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INTERAÇÃO MOLECULAR ENTRE CELULOSE E HEMICELULOSES E SUAS IMPLICAÇOES BIOLÓGICAS E TECNOLÓGICAS

Tese apresentada ao Instituto de Biologi da Universidade Estadual de Campina para obtenção do Título de Doutor en Biologia Vegetal

Orientador: Dr. Marcos Silveira Buckeridge

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"Que toda ausência seja entendida...
e perdoada."

Dedico este trabalho a minha filha Julia Guedes Ubeda de Lima

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RESUMO

As hemiceluloses são polissacarídeos que representam um dos componentes da parede celular vegetal e estão intimamente associados à celulose, definindo as propriedades estruturais na parede além de desempenhar funções na regulação do crescimento e desenvolvimento das plantas. Os xiloglucanos (Xg) e os galactomananos são hemiceluloses presentes tanto em parede primária (função estrutural) como em parede de reserva em sementes de algumas espécies (função de reserva). A interação entre celulose e as hemiceluloses pode ser analisada *in vitro*, possibilitando o estudo e a caracterização dos fatores que atuam sobre esta interação, consequentemente, tornando-se uma ferramenta importante para a compreensão de como o metabolismo e a arquitetura da parede são controlados. Em vista disto, este trabalho teve como objetivos: 1) analisar o efeito de variações na estrutura fina do xiloglucano (diferentes graus de ramificação com galactose e fucose) sobre sua capacidade de interação com celulose; 2) analisar o efeito do tamanho da molécula sobre a capacidade e sobre a energia envolvida nos processos de adsorção e 3) com base na capacidade de interação com celulose, avaliar o potencial das hemiceluloses (xiloglucano e galactomanano) como aditivos para o incremento das propriedades físicas do papel. Os resultados mostraram que, tanto o grau de ramificação como o tamanho da molécula afetam a interação entre Xg e celulose. Enquanto o grau de galactosilação e fucosilação estão relacionados com a força de ligação entre os polissacarídeos, o tamanho da molécula do Xg afeta a sua capacidade (quantidade) de interação à celulose, consequentemente a auto-interação. Estes dados sugerem que, a ausência da fucose nos Xgs de reserva, associada a sua provável síntese a partir de fragmentos de baixo peso molecular, possibilitariam a ligação (de baixa energia) entre as moléculas, permitindo tanto um eficiente empacotamento, como o fácil acesso às enzimas e expansinas durante o processo de desmonte e mobilização das reservas. As regiões fucosiladas do Xg estariam associadas àquelas regiões da parede primária sob maior pressão (alongamento, turgor), ou ainda, na manutenção da arquitetura da parede de reserva em tecidos de sementes que acumulam tais polissacarídeos. Do ponto de vista tecnológico, o uso das hemiceluloses (xiloglucano e galactomanano) são potenciais produtos alternativos para o incremento das propriedades mecânicas do papel.

ABSTRACT

Hemicelluloses are polysaccharides present in plant cell wall that are specifically associated to cellulose. They are related to the structural properties of the wall and act on the regulation of plant growth and development. Xyloglucans (Xg) and galactomannans are hemicelluloses present in primary cell walls, where they have a structural function and in secondary cell walls (as storage polysaccharides) of seeds of some dicotyledonous species. Results of in vitro experiments can be used as important tools to understand how cell wall metabolism and architecture are controlled. In this work, we had the following objectives: 1) to evaluate the effect of variations of the fine structure of Xg (degree of galactosylation and fucosylation) on its binding capacity to cellulose; 2) to analyse the effect of molecular weight of Xg on binding and energy involved in adsorption processes and 3) to assess the potential of hemicelluloses (Xg and galactomannan) as wet-end additives in order to improve the mechanical properties of paper sheets. Our results showed that both the branching degree and the molecular weight of Xg molecules affected the interaction with cellulose. The strength of binding to cellulose was related with the degree of galactosylation and fucosylation. However, the Xg molecular weight strongly affected its binding capacity (amount) and possibly its capacity of self-association. These data suggest that the absence of fucose in storage Xgs, associated to its biosynthesis as low molecular weight fragments, would allow the low energy binding among the molecules thus affording a rather efficient packing of the polymers in the case of the storage wall. It would also facilitate the access of the hydrolases (and maybe expansins) during the period of storage mobilisation. On the other hand, the fucosylated regions, characteristic of primary cell walls of growing tissues, would be associated with regions of the wall under higher pressure. From the biotechnological point of view, the hemicelluloses (Xg and galactomannan) are potential alternative products to be used to improve the mechanical properties of paper.

INTRODUÇÃO

Parede celular

A parede celular é constituída por agrupamentos macromoleculares heterogêneos que circundam as células vegetais. Entre os polímeros presentes, os polissacarídeos representam 80-90% da parede; os outros componentes são as proteínas (estruturais e enzimáticas) e compostos fenólicos como a lignina (Carpita e Gibeaut, 1993).

A parede celular forma a matriz extracelular dos tecidos vegetais e com base nos conhecimentos atuais, ela poderia ser considerada como um outro compartimento celular, onde ocorrem muitas reações que afetam diretamente o metabolismo celular como um todo. A interação, o arranjo e o metabolismo dos componentes da parede celular têm sido objeto de estudo, numa tentativa de esclarecer seu envolvimento em processos biológicos tão diversos como o crescimento e o desenvolvimento dos vegetais, a expansão foliar e a adaptação ao ambiente e o acúmulo de substâncias de reserva na semente (Hayashi, 1989).

Entre as principais funções da parede estão o controle do crescimento, sinalização celular, defesa e porosidade. Como estas funções são exigidas simultaneamente, a parede celular deve ser ao mesmo tempo flexível e resistente, o que requer um fino controle metabólico.

Dois tipos de parede são encontrados em plantas superiores: paredes primárias e paredes secundárias. As paredes primárias são produzidas por células em crescimento, portanto podem se alongar, enquanto que as paredes secundárias não têm essa capacidade (Hayashi, 1989).

As paredes celulares primárias são compostas de um esqueleto de microfibrilas de celulose (~30%), as quais formam a trama da parede denominada de matriz de polímeros, que inclui hemiceluloses (~30%), pectinas (~30%) e proteínas (McCann e Roberts, 1991). As hemiceluloses são depositadas entre as microfibrilas e ligam-se à celulose através de pontes de hidrogênio, formando uma estrutura sólida que se

assemelha à estrutura de concreto armado (Hayashi et al, 1987). Nos modelos mais recentes de parede celular, propõem-se que estes polímeros formem três domínios independentes: celulose-hemicelulose, pectinas e proteínas (McCann e Roberts, 1991; Carpita e Gibeaut, 1993). As microfibrilas de celulose estão, normalmente, arranjadas em padrão helicoidal ordenadas de forma "colestérica" semelhante a um cristal líquido (Roland et al., 1992). Esta organização das microfibrilas é similar a várias matrizes extracelulares animais. Na figura 1, pode-se observar uma comparação entre uma matriz de tecido epitelial de mariposa e células presentes no xilema (esclerócitos), mostrando a semelhança no padrão de organização de diferentes tipos de fibras (protéica - na célula animal e polissacarídica - na célula vegetal).

Carpita e Gibeaut (1993) baseados no fato de que polímeros estruturalmente diferentes poderiam desempenhar funções análogas na parede de diferentes grupos taxonômicos, propuseram a separação da parede em 2 grupos, tipo I e tipo II. A parede celular do tipo II está presente nas monocotiledôneas da família *Poaceae* (Gramineae), onde, a hemicelulose dominante é o arabinoxilano ou o glucano de ligação mista [(1,3)-(1,4)-β-glucano], além de uma baixa quantidade de pectinas. Já a parede tipo I é típica das dicotiledôneas, caracterizando-se por ter altas proporções de pectinas e apresentar o *xiloglucano* como principal hemicelulose. Pode-se observar na figura 2 um modelo esquemático da parede celular primária presente em células de tecidos em crescimento.

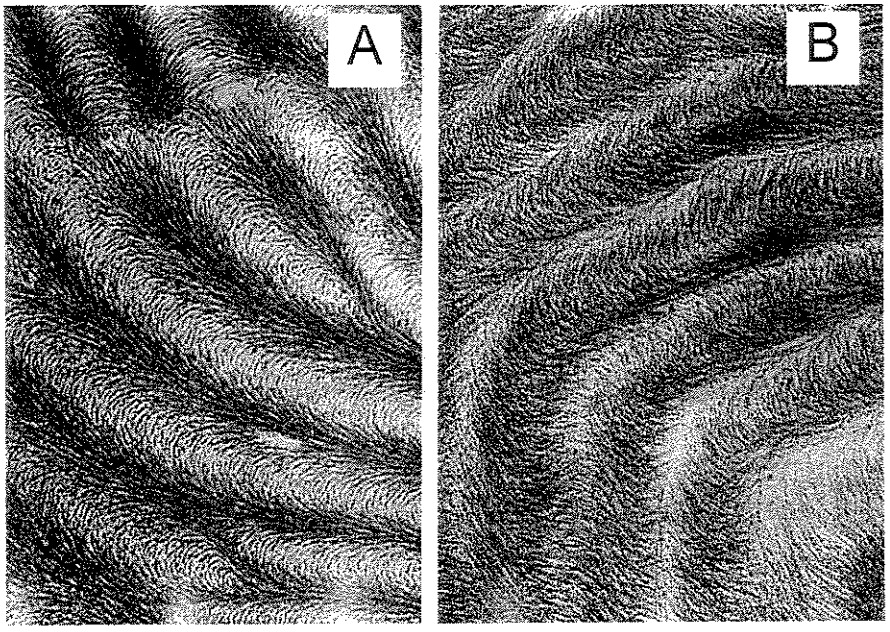


Figura 1. Comparação entre matriz extracelular animal e vegetal. A) tecido epitelial de uma membrana que reveste o embrião de mariposa; B) matriz de célula de xilema. (Roland et al., 1992).

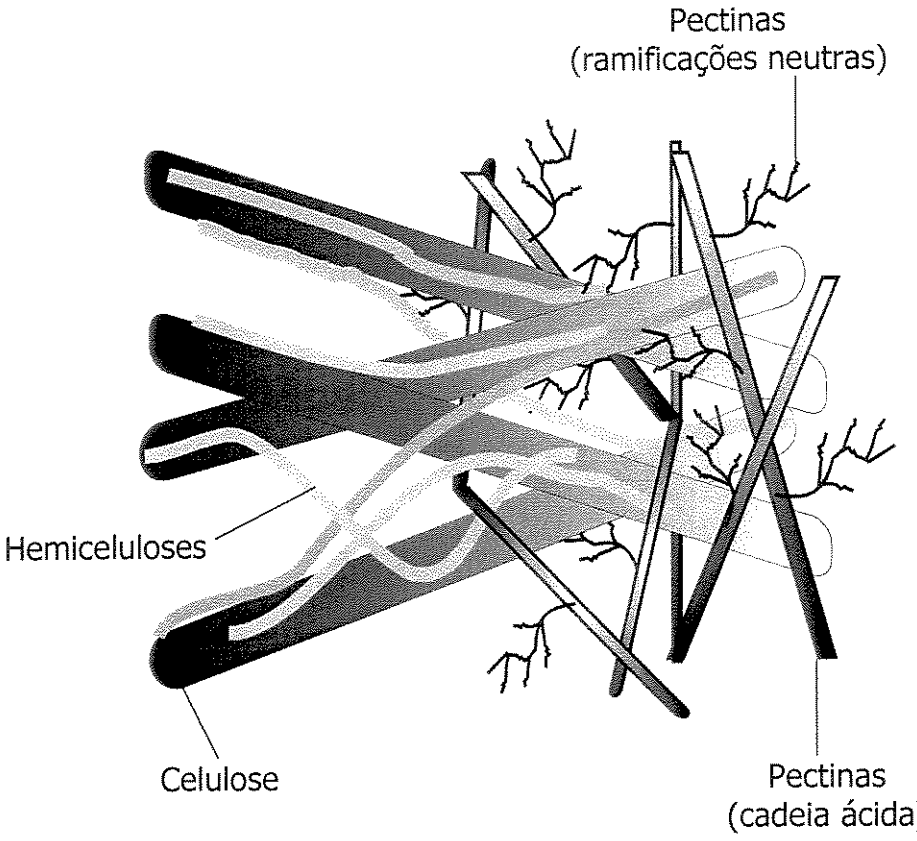


Figura 2. Esquema geral da parede celular vegetal, excluindo o domínio protéico. À esquerda foi retirado o domínio péctico para melhor clareza do desenho. A principal hemicelulose em dicotiledôneas é o xiloglucano e em Poaceae (gramíneas) são os glucuronoarabinoxilanos e os glucanos de ligação mista. O comprimento da cadeia da hemicelulose possibilita que uma mesma molécula estabeleça ligações com mais de uma microfibrila de celulose, formando ligações cruzadas entre as mesmas. (Buckeridge e Tiné, 2001).

Xiloglucanos

Xiloglucanos são polímeros compostos por uma cadeia principal formada por β-D-(1→4)-glucose ramificada com ligações α-(1→6) por unidades de D-xilopiranosídeo ou com o dissacarídeo β-D-galactopiranosídeo-(1→2)-D-xilopiranosídeos (White & Rao, 1953) ou ainda, com o trissacarídeo α-L-fucopiranosídeo (1→2) β-D-galactopiranosídeo (1→2) α-D-xilopiranosídeos (Hayashi, 1989). Utilizando uma celulase microbiana, Kooiman (1960) determinou que os xiloglucanos são formados por unidades (blocos estruturais) de um heptassacarídeo composto por 4 glucoses, 3 xiloses e variações nas substituições com resíduos de galactose. A estrutura detalhada dos blocos de oligossacarídeos que constituem os xiloglucanos de diferentes fontes, incluindo sementes, foi determinada entre os anos 80 e 90, utilizando hidrólise enzimática seguida por análises por metilação, espectrometria de massas e ressonância magnética nuclear. Buckeridge et al. (1992) empregando celulases purificadas de *Trichoderma viride* demonstraram que o xiloglucano das sementes de *Tamarindus indica* e *Copaifera langsdorffii* também são constituídos por quatro subunidades estruturais básicas que estão combinadas em diferentes proporções, dando origem a uma estrutura detalhada que varia com a espécie e mesmo entre as populações da mesma espécie crescendo em diferentes ambientes. Uma variação destas subunidades estruturais foi encontrada no xiloglucano de sementes de *Hymenaea courbaril*, em que além dos blocos com 4 glucoses na cadeia principal, foi demonstrado a existência das unidades formadas por 5 glucoses (além das xiloses e galactoses) (Buckeridge et al., 1997).

A nomenclatura atual para os blocos estruturais do xiloglucano foi proposta por Fry et al. (1993). Glucoses não substituídas são denominadas **G**; glucoses ramificadas com xilose são denominadas **X** e, se a galactose está ligada a xilose, o trissacarídeo é denominado **L**. A letra **F** é utilizada para identificar a fucose ligada à galactose.

Biossíntese

A biossíntese dos polissacarídeos de parede celular requer nucleotídeo-açúcares como doadores de monossacarídeos. As enzimas de biossíntese são traduzidas, montadas no retículo endoplasmático e transportadas para o complexo de Golgi, onde a maioria das reações de biossíntese ocorre. Ao fim do processo de biossíntese e início da produção das vesículas secretoras, os resíduos de fucose e metila são adicionados aos xiloglucanos e pectinas, respectivamente. Os polissacarídeos são secretados para o espaço intercelular onde ocorrerá a *montagem* da parede celular através da orientação das microfibrilas de celulose e constituição dos diferentes domínios polissacarídicos da parede. Como esta *montagem* ocorre ainda é um mistério, mas há indícios de que algumas proteínas possam estar envolvidas no processo (Hayashi, 1989; Gibeaut & Carpita, 1994; Perrin et al., 1999; Gibeaut, 2000).

Diferente dos xiloglucanos e pectinas, a celulose é biossintetizada diretamente a partir da membrana plasmática, onde os nucleotídeo-açúcares (especificamente UDP-glucose) acoplam-se às rosetas (um complexo enzimático, destacando-se a celulose-sintase) e as glucoses são inseridas nas longas cadeias que formam microfibrilas (Delmer, 1987).

Apesar de todo o conhecimento adquirido sobre os processos de biossíntese de polissacarídeos da matriz extracelular vegetal, nenhuma das sintetases foi isolada de tecido vegetal até o presente. O principal problema é que estas enzimas estão associadas às membranas celulares e as tentativas de "solubilização" destas resultam em rápida perda de atividade, mesmo quando detergentes são adicionados aos extratos enzimáticos para "proteger" os domínios hidrofóbicos das proteínas enzimáticas.

Conformação dos xiloglucanos

A montagem e a expansão da parede primária é dirigida pela conformação e dinâmica dos polissacarídeos que devem interagir durante estes processos. A dinâmica destes compostos em solução permite que eles adotem diferentes conformações de baixa energia, tornando muito difícil o estudo estrutural através de cristalografia e técnicas espectroscópicas. Assim, os modelos conformacionais devem se basear principalmente em métodos computacionais, utilizando alguns resultados experimentais obtidos por ressonância magnética nuclear (RMN), possibilitando a análise das variações dinâmicas destes glicanos complexos. Sugere-se que o xiloglucano deva adotar uma conformação de baixa energia, permitindo que todos resíduos ramificadores se virem para um único lado da molécula, deixando a outra face livre para interagir com as microfibrilas de celulose. Levy et al., (1991) sugeriram, através de simulações computacionais, que os aspectos estruturais do xiloglucano relacionados à ramificação fucosilada deste polímero seriam de grande importância para a forma de interação com a celulose. Os dados de modelagem sugerem que os resíduos de fucose sejam responsáveis por tornar a molécula mais linear, enquanto que aqueles oligossacarídeos não-fucosilados seriam mais retorcidos, como mostrado na figura 3.

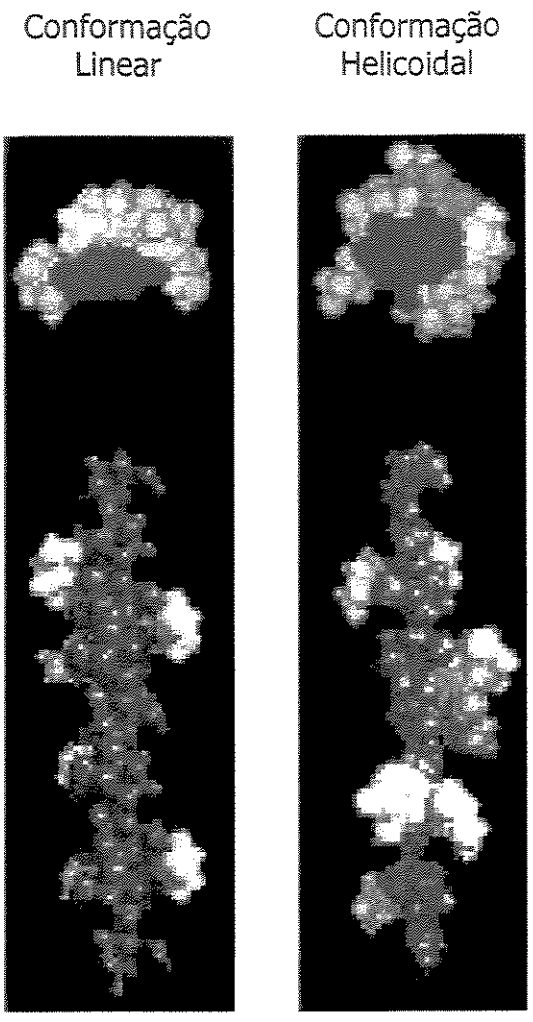


Figura 3. Modelagem computacional da conformação de oligossacarídeos de xiloglucano fucosilados. A conformação linear é provavelmente a forma predominante destes oligossacarídeos em solução, uma vez que aparece num nível mais baixo de energia (não mostrados nesta figura). Descarta-se a predominância da conformação helicoidal para os oligossacarídeos fucosilados por ocorrer em níveis altos de energia, sugerindo que esta forma deva ser encontrada apenas para os oligossacarídeos não-fucosilados. A presença de fucose (magenta) no carboidrato parece ser responsável pela linearidade da molécula, tornando as glucoses (em vermelho) mais expostas para interação, enquanto as xiloses (alaranjado) e as galactoses (verde) ficam agrupadas do outro lado da molécula. (Levy et al., 1991).

Interação xiloglucano–celulose: Aspectos biológicos

Função na parede primária

Os xiloglucanos interagem especificamente com a celulose (Valent & Albersheim, 1974; Hayashi et al., 1987; Vincken et al. 1995). Tal especificidade ocorre devido à similaridade entre a cadeia principal de ambos os polissacarídeos, sendo que as ramificações com xilose não parecem causar alterações conformacionais suficientes que evitem a interação (Levy et al., 1991, 1997). Estes autores sugeriram, através de simulações computacionais, que os resíduos de fucose sejam responsáveis por tornar a molécula mais linear, como mostrado na figura 3. Hayashi et al. (1994) realizaram alguns experimentos com xiloglucano de epicótilo de ervilha (fucosilado) e de sementes de *Tamarindus indica* (não-fucosilado). Os resultados sugeriram que os resíduos fucosilados contribuem para o aumento da afinidade e da constante de adsorção dos xiloglucanos pela celulose.

Devido à capacidade de interação com as microfibrilas de celulose, os xiloglucanos estão intimamente relacionados com as alterações estruturais na parede primária. Estas alterações, que envolvem uma intensa e controlada atividade de hidrolases e transglicosilases, referem-se ao afrouxamento das ligações intra e intermoleculares que permitem o crescimento e alongamento celular (Hayashi, 1989). Além disso, a organização e alinhamento da celulose são atribuídas à presença de moléculas de xiloglucano interagidas, bem como, pelas regiões não interativas entre as microfibrilas. O complexo é responsável pela resistência à pressão de turgor e pelo crescimento anisotrópico das células vegetais (Taiz, 1984; Reis et al., 1994; Baskin, et al., 1999).

O comprimento da cadeia de xiloglucano possibilita que uma mesma molécula estabeleça ligações com mais de uma microfibrila de celulose, formando ligações cruzadas entre as mesmas (Figura 2), o que aumenta ainda mais a resistência da parede às forças externas (Hayashi e Maclachlan, 1984)

Whitney, et al. (1995) demonstraram, através de micrografias obtidas a partir de lâminas de celulose produzidas por culturas de *Acetobacter acetti xylinun* desenvolvidas em meio de cultura com e sem xiloglucano, que a organização das fibrilas de celulose foi obtida apenas em películas produzidas em meio contendo xiloglucano (Figura 4). Isto corrobora as hipóteses de que as hemiceluloses auxiliam no alinhamento, bem como na organização das microfibrilas de celulose, impedindo que grandes agregados de polímeros se formem, colapsando a parede.

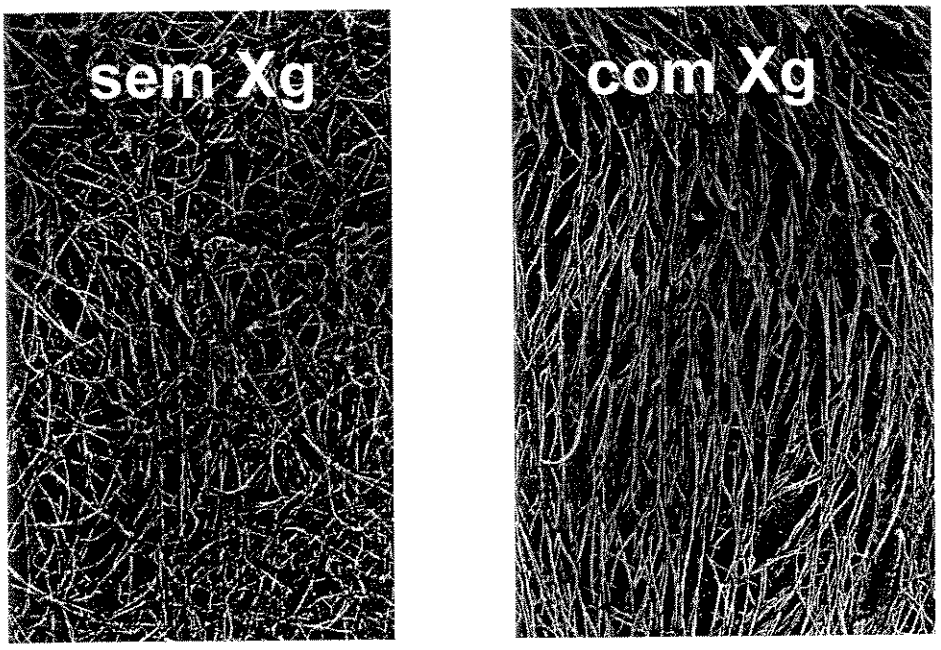


Figura 4. Microscopia eletrônica por crio-fratura (deep-etched freeze fracture) de películas de celulose produzidas por *Acetobacter acetti xylinum* cultivadas em meio de cultura sem e com xiloglucano. (Whitney et al., 1995)

Dados recentes têm colocado em dúvida o papel da fucose como responsável pela alta capacidade de interação dos xiloglucanos à celulose. Mutantes de *Arabidopsis* (*mur1*) são deficientes na síntese *de novo* de L-fucose, o que resultou em plantas anãs com paredes celulares menos rígidas (Reiter et al., 1993). Esta mutação afetou vários polissacarídeos da parede, e o fenótipo observado pode ter sido causado por mudanças estruturais nos componentes pécticos fucosilados, como o rhamnogalacturonano-II, além do xiloglucano. Já Vanzin et al. (2002) obtiveram um outro mutante (*mur2*) no qual foi eliminada a fucosilação específica do xiloglucano na maioria dos órgãos. Apesar desta alteração na estrutura, as plantas do *mur2* mostraram um hábito de crescimento normal e as paredes celulares não sofreram alterações quanto a sua rigidez, indicando que a fucosilação do xiloglucano poderia estar relacionada com eventos que não se referem exclusivamente ao papel estrutural.

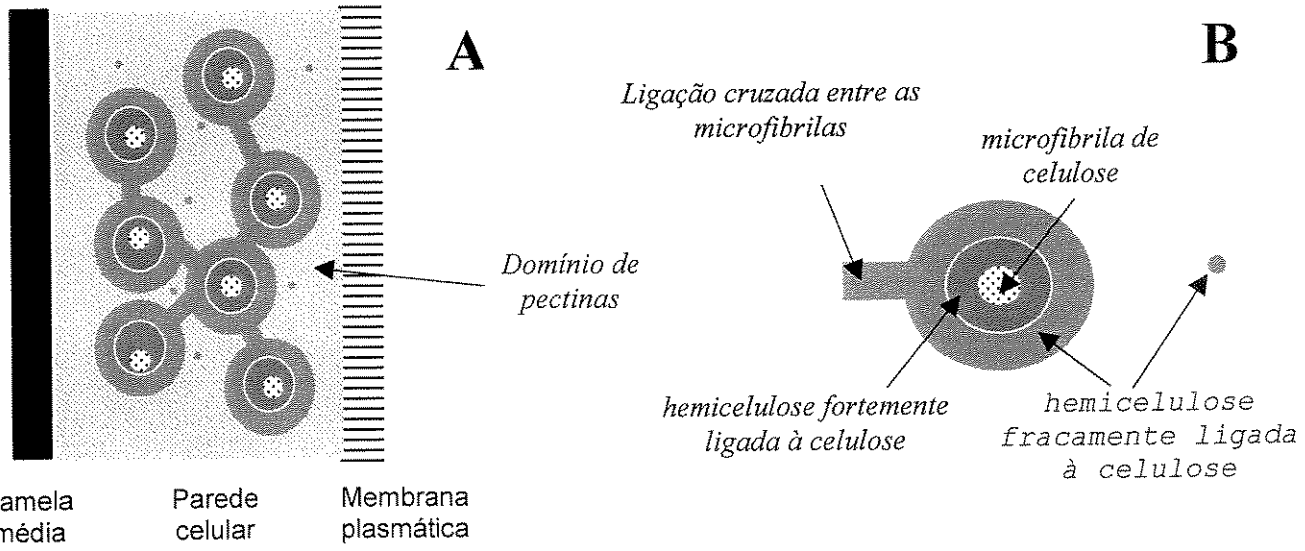
Alguns trabalhos têm sugerido uma outra função para os oligossacarídeos fucosilados, especificamente na atividade biológica, como uma molécula sinal responsável pelo papel anti-auxínico (McDougall & Fry, 1988), que representa a inibição do alongamento de tecidos previamente estimulados por 2,4-D, GA_3 ou H^+ (Warneck & Seitz, 1993). Resultados de experimentos *in vitro* utilizando cultivo de células em suspensão, sugerem que os oligossacarídeos de xiloglucano agem como oligossacarinas, que devem ser hidrolisados enzimaticamente a partir do polímero para atuar como sinalizadores de informações do apoplasto para o simplasto (MacDougall & Fry, 1991). Dunand et al., (2000) identificaram uma proteína de membrana de 62kDa que se liga especificamente ao oligossacarídeo XXFG, sendo um provável receptor.

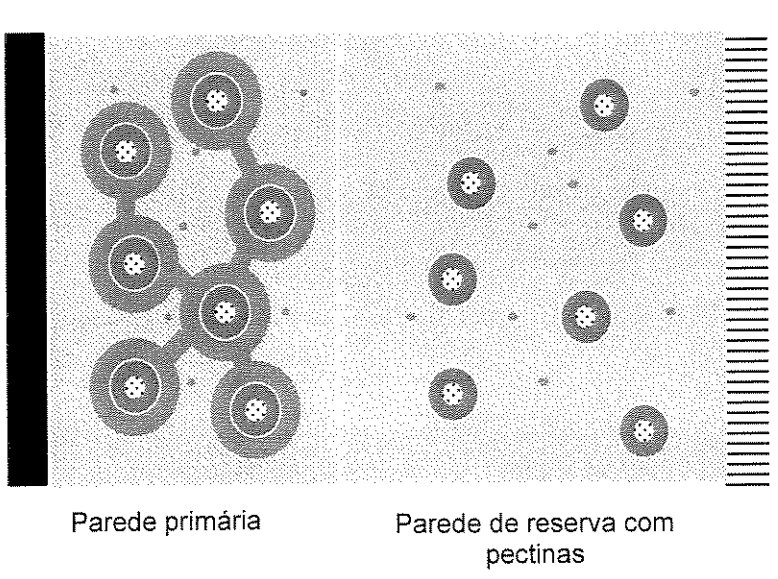
Funções na parede de reserva

Dentre as principais substâncias armazenadas pelas plantas, muitos polímeros de carboidratos foram selecionados durante a evolução como uma das estratégias de adaptação aos diferentes ambientes. Entre os compostos acumulados nas sementes, o xiloglucano destaca-se como o polissacarídeo de reserva de parede celular a ser mobilizado após a germinação, quando seus produtos de desmontagem serão usados

para diferentes propósitos, como a geração de energia e a produção de matéria-prima para a construção de novos tecidos. O xiloglucano (bem como outras hemiceluloses acumuladas em sementes) apresenta a vantagem de ser um polímero osmoticamente inativo, inerte quanto à reatividade química e altamente compactado num "compartimento celular" (parede celular), assemelhando-se ao amido, que também é acumulado e compactado em organelas especiais (amiloplastos) (Buckeridge et al, 2000a). No entanto, os polissacarídeos de reserva de parede celular (xiloglucano, (galacto)manano, galactano) apresentam ao menos uma função paralela à função de reserva de carboidratos. O xiloglucano (acumulado nos cotilédones) e o galactomanano (endospermas) parecem desempenhar um importante papel nas relações hídricas das sementes, controlando o processo de embebição de água no estádio pré-germinativo. Buckeridge et al. (2000b) e Santos (2002) propuseram que estas funções secundárias seriam importantes no mecanismo evolutivo que levou as plantas a utilizarem polissacarídeos da parede celular como reserva de carbono. Estes oligossacarídeos são acumulados na parede celular secundária dos tecidos de reserva das sementes. Sugere-se que esta parede de reserva é uma variação da parede primária, onde um dos domínios (neste caso a hemicelulose) seria depositado (e obviamente, sintetizado) em maior escala em relação aos demais domínios (Figura 5).

A interação xiloglucano-celulose pode não estar diretamente relacionada no que diz respeito à reserva de carboidratos, mas deve-se analisar as características químico-estruturais que afetam a capacidade de auto-interação dos xiloglucanos, que da mesma forma que atua sobre a adsorção à celulose, atua também na capacidade de ligar-se as outras moléculas de xiloglucano. Este fator é marcante no que diz respeito ao empacotamento das reservas durante a deposição das hemiceluloses na parede de reserva.





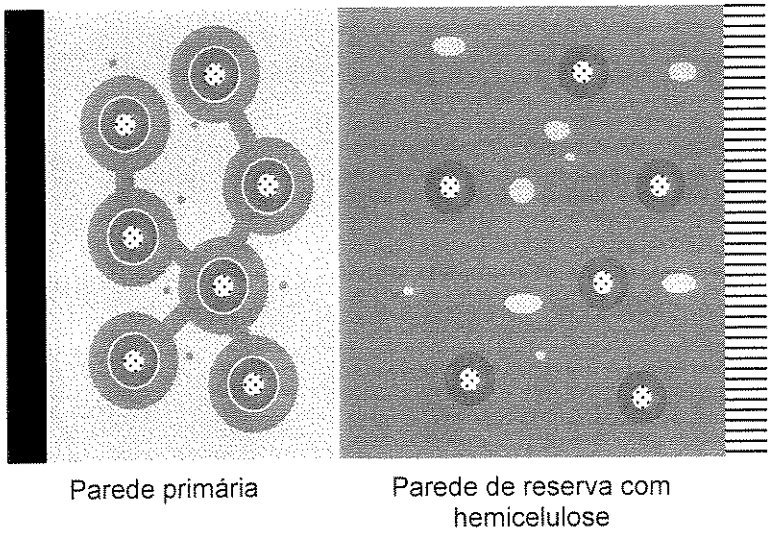


Figura 5 - Representação esquemática da parede celular primária (**A**) em corte transversal. As microfibrilas de celulose estão cobertas por hemicelulose (xiloglucanos, arabinoxilanos ou mananos) que podem estar forte ou fracamente ligadas à celulose (ver **B**). O domínio celulose-hemicelulose está embebido em um domínio péctico (**A**). Em **C** está representada a parede de reserva em que o polímero acumulado é derivado das pectinas (arabinogalactanos em sementes de lupino e de café). Em **D** estão representadas as paredes cujo polímero de reserva é o galactomanano ou o xiloglucano. Estas representações assumem que as "amplificações" de hemiceluloses ou pectinas ainda possuam algumas microfibrilas de celulose juntamente com pequenas quantidades de outros polissacarídeos típicos da parede celular. O domínio protéico, que normalmente representa menos do que 10% da parede celular, foi omitido.

Fonte: Buckeridge et al. (2000a)

Interação xiloglucano–celulose: Aspectos tecnológicos

A capacidade das moléculas de xiloglucano em se ligarem as microfibrilas de celulose através de pontes de hidrogênio, pode ser utilizada como uma interessante ferramenta no aumento da qualidade física de produtos baseados neste polímero, por exemplo, o papel.

As propriedades físicas das folhas de papel estão sob efeito das variações relacionadas à qualidade das fibras (como tipo, tamanho, resistência individual), além da força de adesão entre elas. Estas fibras são células especializadas, com alto teor de lignina e celulose, presentes na madeira das plantas utilizadas pela indústria. Após a extração, estas fibras passam por processos de deslignificação, branqueamento e desfibrilação (refino). Este último passo expõe as fibrilas de celulose presentes nas paredes celulares destas células.

Alguns aditivos como a goma guar (galactomanano de sementes de *Cyamopsis tetragonolobus*) e o amido têm sido amplamente utilizados no processo de produção das folhas, sendo responsáveis pelo aumento da força de coesão entre as fibras (Abson & Brooks, 1985; Blumenthal & Paul, 1994; Sundberg et al., 2000), resultando no aumentando da resistência do papel, principalmente aqueles utilizados como papel cartão e para embalagens em geral.

Da mesma forma, a utilização de xiloglucanos e outros galactomananos de sementes como aditivo para aumentar a qualidade do papel seria uma interessante alternativa para o uso racional das espécies típicas de regiões de mata e cerrado, introduzindo mais uma característica para a utilização sustentável destas áreas. As espécies, já estudadas, que acumulam xiloglucano são *Hymenaea courbaril* (jatobá), *Copaifera langsdorffii* (copaíba), *Tamarindus indica* (tamarindo) e *Tropaeolum majus* (chagas). Uma espécie que apresenta alto potencial para a exploração de galactomananos é a *Dimorphandra mollis*, por apresentar um grau de ramificação (manose:galactose) semelhante à goma guar (Panegassi et al., 2000)

DEFINIÇÃO DO PROBLEMA

As sementes que acumulam carboidratos nos tecidos cotiledonares podem armazenar até 40% da massa seca na forma de xiloglucano presente nas paredes celulares de reserva. Para tal, o empacotamento destes açúcares deve ocorrer de forma muito eficiente, mantendo uma alta densidade sem impedir o acesso das enzimas hidrolíticas durante o processo de crescimento inicial das plantas, quando os polissacarídeos serão desmontados e mobilizados para os diferentes órgãos, gerando energia para os processos metabólicos.

Este empacotamento, que se sobrepõe ao grau de auto-interação dessas moléculas, deve ocorrer durante o período de deposição das reservas ao longo do desenvolvimento dos frutos. Moléculas em que as glucoses estejam mais expostas serão, provavelmente, mais interativas entre si. Os resíduos ramificadores (dissacarídeo xilosil-galactose, no caso de xiloglucano de reserva) seriam os responsáveis por alterações conformacionais, resultando em aumento ou diminuição desta capacidade de interação (como mostrado para galactomananos com diferentes graus de galactosilação – Whitney et al., 1998; Dea e Clark, 1986). Foi demonstrado por Minhoto (2002) que os diferentes complexos xiloglucano-iodo não apresentam o mesmo comportamento físico-químico, sugerindo que as diferenças na estrutura fina do xiloglucano implicam em alterações na conformação molecular, sendo esta refletida na interação com o iodo. Além disso, um outro fator que pode alterar a capacidade de interação do xiloglucano, seria o peso molecular, já que a complexação com lugol (iodo-iodeto de potássio) também é dependente do tamanho da molécula de xiloglucano. Esta característica parece alterar o grau de torção da cadeia principal do polissacarídeo, resultando em variações no grau de exposição das glucoses.

Os xiloglucanos interagem especificamente com celulose e esta adsorção pode ser observada e medida *in vitro*. Assim, ensaios de interação entre xiloglucanos de diversas fontes com celulose microcristalina se tornam ótimas ferramentas para analisar

os efeitos das alterações estruturais sobre a capacidade de interação destas hemiceluloses.

OBJETIVOS

Este trabalho teve, portanto, como objetivos: 1) analisar variações na estrutura fina do xiloglucano (diferentes graus de galactosilação e fucosilação) sobre sua capacidade de interação com celulose; 2) analisar o efeito do tamanho da molécula (utilizando fragmentação enzimática do polímero) sobre a capacidade e sobre a energia (calorimetria) envolvida nos processos de adsorção e 3) avaliar o potencial das hemiceluloses (xiloglucano e galactomanano) como aditivos para o incremento das propriedades físicas do papel.

ARTIGO 1

"Carbohydrate Polymers 46 (2001) 157-163"

INTERACTION BETWEEN CELLULOSE AND STORAGE XYLOGLUCANS: THE INFLUENCE OF THE DEGREE OF GALACTOSYLATION

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ABSTRACT

Xyloglucan (Xg) is a polymer found in primary cell walls of growing tissues and also in seeds of many dicotyledons as storage polysaccharide. In the present work, we used Xgs from seed storage cell walls, which had their fine structure studied, to compare their behaviour under different conditions of interaction with cellulose. The range of variation of pH and temperature did not have a marked effect on the interaction, except for a slightly higher interaction at pH 6.0. Ultrastructural analysis confirmed that binding capacity depends on the surface area of the cellulose. Among different sources of Xg, the binding capacity varied significantly. HPAEC-PAD analysis of the Xg cellulase-limit digest oligosaccharides of bound Xg, suggested that there might be a certain pattern of galactosyl substitution which is related to a higher Xg binding capacity.

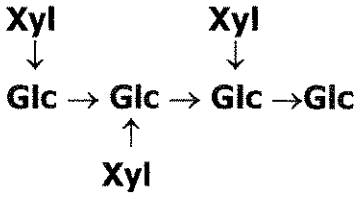
Keywords: cellulose; galactose; xyloglucan; storage cell wall

1. INTRODUCTION

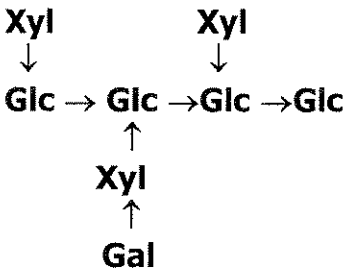
Xyloglucans are cell wall polysaccharides that have a cellulose-like β-(1,4)-glucan backbone to which single-units of α-(1,6)-D-Xyl*p* substituents are attached (branching point named **X**). Some xylosyl residues are further substituted at O-2 by β-D-Gal*p* residues (branching point named **L**) and some of the galactosyl residues may be substituted at O-6 by α-D-Fuc*p* (branching point named **F**) (Hayashi & Maclachlan, 1984; Hayashi, 1989).

Some seeds (especially from legumes) are known to contain large amounts of Xg that are thought to function as storage compounds (Reid, 1985; Buckeridge, Santos & Tiné, 2000). In this type of Xg, fucose is thought to be absent.

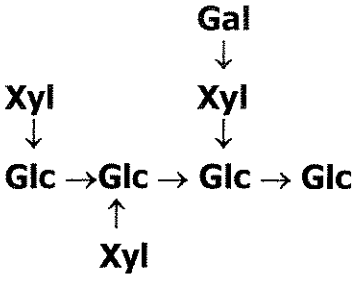
In tamarind seed Xg the pattern of Xyl-substitution is remarkably regular, virtually the whole molecule being composed of repetitive units of Glc_4:Xyl_3 with variable galactosyl substitution (York, Halbeek, Darvill & Albersheim, 1990). A comparative study of the fine structure of seed storage Xgs (*Tamarindus indica*, *Tropaeolum majus* and *Copaifera langsdorffii*) has shown that they are similar in structure, being composed almost entirely of the Glc_4 subunits XXXG, XLXG, XXLG and XLLG (see schedule below).



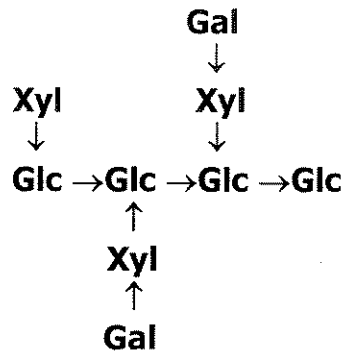
(XXXG)



(XLXG)



(XXLG)



(XLLG)

These subunits are combined in different proportions to give a fine structure that varies according to the species and even to the populations of the same species, reflecting the conformational differences (Buckeridge, Rocha, Reid & Dietrich, 1992).

More recently, Buckeridge, Crombie, Mendes, Reid, Gidley and Vieira (1997) reported that the Xg found in seeds of the tropical legume *Hymenaea courbaril* displays unique structural features. Instead of being based on XXXG only, approximately 50% of the *H. courbaril* Xg is composed of a family of oligosaccharides based on XXXXG.

Xyloglucans are known to interact specifically with cellulose (Valent & Albersheim, 1974; Hayashi, Marsden & Delmer, 1987; Vincken, Keizer, Beldman & Voragen, 1995). The basis for such interaction is thought to be the similarity of the backbone of Xgs with cellulose (both are β-(1,4) linked polymers). In general, it is reasonably well accepted that branching with xylosyl residues do not provoke enough conformational alterations to avoid interaction (Levy, York, Struike-Prill, Meyer & Staehelin, 1991; Levy, Maclachlan & Staehelin, 1997). These and other authors suggested that experimentally that the degree of fucosylation of primary wall Xgs has an important role in rendering Xg more interactive with cellulose. It is thought that fucose residues flatten the main chain of Xg molecules, making them more similar to cellulose and increasing interactivity (Levy et al., 1991; Vincken et al., 1995; Levy et al, 1997).

It has been shown that fucosylated Xgs are comparatively more interactive with cellulose than unfucosylated ones. However, the later have presented variations in the degree of galactosylation depending on the source. Even though these unfucosylated storage Xgs show lower interactivity, the different degrees of galactosylation, or its distribution along the main chain, might have some influence on the interaction with cellulose.

In the present work, we used storage cell walls Xgs from seeds that were extracted under identical conditions and had their fine structure studied, to compare their behaviour under different conditions of interaction with cellulose (paper fibres and microcrystalline). Xyloglucans bound to cellulose were released by hydrolysis with fungal

cellulase and the analysis of their fine structure suggest that there might be a certain pattern of galactosyl substitution which is related to a higher Xg binding capacity.

2. MATERIALS AND METHODS

2.1. Polysaccharides

Microcrystalline cellulose powder was from Avicel-SF; cellulose fibres were kindly provided by "Aracruz Papel e Celulose", Espírito Santo, Brazil. Analysis by acid hydrolysis followed by HPAEC-PAD showed that the composition of the fibres is 99% glucose and 1% xylose. Xyloglucan from primary cell walls of *Phaseolus vulgaris* was kindly supplied by Professor William York from CCRC/University of Georgia, USA. Storage xyloglucans were obtained from seeds of *Hymenaea courbaril* (from immature legumes), *Copaifera langsdorffii*, *Tamarindus indica* and *Tropaeolum majus*. Galactomannan was obtained from seeds of *Sesbania marginata*. The polysaccharides were extracted from cotyledon powders (or endosperm for galactomannan) with water (1% w/v) at 80^0C for 8h with constant stirring. After filtration, 3 volumes of ethanol were added to the aqueous extracts, kept overnight at 5^0C and centrifuged (12,000 g for 15 min at 5^0C). The pellet was partially dried at room temperature and, after ressuspension in water, freeze-dried.

2.2. Binding assays

Optimisation assays were performed in 25mM sodium acetate buffer, 600 μg of Xg and 20 mg of microcrystalline cellulose. Before incubation, cellulose was washed 5 times with distilled water followed by centrifugation. The parameters varied

experimentally were pH (2 to 8) and temperature (5 to 60^{0}C). After incubation the samples were centrifuged (a pulse of 13,000g) and the amount of unbound Xg present in the supernatant was quantified by I_2/KI method (Kooiman, 1960). The proportion of adsorbed Xg was calculated from the difference between the amounts of Xg in the supernatant before and after interaction.

2.3. Scanning Electron Microscopy (SEM) analysis

For the examination of the complexes produced between the polysaccharides the samples (Galactomannan- and Xg-cellulose) were mounted on stubs, freeze-dried, coated with gold (Baltec SCD 050 coater), examined, and photographed in a Philips Scanning Electron Microscope XL20 at an acceleration voltage of 10 kV.

2.4. Analysis of adsorbed xyloglucan

The complexes *Hymenaea* and *Tamarindus* Xgs-cellulose formed after binding assays were washed (3x) with distilled water and ressuspended with 25mM NaOAc pH 6.0. Subsequently, they were subjected to digestion with endo-β-(1→4) glucanase "cellulase" (from *Trichoderma viride* - Megazyme, Australia) during 24h at 30^{0}C. The reaction was stopped by boiling for 2 minutes followed by a pulse of centrifugation (13,000g). Samples of the supernatants were analysed for oligosaccharides by HPAEC-PAD and compared with standards obtained by cellulase hydrolyses of native xyloglucans. HPAEC was performed in a Dionex system DX-500 using a CarboPac PA100 column and detected by pulsed amperometric detection (PAD). The samples were eluted

with a gradient of sodium acetate (from 35 to 75 mM) in sodium hydroxide (88mM) and with a flow of 0.9 mL/min. In order to certify that the enzyme attacked all Xg bound, we performed a further extraction with alkali (NaOH 4M) and detected no recovery of Xg at all. This procedure (treatment with fungal cellulase) permitted an evaluation of the fine structure of the Xg bound to microcrystalline cellulose.

The distribution of galactose residues (galactose distribution index) on either side of the backbone of Xg (see Introduction) was estimated by the ratio among the peak areas of subunits (XLLG+XLXG)/(XLLG+XXLG). The closer to 1 the more uniform is assumed to be the distribution of galactose in the polysaccharide (Buckeridge et al., 1992).

3. RESULTS AND DISCUSSION

3.1. Xyloglucan binding specificity

Figure 1 shows the interaction between storage cell wall Xg from *Tamarindus indica* to microcrystalline cellulose (MC), cellulose fibres (CF) and glass wool (GW). A marked difference was observed in the amount of Xg adsorbed to each type of material. MC adsorbed approximately thrice the amount of Xg adsorbed to CF. The absence of binding to glass wool showed the high specificity of the adsorption of the Xg to cellulose.

The higher binding capacity of Xg to microcrystalline cellulose in comparison with cellulose fibres, might be explained by the differences in surface area and/or chemical

composition of the two types of cellulose. It had already been demonstrated that the binding of Xg to cellulose was dependent on fibre diameter (Hayashi et al., 1987).

3.2. Optimisation of binding assay conditions

Experiments of optimisation were performed for pH and temperature of incubation using T. indica Xg and microcrystalline cellulose. For logistic motives, incubations were performed during 15 minutes, although binding was already maximal within 3 minutes (data not shown).

Within a range from pH 2.0 to 8.0, pH 6.0 showed a slightly better binding capacity (Figure 2A). Below and above of this pH the interaction decreased slightly. Although these data corroborate Vincken et al. (1995), Valent and Albersheim (1974) did not observe alterations in the amount of Xg fragments bound to cellulose changing the pH from 2 to 7.

Although some conformational changes of Xg can be inferred on the basis of the interaction of Xg with iodine in different temperatures (Minhoto, Tiné & Buckeridge, unpublished), experiments performed using a range from 5 to 60°C did not alter significantly the Xg-cellulose binding capacity (Figure 2B).

On the basis of the results described above, the conditions for Xg-cellulose microcrystalline interaction were pH 6, temperature of 30°C and 15 minutes of incubation. These conditions were used for further experiments.

3.3. Visualisation of polysaccharide complexes

Figure 3 shows an ultrastructural analysis by scanning electron microscopy of Xg binding to cellulose. Galactomannan was used as a control. The figures 3C and 3D show the complexes of galactomannan from seeds of *Sesbania marginata* and Xg from seeds of *T. indica* with cellulose fibres, respectively. These fibres have an average width of approximately 14 μm against 10 nm for *in vivo* microfibrils (Baba, Sone, Misaki & Hayashi, 1994), being 1,000 times thicker than cell wall cellulose microfibrils. The complexes formed with MC were not distinguishable from the control samples.

Clusters of hemicellulosic polysaccharide not bound to cellulose (self-association plates) were visualised amidst cellulose fibres for galactomannan (arrows in figure 3C), whereas in a complex formed between Xg and cellulose (Figure 3D) they are not visible. In this case only some "bridges" of fibre-like Xg (arrows) between cellulose fibres can be seen. These bridges are also present in galactomannan-cellulose complexes, but in much lower proportions.

The high degree of self-association for galactomannan molecules, illustrated in figure 3C, suggest that there is a relatively lower affinity between galactomannan and cellulose fibres compared to Xg. The same conditions for binding were used for both polysaccharides. However, we were not able to detect the structures of self-association for Xg-cellulose complexes (figure 3D). This confirms that the interactivity of galactomannan with cellulose is lower than with Xg (Mishima, Hisamatsu, York, Teranishi & Yamada, 1998). In fact, the self-association becomes predominant when galactomannan is in excess. Whitney, Brigham, Darke, Reid and Gidley (1998) showed

that when galactomannan concentration is increased from 0.2 to 0.5% in *Acetobacter* (a bacterium that produces cellulose) culture medium, the galactomannan-cellulose composite was less apparent in spite of an increase in self-association.

3.4. Comparative study of interaction between different seed storage xyloglucans and cellulose

Xyloglucans obtained from seeds of *C. langsdorffii*, *H. courbaril*, *T. majus* and *T. indica* were tested for adsorption to microcrystalline cellulose. Table 1 shows the response in binding capacity of increasing Xg concentrations from different species. It can be seen that the percentage of adsorbed Xg within the species vary slightly, independently of the amount of Xg added. An exception is shown at initial level (10 μg Xg / mg cellulose) where the percentage of adsorption was higher. In general *H. courbaril* Xg showed a greater binding capacity (33.8%) followed by *T. indica* (30.9%), *T. majus* (26.9%) and *C. langsdorffii* (26.6%), respectively.

Primary cell wall Xg from *Phaseolus vulgaris* had an interaction of about 70% (data not shown) against an average of 29% for those storage Xgs studied. The higher binding capacity of the former Xg is related to the presence of fucosyl residues (Levy et al., 1991; Levy et al., 1997).

Buckeridge et al., (1992) analysed the structure of seed storage Xgs from *T. indica*, *C. langsdorffii* and also *H. courbaril* (Buckeridge et al., 1997) and showed that the fine structures of these polymers are composed of 4 structural basic subunits, with the exception of *H. courbaril* that shows a new family of oligosaccharides (XXXXG plus

different degrees of galactosylation in this oligosaccharide). It was also shown by these authors that the proportions among those subunits are combined differently among species, reflecting the differences on the distribution and degree of galactosylation of the molecule. These differences in fine structure might be affecting, at some level, the binding capacity among storage Xgs with cellulose.

Vincken et al., (1995) studied the interaction of cellulose with Xg fragments with different degrees of polymerisation and reported that substitution with galactose probably decreases the ability of fragments to penetrate the smaller pores in cellulose. Similar results were found by Whitney et al., (1998), where they observed that galactomannan and cellulose interaction presented a tendency of increasing as a function of decreasing in the galactosyl residues in galactomannan. Further analyses were performed here to evaluate galactose influence on the Xg binding to cellulose.

3.5. The influence of galactose branching on the binding of xyloglucan to cellulose

HPAEC-PAD analyses showed the patterns of elution profiles of limit digest oligosaccharides from *H. courbaril* (Figure 4A) and *T. indica* Xgs (Figure 4B). Figures 4C and 4D show the variations in the levels of each oligosaccharide after binding to cellulose. The percentages indicate the increasing or decreasing of the bound subunits in relation to native ones.

This fine structural analysis of the xyloglucan that remains bound to cellulose revealed that there might be domains in these storage polysaccharides that are relatively more interactive with cellulose. These regions appear to be richer in XXXG and

XLXG (about 30-50% higher than in native Xg samples) and with a decreasing proportion of XXLG and XLLG (Figures 4C and 4D). These results suggest that the degree of galactosylation might be associated with Xg-cellulose binding. The presence of oligosaccharides *a, b* and *c* from *H. courbaril* Xg (Figure 4A) are also associated with a higher interactivity with cellulose. They are subunits from the XXXXG based polymer (Buckeridge et al., 1997) with a Glc:Xyl:Gal ratio of the 5:4:1 with galactose in different positions (Tiné, Cortelazzo & Buckeridge 1999).

Recently, Santos and Buckeridge (unpublished) found that Xgs from small seeds of *H. courbaril* have lower amounts of galactose than Xg from big seeds. Here, we performed a comparison of the cellulose binding capacity of these two Xg types. The results shown in table 2 indicated that a higher degree of galactosylation of Xg is associated with lower interactivity. Apart from degree of galactosylation, the distribution of galactosyl residues along the main chain (i.e. the fine structure) seems to play a role on binding to cellulose as well. The subunit XXLG was found to be relatively less interactive with cellulose than XLXG for both Xg sources.

The distribution of galactose residues on either side of the backbone of Xg was estimated according to Buckeridge et al. (1992). The data presented in table 3 indicated that the uneven distribution of galactose apparently favours a higher binding capacity. This probably occurs due to the presence of a higher proportion of galactosyl residues at one side of the polysaccharide molecule, which exposes the glucose residues from the other side for interaction with cellulose by hydrogen bonding. On the other hand, a tendency to uniform distribution of galactose would lead to a more twisted

backbone structure, resulting in a lower binding capacity (Levy et al., 1991; Levy et al., 1997).

Dea and Clark (1986) reported that the distribution of galactosyl units along the main chain could have a significant effect on the interactive properties of galactomannans. They showed that galactomannans from different sources with significant differences in the content of galactose (40 and 29%) had similar interaction.

Although the galactosyl distribution index is higher for *C. langsdorffii* (0.92) than for *T. majus* (0.75), their binding capacities were not statistically different. This suggests that at high levels of uniformity (possibly above 0.7) of the galactosyl distribution, no change in the binding capacity occurs. However if this high uniformity was lost, the binding capacity will be increased as well as self-association. Removal of approximately 50% of galactoses results in formation of gel (Reid, Edwards & Dea, 1988; Suisha et al., 1998). This factor could be important for storage cell wall Xg because during seed maturation and storage mobilisation some hydrolytic enzymes (e.g. β-galactosidases) could alter the galactosylation degree of polysaccharides, resulting in alteration of rheological properties of molecules.

Our results showed that the galactosyl residues seem to influence the interaction between Xg and cellulose. However, it is not well understood which factor related to the fine structure could be really affecting the binding capacity, the degree of galactosylation or the distribution of galactosyl residues.

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Table 1. Comparative binding capacity among different sources of xyloglucan with microcrystalline cellulose. Within rows, means followed by different letters are statistically different according to Tuckey ($P<0.05$).

Added Xg (μg/mg cellulose)	Species used			
	H. courbaril	*T. indica*	*C. langsdorffii*	*T. majus*
	Adsorbed Xg (%)			
10	39.3 a	42.1 a	33.0 a	35.8 a
20	32.1 a	27.8 ab	20.7 b	29.8 a
30	29.5 a	27.6 ab	23.5 b	24.9 ab
40	32.2 a	27.9 b	24.0 c	20.1 c
50	35.9 a	29.0 b	31.8 b	23.9 c

Table 2. Comparative binding capacity between Xgs from small and big seeds of *H. courbaril* with microcrystalline cellulose. Their galactosylation degree estimated by the galactose/xylose ratio. The binding assays were performed (4 times) with 20 mg of cellulose and 600 μg in 25mM NaOAc pH 6.0 at 30^{0}C. Within the column, means followed by different letters are statistically different according to Tuckey ($P<0.05$).

Xg sources	Adsorbed Xg (%)	Gal/Xyl ratio
Small seeds	28.8 a	0.22
Big seeds	24.3 b	0.46

Table 3. Comparative binding capacity among Xgs from different sources and their respective galactose distribution index expressed by the ratio between (XLLG+XLXG)/(XLLG+XXLG). Within the column, means followed by different letters are statistically different according to Tuckey ($P<0.05$). The percentages of interactions are averages of the data from table 1.

Species	Galactose distribution index (XLLG+XLXG)/(XLLG+XXLG)	Adsorbed Xg (%)
H. courbaril	0.20	33.8a
T. indica	0.65	30.9b
T. majus	0.75	26.9c
C. langsdorffii	0.92	26.6c

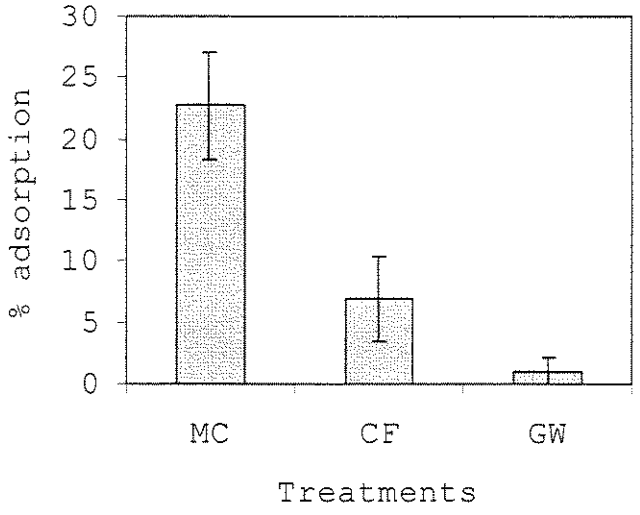

Figure 1. Interaction between *T. indica* Xg with: (MC) microcrystalline cellulose, (CF) cellulose fibres and (GW) glass wool. The binding assays were performed (4 times) with 600 μg of Xg and 20 mg of cellulose at pH 5.0. The bars mean the standard deviation.

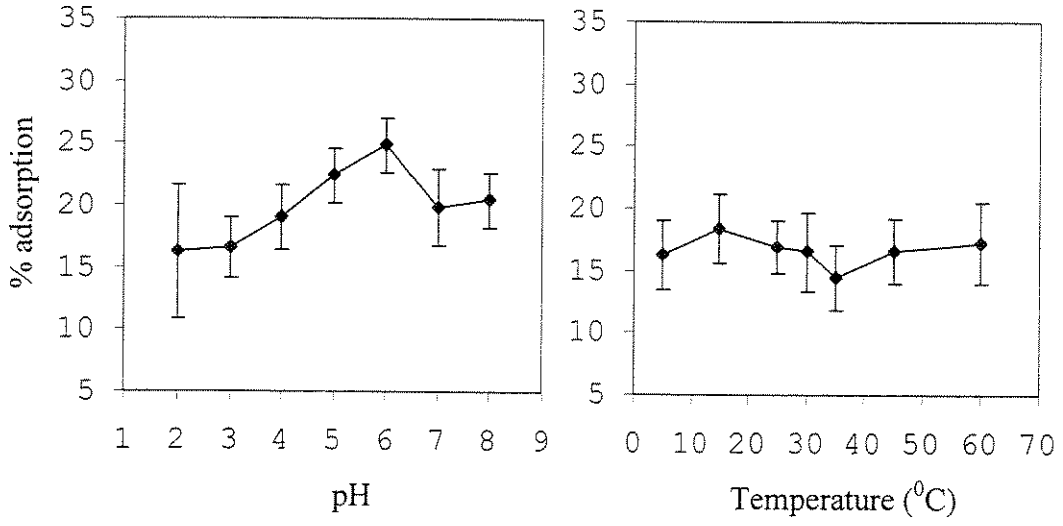

Figure 2. Effects of pH (A)and temperature (B) on binding of *T. indica* xyloglucan to microcrystalline cellulose. The assays were performed (4 times) in 25mM NaOAc, 600 μg Xg and 20 mg of cellulose during 15 minutes. The bars mean the standard deviation.

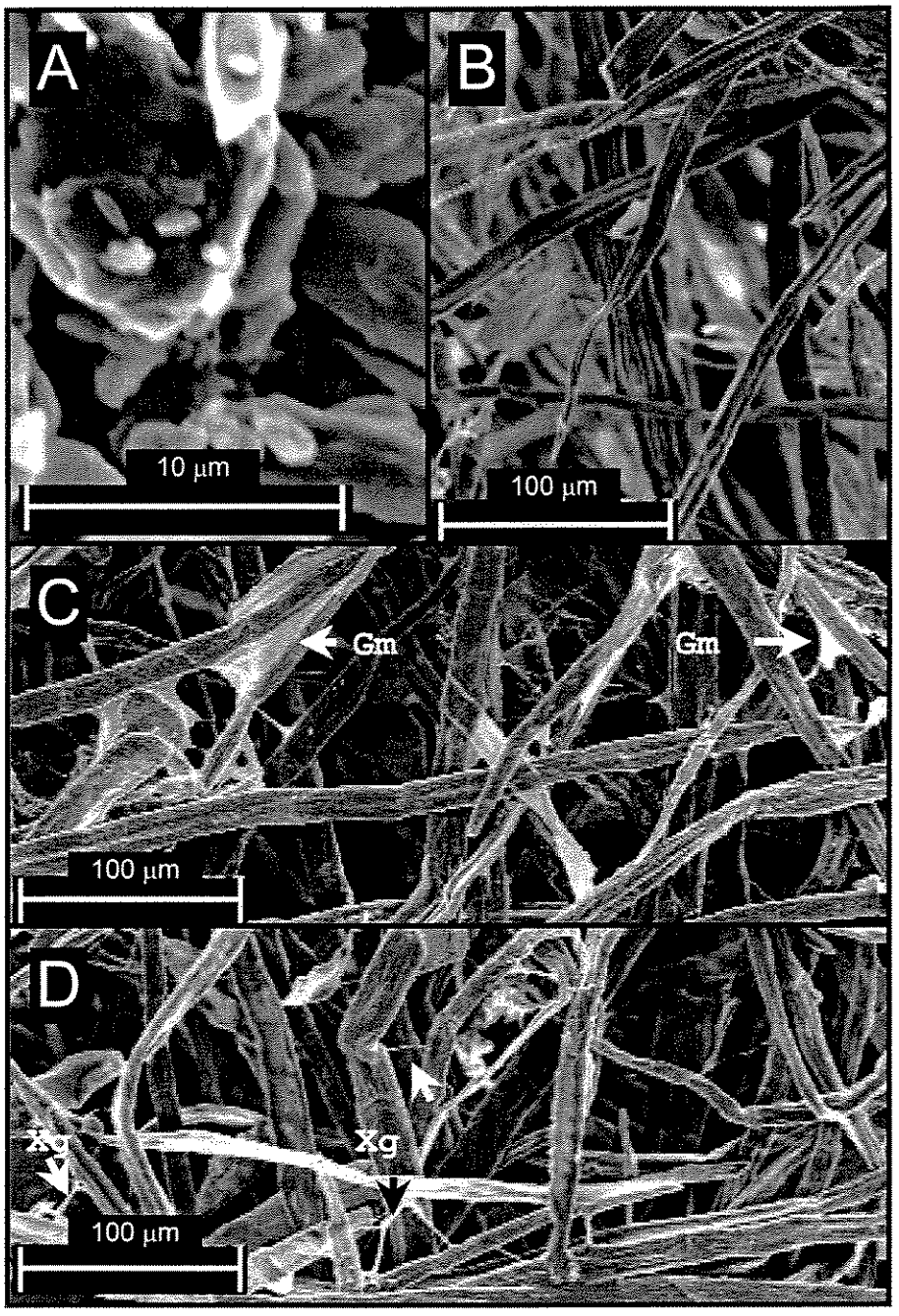

Figure 3. Scanning electron microscopy in microcrystalline cellulose (A); cellulose fibres (B); *Sesbania marginata* galactomannan-cellulose complex (C) and *T indica* xyloglucan-cellulose complex (D). Arrows in (C) show the self-association plates; in (D) they show the "bridges" of Xg among the fibres.

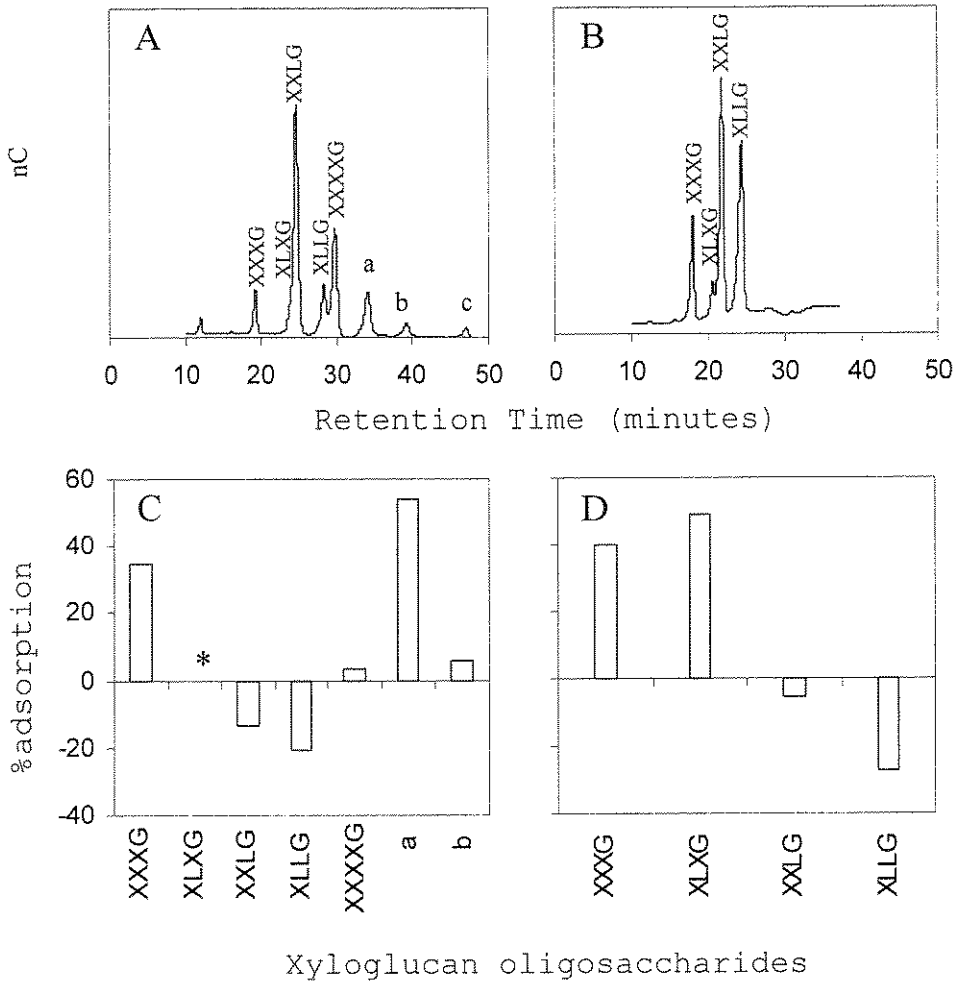

Figure 4. Comparison of the fine structures of native and bound Xgs from *H. courbaril* and *T. indica*. Typical profiles of HPAEC-PAD analysis of *H. courbaril* (A) and *T. indica* Xg (B). (C) and (D) show the variation in the proportions among limit-digest oligosaccharides derived from bound and native Xgs of *H. courbaril* (C) and *T. indica* (D). The peaks *a*, *b* and *c* are subunits with a Glc:Xyl ratio of 5:4. Peak *c* was not detected after interaction to cellulose. (*) The percentage of adsorption of XLXG could not be calculated because it is not detectable in native samples of *H. courbaril* being present only after binding assays.

ARTIGO 2

XYLOGLUCAN-CELLULOSE INTERACTION DEPENDS ON THE SIDECHAINS AND MOLECULAR WEIGHT OF XYLOGLUCAN[1]

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ABSTRACT

Recent papers have brought evidence against the hypothesis that the fucosyl branchings of primary wall xyloglucans (Xg) are responsible for their higher capacity of binding to cellulose. Reinforcement of this questioning has been obtained in this work where we have shown that the binding capacity was improved when the molecular weight (MW) of the Xg polymers was decreased by enzymatic hydrolysis. Moreover, the enthalpy changes associated with the adsorption process between Xg and cellulose, were similar for Xgs with similar MW (but differing in the fine structure such as presence/absence of fucose). On the basis of these results, it can be suggested that the fine structure and MW of Xg will determine the energy and amount of binding to cellulose, respectively. Thus, the occurrence of different domains of fine structure of Xg (eg. the presence of fucose and the distribution of galactoses) might have several different functions in the wall. Besides the structural function in primary wall, these results might have impact on the packing features of storage Xg in cotyledons of seeds, since the MW and absence of fucose could also be associated with the self-association capacity.

KEYWORDS: xyloglucan, cell wall, binding to cellulose, storage, seeds, ITC

INTRODUCTION

Xyloglucans (Xg) are cell wall polysaccharides which have a cellulose-like β-(1,4)-glucan backbone to which single-units of α-(1,6)-D-Xyl*p* substituents are attached. Some xylosyl residues are further substituted at O-2 by β-D-Gal*p* residues and some of the galactosyl residues may be substituted at O-6 by α-D-Fuc*p* (Hayashi & Maclachlan, 1984; Hayashi, 1989). In seeds of many dicotyledons, Xg is found as a secondary cell wall storage polysaccharide (Reid, 1985; Buckeridge et al., 2000), and, in primary walls of growing tissues, Xg is believed to perform a structural role in microfibril orientation and load-bearing network formation (Hayashi, 1989).

These structural functions are due to the linkage between cellulose and Xg by hydrogen bonds, and its binding capacity seems to be affected by the branching residues (Lima & Buckeridge, 2001). Levy et al. (1991), based on computational modelling, suggested that the presence of fucose in primary cell wall Xgs determine a flat conformation to this polymer, being responsible for its capacity to bind to cellulose. Hayashi et al. (1994) performed some binding experiments with pea (fucosylated) and *Tamarindus indica* (non-fucosylated) xyloglucans. Based on their results these authors also suggested that the fucosyl residues contribute to the increase in adsorption affinity and the adsorption constant to cellulose. Lima & Buckeridge (2001) have recently shown by *in vitro* experiments that the degree of galactosylation of storage Xg has a limited effect on the binding capacity to cellulose. The high amounts of galactose branches as well as their distribution along the polymer main chain affected their degree of interaction with cellulose.

Arabidopsis mur1 mutants are defective in the *de novo* synthesis of L-fucose, exhibiting a dwarfed growth habit and decreased wall strength (Reiter et al., 1993). The *mur1* mutation affected several cell wall polysaccharides; therefore, the phenotypes appear to be caused by structural changes in fucosylated pectic components such as rhamnogalacturonan-II, besides xyloglucan polymers (Vanzin et al., 2002).

Vanzin et al. (2002) obtained an *Arabidopsis* mutant (*mur2*) in which xyloglucan fucosylation was eliminated specifically in all major plant organs. Despite this alteration in structure, *mur2* plants show a normal growth habit and wall strength, in contrast with *Arabidopsis mur1* mutants. The normal growth habit and wall strength of *mur2* plants casts doubt on hypotheses regarding the roles of xyloglucan fucosylation in facilitating xyloglucan-cellulose interactions or in modulating growth regulator activity (Vanzin et al., 2002).

In the present report, reinforcement on the hypothesis regarding Xg-cellulose interaction was obtained. We showed that the binding capacity of non-fucosylated Xg was improved to the same level as the fucosylated one as long as they have a similar MW. Moreover, the enthalpy changes from the adsorption processes between Xg and cellulose were similar for Xgs with similar MW, but differed significantly with differences in fine structure such as presence/absence of fucose. Some aspects of our results on biosynthesis and storage packing features are also discussed.

MATERIAL AND METHODS

Polysaccharides

Microcrystalline cellulose powder was obtained from Avicel-SF. Xyloglucan from primary cell wall was obtained from cell suspensions of *Phaseolus vulgaris* (common bean). Cell walls were prepared from 14- and 34-day-old cell suspension cultures according to Braga et al. (1998). Cell walls of suspension cultures were fractionated into pectins with ammonium oxalate buffer and hemicelluloses with 0.5, 1.0 and 4.0 M KOH (Gorshokova et al., 1996).

Storage xyloglucans were obtained from cotyledons of seeds of *Copaifera langsdorffii*, *Tamarindus indica* and three different developmental stages of immature *Hymenaea courbaril* seeds. The polysaccharides were extracted with water (1% w/v) at 80^0C for 8h under constant stirring. After filtration, 3 volumes of ethanol were added to the aqueous extracts, kept overnight at 5^0C and centrifuged (12,000 g for 15min at 5^0C). The pellet was partially dried at room temperature and, after ressuspension in water, freeze-dried.

Production of Xg fragments with various MWs

Partial enzymatic hydrolysis followed by gel filtration chromatography was used to produce Xg fragments with 25, 80, 150, 590 and 1200kDa. *Copaifera langsdorffii* Xg was treated with endoglucanase (cellulase from *Trichoderma viride* - Megazyme) during 5, 10, 15 and 30 minutes of incubation at 30^0C. Subsequently, the samples were submitted to gel filtration in Sepharose 6B and eluted with 50mM McIlvaine buffer pH 5.2. A

standard curve was performed based on the hydrolysis products to obtain a relationship between MW and incubation time.

HPAEC analysis

Aliquots of hemicellulosic fractions from bean cell wall and storage xyloglucans were extensively hydrolysed with cellulase (Megazyme) in 0.1 M sodium acetate buffer pH 5.5 for 24 h at 30°C. Xyloglucan oligosaccharides were analysed by HPAEC-PAD in a Carbo-Pac PA-100 column using a gradient of sodium acetate (35 to 70 mM) in 88 mM NaOH with flux of 0.9 $ml.min^{-1}$.

Binding assays

The assays were performed in 25mM sodium acetate buffer pH 6.0 at 30°C during 15 minutes of incubation (Lima & Buckeridge, 2001). Thirty μg of Xg were added per mg of microcrystalline cellulose (previously washed 5 times with distilled water). After incubation the samples were centrifuged (a pulse of 13,000g) and the amount of unbound Xg present in the supernatant was quantified by the phenol-sulphuric acid method (Dubois et al., 1956). The proportion of adsorbed Xg was calculated from the difference between the amounts of Xg in the supernatant before and after interaction.

Isothermal Titration Calorimetry (ITC)

Samples of bean (fucosylated) Xg and *Tamarindus indica* Xg (non-fucosylated) were submitted to interaction with cellulose and the enthalpy changes involved in adsorption processes were recorded.

In this experiment the heat (energy) exchanged during adsorption process was measured and compared among the different samples. Solutions of xyloglucans solubilised in water (8mg.mL^{-1}) were added into the ITC cell (maximum capacity of 4.0 mL) containing a cellulose suspension (~0.2g diluted in 2mL of distilled water) by 9x30 μL injections at 60min intervals under constant stirring (110rpm). A control experiment was performed under identical conditions by injecting Xg into the ITC cell on distilled water alone. Heats of adsorption were calculated by subtracting the correspondent heats of dilution (from the control experiment). Enthalpy changes associated with the interaction were calculated based on the amount of Xg adsorbed to cellulose (kJ.g^{-1}). This experiment was performed at 25^{0}C using TAM 2277 (Thermal Activity Monitor) microcalorimeter.

RESULTS

Production of Xg fragments

Although alcoholic precipitation could be used to produce fragments with very low MWs (from 1.3 to 5.1kDa) (Silva, C.O. personal communication), these small polymers do not interact with cellulose. Thus, we decided to perform controlled enzymatic hydrolysis with cellulase, which was an efficient tool to obtain Xg with wide range of MW. Fragments of *C. langsdorffii* Xg with 25, 80, 150, 590 and 1200kDa and fragments of *T. indica* Xg with 240kDa were obtained based on the standard curve shown in Figure 1. The use of Xg from different sources is not expected to affect the results because their fine structure is similar (Buckeridge et al., 1992). The partial hydrolysis method is

therefore useful to produce molecular weight markers to gel filtration chromatography, achieving a range of MW broader than that normally provided by commercial markers (dextrans).

The fine structure of polysaccharides is shown in Table 1 and the proportions of the structural blocks (oligosaccharides) can be seen. Besides the presence of fucosylated oligosaccharide (XXFG) only in primary wall bean Xg, it presented a polydispersion higher than storage polysaccharides (data not shown).

Comparison of the capacity of binding

Fucosylated bean Xg and various fragments of non-fucosylated *Copaifera* Xg were submitted to interaction with cellulose under the same assay conditions. The results in Figure 2 shows that the lower the MW the higher was the capacity of binding (%) to cellulose. The fucosylated bean Xg presented a MW around 200kDa and its adsorption was similar to that of non-fucosylated storage Xg fraction with 150kDa. Yet, the Xg fragments with MW lower than 150kDa showed higher interaction with cellulose than the fucosylated ones. These results are not in agreement with those obtained by Hayashi et al. (1984), who have suggested that the fucosyl residues contribute to the increase in adsorption affinity and adsorption constant to cellulose with the increase in MW.

ITC results

The enthalpy related to the adsorption processes was measured in an experiment where Xg solutions were added to a cellulose suspension. This enthalpy represents the balance of energy among all processes involved, such as dilution of polymers during injections, H-bond formation and dissociation between H_2O and saccharides, H-bond formation between Xg and cellulose, H-bond formation among Xg molecules themselves and conformational changes of polymers.

The ITC results are shown in Figure 3. The initial values of enthalpy for *T. indica* Xg (intact and 240kDa) were quite similar at low Xg concentration, whereas bean Xg data started at more negative values. The heat loss for bean Xg diminished with increase of Xg concentration while the heat increased for 240kDa-Xg with Xg addition. From 0.6 $g.L^{-1}$ of Xg added the low MW polymers (240kDa and bean Xg) reached the plateau at negative values, whereas intact *T. indica* Xg kept on the initial value (near zero). The balance of energy during injection of low MW Xg on cellulose resulted in a predominance of exothermic processes. It means that there is more energy involved in adsorption processes between low MW Xg and cellulose than with high MW ones, revealing that enthalpy contribution to binding is more favourable for the former. As these data were calculated on the basis of the amount of Xg adsorbed to cellulose, we can not affirm that the differences in observed energy were due to the different capacity of binding to cellulose. The more negative values observed at lower concentrations for bean Xg sample suggest that it binds firstly to more energetic sites of cellulose, releasing more energy. However, the pattern of response for 240KDa Xg was not so

simple, since the initial value for adsorption of 240KDa Xg was positive. This means that energy is initially absorbed during adsorption, being subsequently released (exothermic process) in values that increase with the increase in surface coverage. One possible explanation for the increased enthalpy of binding with Xg addition may be a cooperative binding, when interactions with other unfucosylated Xg molecules already adsorbed onto cellulose surface turn the interaction into more enthalpically favourable. In other words, processes of self-association could be happening as Xg was added.

Interaction between xyloglucans and cellulose in water should involve the rupture and formation of a large number of H-bonds. For such complex processes, a negative enthalpy change (exothermic process) represents an energy release due to the formation of higher number of H-bonds or more energetic ones, as is the case for low MW Xgs in comparison with intact *T. indica* Xg. For their macroscopic nature, calorimetric measurements alone are not capable of differentiating between the two hypotheses.

Molecular weight changes during xyloglucan deposition in developing legume seeds

In order to understand some aspects of the assembly of the cell wall storage non-fucosylated xyloglucan in legume seeds, we collected seeds of *Hymenaea courbaril* during fruit development at three different stages of xyloglucan deposition. Figure 4 shows the results of gel filtration chromatography of Xg extracted with hot water from cotyledons during deposition. The MW of storage Xg increased gradually from about 150kDa (initial stage – Jul03) to >2000kDa (final stage – Sept06) as the amount of Xg

varied from 3 to 40% (w/w) in cotyledons, respectively (data not shown). It is noteworthy that the process of assembly of the wall increased rather slowly within an interval of three months, suggesting that either Xg chain elongation is very slow or that Xgs are synthesised as relatively low MW molecules and further assembled as much larger complexes (>2000kDa) in the storage wall.

DISCUSSION

Put together the results of Xg-cellulose binding assays (Figure 2) and the ITC (Figure 3), the data suggest that the molecular weight of Xg has a marked effect on capacity of binding to cellulose and the branching with fucose of primary wall Xg seems not to be a key factor in this capacity, corroborating the data of Vanzin et al. (2002).

However, the *energy* (enthalpy) of the binding to cellulose could be altered by the degree of fucosylation whereas the *capacity* of binding (amount of Xg adsorbed) seems to be related to MW. Therefore, depending on the cell metabolism or the specific region in the wall where xyloglucan is located, the degree of Xg fucosylation may be altered to strengthen the cell wall structure. This hypothesis has been confirmed by recent data from Tiné (2002) who found that the storage walls of cotyledons of *Hymenaea courbaril* are in fact enveloped between two primary walls containing fucosylated xyloglucan.

The fact that small Xg fragments have a higher capacity of binding to cellulose than high MW polymers might have an impact on aspects of the biosynthesis mechanism, mainly of storage Xgs. It has been extensively shown (Buckeridge et al.,

1992, Franco et al. 1996, Buckeridge et al., 2000, Lima & Buckeridge, 2001) that storage Xgs are non-fucosylated and have a high molecular weight (above 2000kDa). It has been observed that in seeds from species that accumulate Xg it is stored in very high proportions in thick storage walls (ca. 40% of the dry mass of cotyledons for *Hymenaea courbaril* seeds for example) (Tiné et al., 2000). Considering the fact that these walls have no or very low proportions of cellulose, a question rises on how can such walls be assembled. The answer is probably related to the fact that packing has to be highly efficient, involving self-association among the polymers, but at the same time the packing can not be too tight, because it might otherwise prevent the process of polysaccharide mobilisation.

If the self-association capacity is low for high MW Xg, a high packing efficiency would not be expected. Therefore, the biosynthesis of storage Xg should be performed firstly by small fragments inside the Golgi vesicles, being subsequently deposited in the extracellular matrix. In this context, the absence of fucose in storage Xg might be associated with an improvement of self-association, since the fucosylated Xg seems not to self interact (Figure 3). As the binding capacity of low MW Xg fragments is higher, they could be associated to each other (adsorption) and/or linked by transglycosylation processes (Nishitani, 1998).

Data on the Figure 4 show the increase of MW of Xg extracted from cotyledons of *Hymenaea courbaril* seeds during storage polysaccharide deposition. It was observed that the MW increased from about 150kDa to >2000kDa and the amount of Xg varied from 3 to 40% (w/w) in cotyledons, respectively (data not shown). In conjunction with

the observation by Santos & Buckeridge (unpublished data) of synthesis of small fragments of storage Xg (4 structural blocks ~4.5kDa) during storage Xg mobilisation in cotyledons of *H. courbaril*, our results support the hypothesis that the Xg might be synthesised as low MW molecules, which are latter assembled *in muro*. How it happens remains to be investigated.

Thus, our findings that 1) amount of binding of Xg to cellulose increases with lower MW of the former and 2) storage Xg appears to be produced firstly as low MW molecules which are assembled as bigger complexes at the end of polysaccharide deposition, might reflect the existence of self-association between Xg molecules as a way to maintain storage wall integrity, but still maintaining a higher solubility and availability for enzyme attack during storage mobilisation.

Altogether, our results suggest that the fine structure and MW of different xyloglucans will determine the energy and amount of adsorption, respectively. Thus, the presence of domains given by the occurrence of different fine structures of xyloglucans (eg. the presence of fucose and the distribution of galactoses) highlights the importance of these molecular features for the structure-function relationships of xyloglucans in the cell walls of plants.

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Table 1. Proportions of peak area from chromatogram analysis of limited digest oligosaccharides based on XXXG block. The oligosacharides were obtained by enzymatic hydrolysis (cellulase – Megazyme) and submitted to HPAEC.

Oligosaccharides	Xyloglucans source		
	P. vulgaris (proportions)	*T. indica* (proportions)	*C. langsdorffii* (proportions)
XXXG	1.00	1.00	1.00
XLXG	0.45	0.30	0.80
XXLG	0	1.50	2.00
XLLG	0.21	1.30	4.00
XXFG	0.28	0	0

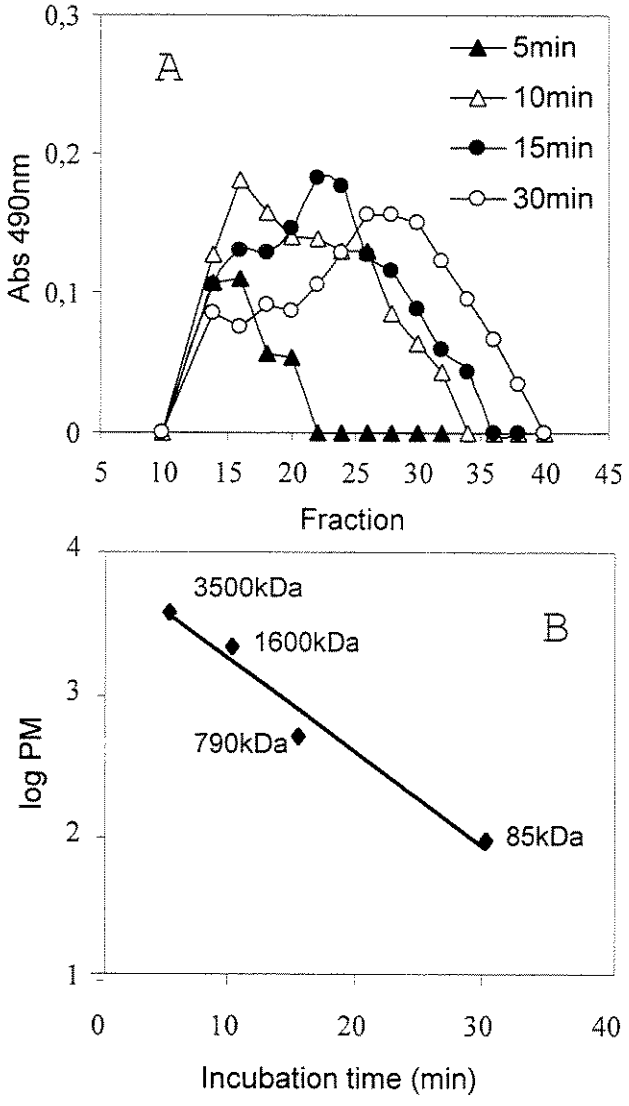

Figure 1. Production of Xg fragments by partial enzymatic hydrolysis **A**) *Copaifera langsdorffii* Xg submitted to gel filtration (Sepharose 6B) after an enzymatic hydrolysis (*Trichoderma viride* cellulase) for 5, 10, 15 and 30 minutes of incubation at 30^0C. **B**) Correlation between MW and incubation time of hydrolysis, r^2=0.967.

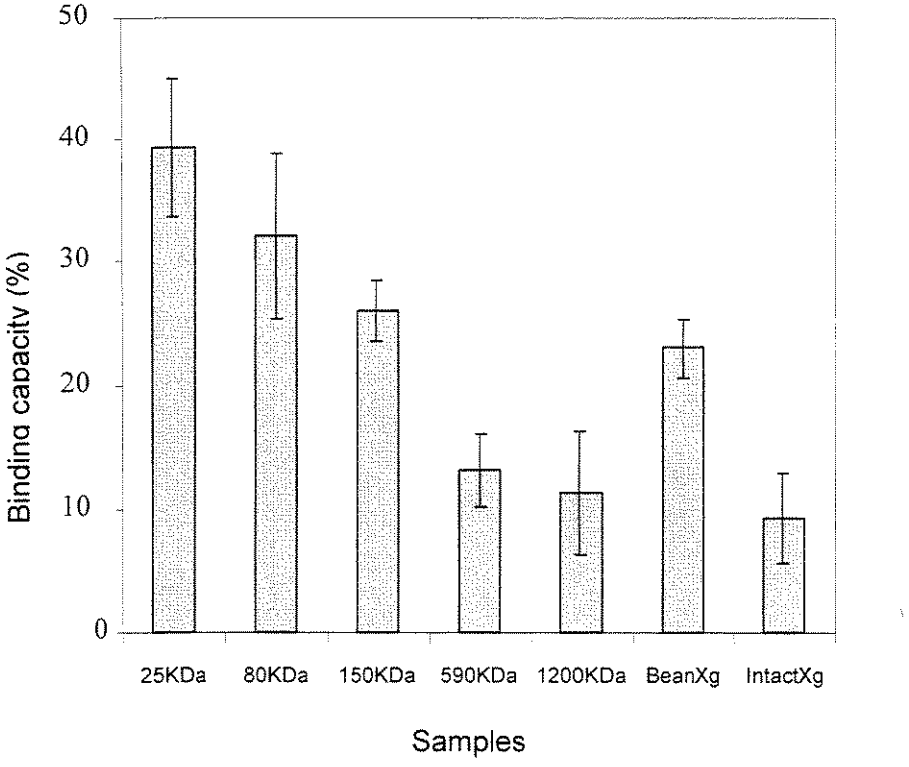

Figure 2. Relationship between molecular weight of Xg and the capacity of binding (%) to cellulose. *Copaifera langsdorffii* Xg fragments were obtained by enzymatic hydrolysis (*Trichoderma viride* cellulase) and gel filtration (Sepharose 6B). Intact Xg showed MW>2000kDa and bean Xg is a fucosylated primary wall Xg from cell suspension. Bars are standard deviation of 3 replicates.

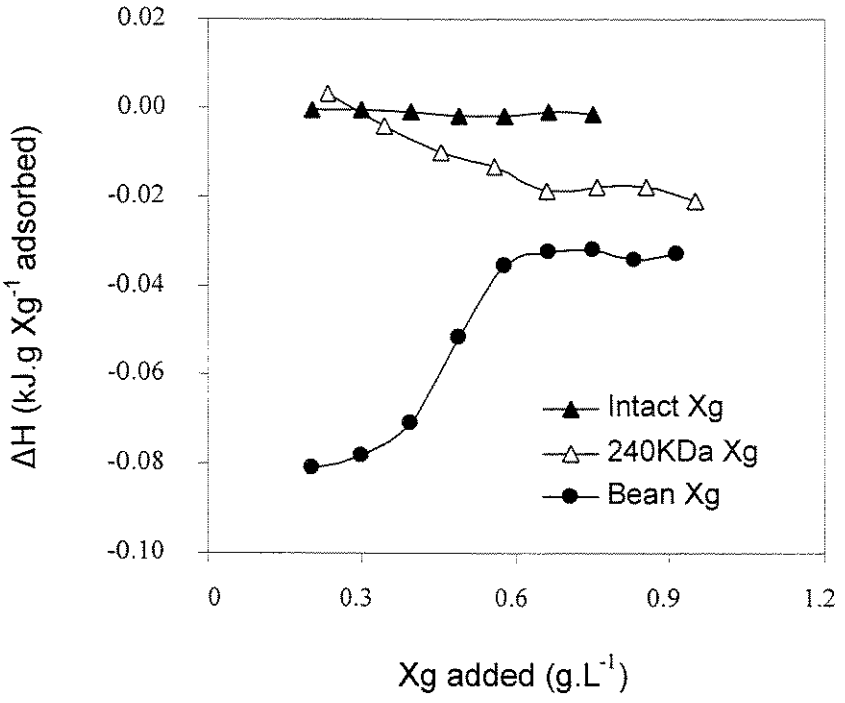

Figure 3. Isothermal calorimetry experiment involving adsorption reactions between Xg and cellulose. Solutions of Intact tamarind Xg (>2000kDa), 240kDa Xg (fragment of tamarind Xg) and bean Xg (~200kDa fucosylated Xg) were solubilised in water (8mg.mL^{-1}) and added into cellulose suspension under constant stirring. Control sample was performed by injection of Xg into the ITC cell with water alone.

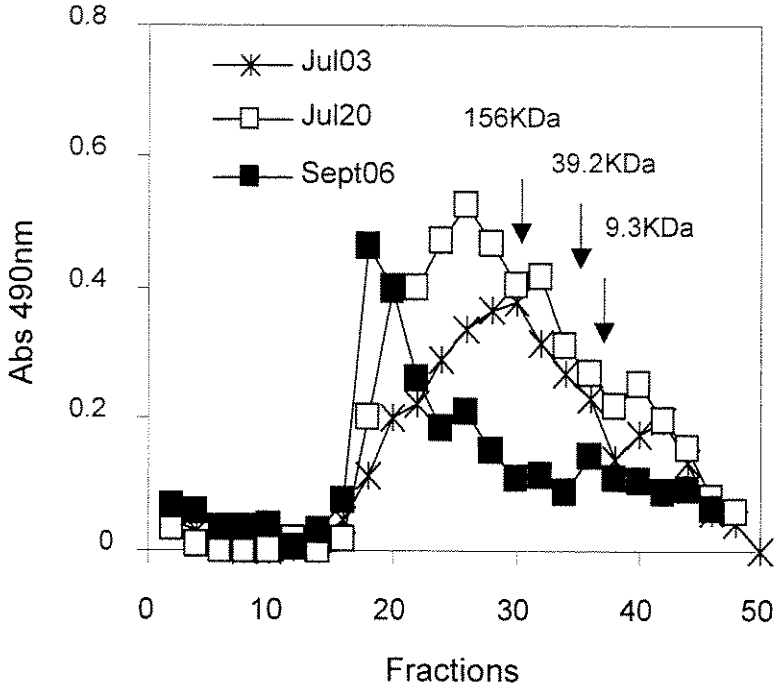

Figure 4. Gel filtration chromatography (Sepharose 6B) of Xg samples extracted from cotyledons of seeds of *Hymenaea courbaril* during storage Xg deposition and fruit development. The fruits were collected in Jul03 (initial stage of deposition), Jul20 and Sept06 (final stage). The MW marker dextrans were used for Sepharose 6B calibration.

ANEXO DO ARTIGO 2

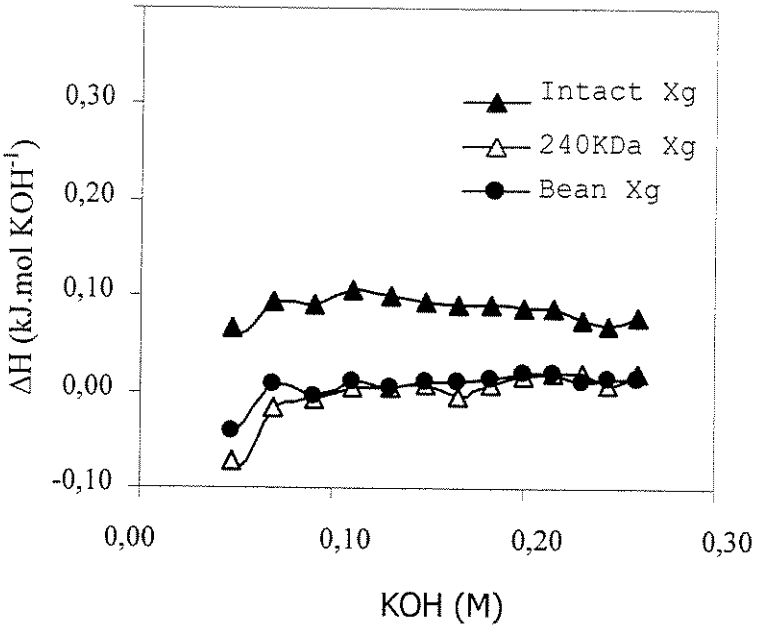

Figure. Isothermal calorimetry experiment involving dissociation reactions of the complex Xg-cellulose. 1M KOH was added to the ITC cell on the Xg-cellulose complex diluted in 2mL of water under constant stirring. The complexes were previously performed using Intact tamarind Xg (>2000kDa), 240kDa Xg (fragment of tamarind Xg) and Bean Xg (~200kDa fucosylated Xg). Control sample was performed under identical conditions by injection of KOH into the ITC cell with cellulose suspension alone.

RESULTADO OBTIDO MAS QUE NÃO FARÁ PARTE DO ARTIGO

Este experimento foi realizado com o objetivo de se medir a energia de dissociação entre Xg e celulose, através da adição de KOH sobre o complexo formado entre estes dois polissacarídeos, sendo comparados com os dados obtidos da adição de KOH sobre suspensão de celulose (amostra controle).

Devem-se levar em conta algumas observações importantes no que concerne esta análise: 1) A adição de KOH sobre o complexo Xg-celulose envolve a ruptura das pontes de H entre os polissacarídeos além de romper as pontes de H internas da celulose, degradar os finais redutores dos polissacarídeos e formar novas pontes com as hidroxilas livres dos polímeros; 2) a amostra controle que envolve a adição de KOH sobre uma suspensão de celulose, não é suficiente para anular todos os processos envolvidos nas amostras com o complexo Xg-celulose, limitando os resultados obtidos.

Portanto, serão apenas destacados alguns pontos na comparação entre as amostras que, ainda assim, corroboram as hipóteses levantadas no artigo:

- As amostras de Xg (fucosilados ou não) com pesos moleculares similares (~200KDa) responderam de forma quase idêntica à adição de KOH
- Os valores de entalpia mais positivos (processo endotérmico) para as amostras de Xg intacto (>2000KDa) poderiam estar relacionados com uma maior ruptura de pontes de H da celulose, considerando que tenha havido uma menor área de cobertura deste polímero pelo Xg, permitindo um ataque mais intenso do KOH. Obviamente existe a possibilidade da ligação entre Xg intacto e celulose ter sido mais forte, resultando em maior entalpia para dissociação, no entanto, a figura 3 apresentada no artigo mostrou que a energia de adsorção para Xg intacto foi menor.

ARTIGO 3

SEED STORAGE HEMICELLULOSES AS WET-END ADDITIVES IN PAPERMAKING[1]

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ABSTRACT

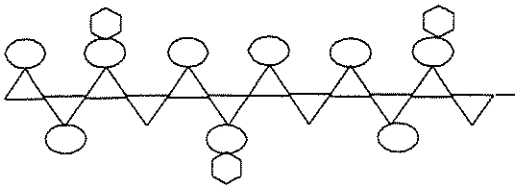
Xyloglucans and galactomannans are examples of hemicelluloses that can be accumulated in seeds of many plants, being extensively studied and used for industrial applications. Guar gum and starch are polysaccharides currently used as wet-end additives in papermaking, whereas xyloglucans have never been documented as improved in paper quality. In this work we show that different types of xyloglucans improved the mechanical properties of paper sheets without affecting the optical ones. Addition of 1% (w/w) of hemicelluloses on cellulosic pulp was able to increase in about 30% the mechanical properties such as burst and tear indexes. Seeds of several species could be used as source for the production of wet-end additives, since the results did not vary with the source of polysaccharides. Even if the utilisation of these hemicelluloses will not cost less than starch or guar gum, it might represent an important strategy for sustainable use of rainforest species.

KEYWORDS: hemicellulose, xyloglucan, galactomannan, papermaking, wet-end additives, industrial application, seed.

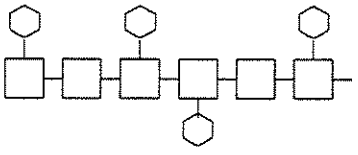
INTRODUCTION

Hemicelluloses are plant cell wall polysaccharides closely associated to cellulose. Unlike the former, the cellulose is formed only by β-(1,4) glucosyl linkages in a linear backbone whereas hemicelluloses are branched polymers composed of several monosaccharides, which confer to this class of cell wall polysaccharides a higher level of complexity. Xyloglucans and galactomannans are the principal hemicelluloses found in the primary cell wall of dicotyledonous plants, where their interaction with cellulose plays a key role in the properties of the wall. In secondary walls of seeds from several species these hemicelluloses serve as storage polysaccharides. (Buckeridge et al., 2000a).

Xyloglucan has a cellulose-like glucan backbone to which units of xylose and xylosyl-galactose disaccharides are attached to the main chain as seen below.



XYLOGLUCAN



GALACTOMANNAN

△ glucose

○ xylose

⬡ galactose

□ mannose

Xyloglucans with different structural features are known to interact specifically with cellulose (Vincken et al., 1995; Lima & Buckeridge, 2001). The basis for such interaction is thought to be the similarity of the backbone of xyloglucans to cellulose (both β-1,4 linked glucans). This binding capacity seems to be altered by the degree of galactosylation and/or by the distribution of galactosyl residues along the main chain (Lima & Buckeridge, 2001).

Galactomannans (schematically shown above) are composed of a linear backbone of β-(1→4) linked mannose, branched with single units of galactosyl residues. This polysaccharide has a wide occurrence as a storage compound in seeds of leguminous species (Dea & Morrison, 1975; Buckeridge et al., 2000b).

The accumulation of hemicellulose in seeds can reach ca. 40% of seed dry mass (xyloglucan in cotyledons of *Hymenaea courbaril* and galactomannan in endosperms of *Dimorphandra mollis*, for example) (Buckeridge et al., 2000a). These hemicelluloses differ strongly from polysaccharides extracted from wood pulps. In softwood hemicelluloses, glucomannans and galactoglucomannans are present in large proportions, whereas glucuronoxylans dominate in the hardwood hemicelluloses (El-Ashmawy et al., 1973; Sjostrom, 1981).

It has been demonstrated that the presence of soft or hardwood hemicelluloses in the cellulosic pulp can improve some features of papermaking. The time and energy utilised to achieve a required fibrillation level can be diminished during the refining process in the presence of hemicellulose. The plasticity and the high superficial area conferred by hemicelluloses result in an increased binding among the fibres and a

higher tensile strength in the paper sheet. However, high amounts of hemicelluloses seem to be deleterious to the mechanical properties of the paper due to a decrease in the individual fibre resistance and to the optical properties due to the low opacity in the paper sheet (Senai/IPT, 1988).

The information cited above were obtained from works that analysed only wood hemicelluloses based on xylan and mannan groups. Other wet-end additives, such as guar gum (galactomannan) and starch, are often used on account of their adsorption behaviour. These additives improve the mechanical properties of paper by regulating the state of flocculation in the cellulosic fibre suspension during the sheet-forming process.

The effects of guar gum, glucomannan and starch derivatives on the paper properties are well documented (Abson & Brooks, 1985; Blumenthal & Paul, 1994; Sundberg et al., 2000). However, the utilisation of xyloglucans as wet-end additives is firstly shown in this work. The data obtained here showed that the use of storage hemicelluloses (such as xyloglucan and galactomannan) might improve some of the mechanical properties of paper sheet without altering the optical ones.

MATERIAL AND METHODS

Plant Material

Hemicelluloses: Storage xyloglucans were obtained from seeds of *Hymenaea courbaril* L. (jatoba), *Copaifera langsdorffii* Desf.(copaiba), *Tamarindus indica* L.(tamarind) and *Tropaeolum majus* L. (nasturtium). Galactomannan was obtained from seeds of

Dimorphandra mollis. The polysaccharides were extracted from cotyledon powders (or endosperm for galactomannan) with water (1% w/v) at 80^0C for 8h with constant stirring. After filtration, 3 volumes of ethanol were added to the aqueous extracts, kept overnight at 5^0C and centrifuged (12,000 g for 15 min at 5^0C). The pellet was partially dried at room temperature and, after ressuspension in water, freeze-dried.

Methods

Sheet formation: Five sheets were formed for each tested treatment according to ABTCP norms (Brazilian Technical Association of Pulp and Paper). These sheets were made to have a grammage of ~60g/m^2 and 0.110mm thick. The pulp was obtained from wood of *Eucalyptus* sp by kraft process being subsequently delignified and bleached. The refining process was performed to achieve ~30^0SR. Aqueous solutions of hemicelluloses (xyloglucans and galactomannan) were added to the pulp fibre during homogenisation before sheet formation.

Properties analysed: Burst Index- BI (ABTCP P8/1994), Tear Index - TI (ABTCP P9/1994), Porosity (ABTCP P11/1994), Capillarity (SCAN-P 13:64) and Water Retention Value – WRV (LCP 01 pp-96), Brightness (ABTCP P16/1994) and Opacity (ABTCP P18/1994). Tensile Index, Tensile Energy Absorption – TEA and Specific Elastic Modulus – SEM, were performed automatically (Instron Corporation-Series IX system 1.09).

The samples were kept and analysed under controlled environment (temperature of 23±1^{0}C and RH of 50±1%) according to ABTCP (P4/1994).

In order to determine the optimal concentration and time of homogenisation for testing all the hemicelluloses used in this work, xyloglucan from seeds of *T. indica* was used for preliminary assays.

Variations tested: 1) the amount of hemicellulose added (0-10% w/w – hemicellulose/pulp fibre); 2) the time of homogenisation; 3) refining or non-refining of pulp fibres; 4) the addition of hemicellulose before and after the refining process and 5) the effects of different hemicelluloses added.

RESULTS AND DISCUSSION

Optimisation of assays

Aqueous solutions of xyloglucan were added to the cellulosic pulp at 0, 0.25, 0.50, 1.0, 2.5, 5.0 and 10% (w/w – xyloglucan/pulp fibre) (Figure 1). The additions were performed during the homogenisation process of the pulp just before sheet formation. Excluding the tensile (Figure 1B) and tear (Figure 1C) indexes analyses, the further mechanical parameters reached the maximum improvement at 1% of added xyloglucan. The tensile and tear indexes were maximal at 5% of added xyloglucan (Figures 1B, 1C).

The time necessary for complete homogenisation of hemicelluloses and pulp fibres was also tested. There was no significant difference in the mechanical properties when the solutions were kept under homogenisation either for 1 or for 18h (Figure 2). This suggests that the hydrogen bonding seems to occur immediately after the mixture

of polysaccharides, as already shown by Rojas & Neuman (1999) and Lima & Buckeridge (2001).

The optical properties analysed (brightness and opacity) were not affected by the presence of xyloglucan or galactomannan, even when the mixtures were made with high hemicellulose concentrations (10%, data not shown).

Hence, the subsequent analyses were performed at a final hemicellulose concentration of 1% (w/w) with 20min of homogenisation.

Effects of hemicelluloses in a non-refined pulp

The addition of xyloglucan or galactomannan to the non-refined pulp fibres did not affect the mechanical properties (Figure 3) in comparison to refined pulp without wet-end additives. The addition of galactomannan from *D. mollis* promoted a slight improvement which was irrelevant compared to the control of refined pulp. The values obtained by the treatments of non-refined pulp were about 80% lower than the refined one, even when the hemicelluloses were added during the sheet formation. The refining of pulp fibres is a mechanical process (beating) that increases the number of bond-forming sites on the fibre surface as well as the number of interfibre bonds due to the higher superficial area produced. The absence of effect of hemicelluloses on non-refined pulp could be explained by the lower capacity of binding to those cellulose fibres, resulting in paper sheets with lower values of mechanical properties.

Figure 4 shows the effect on the mechanical properties of the addition of xyloglucan before and after the refining process. It was observed that the addition of

hemicellulose after beating, specifically during the homogenisation of the pulp, had better effect on the properties analysed, excepted for TEA and SEModulus. The data obtained from the pulp refined in the presence of xyloglucan showed an intermediate level (Figure 4). It has already observed that the presence of xylan-based hemicellulose (from wood) during beating could also improve some properties of the resulting pulp (Senai/IPT, 1988).

The results showed that the addition of wet-end additives, such as xyloglucans or galactomannans, achieve a better response when they are added after refining, possibly due to a higher H-bonding formation between cellulose and hemicellulose.

Comparison of effects of different hemicelluloses

Xyloglucans from seeds of *H. courbaril*, *T. majus*, *T. indica* and *C. langsdorffii*, and galactomannan from *D. mollis* were added to the cellulosic pulp during paper sheet formation. Their mechanical and optical properties were analysed and compared. Xyloglucans from different sources have different fine structural features such as degree of galactosylation and galactose distribution along the polymer main chain (Buckeridge et al. 2000a). The galactomannan from seeds of *D. mollis* presents a mannose:galactose ratio of 2.1 (Panegassi et al., 2000), similar to guar gum (*Cyamopsis tetragonolobus* galactomannan), which is commonly used as wet-end additive to papermaking.

Veluraja et al. (1998) showed that tamarind xyloglucan could be used as a good adhesive in a composite with cellulosic rich sisal fibres. These authors used a high concentration of xyloglucan (2g of fibre per 200mL of 3% xyloglucan), therefore, their

data could neither be compared to the ones obtained in this work nor to be used at industrial level.

Figure 5 shows that all hemicelluloses tested had some effect on the mechanical properties of the paper sheets. Optical features were not affected by the presence of the wet-end additives studied (data not shown). With the exception of porosity (Figure 5E), capillarity (Figure 6A) and water retention value (WRV) (Figure 6B) the other properties analysed increased as a consequence of the addition of hemicelluloses. For example, it can be highlighted that the burst and tear indexes are probably related to the bonding strength among the fibres. In spite of the fact that hemicelluloses have a high capacity to retain water due to their hydrodynamic properties (Buckeridge et al., 2000a; Buckeridge et al., 2000b) the amount of hemicelluloses added to the paper sheet was not enough to change the WRV (Figure 6B).

On the basis of the results described above, the role of hemicelluloses as wet-end additives might be the improvement of mechanical properties rather than increasing the water adsorption capacity. Similar data were obtained in an experiment where deacetylated glucomannan was added to fibre suspension, resulting in an increasing of the strength properties of the paper sheets (Sundberg et al., 2000).

Among the hemicelluloses tested, *T. majus* xyloglucan seems to have the lowest effect on porosity, burst and tear indexes (Figures 5A, 5C, 5E) and galactomannan effects were similar to other xyloglucan.

Table 1 summarises the main results obtained in this work. The burst and tear index had the highest improvement after addition of hemicelluloses (28 and 30%

respectively). As these properties are highly affected by the bonding strength among the fibres, our observations indicate that bonding strength was strongly affected by xyloglucans and galactomannan. Besides higher adhesion among the fibres, the presence of hemicelluloses also helps in the retention of fines as observed by the increase (3%) in the Apparent Specific Weight (ASW – kg/m^3).

Analysis related to tensile strength (Table 1) as tensile index, tensile energy absorption and specific elastic modulus showed lower improvement (11, 19 and 3% respectively) compared to the control samples.

In general, the improvement observed on the mechanical properties of paper sheets shows that the gums analysed in this work have some technical features as wet-end additive and possibly they might be used in papermaking, including the xyloglucans that have been poorly cited as wet-end additive in the literature. Different types of hemicellulose (from rice straw pulp and hardwood pulp) were tested by Mobarak et al. (1973). These authors showed that hemicelluloses added as an additive were more effective than hemicelluloses in situ (in the pulp) as a strength promoter. Moreover, it has been demonstrated that raising the hemicellulose content by addition was more effective on paper strength promotion than raising the hemicellulose content in the pulp through adjusting pulping conditions (Mobarak et al., 1973).

As the effects among the different hemicelluloses used in this work did not vary greatly, neither affected the optical properties, these polymers could be extracted from various sources and used as a pooled sample, improving the yielding of the hemicellulose production for industrial use.

Seeds of several species from tropical rainforest as those studied here could be used as source for production of wet-end additives. Even if the utilisation of these products will not cost less than starch or guar gum, it might represent an important strategy for sustainable use of Brazilian rainforest species.

ACKNOWLEDGEMENTS

We wish to thank FAPESP for financial support, MSc Liliana Martim for technical assistance, Dr Marco A.S. Tiné, Dr Sonia M.C. Dietrich, Dr Watson Loh and Dr Claudia Haddad for critical reading.

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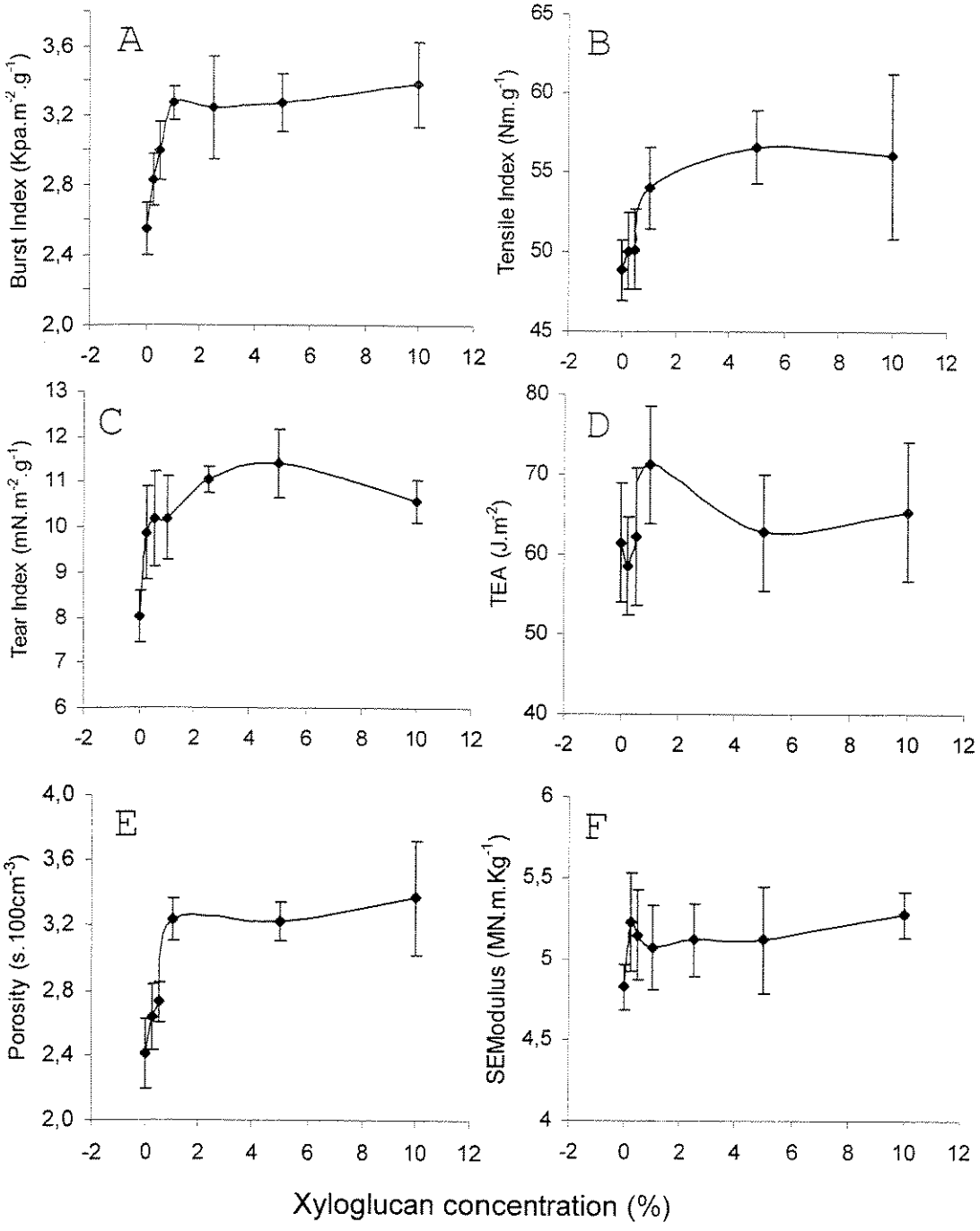

Figure 1. Optimisation of the concentration of hemicellulose added to cellulosic pulp. The assays were performed with *Tamarindus indica* xyloglucan at 0, 0.25, 0.50, 1.0, 5.0 and 10% (w/w) final concentrations. Methods and analysis were performed according to ABTCP norms. TEA – Tensile Energy Absorption; SEModulus – Specific Elastic Modulus. Bars represent the standard deviation.

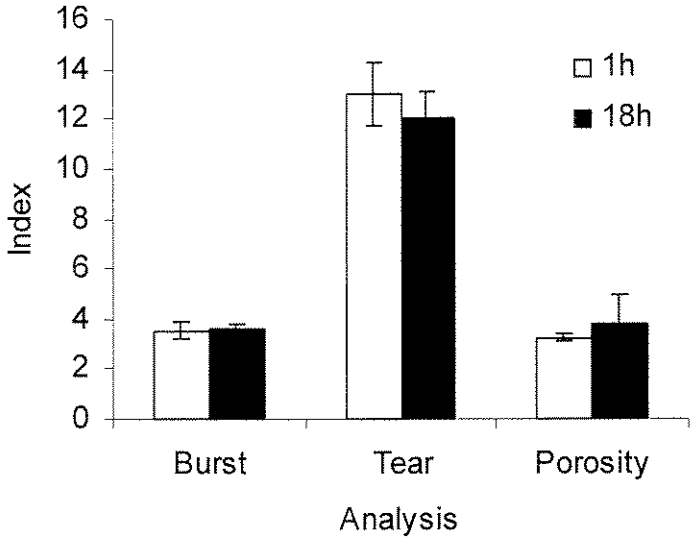

Figure 2. Optimisation of time for homogenisation of hemicellulose and cellulosic pulp. The assays were performed with *Tamarindus indica* xyloglucan at a final concentration of 1% (w/w). Methods and analyses were performed according to ABTCP norms. Bars represent the standard deviation.

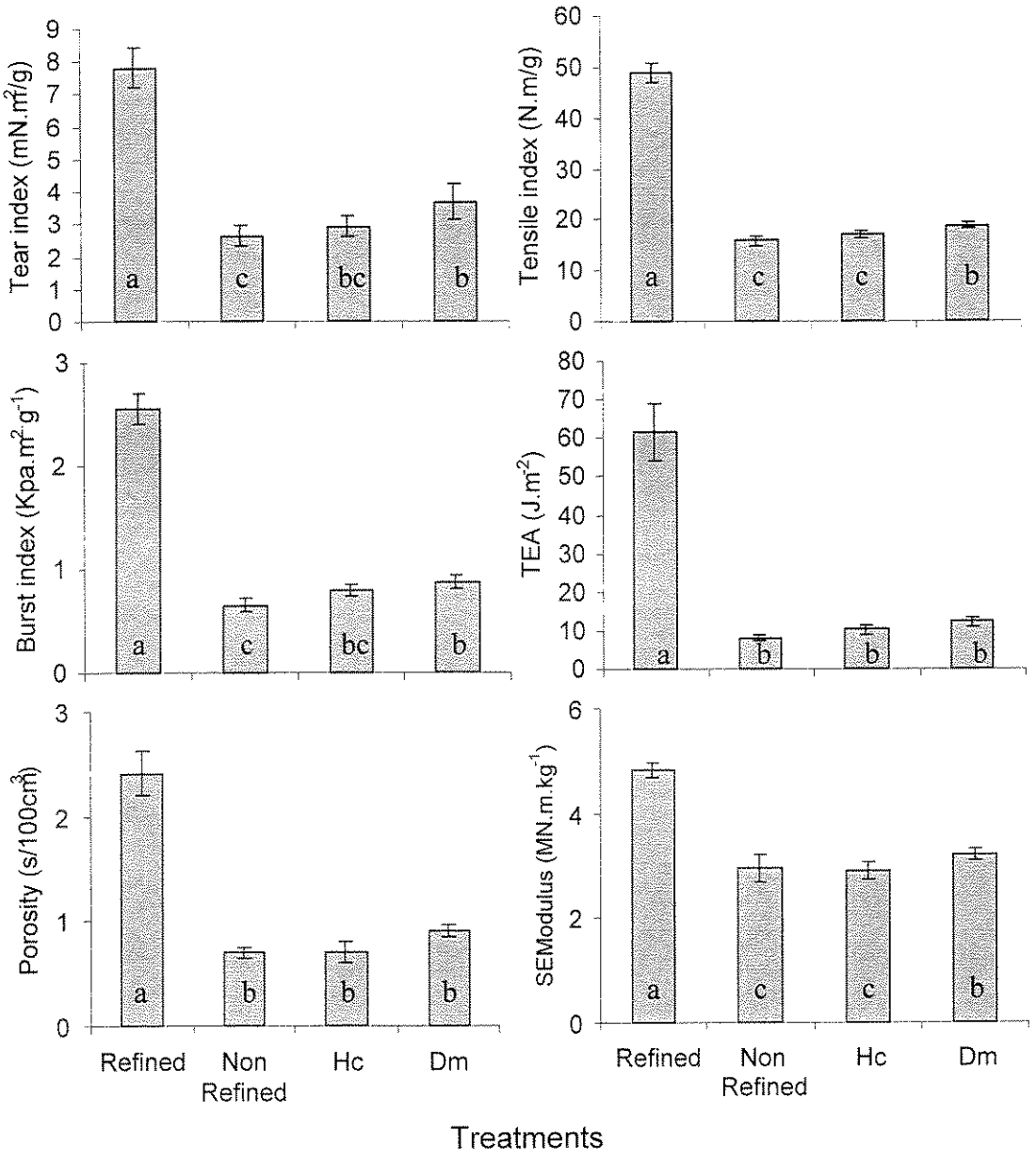

Figure 3. Effects of hemicelluloses on non-refined pulp. Refined pulp without hemicelluloses was the control sample. **Hc** – non-refined pulp with *Hymenaea courbaril* xyloglucan; **Dm** – non-refined pulp with *Dimorphandra mollis* galactomannan. The assays were performed with hemicelluloses at a final concentration of 1% (w/w). Methods and analysis were performed according to ABTCP norms. TEA – Tensile Energy Absorption; SEModulus – Specific Elastic Modulus. The bars represent the standard deviation. Different letters represent statistical differences according to Tukey test ($P<0.05$).

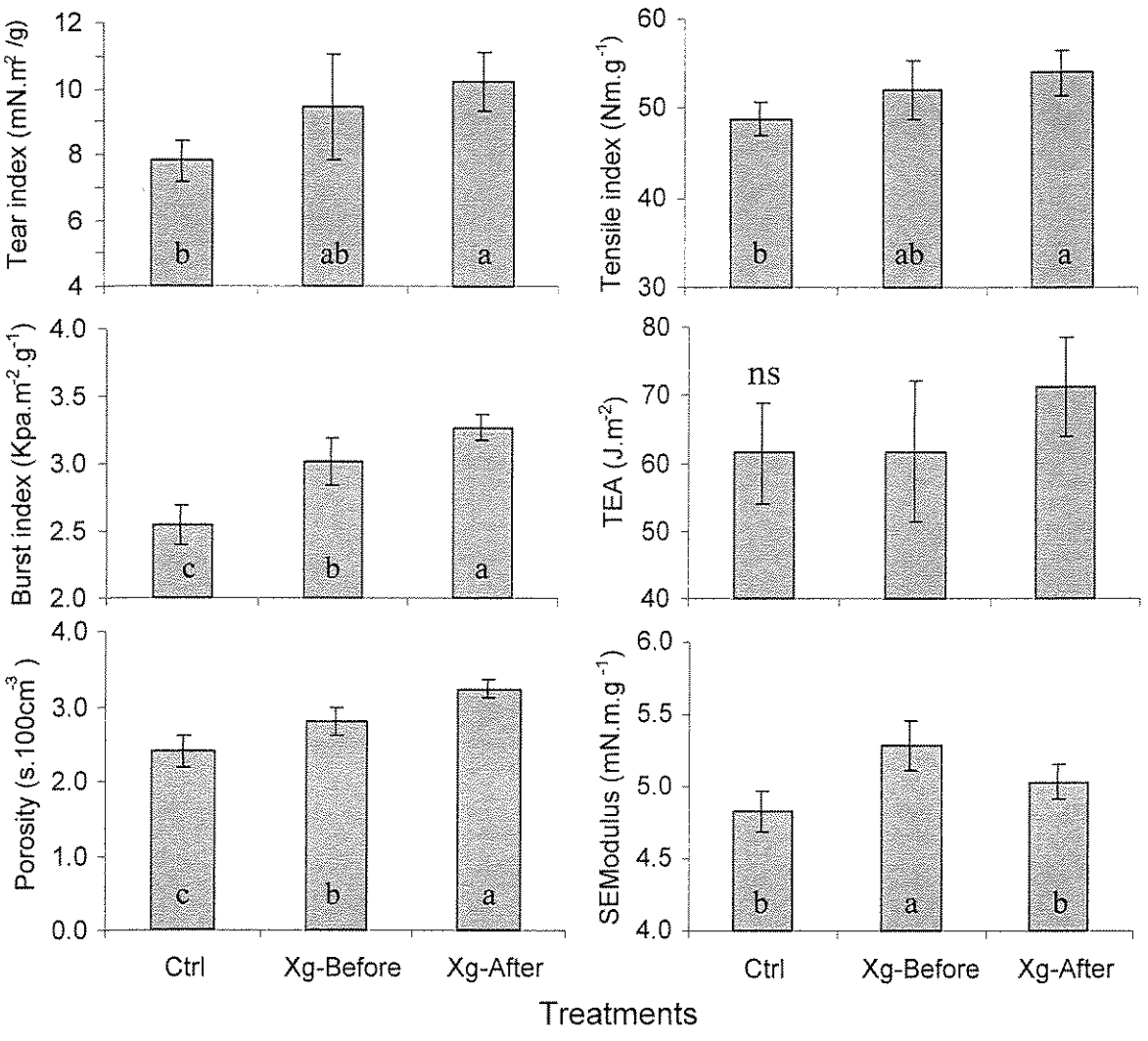

Figure 4. Effect of the addition of *Tamarindus indica* xyloglucan on the mechanical properties before and after the refining process. Control samples (Ctrl) were performed without hemicelluloses. The assays were performed with hemicelluloses at final concentration of 1% (w/w). TEA – Tensile Energy Absorption; SEModulus – Specific Elastic Modulus. The bars represent the standard deviation. Different letters represent statistical difference according to Tukey test ($P<0.05$). ns – not significant.

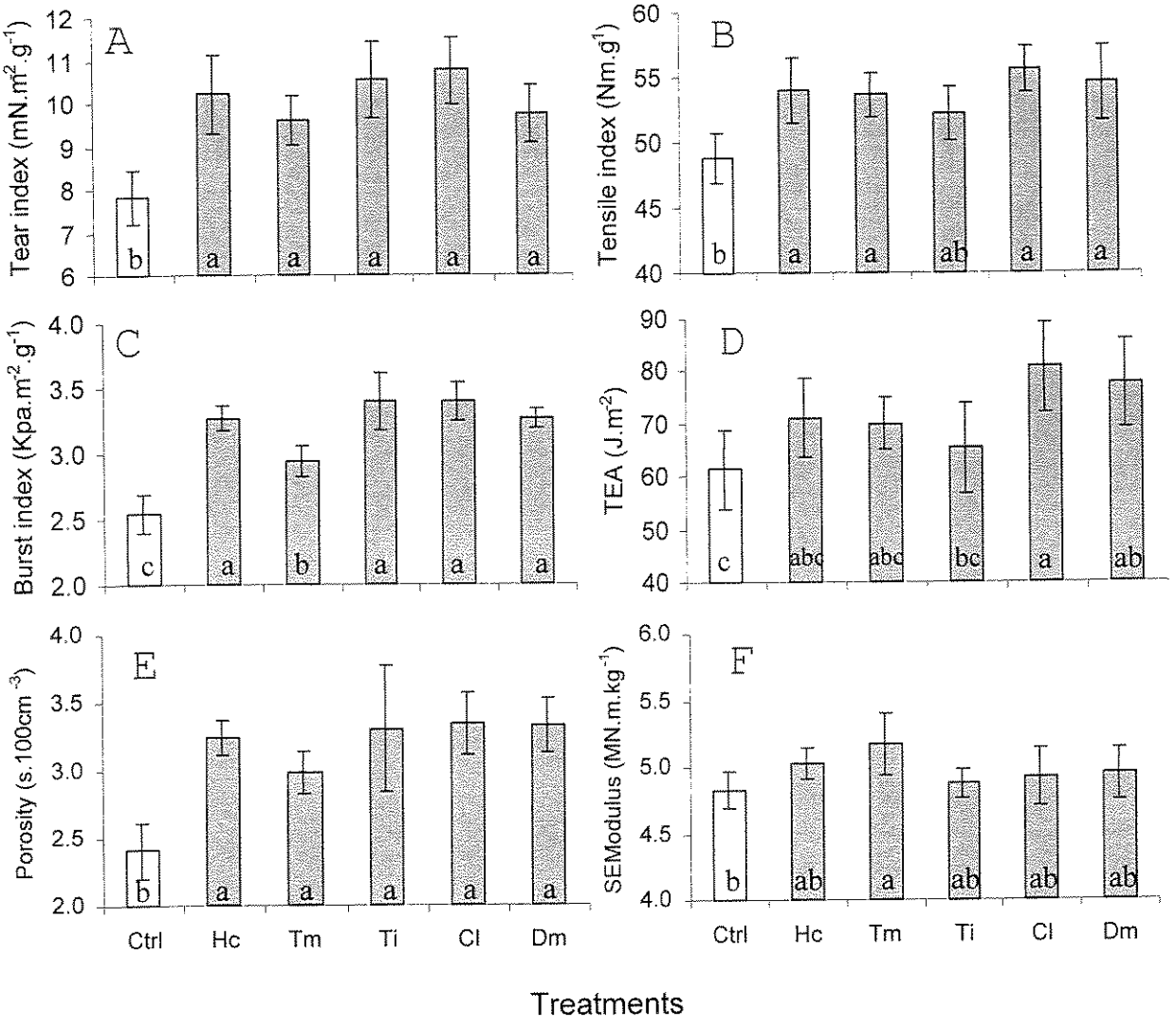

Figure 5. Comparison among different hemicelluloses added to the cellulosic pulp. The assays were performed with xyloglucans from Hc (*Hymenaea courbaril*), Tm (*Tropaeolum majus*), Ti (*Tamarindus indica*), Cl (*Copaifera langsdorffii*) and galactomannan from Dm (*Dimorphandra mollis*) at final concentration of 1%. TEA – Tensile Energy Absorption; SEModulus – Specific Elastic Modulus. The bars represent the standard deviation. Different letters represent statistical difference according to Tukey test ($P<0.05$).

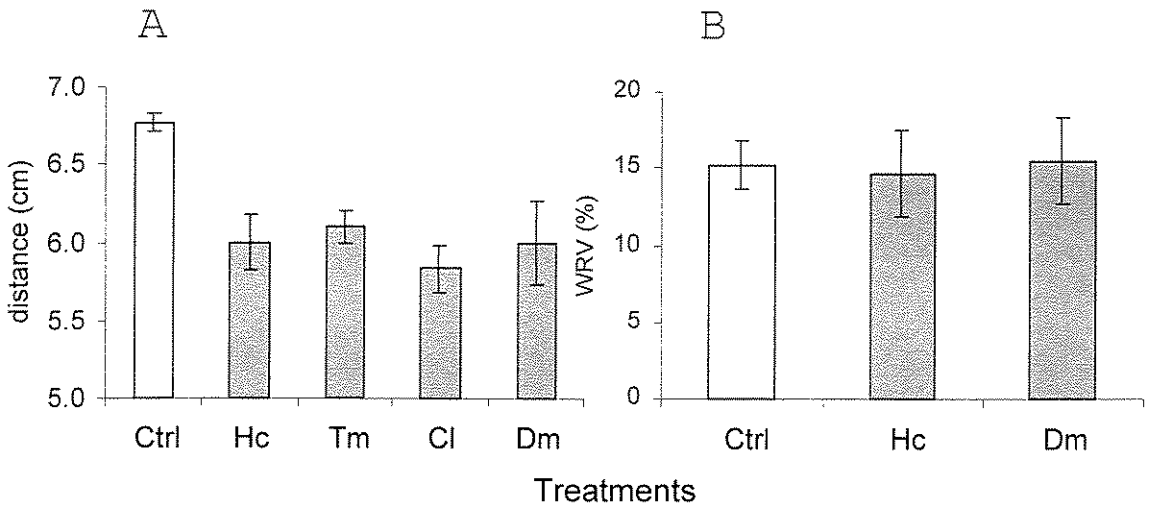

Figure 6. Capillarity (given by distance moved by water in cm) and Water Retention Value (WRV) of sheets produced with different hemicelluloses, Hc (*Hymenaea courbaril*), Tm (*Tropaeolum majus*), Cl (*Copaifera langsdorffii*) and galactomannan from Dm (*Dimorphandra mollis*) at 1% final concentration.

Table 1. Improvement (%) on properties due to the addition of hemicelluloses to paper sheets. The assays were performed with xyloglucans from Hc (Hymenaea courbaril), Tm (*Tropaeolum majus*), Ti (*Tamarindus indica*), Cl (*Copaifera langsdorffii*) and galactomannan from Dm (*Dimorphandra mollis*) at 1% final concentration. Ti (Tear index), Bi (Burst index), TEA (Tensile Energy Absorption), SEM (Specific Elastic Modulus), ASW (Apparent Specific Weight), Porosity and Capillarity were analysed. Negative values mean a percentual decrease in those properties due to the presence of hemicelluloses in comparison to the control samples.

Hemicellulose	*Ti*	*Bi*	*Porosity*	*Tensile Index*	*TEA*	*SEM*	*ASW*	*Capillarity*
Hc	28	31	-34	10	16	4	4	-12
Tm	15	23	-24	10	14	7	3	-10
Ti	33	35	-37	7	6	1	2	-
Cl	33	38	-39	14	31	2	4	-14
Dm	28	25	-38	12	26	2	3	-12
Average	*28*	*30*	*-34*	*11*	*19*	*3*	*3*	*-12*

DISCUSSÃO

Modelos de estudo da parede celular

A estrutura celular e o crescimento anisotrópico estão diretamente relacionados com os componentes da parede celular, suas disposições e o metabolismo de síntese e degradação a que eles estão submetidos. A parede primária, relacionada com tecidos jovens em crescimento, pode ser dividida em 2 tipos (tipo I e tipo II) de acordo com a composição dos polissacarídeos que formam a matriz extracelular. Na parede do tipo II a hemicelulose dominante é o arabinoxilano ou o β-glucano [(1,3)-(1,4)-β-glucano], além de uma baixa quantidade de pectinas. Já a parede tipo I é típica das dicotiledôneas, caracterizando-se por ter altas proporções de pectinas e por apresentar o *xiloglucano* como principal hemicelulose. Este polissacarídeo está associado às funções estruturais da parede devido sua capacidade de interagir especificamente com a celulose.

Vários têm sido os trabalhos que procuram entender a complexidade da parede celular utilizando modelos bem caracterizados para a análise da estrutura primária da matriz extracelular. No entanto, esses modelos são geralmente oriundos de tecidos vegetais sob condições de estresse luminoso para produzir tecidos estiolados podendo gerar situações que não sejam encontradas nas mesmas proporções que aquelas vistas sob as condições ambientais mais comuns de luminosidade, temperatura, etc.

Modelos de estudo de parede celular onde são encontradas amplificações de um dos domínios (hemiceluloses ou pectinas), estão sendo utilizados a pelo menos 2 décadas, produzindo uma gama de resultados que parecem ser razoáveis para extrapolação daquilo que acontece em vários outros tecidos, sem, no entanto, estar sob condições de estresse (luminosidade, por exemplo). Espécies onde os cotilédones ou endospermas das sementes acumulam polissacarídeos de parede celular como reserva de carboidratos representam modelos de tais estudos. Além da possibilidade de obter maiores conhecimentos sobre o metabolismo da parede celular primária, estes trabalhos

têm um grande impacto no que diz respeito à utilização dos carboidratos de reserva durante o crescimento inicial de plantas de diferentes biomas.

Os estudos da interação entre xiloglucanos e celulose realizados no presente trabalho (divididos em 3 partes), mostraram um pouco da amplitude que se pode alcançar com experimentos onde são utilizados polissacarídeos obtidos de sementes.

Influência da interação xiloglucano-celulose na parede primária

Efeito do grau de galactosilação do xiloglucano

Os dados obtidos sobre a influência da ramificação galactosilada mostrada para xiloglucanos de alto peso molecular, podem servir como base teórica para entender algumas características estruturais observadas na parede primária.

Existem regiões da molécula do xiloglucano que se ligam a duas microfibrilas de celulose formando uma ponte entre elas (figura 2 da introdução), sendo responsáveis pelo impedimento de colapso da parede, evitando que grandes aglomerados de celulose se formem. Para que isso seja possível, estas regiões devem ser menos interativas. Uma possibilidade seria a produção de um polissacarídeo com regiões mais enoveladas, onde as glucoses da cadeia principal estariam inacessíveis, portanto, com menor número de sítios de interação com a celulose. Como visto no primeiro trabalho (páginas 18 a 42), um alto grau de galactosilação, ou ainda, uma disposição das galactoses ao longo do polímero de forma acertada, seria prováveis ferramentas que poderiam ser utilizadas durante a biossíntese, produzindo tais regiões menos interativas (Tabelas 1 e 3; Figura 4 do artigo 1).

Um outro fato interessante é que estas regiões galactosiladas são menos suscetíveis ao ataque de endoglucanases (Tiné et al., 2002). Estes autores mostraram que durante a hidrólise enzimática (utilizando celulase fúngica) os primeiros blocos estruturais liberados do polímero foram aqueles sem galactose (XXXG), apesar da grande disponibilidade de oligossacarídeos galactosilados (XLLG). Esta resistência (inicial) indica que além do grau de interatividade, as galactoses desempenham uma função de controle durante o processo de hidrólise por endoenzimas (*in vivo* esta hidrólise é realizada por Xiloglucano Endo Transglicosilases – XET).

As *expansinas* são proteínas que atuam sobre os xiloglucanos durante os processos relacionados ao alongamento e crescimento celular. Algumas trabalhos sugerem que elas rompam as pontes de H entre xiloglucano e celulose (McQueen-Mason e Cosgrove, 1994; Cosgrove, 1998), para isso, elas precisam identificar onde começam tais ligações. Considerando que as regiões mais galactosiladas do xiloglucano estão naquelas regiões "pontes" entre as microfibrilas de celulose, estas galactoses poderiam estar desempenhando, portanto, um papel de sinalizador para as expansinas.

Efeito do Peso Molecular

Os dados obtidos no segundo artigo (paginas 43 a 66), mostraram que a interação entre xiloglucano e celulose é fortemente favorecida por moléculas menores. Tal característica estrutural chega a ser tão importante quanto o grau de ramificação com fucose (muitos autores propõem que a capacidade de interação do xiloglucano está intimamente relacionada com a presença de resíduos fucosilados), já que numa comparação entre xiloglucanos fucosilados e não-fucosilados, com peso molecular médio similar, eles apresentaram semelhante capacidade de interação com celulose (Figura 2 do artigo 2). Além disso, a energia de adsorção entre estes polímeros (considerando os dados de entalpia) foi variável de acordo com seus pesos moleculares e a suas ramificações (Figura 3 do artigo 2). O xiloglucano fucosilado apresentou maior energia de ligação, principalmente nas primeiras interações, mesmo quando comparado às interações dos fragmentos de xiloglucanos não-fucosilados de peso molecular similar (Xg 240kDa). Isto deve favorecer a manutenção da arquitetura da parede, se considerarmos que quanto maior a força para adsorção, maior será a força para dissociação. Além disso, a energia de ligação pode estar correlacionada com a função dos xiloglucanos em orientar as microfibrilas de celulose.

Sugere-se, portanto, que a estrutura da parede celular pode ser alterada de forma controlada variando o grau de ramificação com fucose, sem alterar significativamente o tamanho das moléculas. Isto resultaria numa alteração da *força* (energia) de ligação entre xiloglucano e celulose sem alterar *a capacidade* de interação (quantidade adsorvida) entre eles.

Com base nesses dados, podemos sugerir que, topologicamente os oligossacarídeos fucosilados devem ser encontrados nas regiões da parede que necessitem maior rigidez. Já, as regiões que irão se distender deverão apresentar, provavelmente, menor grau de fucosilação, não impedindo a interação, mas permitindo uma maior flexibilidade devido a menor energia requerida nos processos de ligação e ruptura das pontes de H.

Influência da interação xiloglucano-celulose na parede de reserva

Efeito do grau de ramificação

Considerando o acúmulo de xiloglucanos nas paredes de reserva (secundárias) em cotilédones de sementes de várias espécies, pode-se identificar um importante papel para as galactoses presentes no polímero. O alto grau de galactosilação diminui a interatividade dos xiloglucanos em relação aos polímeros menos galactosilados, sem, no entanto, impedir a ocorrência de auto-interação. Assim, o empacotamento dos polissacarídeos durante a deposição de reservas na formação dos frutos, não formará aglomerados insolúveis (ou quase insolúveis), como aqueles formados por mananos (cadeia de manose β-1,4 ligadas), em sementes de café, por exemplo.

Embora poucos xiloglucanos de reserva tenham sido caracterizados, é comum a todos eles a ausência da ramificação com fucose, sendo esta exclusiva na parede celular primária. Esta perda da fucose poderia ser uma forma de evitar o efeito biológico (anti-auxínico) dos oligossacarídeos, separando os mecanismos de sinalização celular da degradação do xiloglucano. Uma outra alternativa seria a demarcação química entre duas populações de xiloglucano: de reserva e estrutural (Tiné, 2002) presentes na mesma célula, onde a fucosilação é característica dos polissacarídeos estruturais. Baseando-se nas análises de interação xiloglucano-celulose, podemos lançar mão de outra hipótese para a ausência de fucose nos polissacarídeos de reserva: como os resultados mostrados sugerem que a interação do xiloglucano fucosilado envolve muita energia e não parece ocorrer auto-interação, a ausência da fucose seria responsável em permitir a ligação (de baixa energia) entre as moléculas, permitindo tanto um eficiente

empacotamento, como o fácil acesso às enzimas e expansinas durante o processo de desmonte e mobilização.

Efeito do peso molecular

A característica de maior interação em função do menor peso molecular, deve favorecer a eficiência de empacotamento das reservas, já que a auto-interação também é favorecida. Isso permite, por exemplo, que sementes como de jatobá (*Hymenaea courbaril*) possam acumular até 50% de carboidratos nos cotilédones, o que representa aproximadamente 2 g de material de reserva por semente.

Parece existir um balanço entre a necessidade de acúmulo de grandes quantidades de carboidratos através da biossíntese de pequenos fragmentos não fucosilados, e a solubilidade (dada pelo grau de galactosilação) necessária para a mobilização dos açúcares durante o crescimento inicial das plantas.

Deposição e montagem dos xiloglucanos durante a biossíntese

No aspecto biológico, este trabalho abre perspectivas no que tange os processos de biossíntese de xiloglucanos, não àquilo que se refere aos modos e mecanismos de ação enzimática, mas sim, nos produtos que são depositados e montados na parede celular.

Com base nos resultados do segundo artigo em que foi mostrada a influência do tamanho da molécula sobre a capacidade de interação, pode-se pensar que os xiloglucanos inicialmente biossintetizados no Complexo de Golgi e depositados na parede celular possam ser polímeros de baixo peso molecular. Duas hipóteses podem ser propostas quanto ao processo de montagem do xiloglucano na parede. A primeira seria a formação de um polímero muito extenso produzido através da transglicosilação destes pequenos fragmentos. Para isso, deveria ser identificada uma XET (Xiloglucano Endo-Transglicosilase) capaz de formar tais polissacarídeos durante a deposição dos materiais na parede. Em ensaios preliminares nós identificamos uma XET presente em cotilédones de sementes imaturas de jatobá, mas sua atividade "transglicosilítica" não pôde ser verificada devido a problemas metodológicos. No entanto, a existência de uma

XET com atividade de transglicosilação nestes tecidos (durante o processo de mobilização de resevas após a germinação) foi encontrada por Alcântara (2000). Além disso, a especificidade desta enzima pelo xiloglucano de *H. courbaril* foi demonstrada por Minhoto (2002).

A segunda hipótese seria a de que o xiloglucano (normalmente com PM>2000KDa) seja formado por agrupamentos de várias moléculas menores auto-interagidas, resultando numa "microfibrila de xiloglucano", semelhante à celulose (que é formada por 36 cadeias de glucose ligadas lateralmente umas às outras por pontes de H). No entanto, as ligações laterais da microfibrila de xiloglucano não seriam nas mesmas proporções (comparando-se à celulose) devido ao seu alto grau de ramificação, impedindo a formação de grandes agregados com estrutura cristalina.

Ainda não se sabe o que define o grau de galactosilação e sua disposição ao longo das moléculas, durante o processo de biossíntese em xiloglucanos. Já Edwards et al., (1992) mostraram que durante o processo de deposição de reservas de galactomanano (em sementes de *Senna occidentallis*), após a fase de biossíntese, ocorre a desgalactosilação pela atividade da α-galactosidase, atuando no polissacarídeo. É improvável que isso ocorra com xiloglucanos, uma vez que não identificamos nenhuma β-galactosidase capaz de hidrolisar as galactoses presentes no polímero (Tabela 1). Apenas quando os xiloglucanos foram hidrolisados por endoglucanases (produzindo fragmentos com no máximo 4 blocos estruturais) é que se detectou atividade da galactosidase (Silva, 2001). Ou seja, existe a possibilidade de haver desgalactosilação em xiloglucanos, de forma similar àquela vista para galactomananos se, e apenas se, o processo de biossíntese produzisse fragmentos de baixo peso molecular.

As discussões acima mostram que os estudo com parede celular a partir de sementes que acumulam xiloglucano, podem servir para analisar diversos aspectos pertencentes tanto ao metabolismo como à estrutura da parede. Isto é possível por que durante o processo evolutivo destas espécies que acumulam polissacarídeos de parede celular, todo (ou grande parte) do sistema protéico-enzimático foi transferido da parede

primária para a secundária (reserva) carregando todas as informações necessárias para a manutenção do sistema de síntese e degradação (Buckeridge et al, 2000a).

Tabela 1. Atividade de β-galactosidases sobre substrato sintético (*p*-nitrophenyl-β-galactosideo) e xiloglucano de reserva (oligossacarídeos galactosilados e polissacarídeo de alto peso molecular). As enzimas de origem vegetal foram obtidas de cotilédones coletados durante a fase de mobilização das reservas. As enzimas de *Aspergillus oryzae*, *Escherichia coli* e fígado bovino foram obtidas da SIGMA. As dosagens de atividade sobre xiloglucano foram monitoradas por cromatografia de troca aniônica (HPAEC-PAD, HPLC-Dionex) e os perfis cromatográficos foram comparados com as amostras controle. XLXG e XLLG representam os oligossacarídeos de xiloglucano com 1 e 2 galactoses, respectivamente. Dados cedidos por Silva, A.M. (*) e Alcântara et al. (1999)

Extratos	**Atividade sobre substrato sintético (μmol pNP.min^{-1}.mL^{-1})**	**Atividade sobre oligossacarídeos de xiloglucano**	**Atividade sobre polissacarídeo**
A. oryzae	23000	XLXG e XLLG	nenhuma
E. coli	802	nenhuma	nenhuma
Fígado bovino	4500	XLXG e XLLG	nenhuma
Hymenaea courbaril	16	XLLG	nenhuma
Tropaeolum majus	1300*	XLXG e XLLG	nenhuma
Copaifera langsdorffii	240**	XLXG e XLLG	nenhuma

* Doutoranda do Depto. de Biologia Celular da UNICAMP

Aplicações tecnológicas baseadas na interação xiloglucano-celulose

No aspecto de ciência aplicada, nossos resultados podem gerar produtos novos e alternativos para a indústria de papel, uma vez que as hemiceluloses se ligam fortemente às fibras de celulose. Os xiloglucanos e os galactomananos são aditivos potenciais para incrementar as propriedades mecânicas das folhas de papel, chegando a aumentar em até 30% alguns índices físicos analisados. Estes polissacarídeos agem como adesivos das fibras, aumentando a coesão entre elas permitindo os incrementos observados. Papéis para embalagens e papéis-cartão podem ser beneficiados com o uso destas hemiceluloses. O amido e a goma guar (galactomanano de *Cyamopsis tetragonolobus*) já são utilizados com estes propósitos de conferir maior resistência mecânica.

O galactomanano de *Dimorphandra mollis* e os xiloglucanos de *Hymenaea courbaril* e *Copaifera langsdorffii* podem ser extraídos de sementes de espécies típicas de regiões de mata e cerrado, introduzindo mais uma característica para a utilização sustentável destas áreas. Outras fontes de xiloglucano estudadas foram *Tropaeolum majus* e *Tamarindus indica*.

As diferenças na estrutura fina destes polissacarídeos de reserva de parede celular não foram suficientes para causar variação significativa no incremento das propriedades mecânicas analisadas, permitindo a utilização conjunta das gomas das diferentes fontes (Figura 5 do artigo 3).

Atualmente, as indústrias de papel estão buscando nos materiais para embalagem aquele que apresente maior resistência, maior leveza e apresente menor densidade. Isso implica em menor volume e quantidade de material utilizado em embalagens, resultando num menor custo com transporte e menor produção de resíduos sólidos. Nossos resultados abrem possibilidades de estudo de caracterização de diferentes hemiceluloses que possam ser utilizadas com a finalidade de alcançar tais objetivos, focando os interesses financeiros (para a indústria) e os interesses ao meio ambiente (uso sustentável).

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