em até 1700%) a % de alongamento embora também tenha reduzido a resistência á tração. Evitou a cristalização de sacarose quando utilizada em 0.4%, na presença de até 1.2% de sacarose. Embora não tenha afetado a microestrutura dos filmes, resultou em recristalização da

fécula de mandioca durante a estocagem. Sua utilização em conjunto com a sacarose para melhorar a % de alongamento de filmes obtidos de amido de mandioca é altamente recomendável.

11) Nenhum aditivo da etapa de otimização afetou significativamente a permeabilidade ao vapor d'água, a atividade de água e a solubilidade em água. Entretanto, comparando as amostras com o controle, houve um aumento na atividade de água, solubilidade em água e uma diminuição na permeabilidade ao vapor d'água.
12) Caso a permeabilidade ao oxigênio seja indesejada, filmes adicionados com 0,7% de sacarose, 1,4% de açúcar invertido e 0,3% de fosfato de sódio são a melhor alternativa e apresentam boa relação entre a força de tensão (>60 kgf/cm^2) e a % alongamento (>60%).
13) Duas temperaturas de transição vítrea foram observadas nos filmes da etapa de otimização, indicando separação de fase.
14) Durante a análise de DSC foram observados 2 picos, um endotérmico, provavelmente relacionado à degradação do material e outro exotérmico, provavelmente relacionado à sua cristalização.
15) A adição de sacarose e açúcar invertido acarretou em melhorias nos filmes obtidos de fécula de mandioca. Embora ainda possuam alta permeabilidade ao vapor d' água quando comparados aos filmes tradicionais, os filmes elaborados representam alternativas potenciais devido ao seu baixo custo e ao fato de serem elaborados com matérias-primas advindas de produtos de grande importância para a agricultura brasileira, como cana de açúcar e fécula de mandioca.

Sugestões de Continuidade

1. Embora o óleo de soja não tenha sido incorporado pela rede estrutural do filme, outros aditivos lipídicos deveriam ser testados na tentativa de aumentar a resistência ao vapor d'água dos filem obtidos a partir de fécula

de mandioca. Tal melhoria aumentaria a eficácia dos filmes propostos neste estudo.

2. Neste estudo somente filmes flexíveis, elaborados por moldagem, foram investigados. Outras técnicas para produção de embalagens biodegradáveis (como extrusão ou a combinação de moldagem e pressão) deveriam ser testadas para a formulação proposta. Pelos resultados obtidos na literatura, a extrusão de amido para produção de embalagens biodegradáveis resulta, em geral, em materiais com menor permeabilidade ao vapor d'água quando comparado aos filmes flexíveis obtidos de amido.

3. Embora a caracterização dos materiais tenha sido feita, não houve tempo para testes dos filmes como embalagem de produtos. Produtos contendo baixa atividade de água poderiam ser embalados e sua vida de prateleira acompanhada durante estocagem sob diferentes condições de umidade e temperatura.

Apêndice

Apêndice 1:

Matérias-Primas: Caracterização

1. Matérias-Primas: Caracterização

A caracterização físico-química das matérias-primas utilizadas estão apresentadas a seguir. O mesmo lote de matéria-prima foi utilizado para todos os experimentos.

Fécula de Mandioca

A caracterização da fécula de mandioca foi realizada pelo Centro de Raízes e Amidos Tropicais (CERAT), Faculdade de Agronomia, UNESP-Botucatu. A análise de umidade foi realizada em estufa a 105˚C/24hs (Instituto Adolfo Lutz, 1986); a de cinzas, por combustão em forno incinerador (AOAC, 1995). Lipídios totais foram determinados por Soxleth (AOAC, 1995), proteínas foram determinadas pelo método de Kjeldahl, utilizando 6,25 para calcular a proteína bruta (AOAC, 1995). O teor de fibras foi determinado por hidrólise ácida, seguida de hidrólise alcalina (AACC, 1975) e a densidade, pelo método de deslocamento de xileno em picnômetro (25˚C) (Schoch & Leach, 1964). O teor de amido total foi determinado por hidrólise enzimática, metodologia ISO-6647 (International Organization for Standardization, 1987a), calculando-se o teor de açúcares totais pelo método Somogy, adaptado por Nelson (1944). O teor de amilose foi quantificado por metodologia ISO-6647 (International Organization for Standardization, 1987b)

A Tabela 1 apresenta a caracterização da matéria-prima segundo o laudo apresentado pelo CERAT.

Tabela 1. Caracterização físico-química da fécula de mandioca segundo laudo fornecido pelo CERAT-UNESP.

	Fécula Mandioca
Umidade *(g/100g m seca)*	14,86
Fibras *(g/100g m seca)*	0,52
Lipídios totais *(g/100g m seca)*	0,15
Cinzas *(g/100g m seca)*	0,23
Proteínas *(g/100g m seca)*	0,24
pH	0,15
densidade	0,23
Amido total *(%)*	87,60
Teor de amilose *(%)*	16,02
Açúcares redutores *(%)*	0,21

Sacarose e Açúcar Invertido.

Os laudos fornecidos pelos fabricantes da sacarose analiticamente pura (Sinth, Brasil) e comercial (Copersucar, Brasil) estão apresentados na Tabela 2.

Tabela 2. Caracterização físico-química da sacarose analiticamente pura (P.A.) e comercial, segundo laudo fornecido pelos fabricantes.

	Sacarose P.A.	**Sacarose Comercial**	**Açúcar Invertido**
Açúcares redutores	0,0%	0,0%	72%
acidez	c/ acético 0,0008 meq/g	na*	na*

na: não analisado pelo fabricante.*

Análise de cromatografia líquida (HPLC) foi realizada para a determinação e quantificação dos monossacarídeos glicose e frutose e do dissacarídeo sacarose, presentes no açúcar invertido, sacarose comercial e sacarose analiticamente pura, utilizados nesta tese. As amostras foram diluídas em água destilada e deionizada, e filtradas, foram diretamente injetadas em HPLC, coluna (7,9 mm x 30 cm) SCR-101P, Shimatzu, Co. A fase móvel utilizada foi água destilada e deionizada, com fluxo de 0,6 mL/min, T=80°C, utilizando detector de índice de refração.

Para a quantificação das amostras foram utilizadas curvas padrão de sacarose, frutose e glicose (analiticamente puras, SIGMA) com 6 pontos cada. As equações obtidas das curvas padrão, seus respectivos R^2, e a quantificação obtida para as matérias-primas estão apresentados na Tabela 3.

Tabela 3. Equações das curvas padrão para sacarose, glicose e frutose. Também seus respectivos R^2, e a quantificação por HPLC.

	Glicose (%)	**Frutose (%)**	**Sacarose (%)**
Sacarose P.A.	nd*	nd*	99,72
Sacarose Comercial	nd*	nd*	99,68
Açúcar Invertido	41,58	26,94	26,13
Equação curva padrão (R^2)	y = 141297x	y = 77224x	y = 215728x
R^2	0,9854	0,9927	0,9992

nd: não detectado.*

As Figuras 1, 2 e 3 apresentam os cromatogramas obtidos para a sacarose analiticamente pura (Sinth, Brasil), sacarose comercial (marca A) e açúcar invertido (marca B).

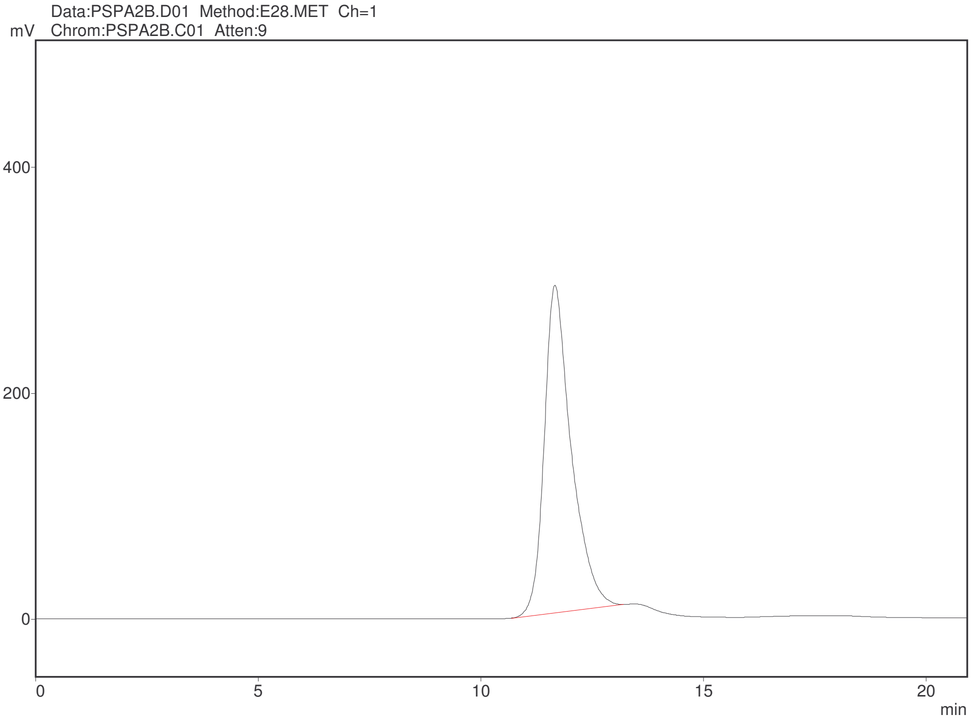

Figura 1. Composição da sacarose analiticamente pura, obtida por análise de HPLC.

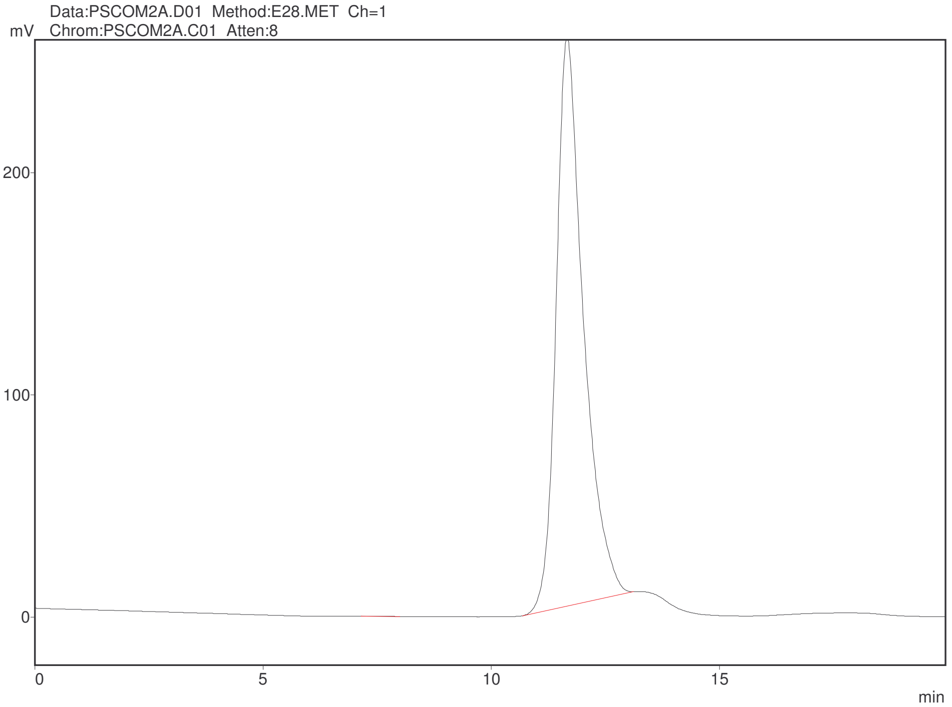

Figura 2. Composição da sacarose comercial, obtida por análise de HPLC.

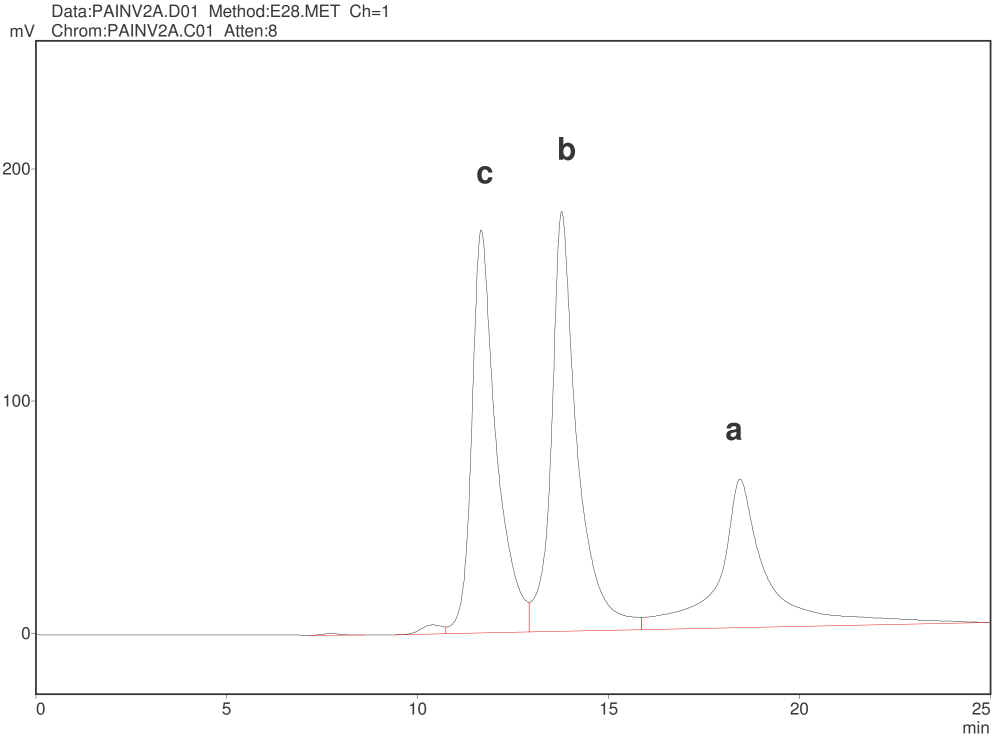

Figura 3. Composição do açúcar invertido, obtida por análise de HPLC com picos de (a) frutose, (b) glicose e (c) sacarose.

Gelatina

Neste estudo foi utilizada gelatina (tipo B), cuja composição físico-química está apresentada na Tabela 4, segundo laudo fornecido pelo fabricante (LINER DAVIS GELATIN, Brasil).

Tabela 4. Caracterização físico-química da gelatina utilizda, segundo laudo fornecido pelo fabricante.

	Gelatina tipo B
Lote	MG1765
Bloom	245 g
cinzas	0,66
umidade	9,5%
viscosidade	32 cp
turbidez	39,8
granulometria	30 mesh

Goma Xantana acetilada e desacetilada

A goma xantana (Rhodigel 200), sem nenhuma desacetilação, foi doada pela Rhodia Brasil, e a goma xantana desacetilada (tipo K0B619), pela CP Kelco Brasil. Os laudos fornecidos pelos fabricantes estão apresentados na Tabela 5.

Tabela 5. Caracterização físico-química da goma xantana sem desacetilação (acetilada) e desacetilada, segundo laudo fornecido pelos fabricantes. Acetilação calculada, de acordo com metodologia (TAN *et al.*,1998).

	Xantana acetilada	**Xantana desacetilada**
código comercial	Rodigel ultra	K0B619
Lote	992841	1C0351A
viscosidade	1,200 – 1,600 mPa.s (cP)	1,200 – 1,900 mPa.s (cP)
Arsênico	Max 2mg/Kg	Max 3mg/Kg
Total metais pesados	Max 20 mg/Kg	Max 20 mg/Kg
pH	6 - 8	6 - 8
Limites microbiológicos		
bactérias	Max 2000 ufc/g	Max 2000 ufc/g
Leveduras	Max 100 ufc/g	Max 100 ufc/g
Fungos	Max 100 ufc/g	Max 100 ufc/g
coliformes	nd.*	Negativo pelo MPN
E. Coli	Ausênica em 25 g	Ausênica em 25 g
Salmonela	Ausênica em 25 g	Ausênica em 25 g
Staphylococcus aureus	Ausênica em 25 g	Ausênica em 25 g
Pseudomonas aeruginosa	Ausênica em 25 g	Ausênica em 25 g
(%) acetilação calculada	5,75%	3,35%

nd.: não declarado pelo fabricante.*

Análise de titulometria (TAN *et al.*,1998) foi realizada para verificar o teor de desacetilação entre as amostras. Os resultados das análises, também apresentados na Tabela 5, mostram um teor de desacetilação de 41,73% entre as amostras.

Propileno Glicol

A caracterização físico-química do propileno glicol analiticamente puro é apresentada na Tabela 6, cujo laudo foi fornecido pelo fabricante (Sinth Brasil).

Tabela 6. Caracterização físico-química do propileno glicol segundo laudo fornecido pelo fabricante.

	Propileno Glicol
PM	76,10
Umidade	0.2%
densidade	1,035-1,037
Impurezas máximas	
Ácido CH_3COOH	0,006%
Cloretos	0,01%
Sulfatos	0,002%
Metais pesados	0,0005%
Ponto ebulição	180°-188°C

Fosfato de sódio monobásico

A caracterização físico-química do fosfato de sódio monobásico (analiticamente puro) é apresentada na Tabela 7, cujo laudo foi fornecido pelo fabricante (Sinth, Brasil).

Tabela 7. Caracterização físico-química do fosfato de sódio monobásico, segundo laudo fornecido pelo fabricante.

Fosfato de Sódio Monobásico	
Titulação com ácido base	99,0%
pH (25°C, solução 5%)	4,1 – 4,5
Impurezas	0,01655%
Material insolúvel ppt pelo Ca e NH_4OH	0,01%

Óleo de soja

A caracterização físico-química do óleo de soja comercial é apresentada na Tabela 8, cujo laudo foi fornecido pelo fabricante (Cargill Agrícola S.A., Brasil).

Tabela 8. Caracterização físico-química do óleo de soja comercial, segundo laudo fornecido pelo fabricante.

Óleo de soja	p/ 15 mL
Lipídios totais	14g
Lipídios saturados	2g
Lipídios monoinsaturados	4g
Lipídios polinsaturados	8g
Vitamina E	2mg
pH	

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Apêndice 2:

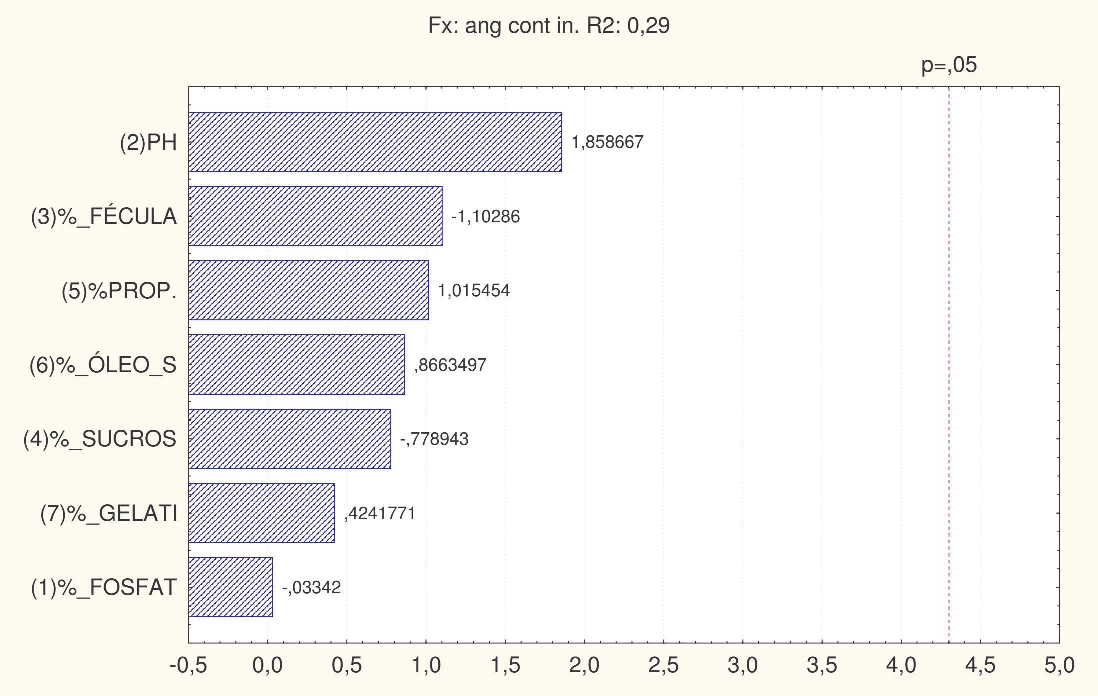
Gráficos Pareto:

Caracterização de filmes à base de fécula e goma xantana

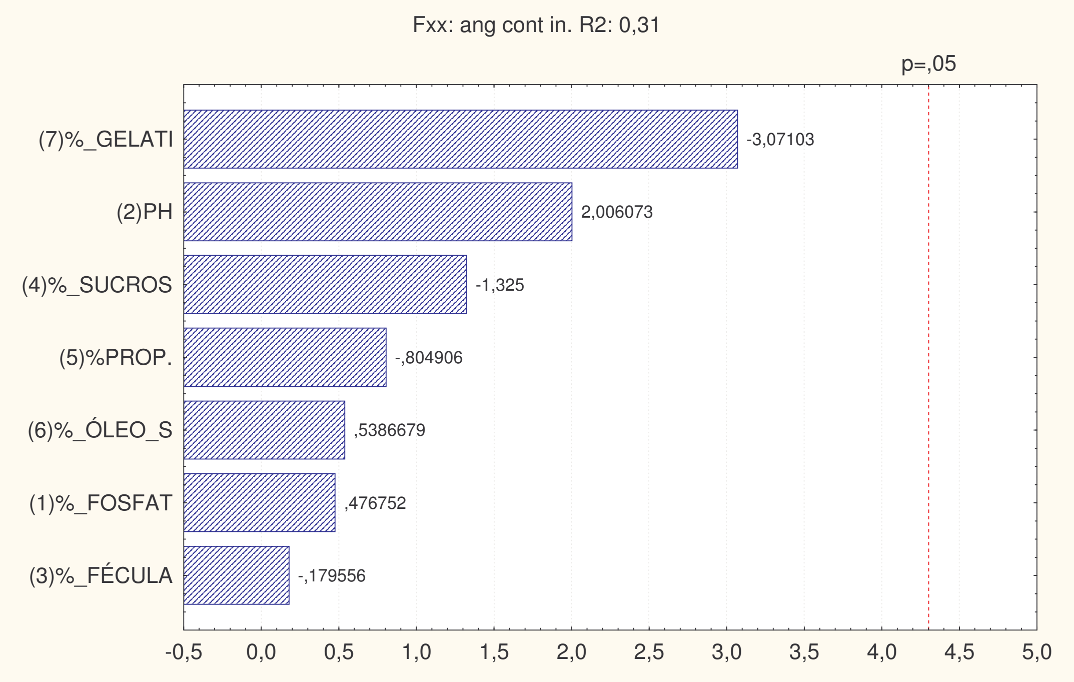
1. Gráficos Pareto dos filmes à base de fécula e goma xantana

Para os filmes à base de fécula de mandioca e goma xantana foram utilizados símbolos conforme os blocos estatísticos utilizados. Para bloco 1, referente aos capítulos 2 e 3, contendo sacarose goma xantana acetilada foi utilizado o símbolo Fx. Para o bloco 2, referente aos capítulos 2 e 3, contendo goma xantana desacetilada foi utilizado o símbolo Fxx.

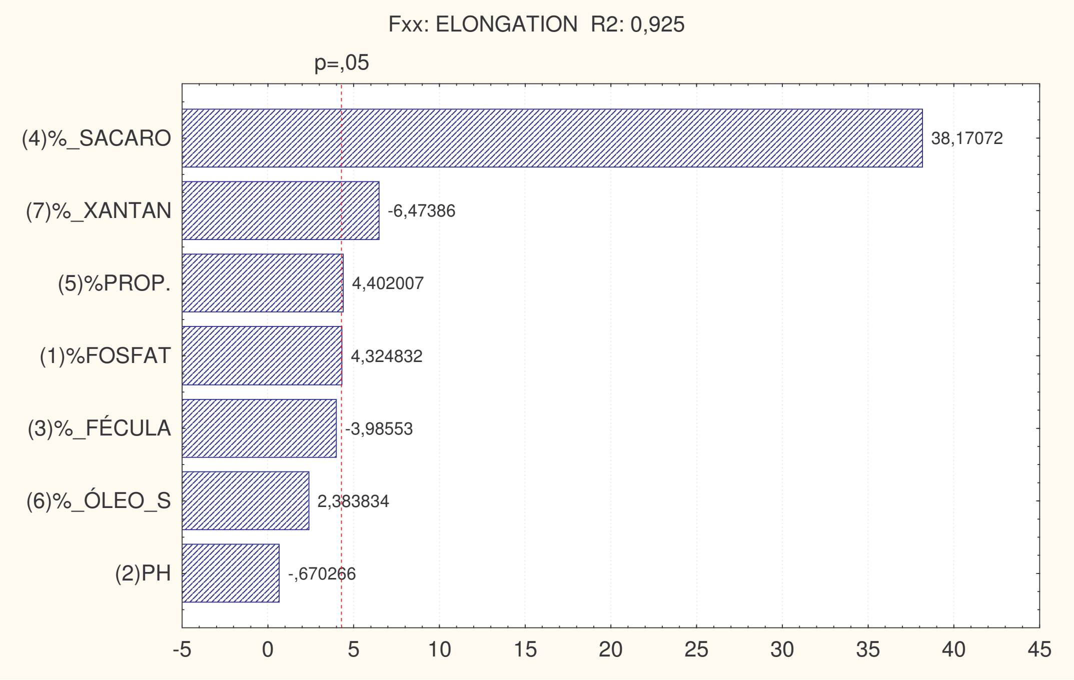
1.1. Gráfico Pareto para ângulo de contato.



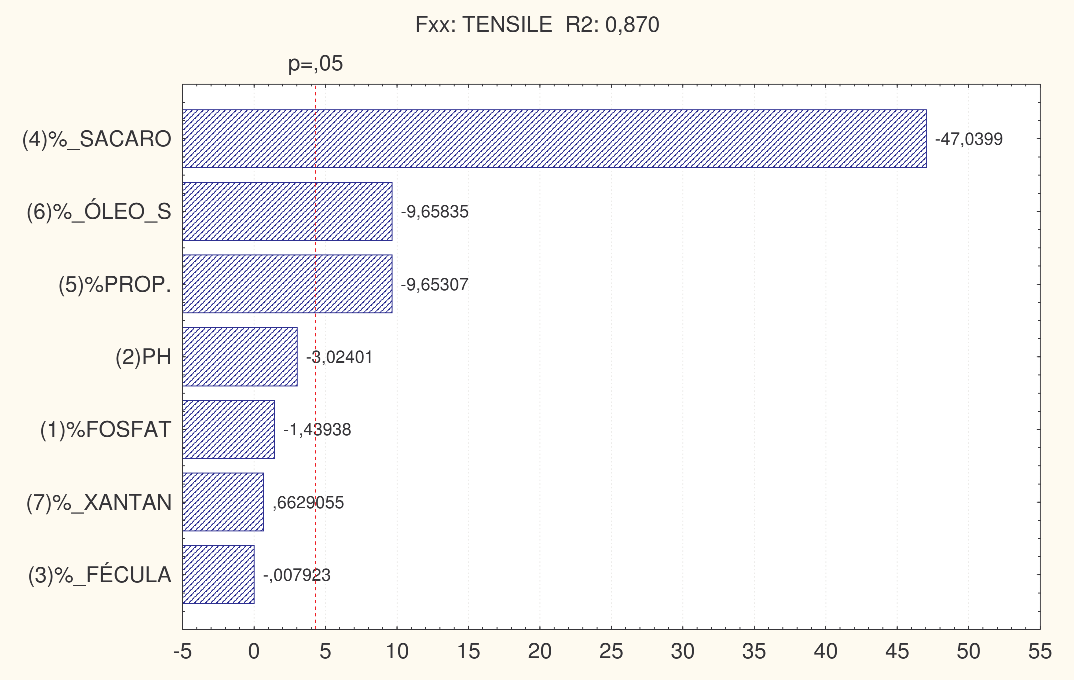
1.2. Gráfico Pareto para ângulo de contato.



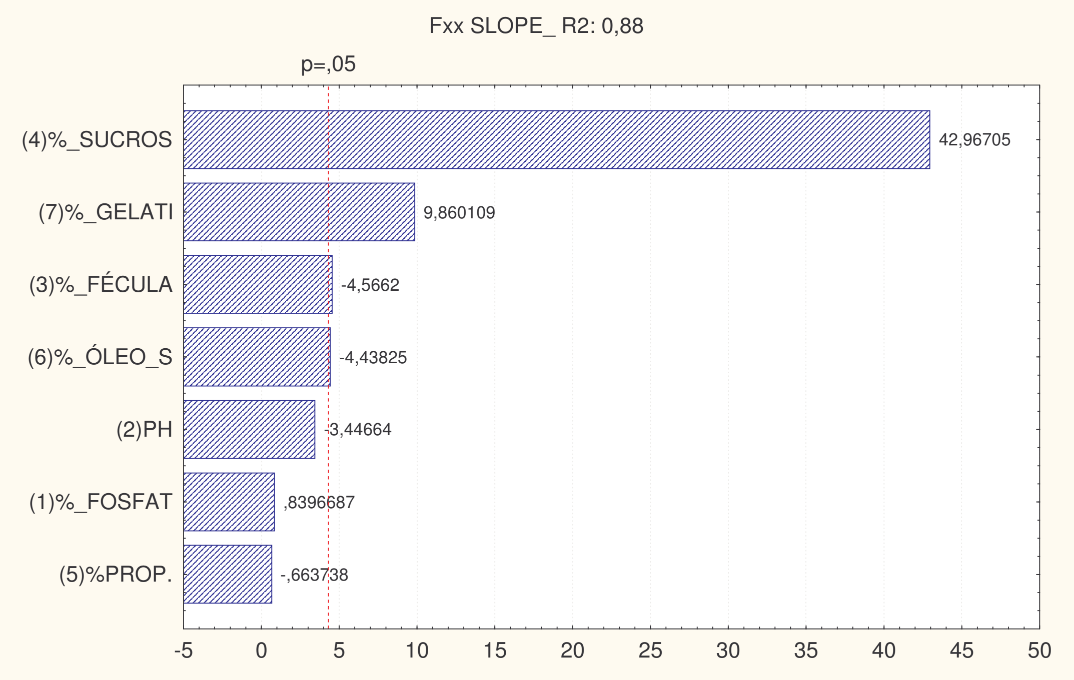
1.3. Gráfico Pareto para alongamento.



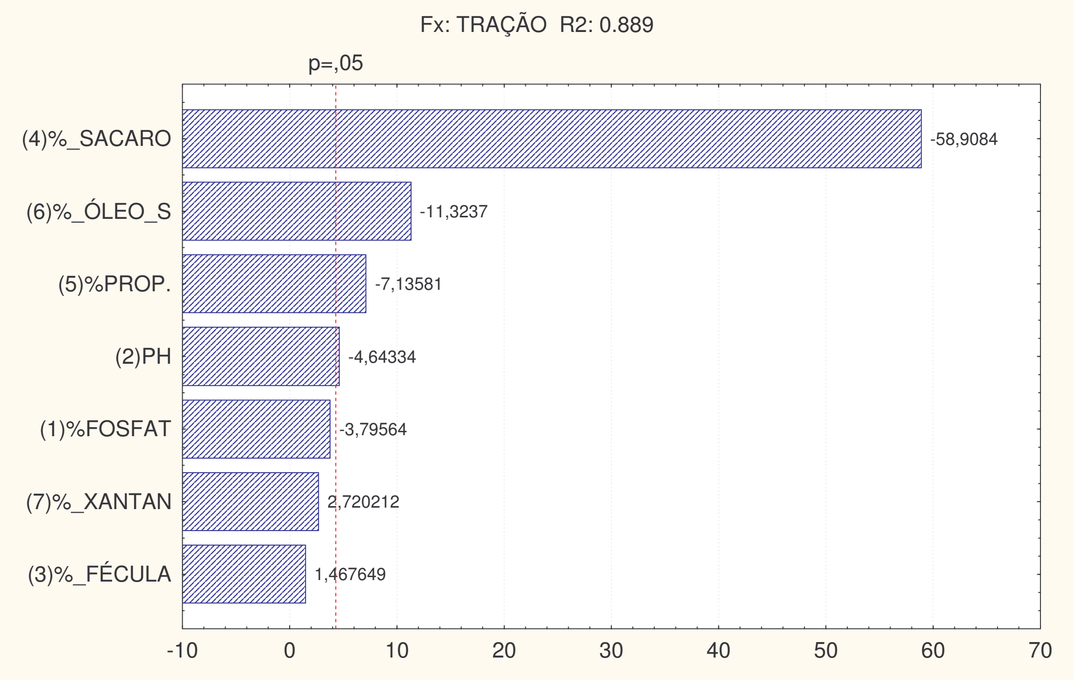
1.4. Gráfico Pareto para tração.



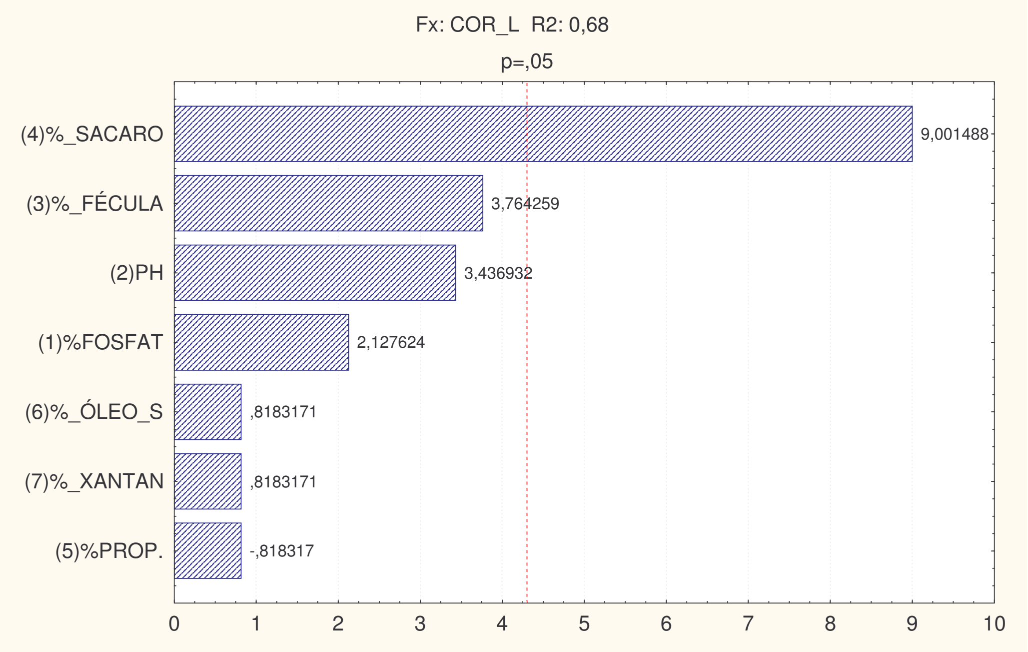
1.5. Gráfico Pareto para ângulo de contato (slope)



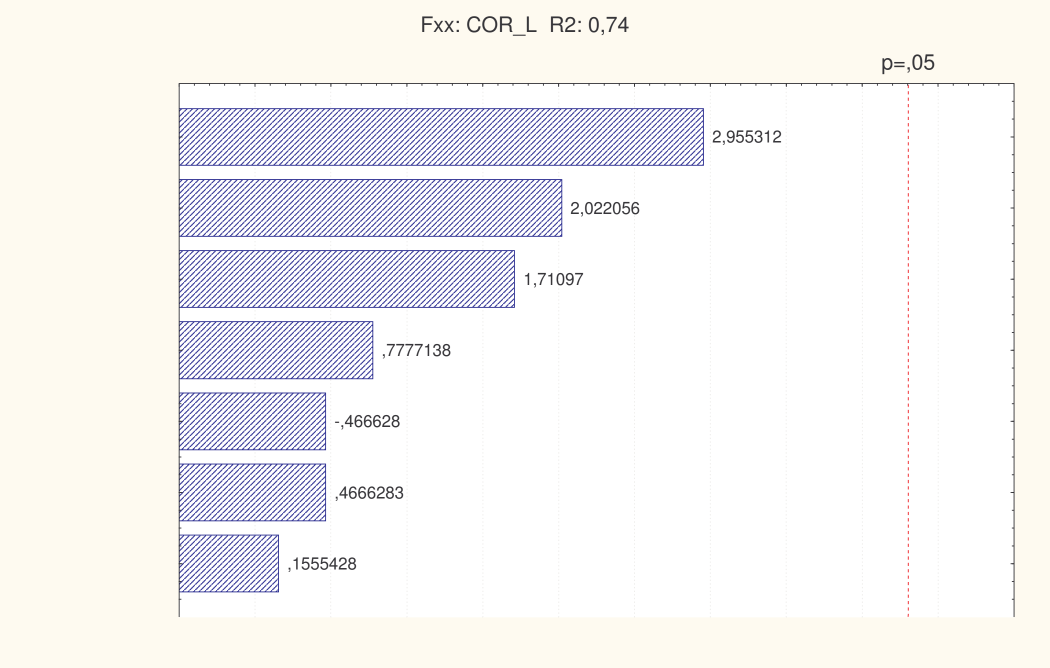
1.6. Gráfico Pareto para tração



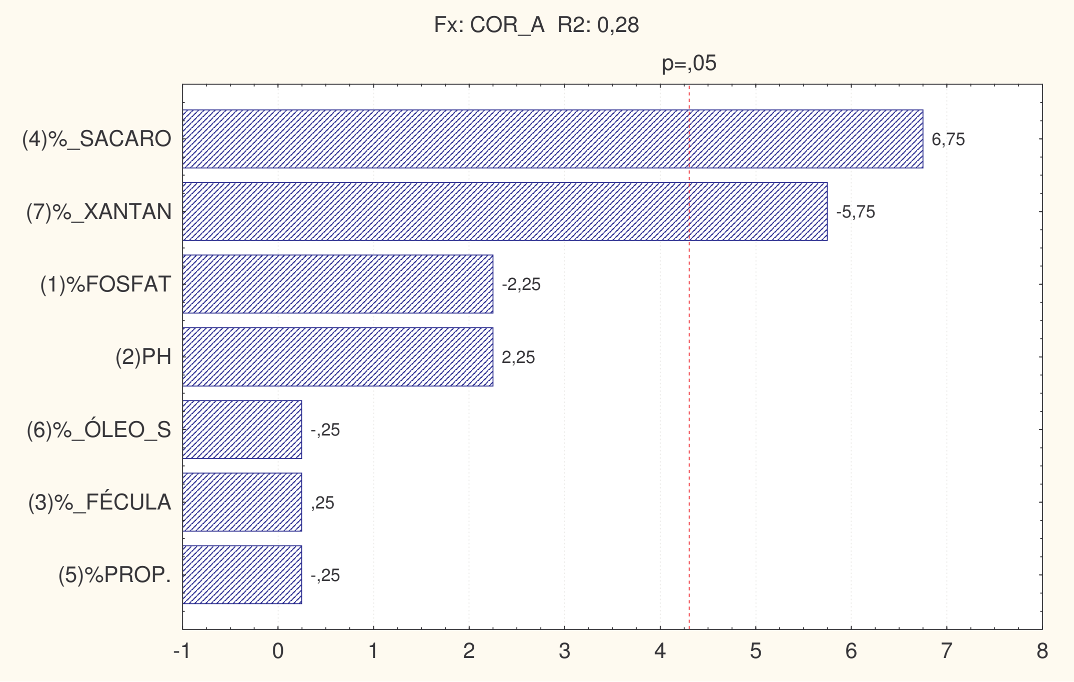
1.7. Gráfico Pareto para cor "L".



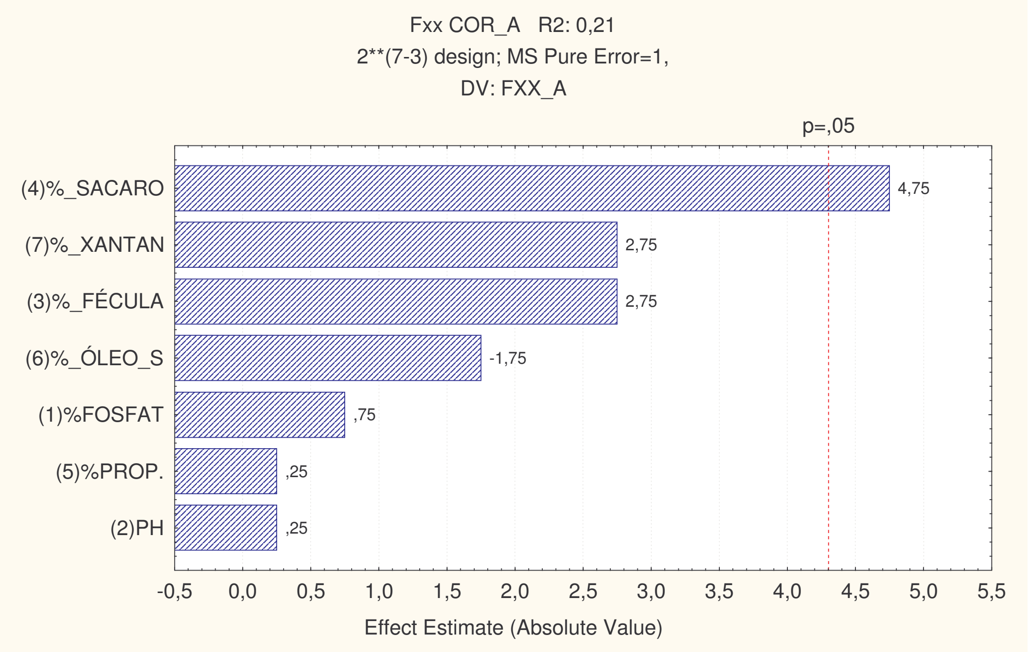
1.8. Gráfico Pareto para cor “L”.



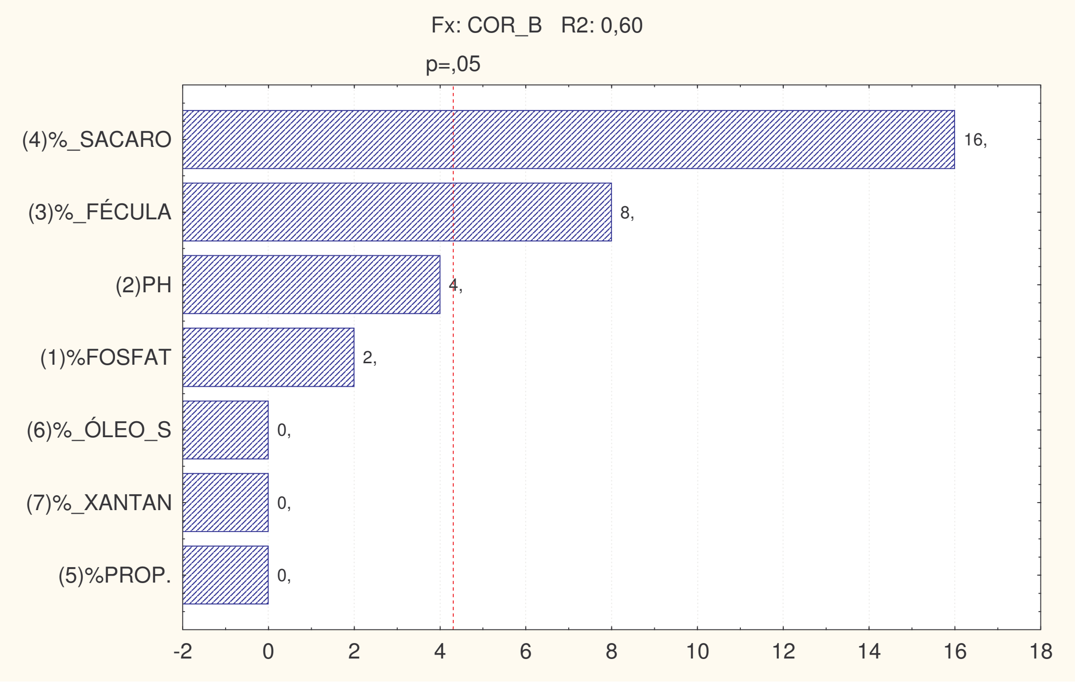
1.9. Gráfico Pareto para cor “a”.



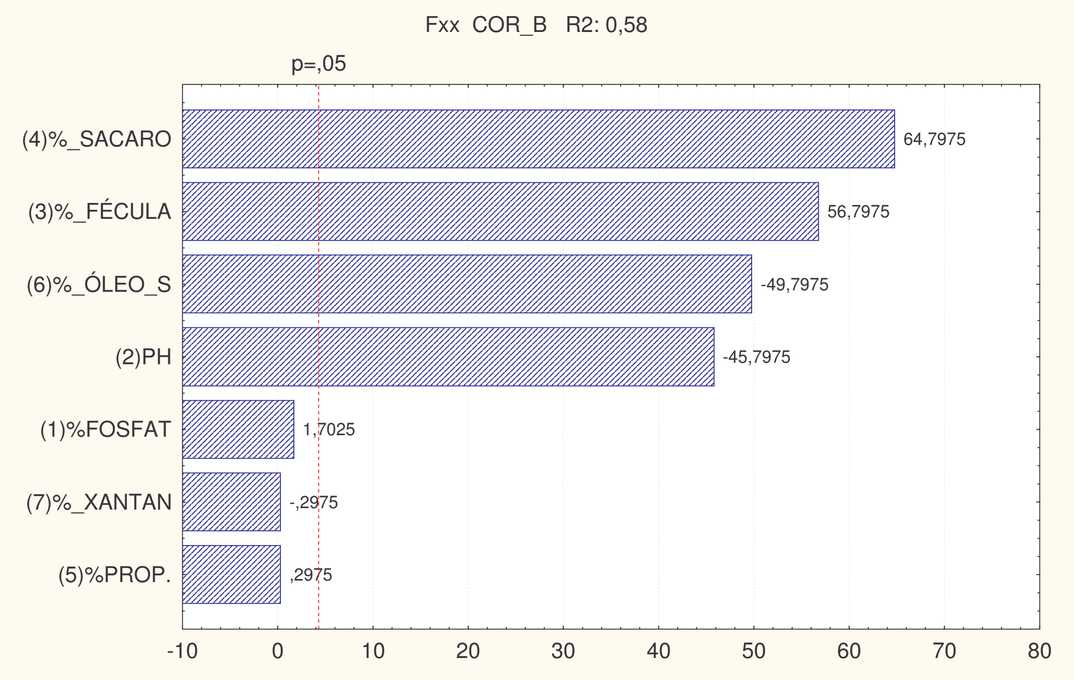
1.10. Gráfico Pareto para cor “a”.



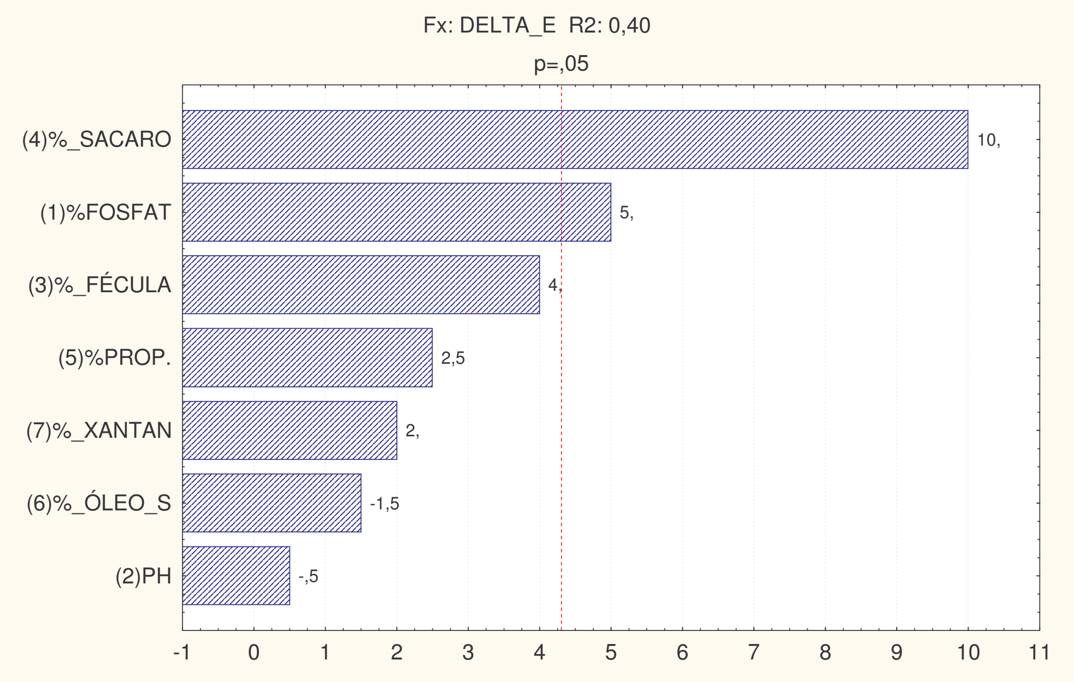
1.11. Gráfico Pareto para cor “b”.



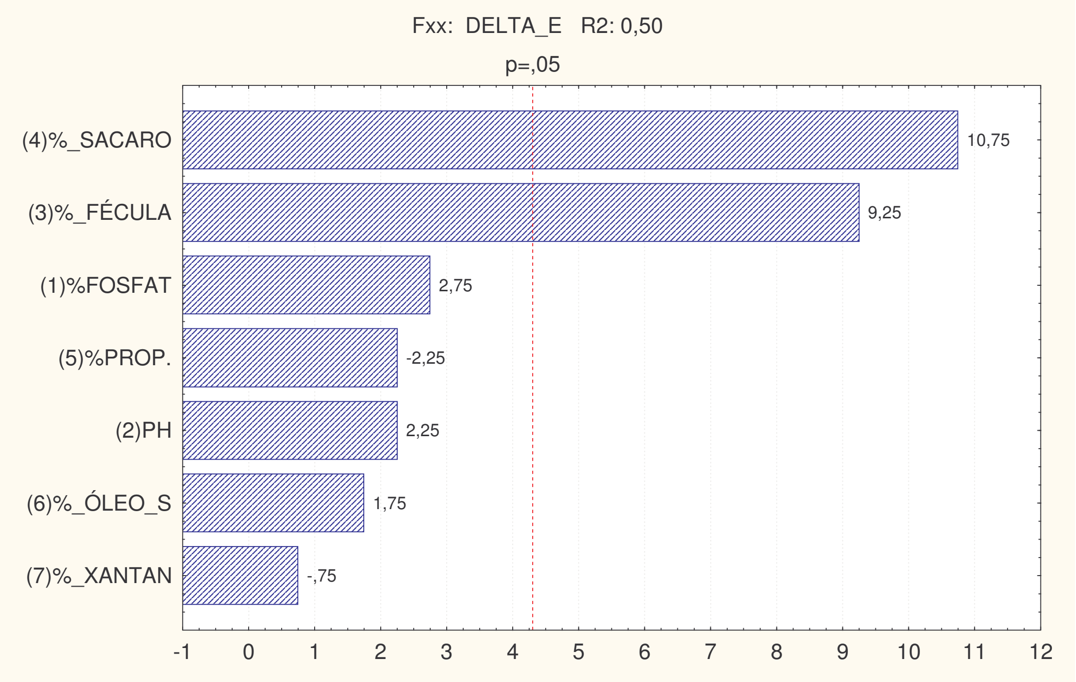
1.12. Gráfico Pareto para cor “b”.



1.13. Gráfico Pareto para diferença total de cor



1.14. Gráfico Pareto para diferença total de cor



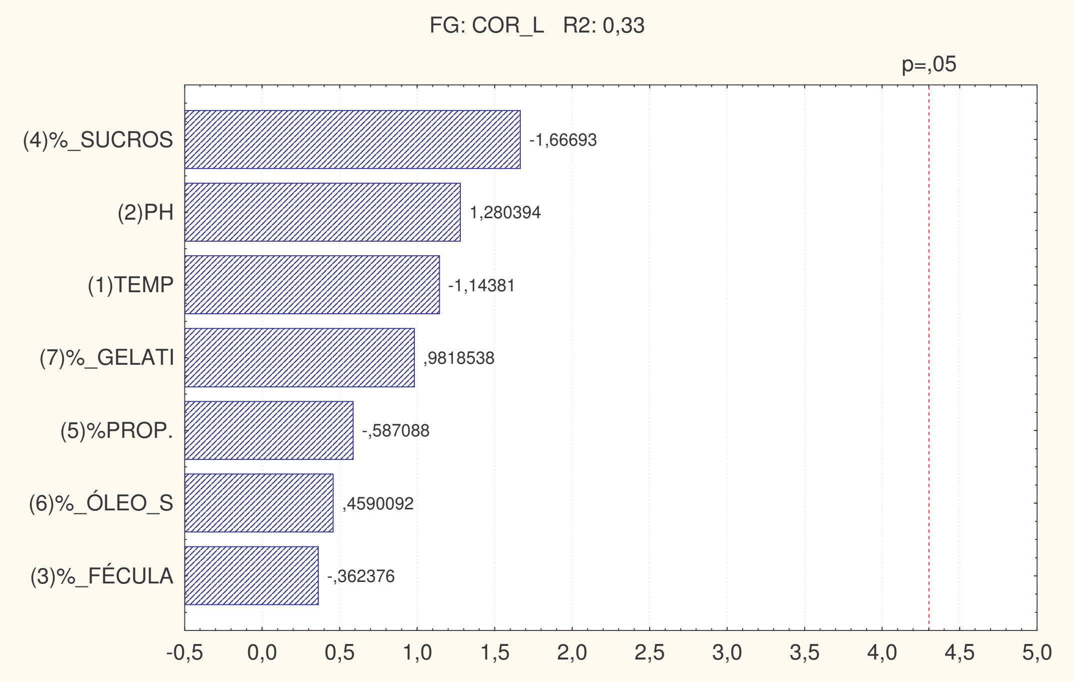
Apêndice 3:

Gráficos Pareto para filmes à base de fécula e gelatina

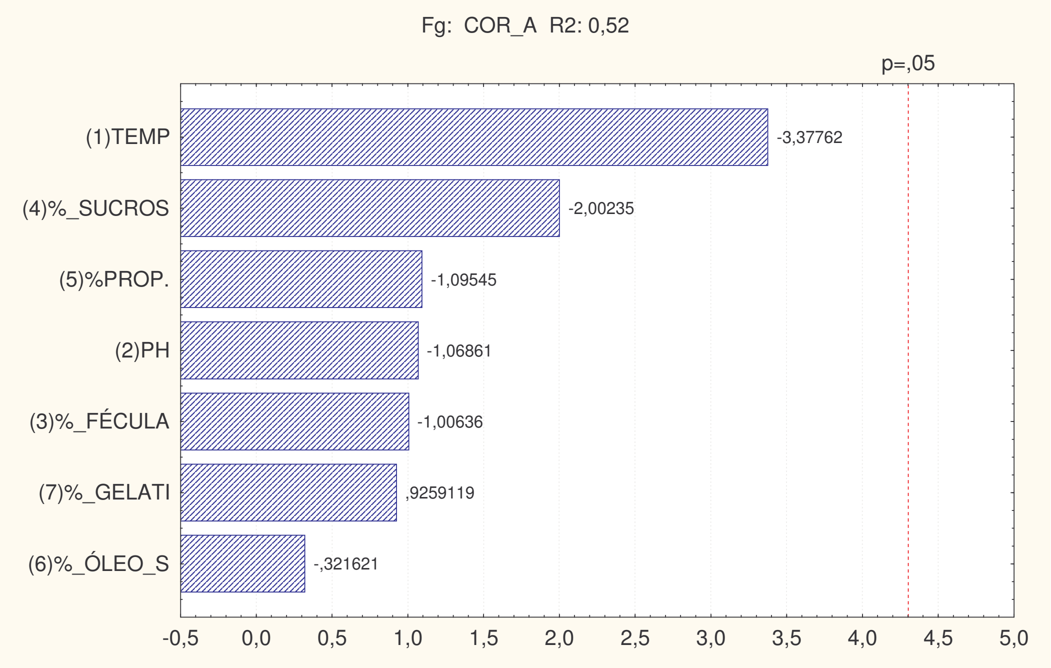
1. Gráficos Pareto dos filmes a base de fécula e gelatina

Para os filmes à base de fécula de mandioca e gelatina foram utilizados símbolos conforme os blocos estatísticos utilizados. Para bloco 1, referente ao capítulo 4, foi utilizado o símbolo Fg. Para o bloco 2, referente aos capítulos 5 e 6, contendo sacarose foi utilizado o símbolo Fgg. Para o bloco 3, referente aos capítulos 5 e 6, contendo açúcar invertido foi utilizado o símbolo Fig.

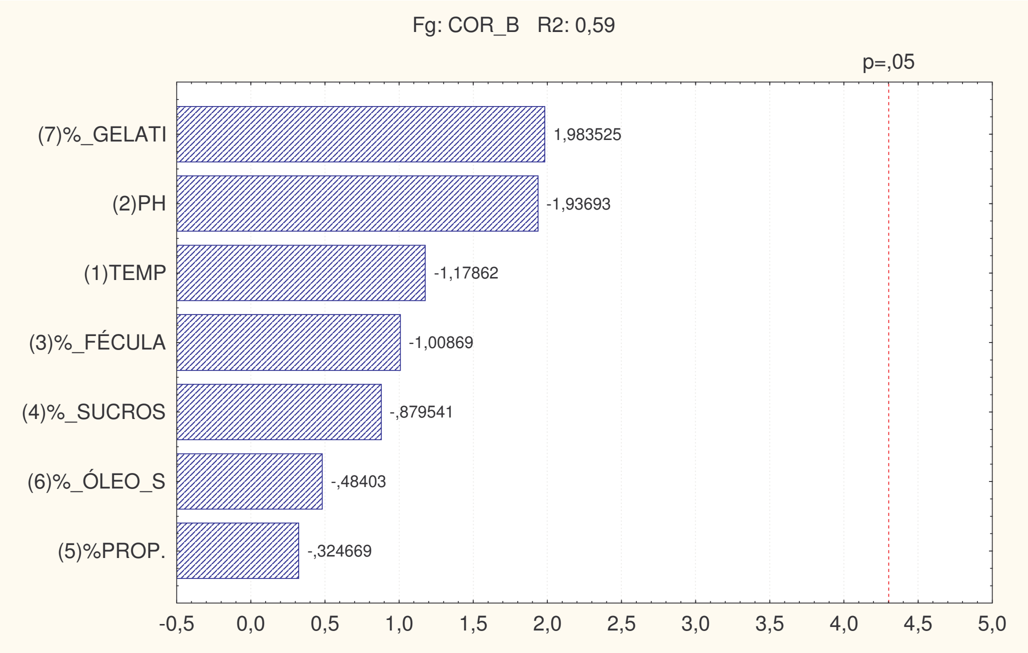
1.1. Gráfico Pareto para cor "L".



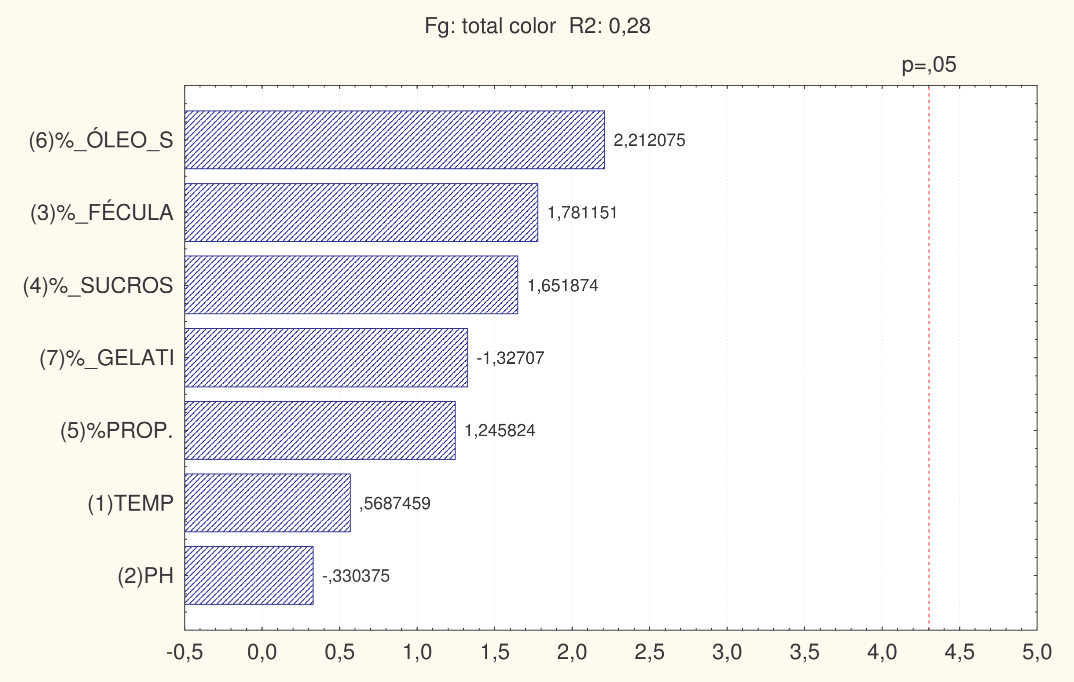
1.2. Gráfico Pareto para cor “a”.



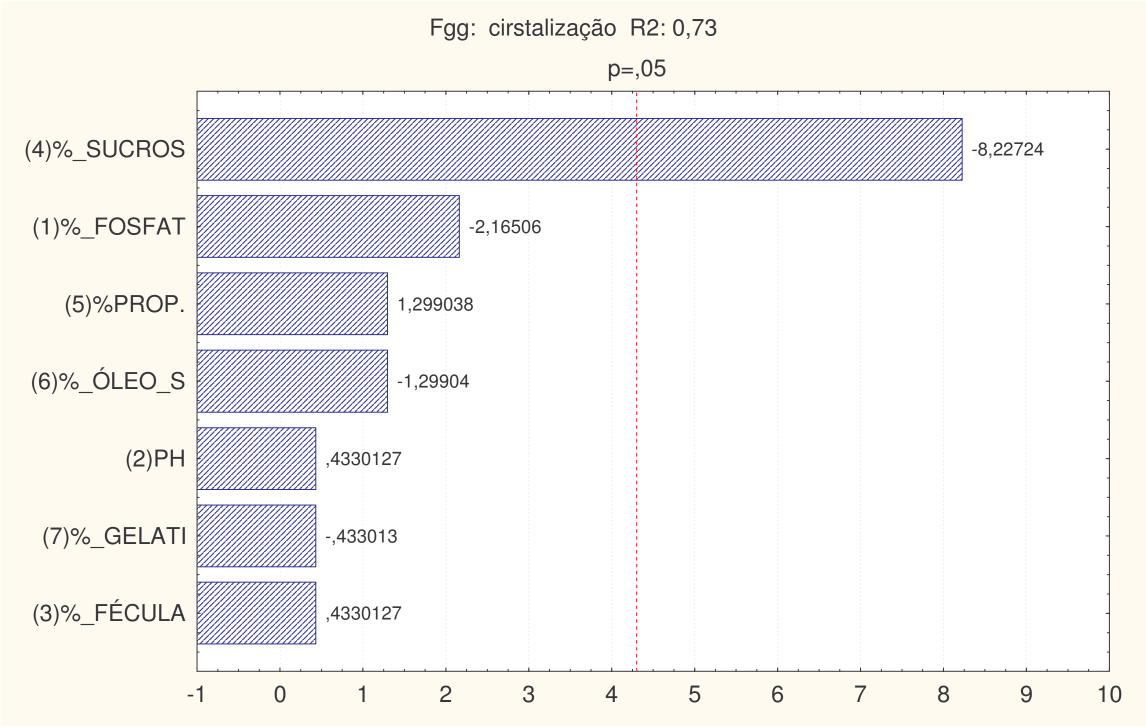
1.3. Gráfico Pareto para cor “b”.



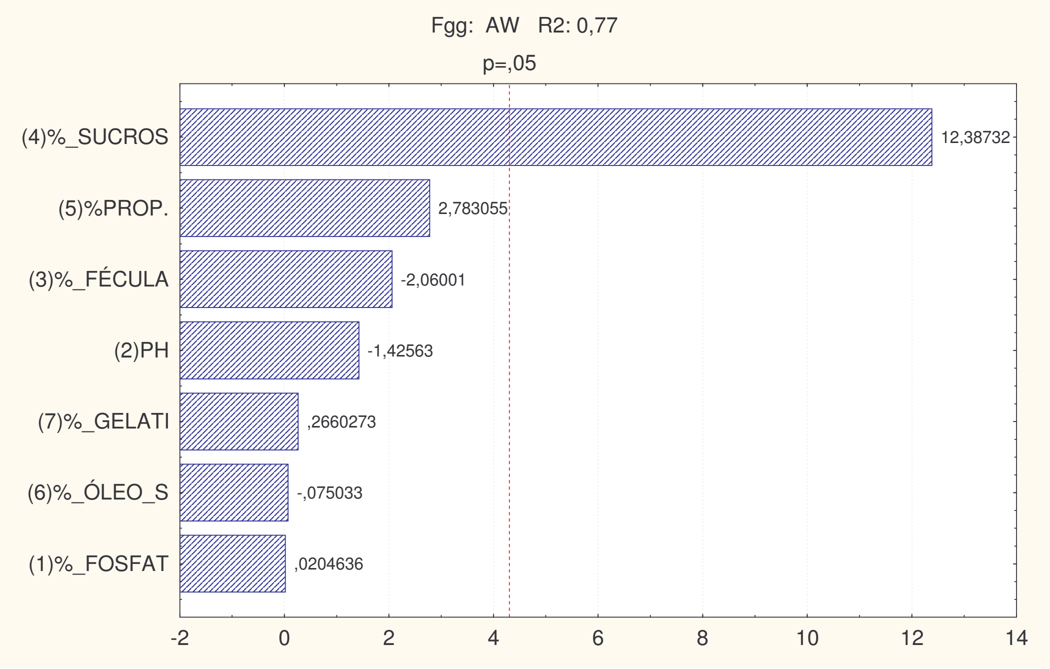
1.4. Gráfico Pareto para diferença total de cor



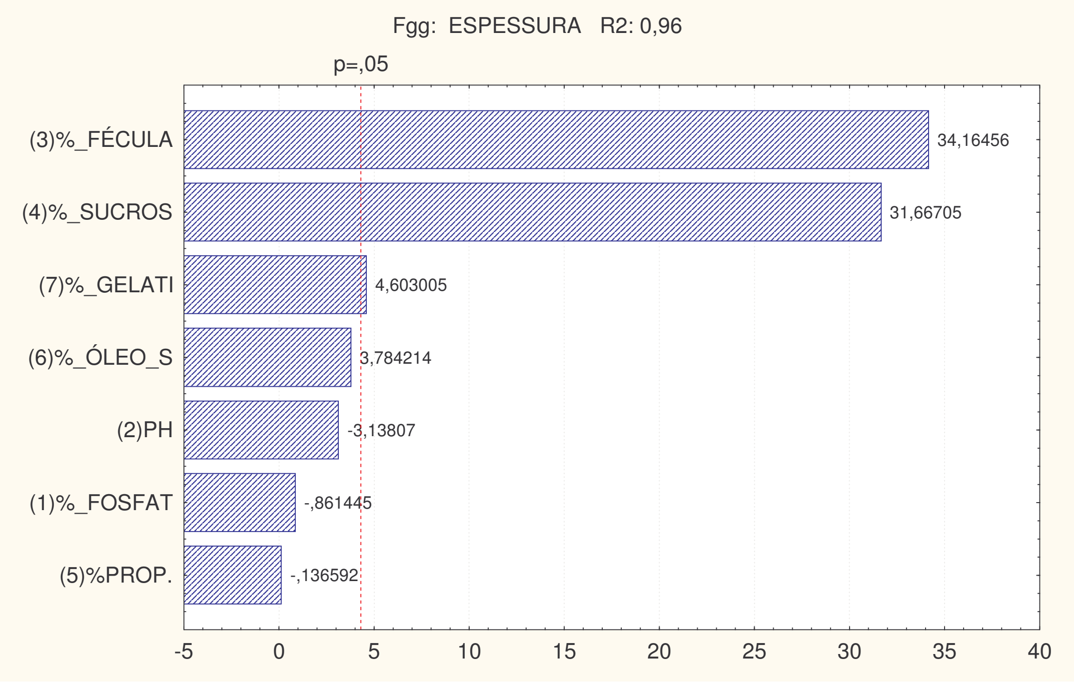
1.5. Gráfico Pareto para cor "L".



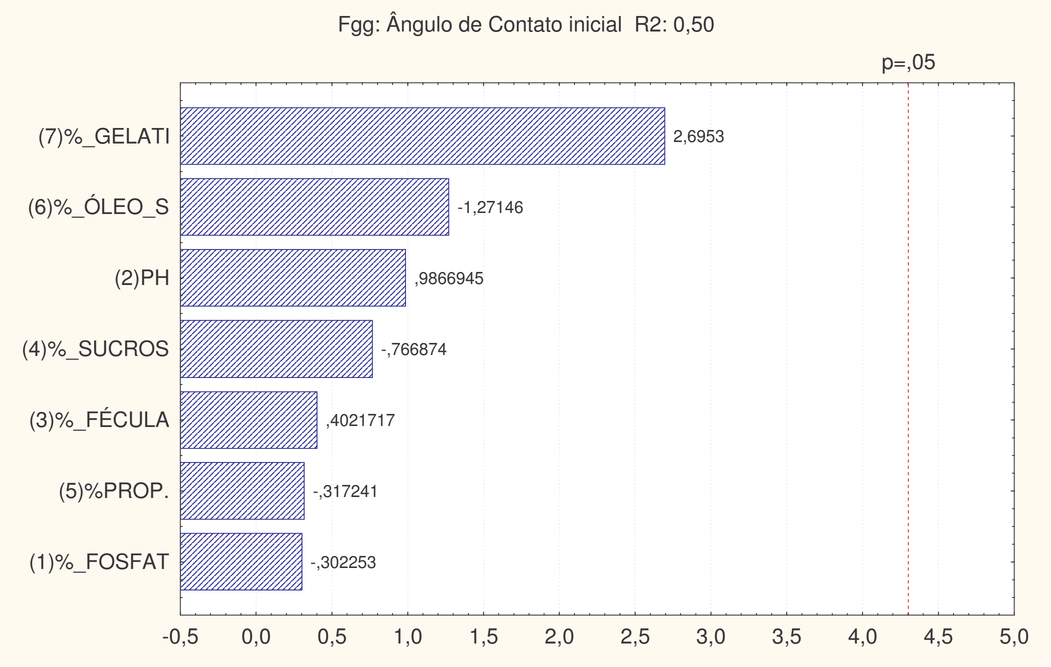
1.6. Gráfico Pareto para aw.



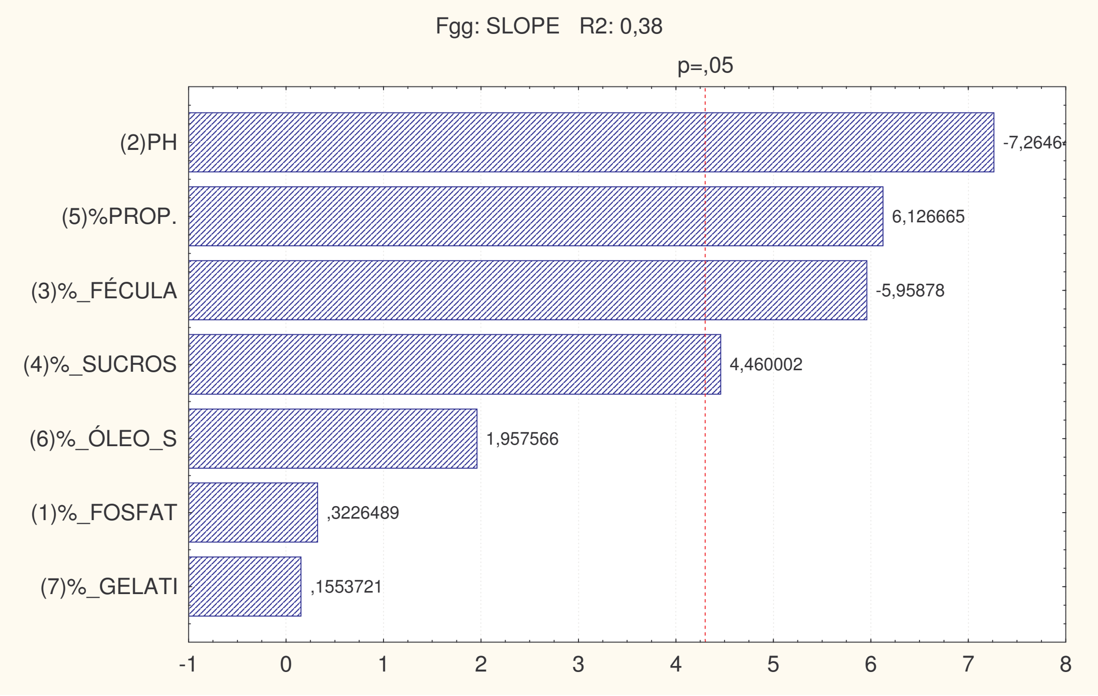
1.7. Gráfico Pareto para espessura



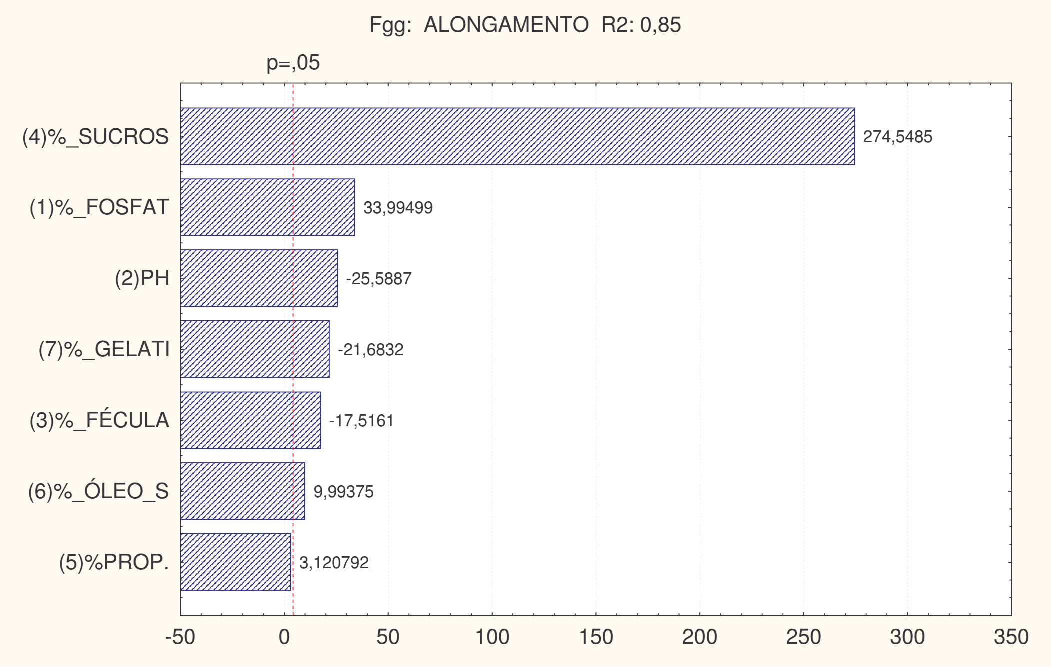
1.8. Gráfico Pareto para ângulo de contato



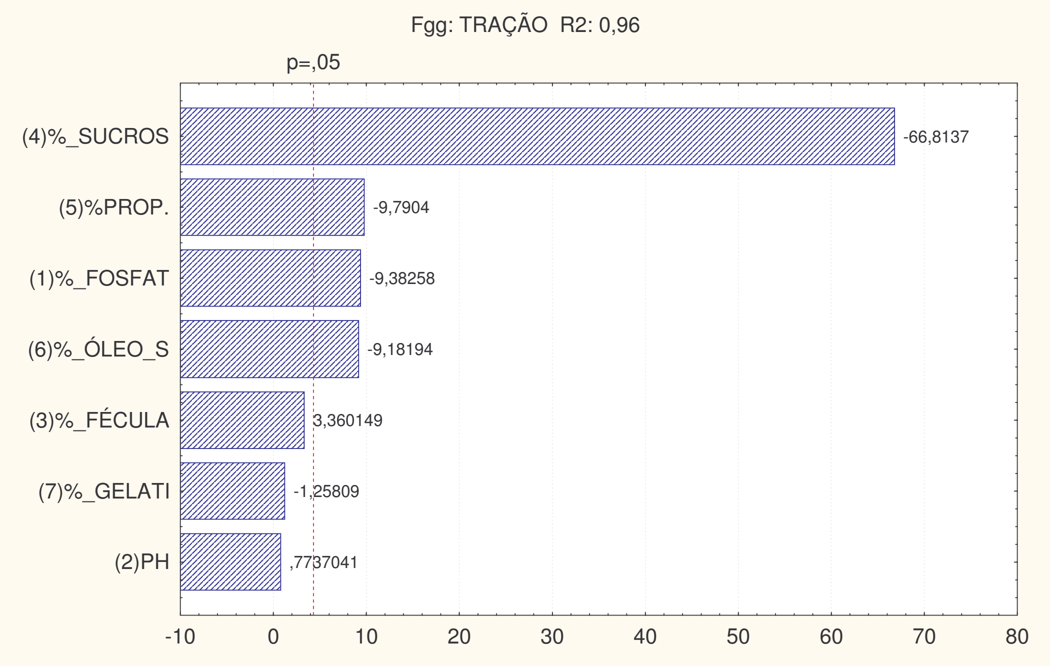
1.9. Gráfico ângulo de contato (slope)



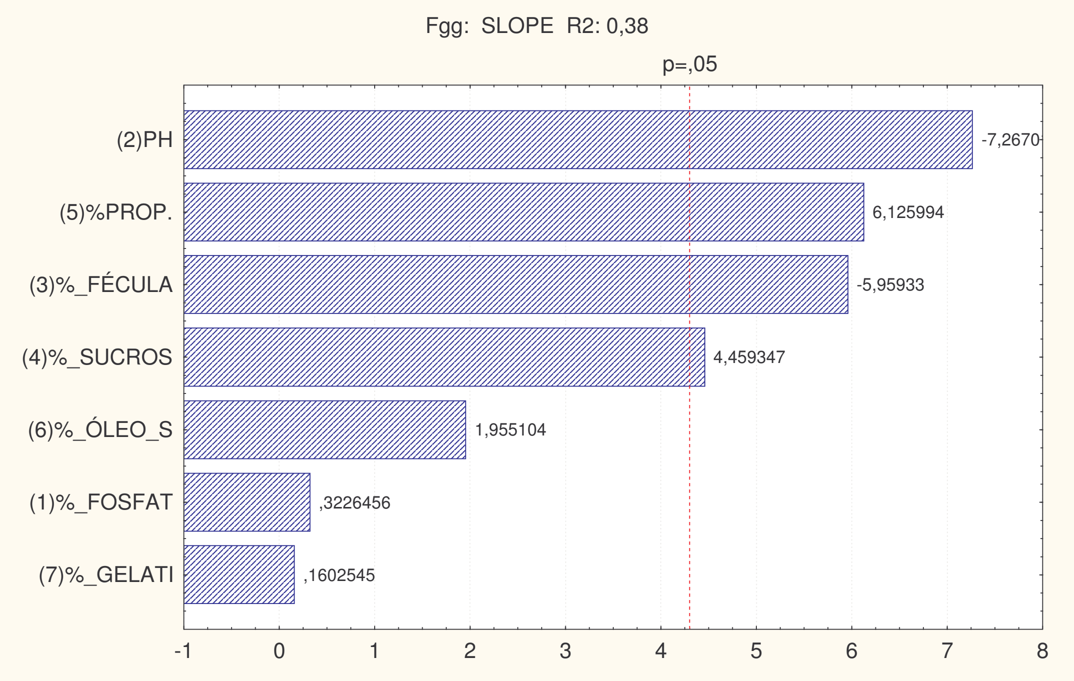
1.10. Gráfico Pareto para alongamento



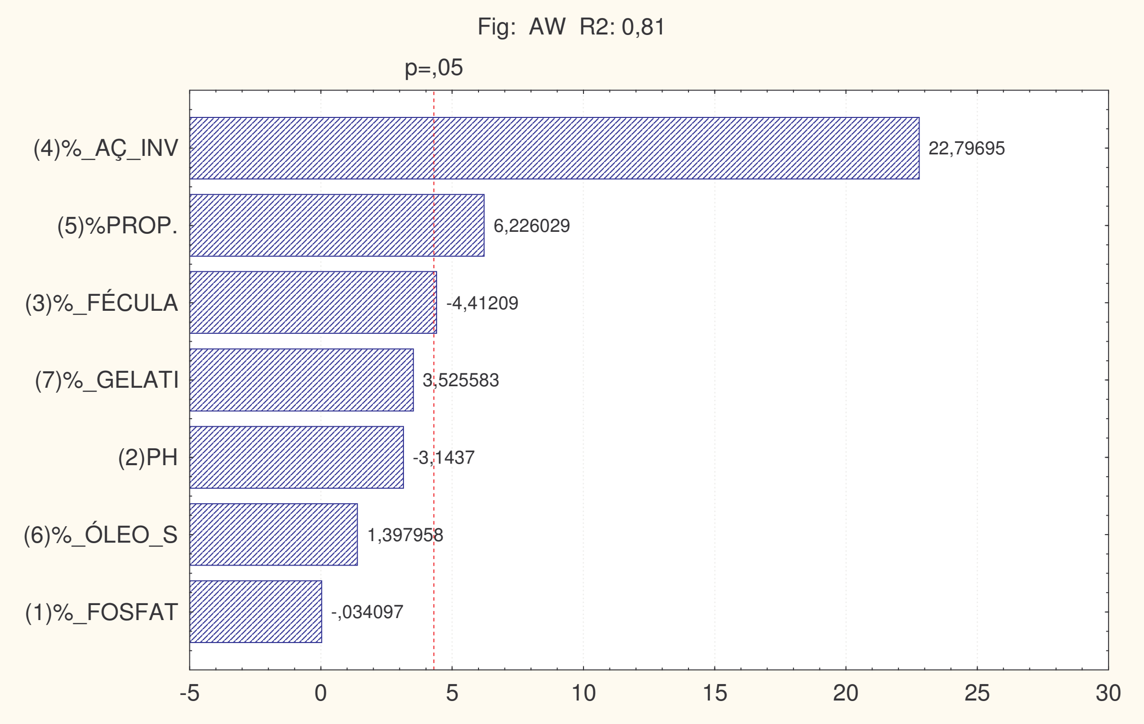
1.11. Gráfico Pareto para tração



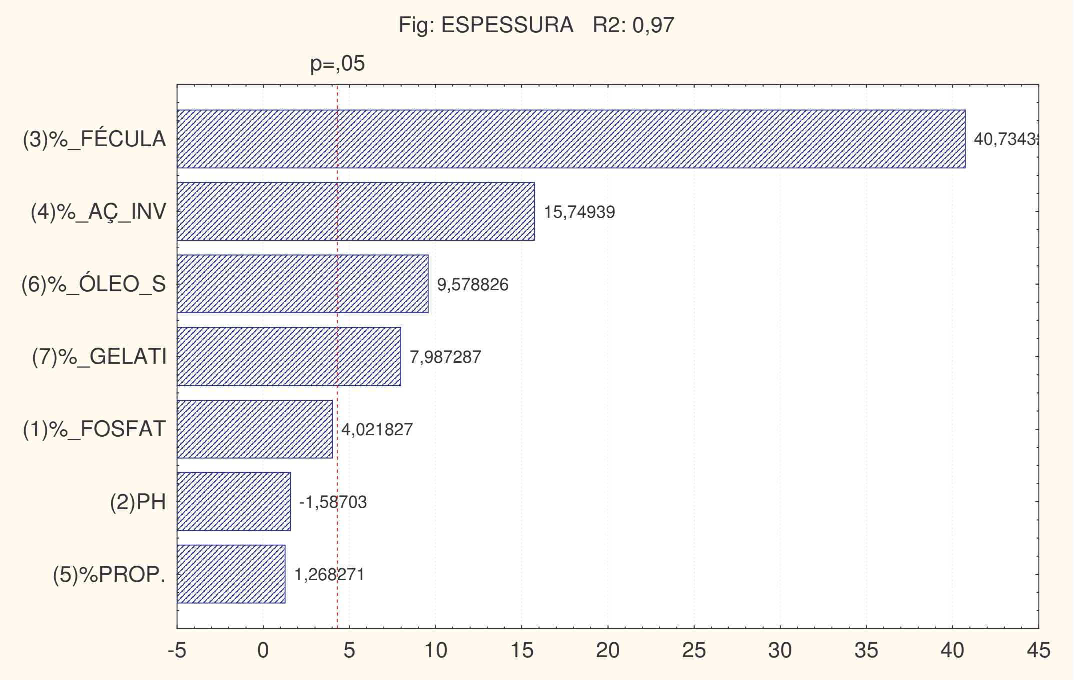
1.12. Gráfico Pareto para ângulo de contato (slope)



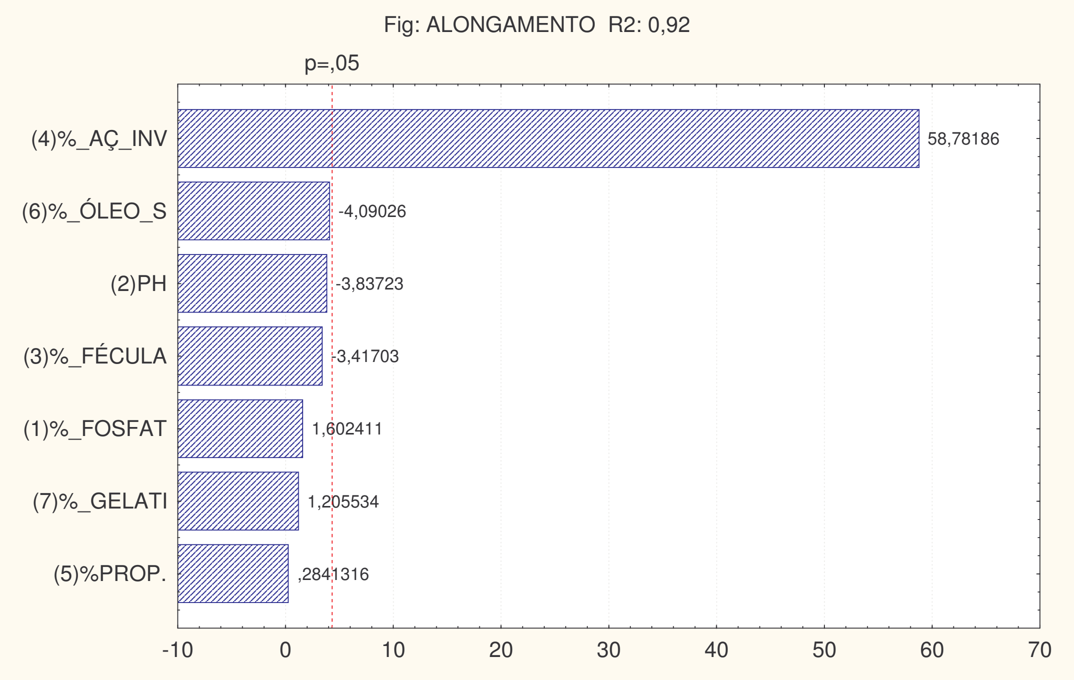
1.13. Gráfico Pareto para espessura



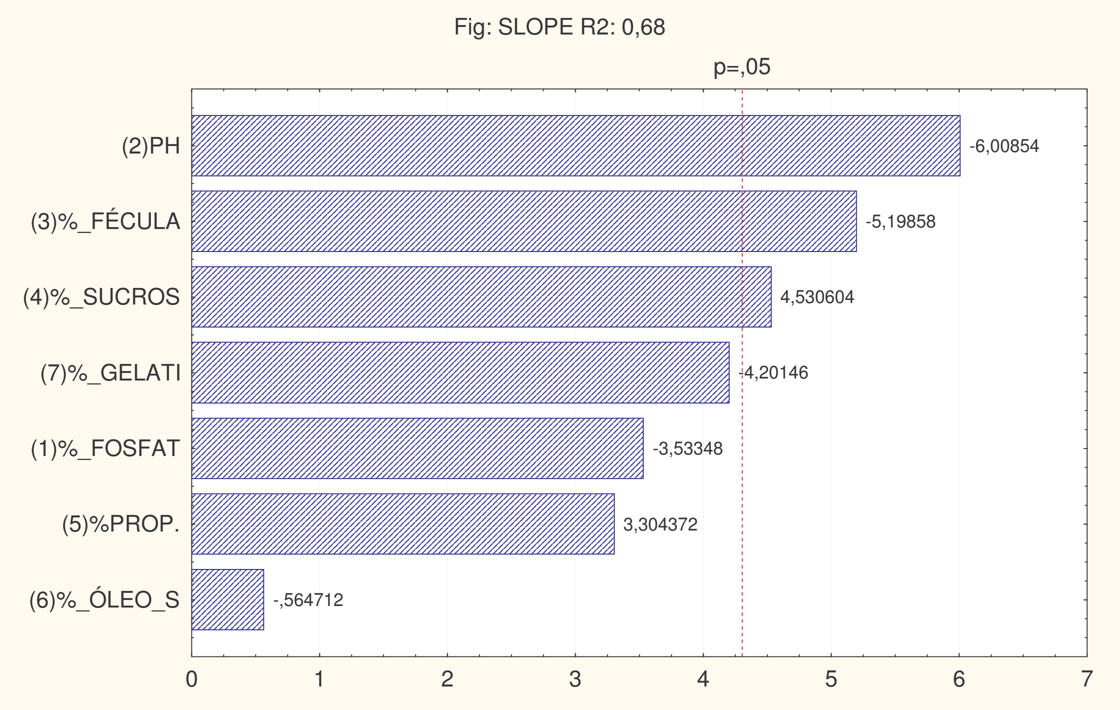
1.14. Gráfico Pareto para espessura



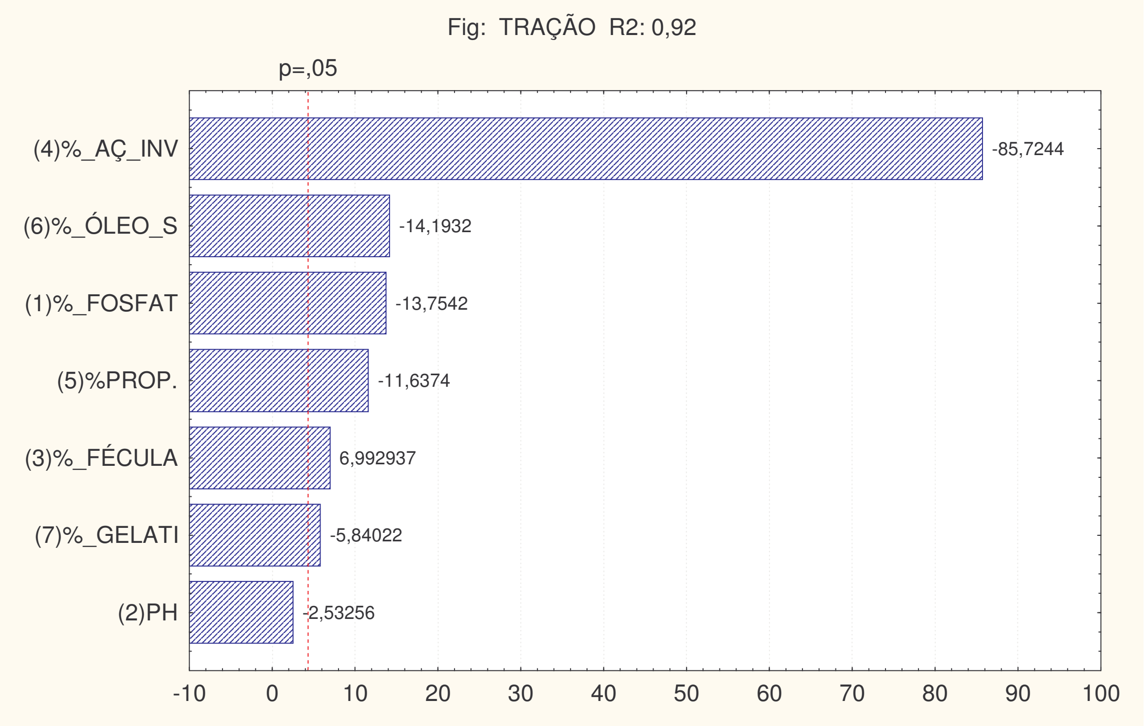
1.15. Gráfico Pareto para alongamento



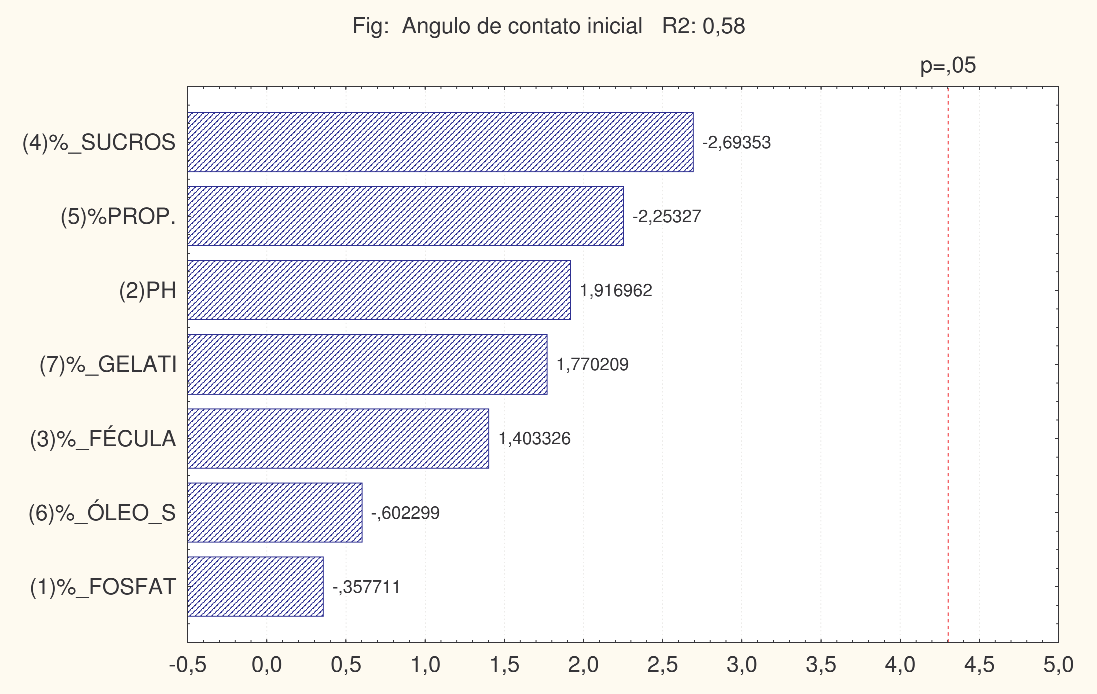
1.16. Gráfico Pareto para tração



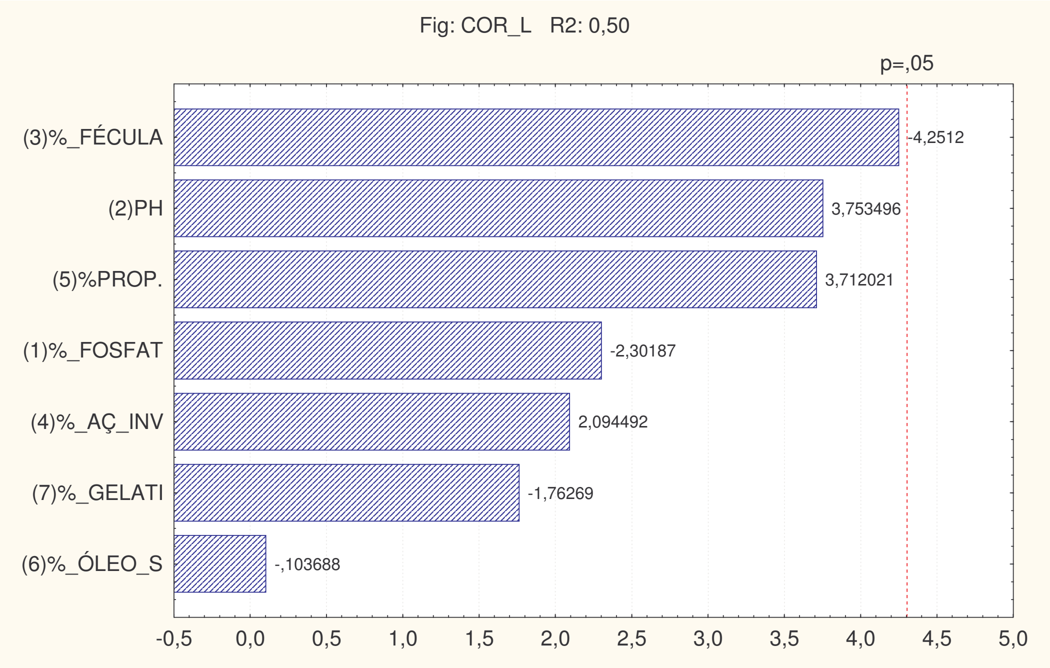
1.17. Gráfico Pareto para tração



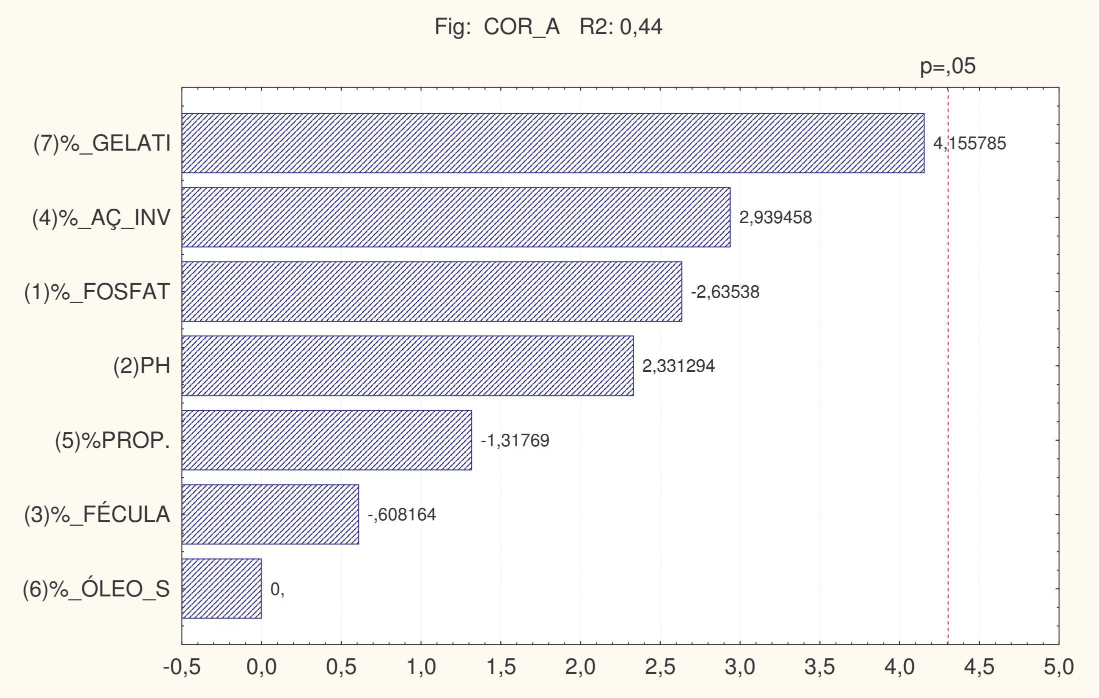
1.18. Gráfico Pareto para ângulo de contato



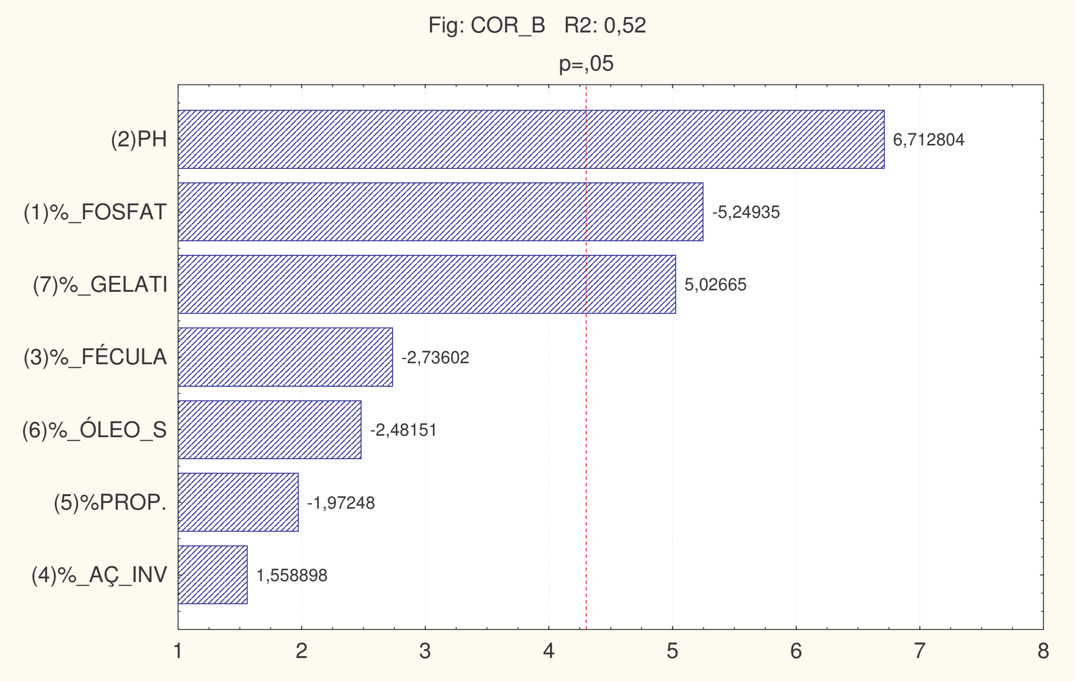
1.19. Gráfico Pareto para cor "L"



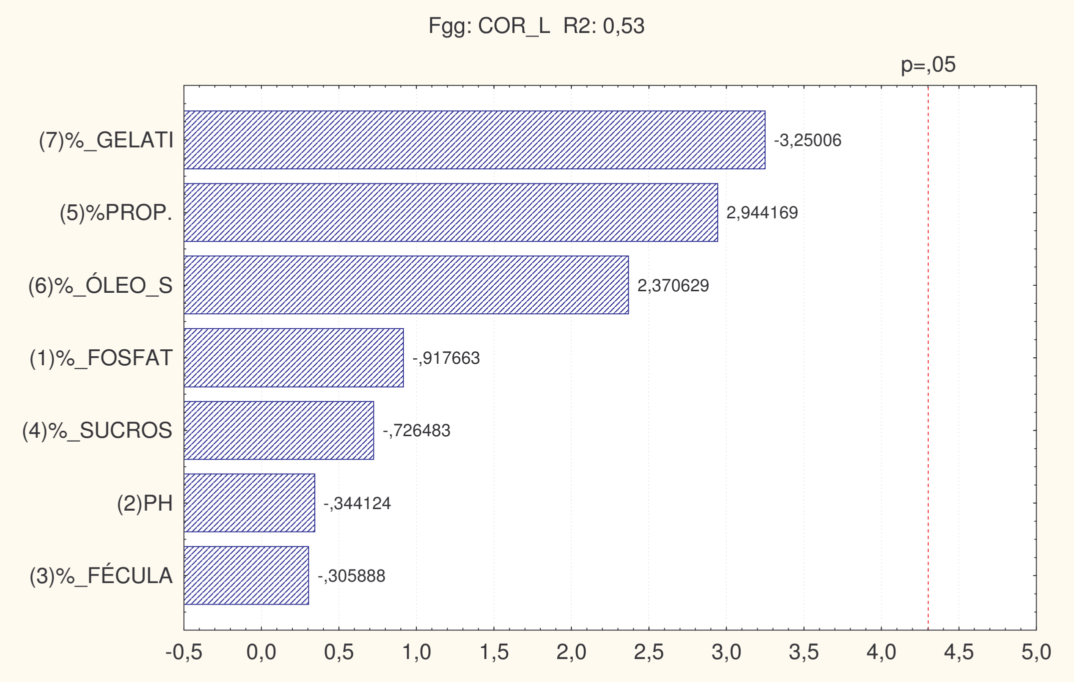
1.20. Gráfico Pareto para cor "a"



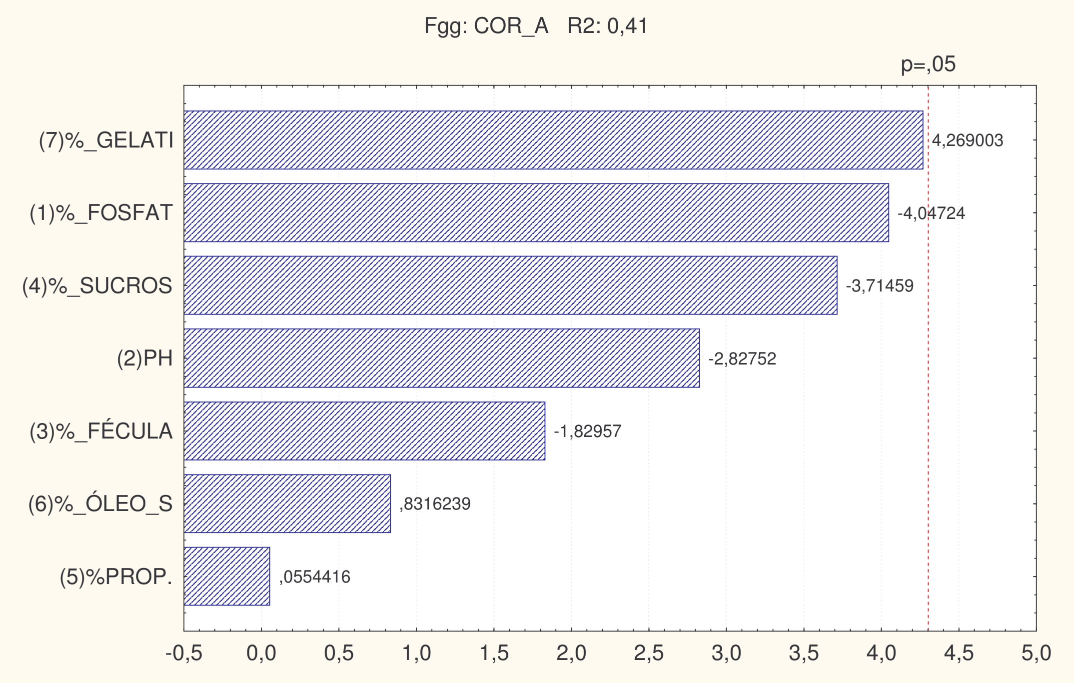
1.21. Gráfico Pareto para cor "b"



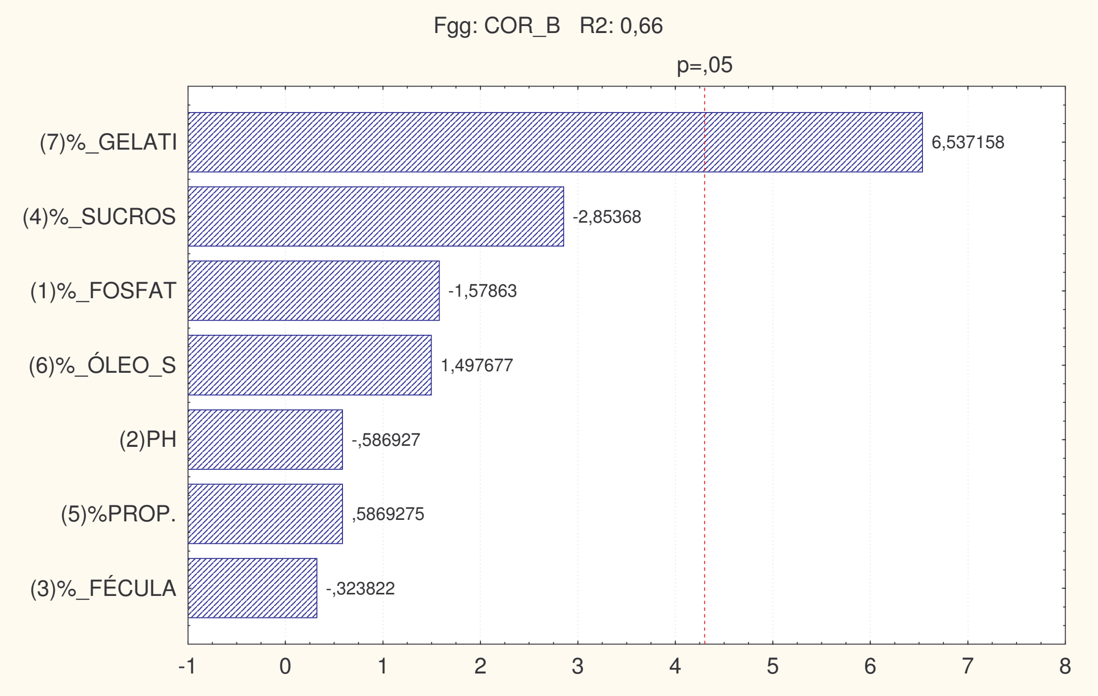
1.22. Gráfico Pareto para cor "L"



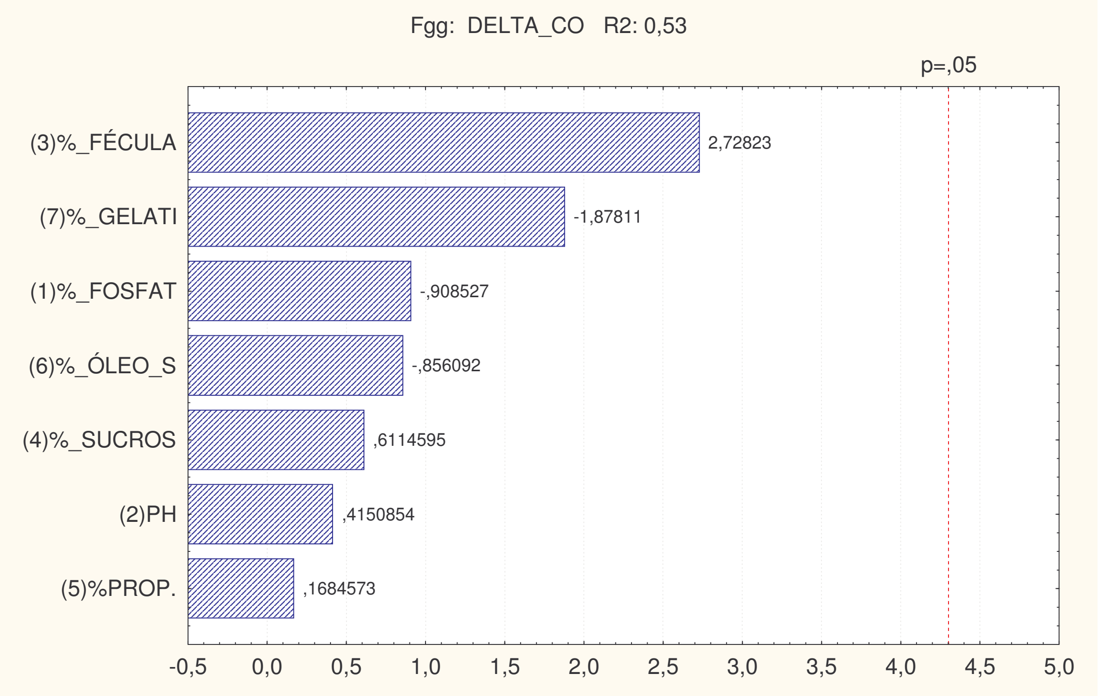
1.23. Gráfico Pareto para cor "a"



1.24. Gráfico Pareto para cor “b”



1.25. Gráfico Pareto para diferença total de cor



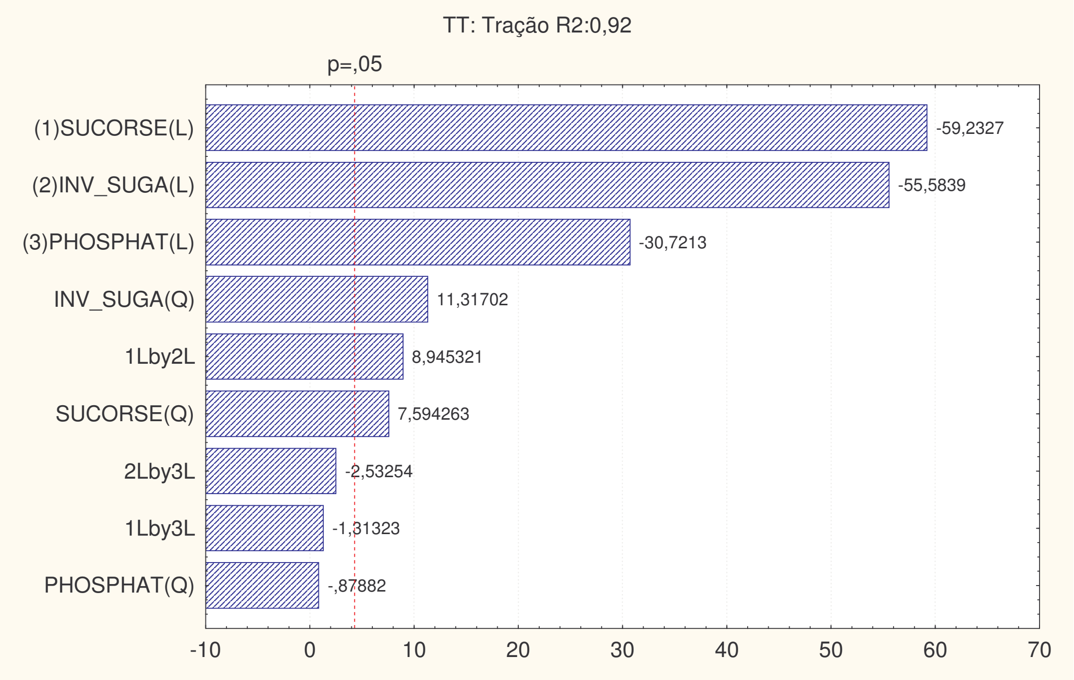
Apêndice 4:

Gráficos Pareto para os filmes da etapa de otimização

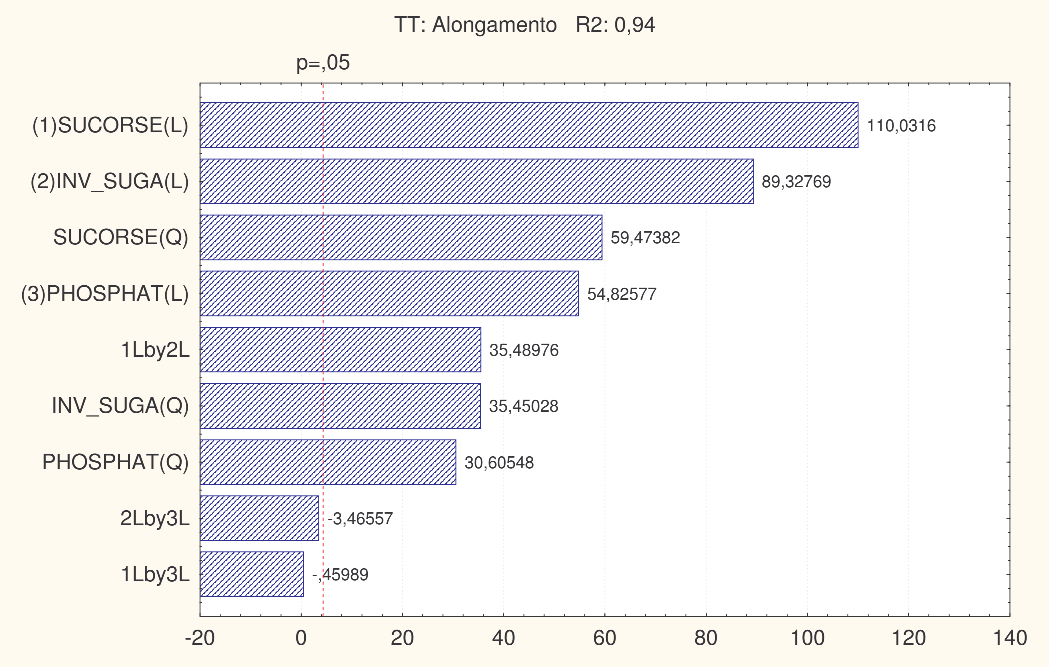
1 Gráficos Pareto dos filmes da etapa de otimização

Para os filmes da etapa de otimização foi utilizado o símbolo TT.

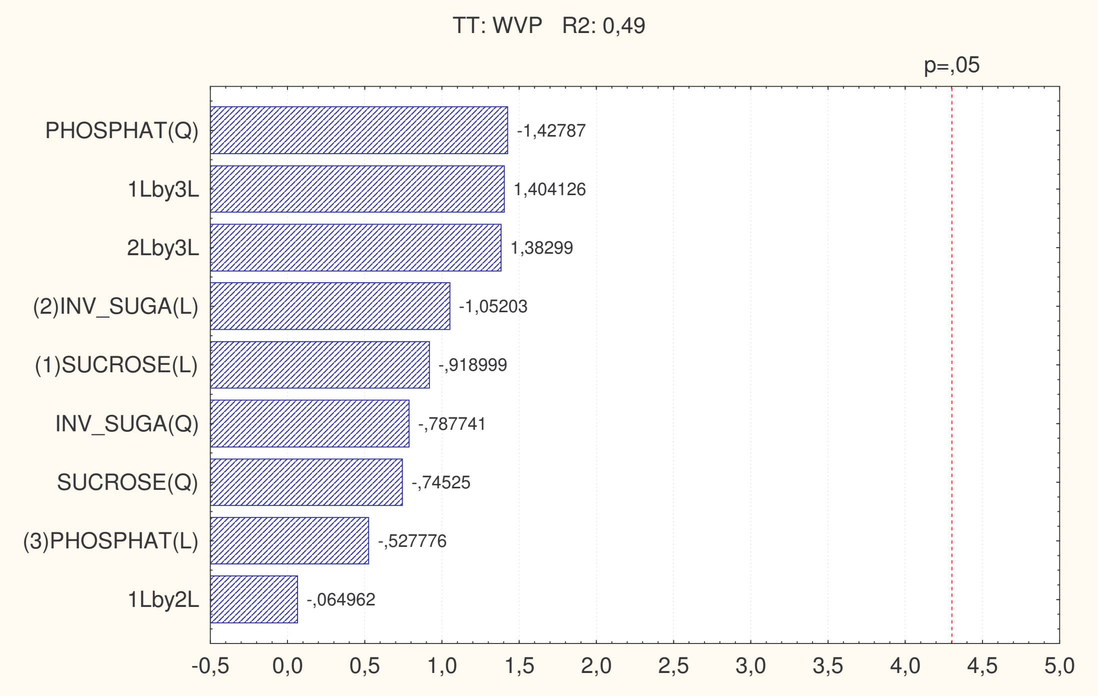
1.1. Gráfico Pareto para tração



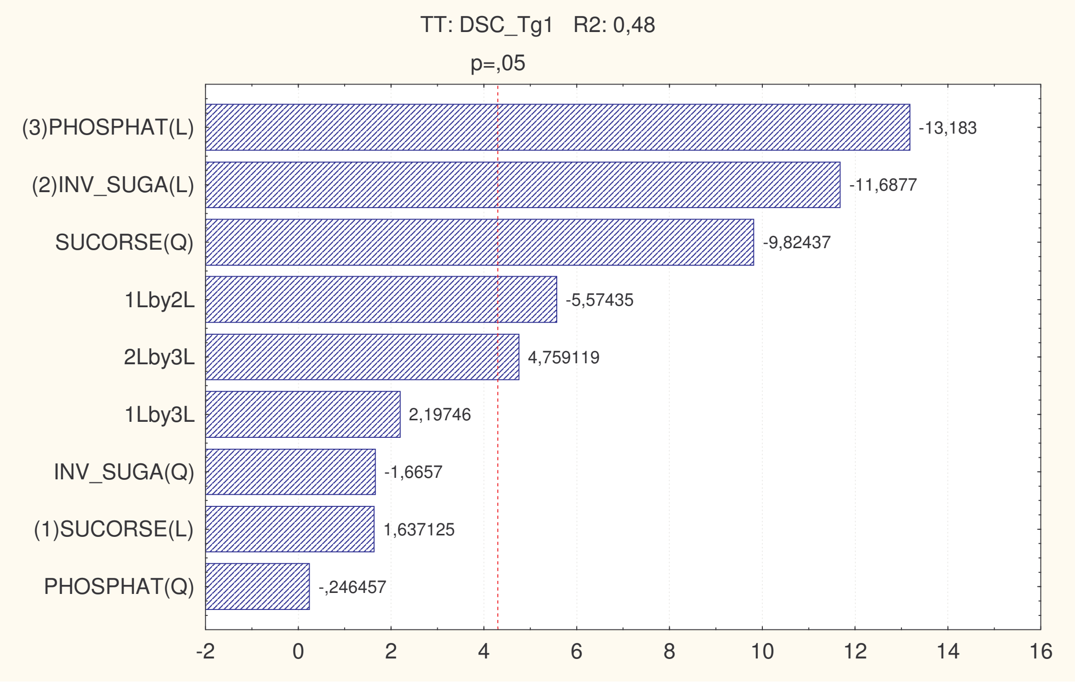
1.2.Gráfico Pareto para alongamento



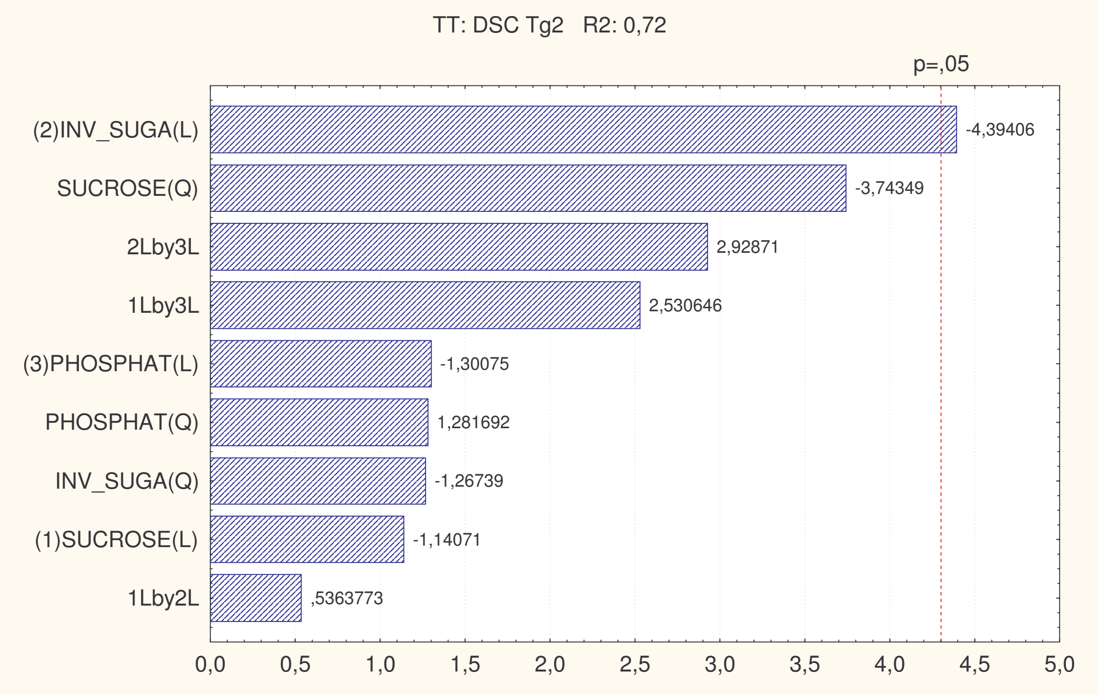
1.3.Gráfico Pareto para permeabilidade ao vapor d´água



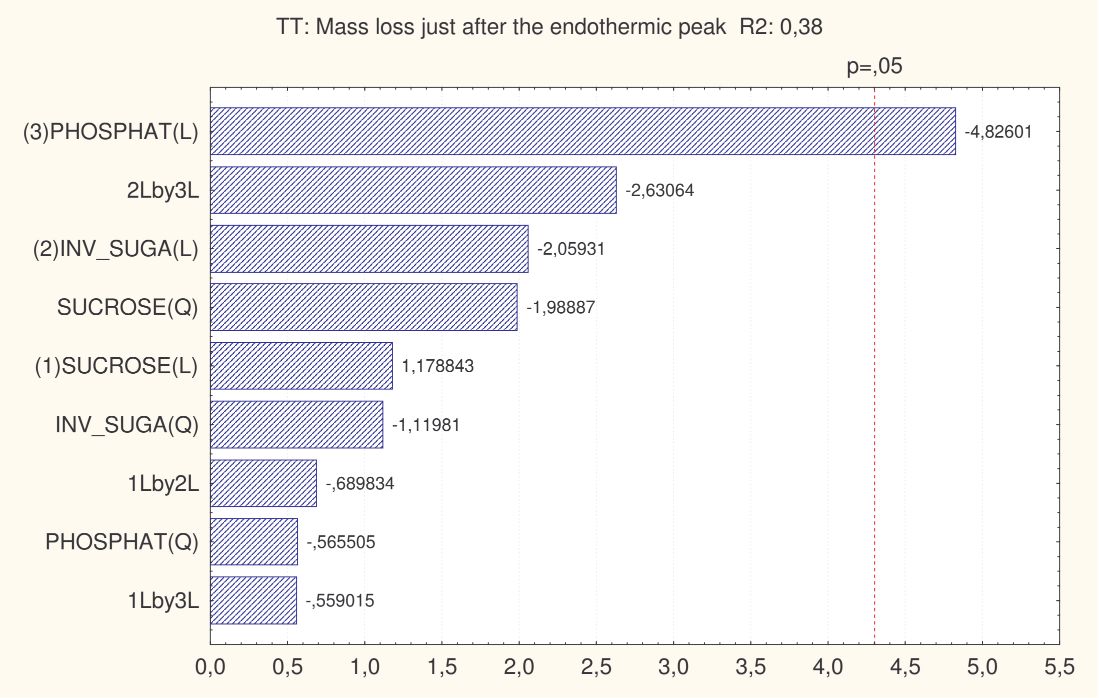
1.4.Gráfico Pareto para Tg, 1 do DSC



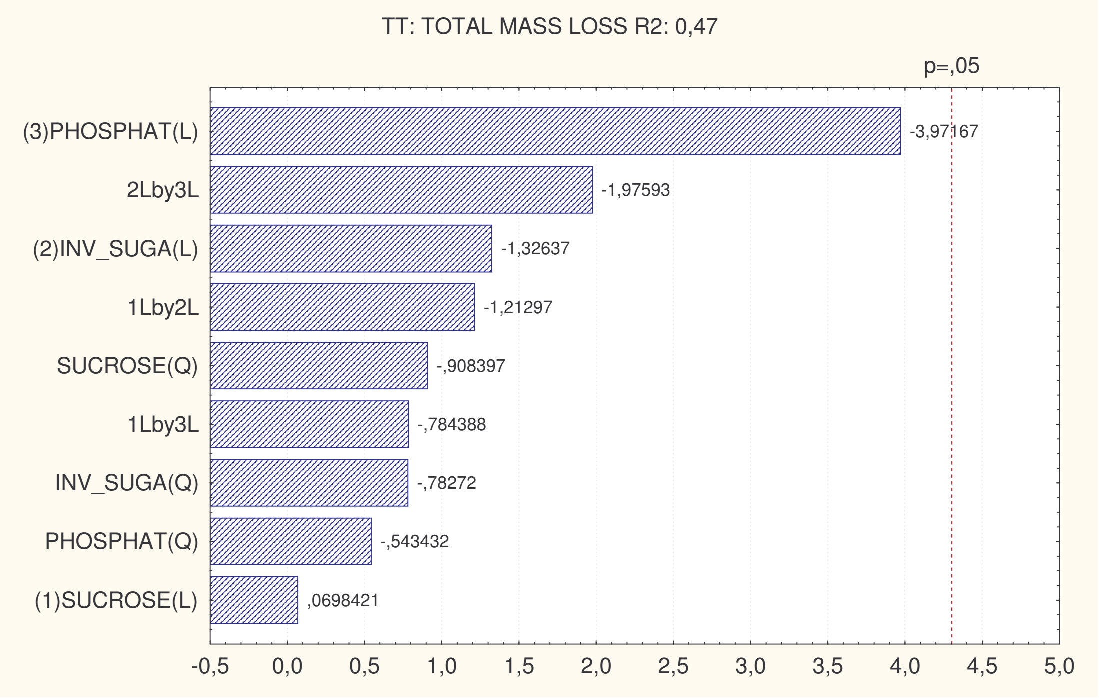
1.5.Gráfico Pareto para Tg, 1 do DSC



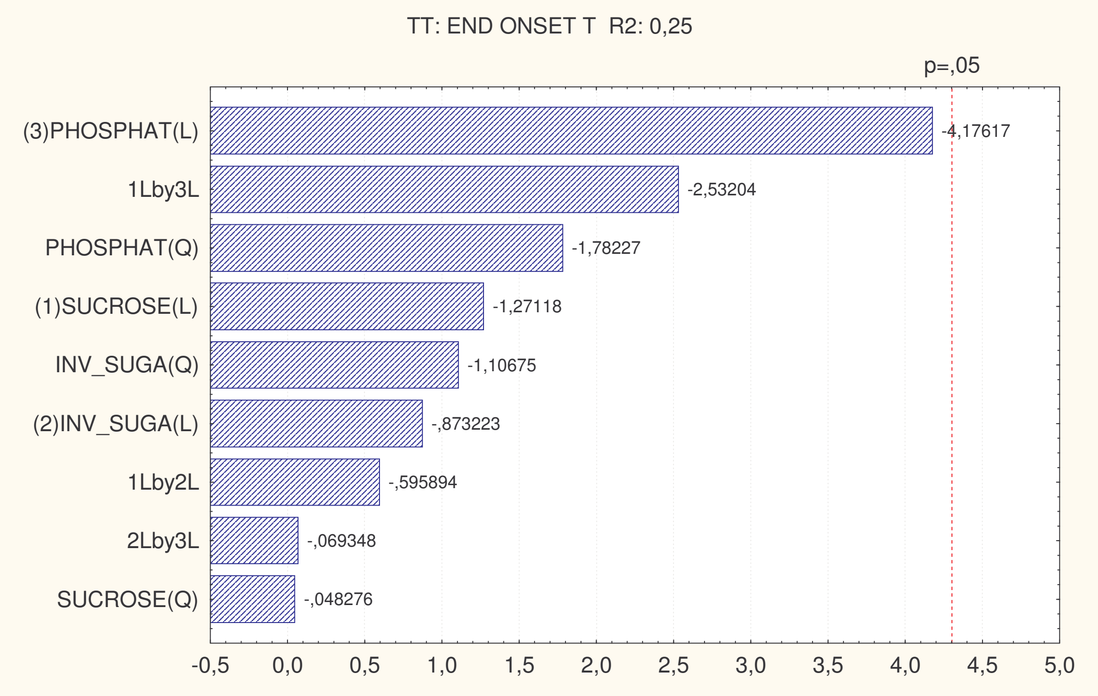
1.6.Gráfico Pareto para perda de massa logo após pico endotérmico



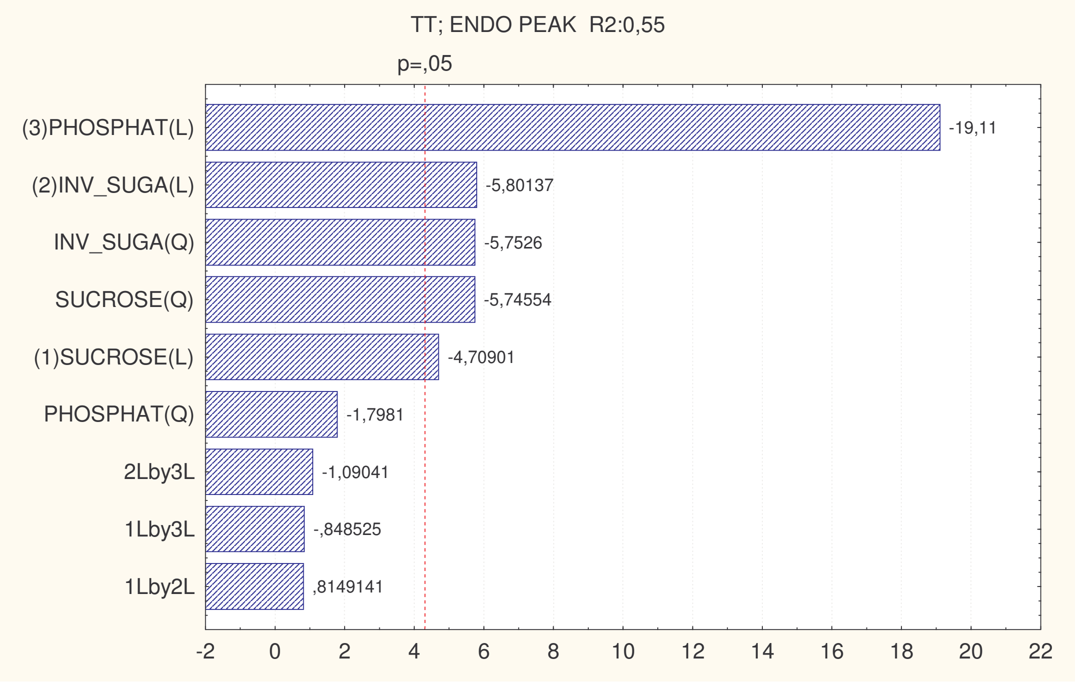
1.7.Gráfico Pareto para perda de massa total



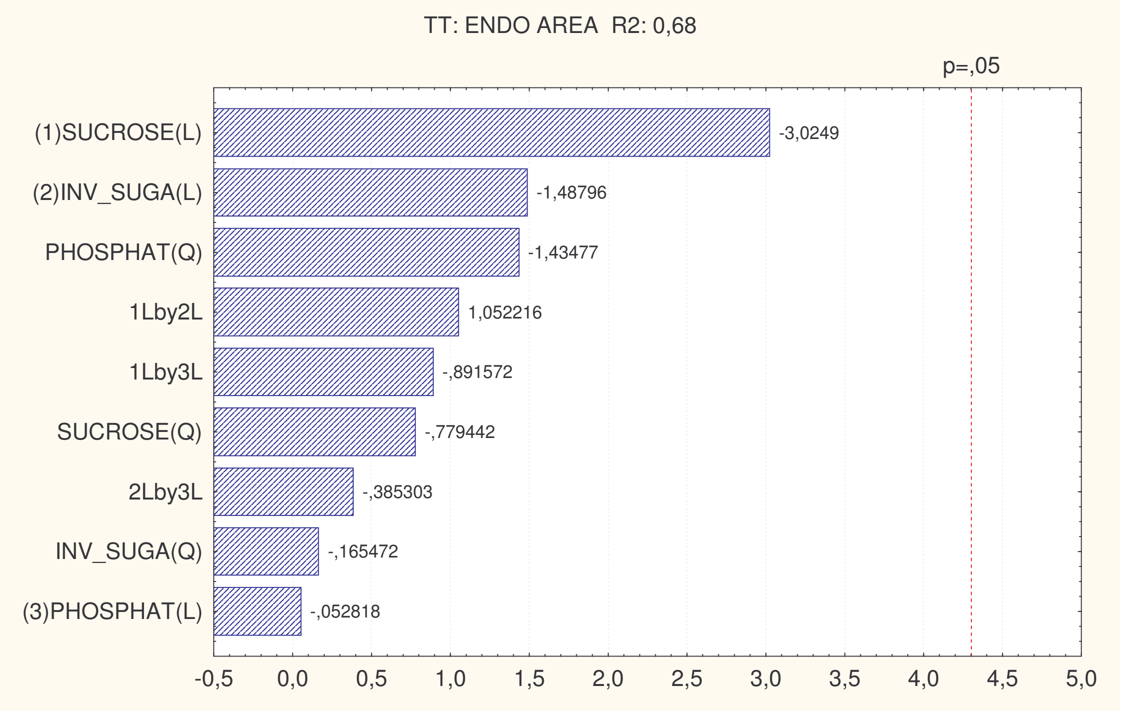
1.8.Gráfico Pareto para onset do pico endotérmico



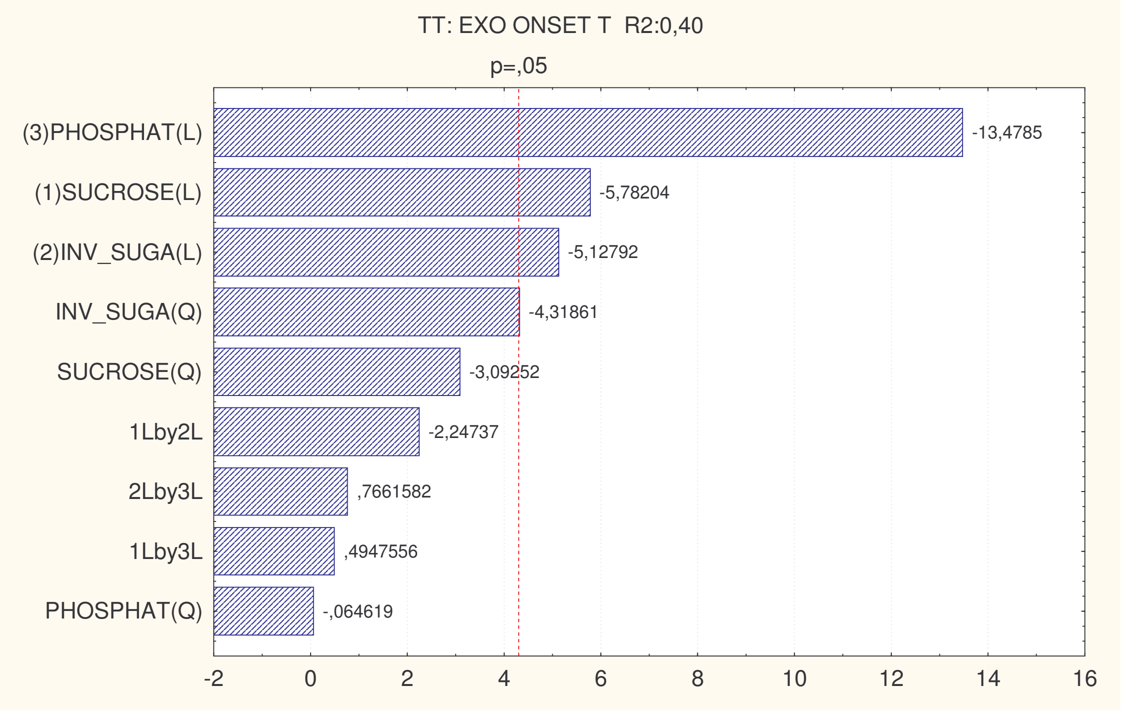
1.9.Gráfico Pareto para temperatura média do pico endotérmico



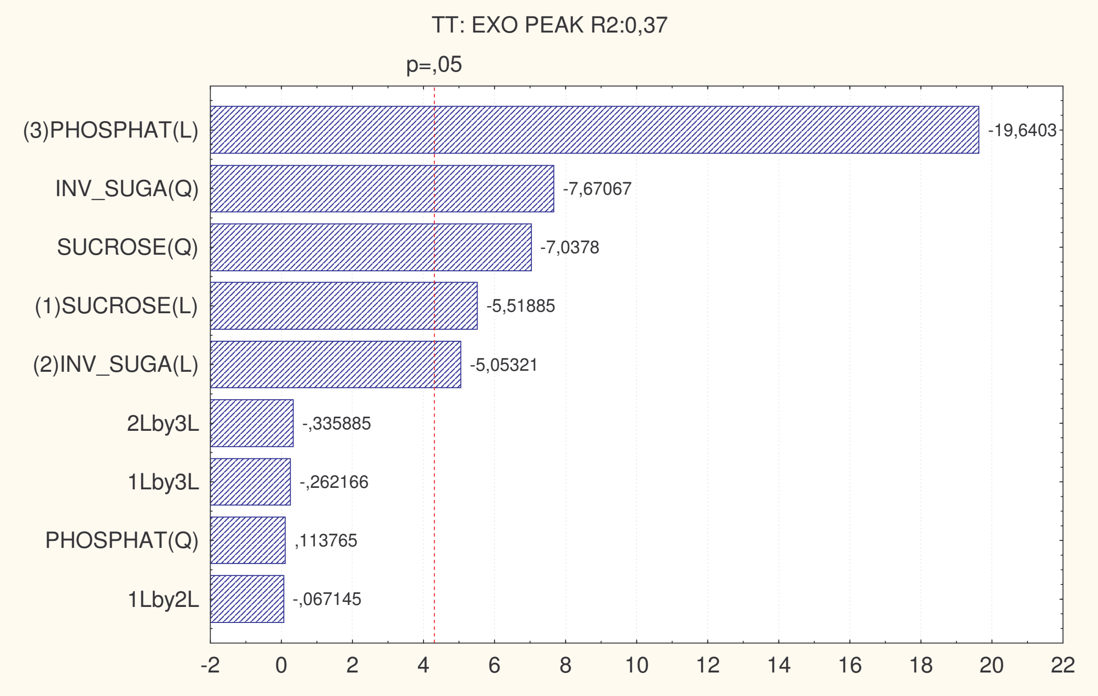
1.10.Gráfico Pareto para área do pico endotérmico



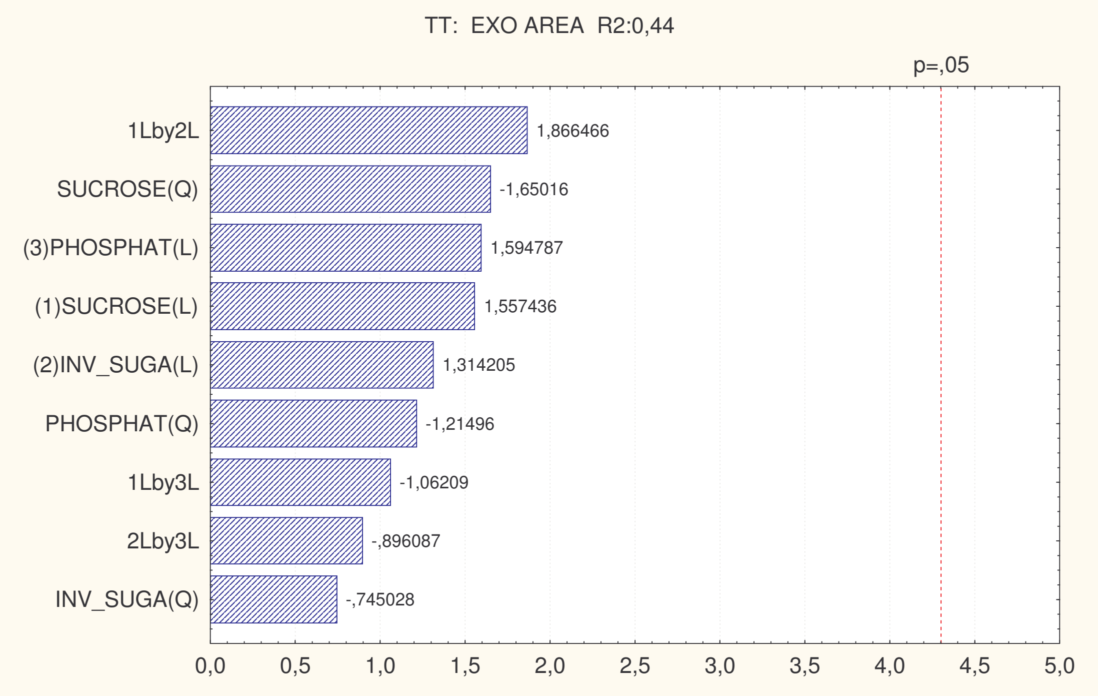
1.11.Gráfico Pareto para onset do pico exotérmico



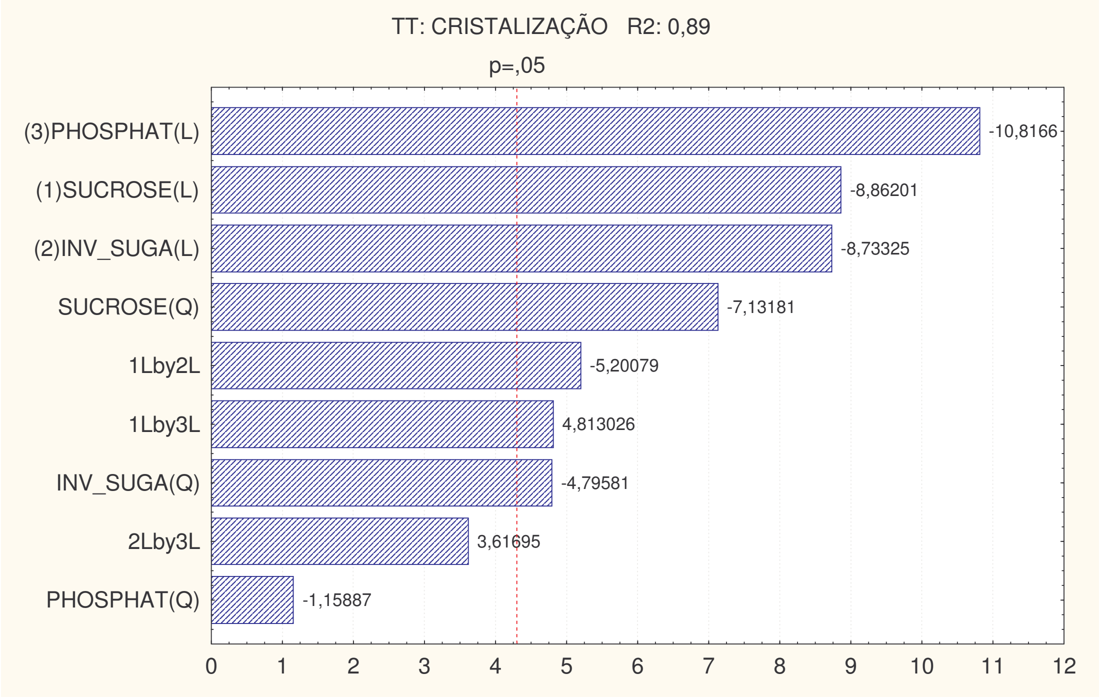
1.12.Gráfico Pareto para temperatura média do pico exotérmico



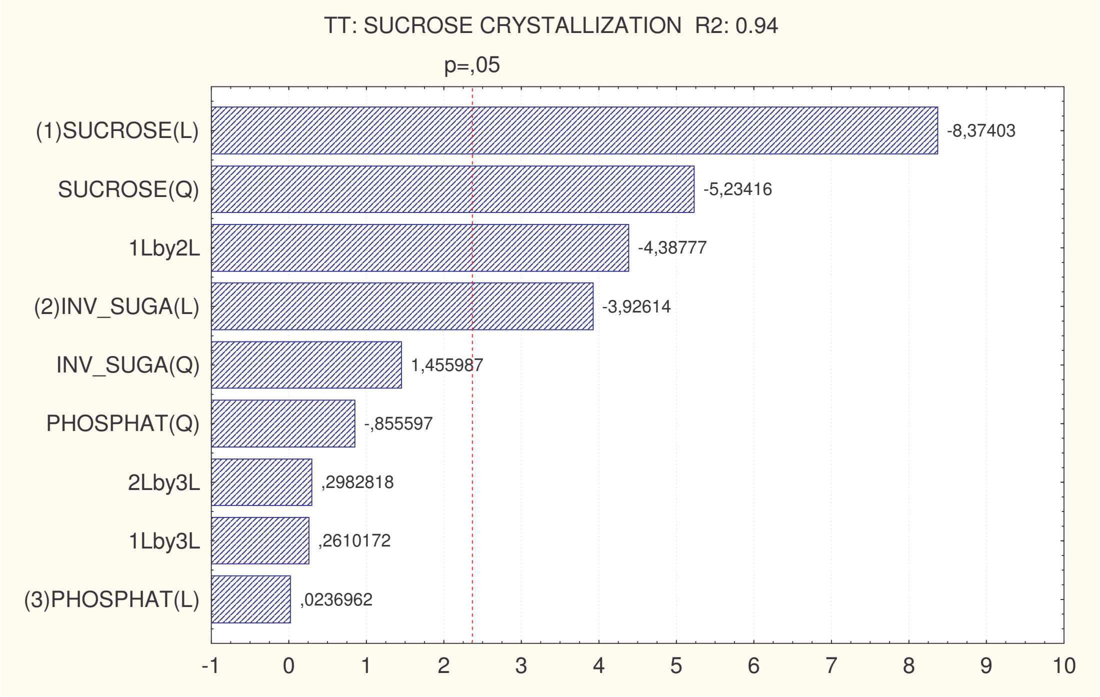
1.13.Gráfico Pareto para área do pico exotérmico



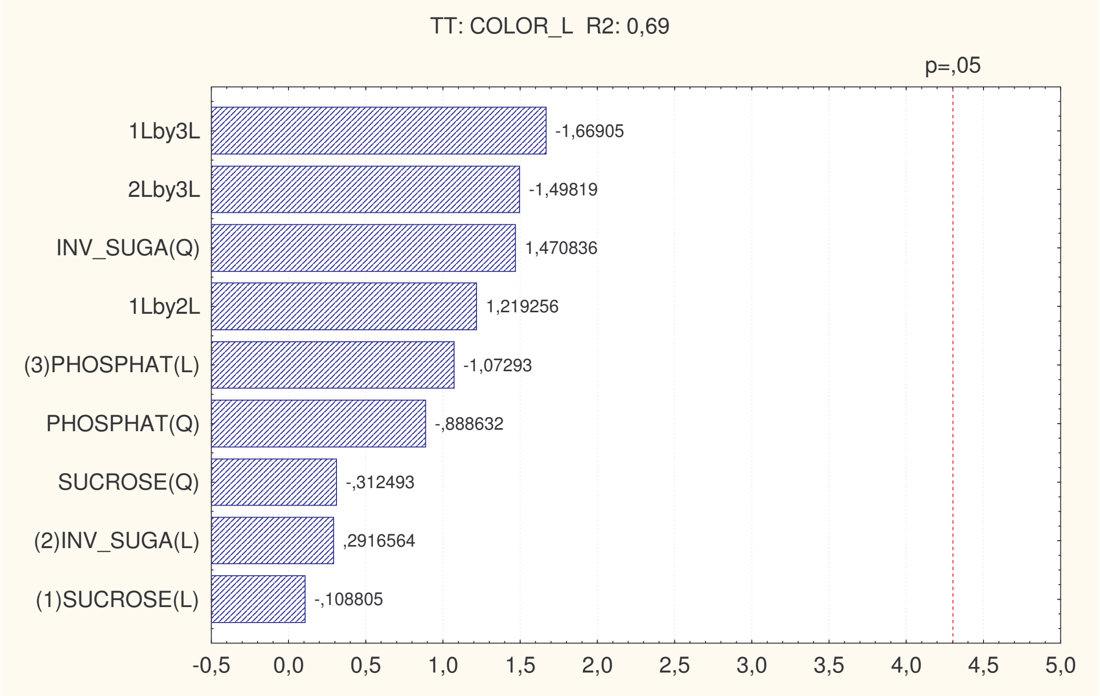
1.14.Gráfico Pareto cristalização total



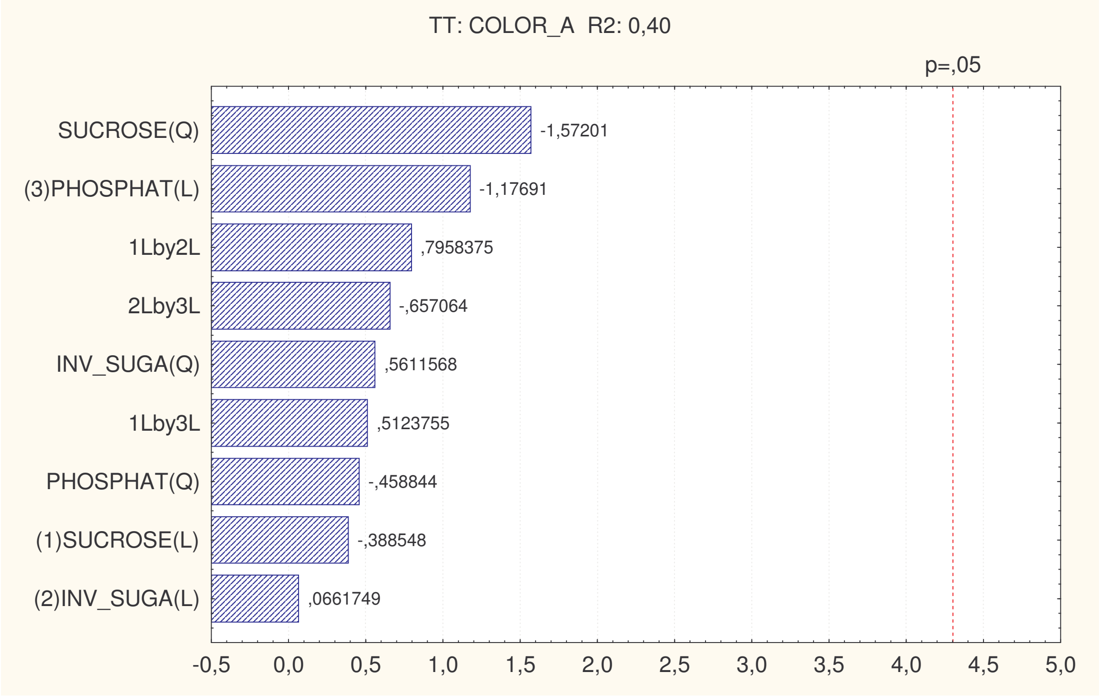
1.15.Gráfico Pareto cristalização apresentada por sacarose



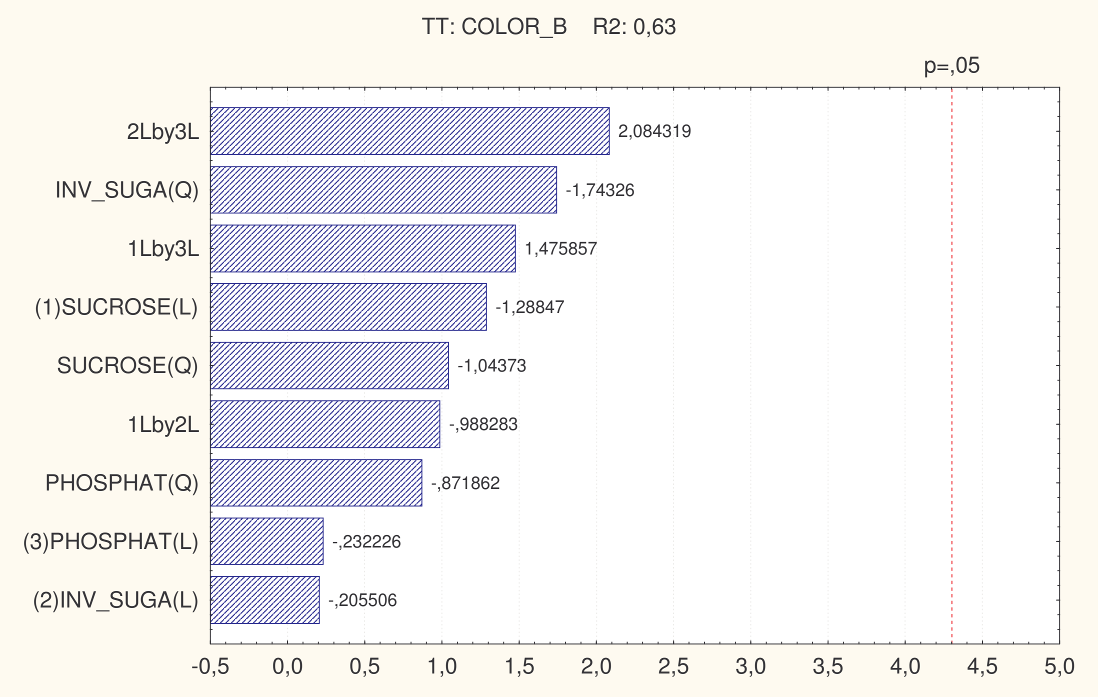
1.16. Gráfico Pareto para cor “L”



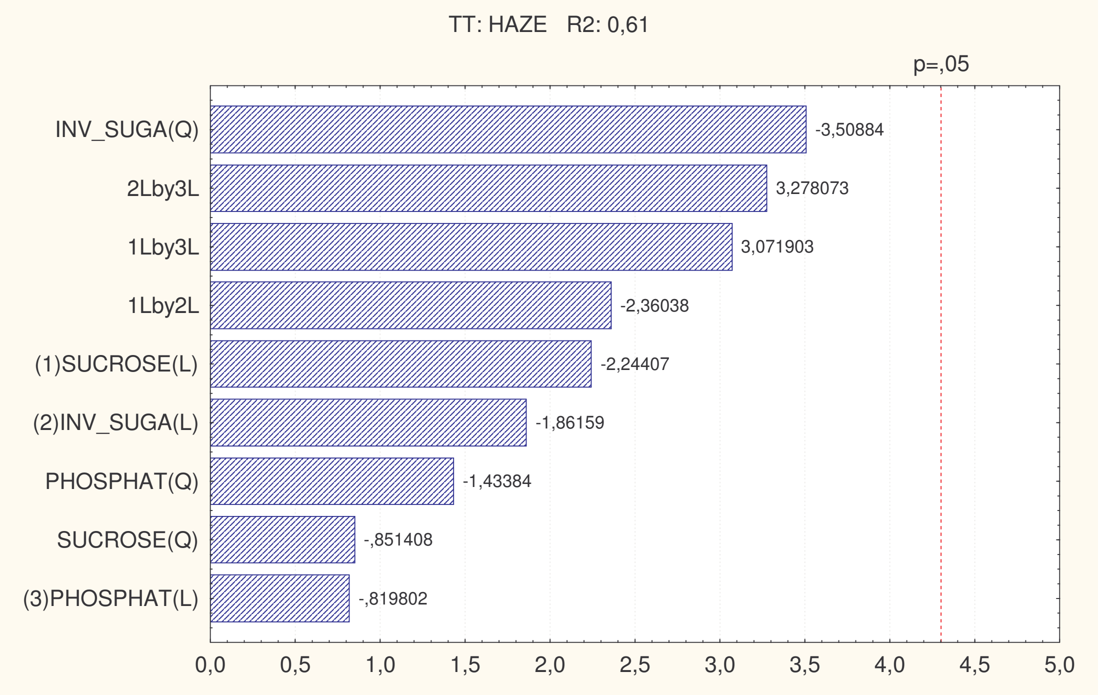
1.17. Gráfico Pareto para cor “a”



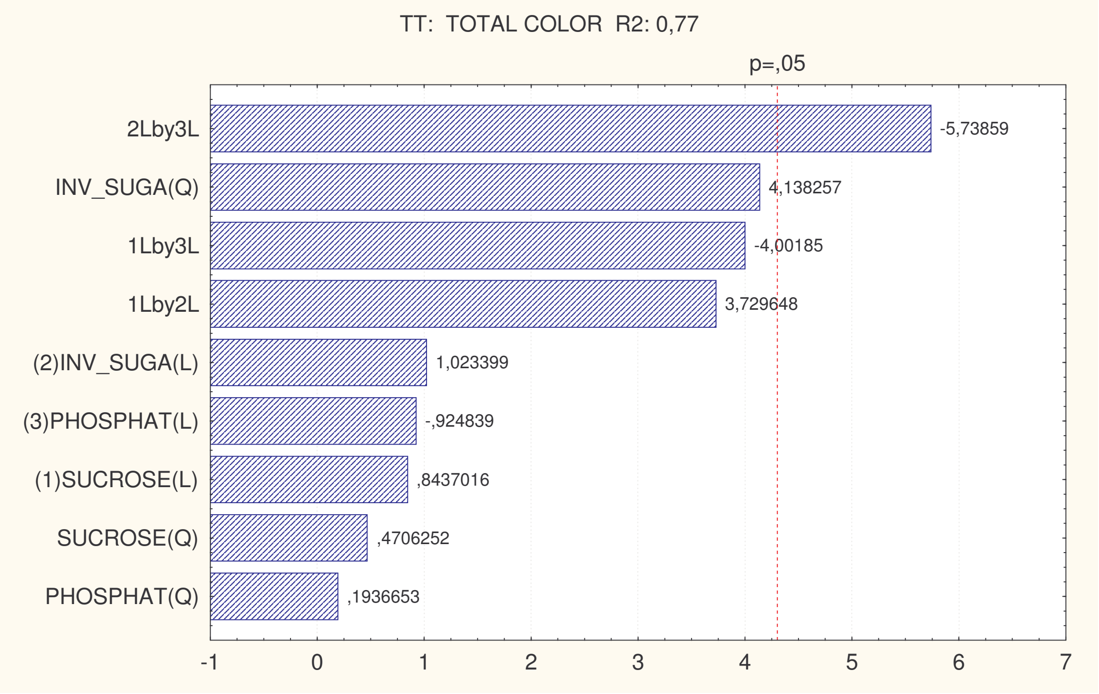
1.18.Gráfico Pareto para cor "b"



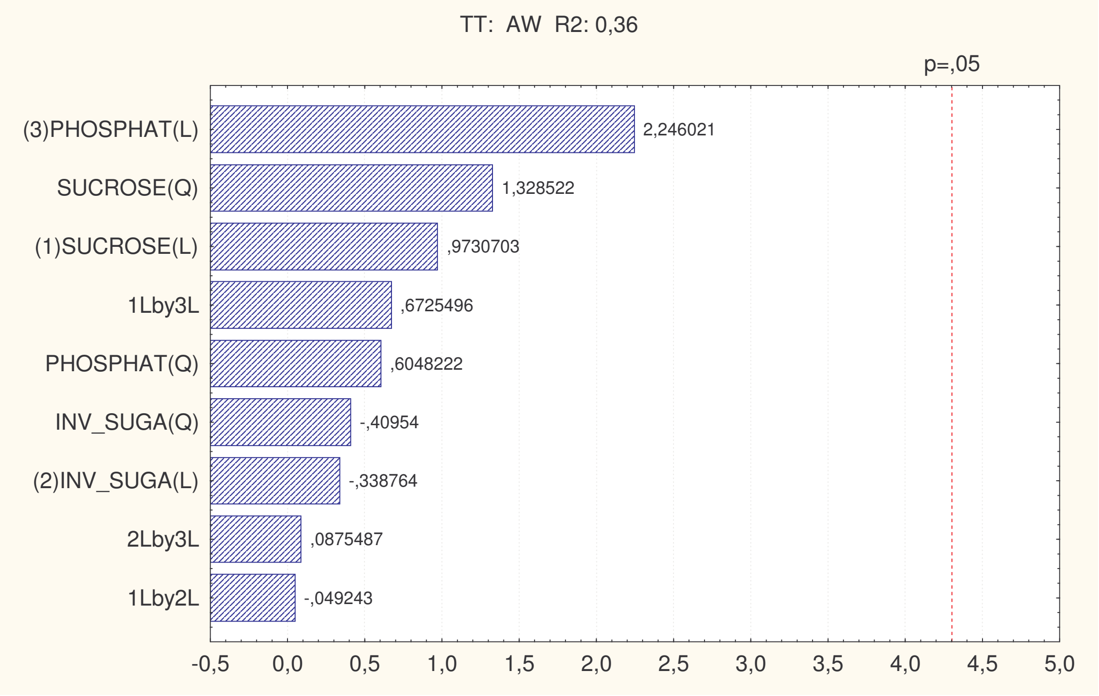
1.19.Gráfico Pareto para haze



1.20.Gráfico Pareto para diferença total de cor



1.21.Gráfico Pareto para aw



1.22.Gráfico Pareto para solubilidade