UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ENGENHARIA DE ALIMENTOS

MÁRIA HERMINIA FERRARI FELISBERTO

Characterization and evaluation of flour and starch of young culm of *Dendrocalamus asper*, *Bambusa tuldoides* and *Bambusa vulgaris* for cookie application

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Thesis presented to the Faculty of Food Engineering of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Food Technology

Tese apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutora em Tecnologia de Alimentos

Supervisor/Orientador: Maria Teresa Pedrosa Silva Clerici

ESTE EXEMPLAR CORRESPONDE À VERSÃO FINAL DA TESE DEFENDIDA PELA ALUNA MÁRIA HERMINIA FERRARI FELISBERTO, E ORIENTADA PELA PROFA. DRA. MARIA TERESA PEDROSA SILVA CLERICI

CAMPINAS
2018

Agência(s) de fomento e nº(s) de processo(s): FAPESP, 2015/19637-4; CAPES

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Engenharia de Alimentos
Claudia Aparecida Romano - CRB 8/5816

F335c

Felisberto, Mária Herminia Ferrari, 1986-
Caracterização e avaliação da farinha e amido dos colmos jovens de *Dendrocalamus asper*, *Bambusa tuldoides* e *Bambusa vulgaris* para aplicação em biscoito tipo cookie / Mária Herminia Ferrari Felisberto. – Campinas, SP : [s.n.], 2018.

Orientador: Maria Teresa Pedrosa Silva Clerici.
Tese (doutorado) – Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos.

1. Amido. 2. Bambu. 3. Ingredientes. 4. *Poaceae*. 5. Sustentabilidade. I. Clerici, Maria Teresa Pedrosa Silva. II. Universidade Estadual de Campinas. Faculdade de Engenharia de Alimentos. III. Título.

Informações para Biblioteca Digital

Título em outro idioma: Characterization and evaluation of flour and starch of young culm of *Dendrocalamus asper*, *Bambusa tuldoides* and *Bambusa vulgaris* for cookie application
Palavras-chave em inglês:
Starch
Bamboo
Ingredients
Poaceae
Sustainability
Área de concentração: Tecnologia de Alimentos
Titulação: Doutora em Tecnologia de Alimentos
Banca examinadora:
Maria Teresa Pedrosa Silva Clerici [Orientador]
Antonio Ludovico Beraldo
Ivo Mottin Demiate
Lucimara Mach Côrtes Cordeiro
Marney Pascoli Cereda
Data de defesa: 21-05-2018
Programa de Pós-Graduação: Tecnologia de Alimentos

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A ata da defesa com as respectivas assinaturas dos membros encontra-se no processo de vida acadêmica do aluno.

Tenha coragem de seguir o que seu coração e sua intuição dizem. Eles já sabem o que você realmente deseja. Todo o resto é secundário.

Steve Jobs

AGRADECIMENTOS

É muito difícil expressar o meu sentimento de gratidão por todos aqueles que estiveram presentes ao longo da minha caminhada do doutorado.... Muitos já me acompanhavam e torciam por mim ao logo da minha vida, e outros muito especiais entraram na minha vida nestes últimos quatro anos, e os levarei pra sempre no coração. Mas primeiramente eu agradeço a Deus, não só pela oportunidade que tive, mas também por sempre ter me mostrado que eu era capaz e que conseguiria vencer mais este obstáculo.

Um agradecimento especial à minha família e meu porto seguro, pois sem eles, eu nada seria!! À minha "pãe", Vera Lúcia, o meu muito obrigada por ser um exemplo de mulher, de superação e de determinação. Obrigada pelo apoio financeiro, pelas palavras de carinho e pela atenção de sempre! Ao meu amigo, anjo da guarda e irmão, Mario Antonio, que mesmo distante sempre esteve tão perto e presente em todos os meus passos! Às minhas peruas lindas, Tia Zezé e Vovó Angélica, pela ajuda financeira, emocional e por todas as orações e palavras de conforto. Muito obrigada pelo carinho, dedicação e confiança que todos vocês depositaram em mim.

Ao meu noivo, Moysés, por estar sempre ao meu lado, torcendo a cada vitória e me apoiando nos momentos difíceis. Obrigada pela paciência, carinho e pelos momentos de descontração. Ter você ao meu lado faz tudo parecer mais simples e fácil de resolver. Muito obrigada Paixão!!!

Ao querido LabCer e seus agregados....estou sem palavras para expressar o quão grata eu sou....!!! Mais do que um local de trabalho, este laboratório foi minha casa, meu abrigo e meu porto seguro, física e emocionalmente!, Por todas as pessoas que por ali passaram e lá ainda estão, tenho imensa gratidão. Gostaria de expressar o meu muito obrigada ao Marcio, Georgia, Lud, Lara, Fernanda, Amanda Nogueira, Thaísa, Amanda Rios, Elisa, Ulliana, Aline, Flávio, Zé, Izilda, Michele, Ana, Carla, Gisela, Rebeca, Alessandra, Bianca, Ana Paula, Wellington, Alexandre, Ana Laura, Lucas, Mateus, Carol, Gustavo e Edna.

Às meninas Nayara, Nathanny, Patrícia, e ao menino de ouro, Thiago, por abraçarem o desafio do bambu junto comigo. Muito obrigada pelas várias horas trabalhadas sempre com um sorriso no rosto. Obrigada pela companhia durante os almoços, no bandejão ou na feirinha, e pelos momentos de descontração, mesmo no auge do stress.

O meu muito obrigada aos amigos do café, Kazumi, Diana, Dri, Leandro e Teresinha! Foram muitas risadas e desabafos acompanhados sempre de um café fresquinho. Obrigada pelo ombro amigo, pelo ouvido alugado, pelas palavras de apoio e pelos puxões de orelha.... Nossa amizade começou ali, e seguirá para onde eu for.

Um agradecimento especial à professora Maria Teresa, primeiramente por ter aceitado me orientar. Obrigada por compartilhar seus conhecimentos, por sempre incentivar *a criação e por acreditar no meu trabalho. Muito obrigada pela sua orientação, disponibilidade, atenção e confiança.*

Tenho muito para agradecer também ao professor Beraldo por me apresentar este maravilhoso universo do bambu, por apoiar e incentivar nosso projeto desde o início. O senhor foi um exemplo de dedicação e superação, de que quando a gente gosta do que faz, não importa onde, quando e nem como, simplesmente a gente faz!

Gostaria de agradecer à banca avaliadora, professores Drª Lucimara Cordeiro, Drª Marney Cereda, Drª Célia Maria Franco, Dr Ivo Demiate, Dr Manuel Oviedo e Dr Beraldo por todos os comentários e observações pertinentes que só acrescentaram ao trabalho e ao meu crescimento profissional.

Um agradecimento em especial à Flávia, Mariana e Márcia, por me receberem em seu local de trabalho e compartilharem todo conhecimento!

Aos amigos que em Campinas fiz pelos maravilhosos momentos de descontração, e aos velhos amigos, que mesmo à distância sempre estiveram na torcida, Ana Carol, Djalma, Tati Paiva, Sílvia Agibert, Gerson Luiz, Renata Caroline, Milhão, Robledinho, Ana Alice, ...

À FAPESP, CNPq e CAPES pelo apoio financeiro. À UNICAMP, e em especial ao Departamento de Tecnologia de Alimentos/FEA e seus funcionários, por permitirem a realização desta pesquisa, e por me acolherem tão bem. Obrigada a todos os professores do DTA, pela disponibilidade e atenção de sempre, e à secretária Cláudia por estar sempre pronta a me socorrer, pela atenção, disponibilidade e gentileza de sempre.

Muito Obrigada!!

RESUMO

O bambu é uma gramínea de cultivo simples, dispersa em nível global e que dispensa o replantio e a utilização de insumos agrícolas. Seus brotos são bastante utilizados pela culinária e para extração de fibras, enquanto que o colmo jovem, apesar do seu potencial para alimentação humana por conter amido e fibras, ainda não é explorado. Assim, o objetivo deste trabalho foi produzir farinha de três espécies de colmo jovem de bambu (*Dendrocalamus asper*, *Bambusa tuldoides* e *B. vulgaris*) e avaliar suas características físico-químicas e tecnológicas, visando aplicações alimentícias. Os colmos jovens de bambus foram colhidos com 36 meses de idade, secos e processados em farinhas, que apresentaram coloração levemente amarelada e baixos teores de umidade, proteínas, lipídeos e cinzas. Com relação ao teor de carboidratos, apresentaram potencial para extração de fibras (> 64 g/100 g), sendo que as espécies *B. vulgaris* e *D. asper* apresentaram adicional potencial para extração de amido (> 8 g/100 g). Com base nestes resultados, foi realizada a extração do amido do colmo jovem das três espécies de bambu, e avaliaram-se suas características morfológicas, estruturais e físico-químicas. Todos os amidos apresentaram coloração branca clara, ligeiramente amarelada, grânulos com formato poliédrico e diâmetro <7,0 µm. Os amidos apresentaram maior proporção de cadeias de amilopectina com DP 13-24, polimorfismo tipo-A e temperatura de gelatinização superior a 80 ºC. A distribuição molecular de tamanho de amilopectina e amilose não variou entre os amidos das diferentes partes do colmo e nem entre as espécies de bambu. Com base nos resultados obtidos para caracterização das farinhas, selecionou-se a farinha do *D. asper* para aplicação em formulações de *cookies,* com substituição parcial da farinha de trigo (15%) pela farinha do colmo jovem de bambu (FCJB). Utilizou-se um delineamento linear (2^2) com duas variáveis independentes (redução de 0, 25% e 50% dos teores de açúcar e/ou de gordura) e três pontos centrais e os resultados das características tecnológicas foram avaliados por metodologia de superfície de resposta ($p \leq 0,10$, $R^2=0,8$). Observou-se que a redução do teor de açúcar e de gordura em 50% não alterou as características de volume, cor, textura e aw dos *cookies*, e que a redução do açúcar em 50%, independentemente do teor de gordura adicionado, levou à redução do diâmetro dos cookies após o forneamento. *Cookies* F2 (redução de 50% de açúcar, 0% de gordura) e F3 (redução de 50% de gordura e 0% de açúcar) foram selecionados para avaliação da estabilidade ao longo do armazenamento e da composição nutricional. Uma formulação controle, sem redução de açúcar e gordura, e sem a substituição parcial da farinha de trigo pela FCJB também foi elaborada. Os teores de umidade (< 4,27 g/100 g) e de aw (0,39) dos biscoitos mantiveram-se na faixa recomendada para manutenção da crocância. Os resultados obtidos indicaram que tanto a FCJB quanto os amidos podem ser utilizados em vários processos industriais alimentícios, melhorando o aporte nutricional do produto e corroborando com o apelo de saudabilidade, atualmente em alta pelos consumidores, podendo de agregar valor econômico a esta matéria-prima.

Palavras-chave: amido, bambu, ingredientes, *Poaceae* e sustentabilidade.

ABSTRACT

Bamboo is a simple growing grass, which develops globally, and does not require replanting and the use of agricultural inputs. Its shoots are widely used both for cooking and for fiber extraction, while the adult culm is not yet exploited, despite its potential for human food due to its starch and fibers contents. Thus, the objective of this study was to produce flour from three young bamboo culm species (*Dendrocalamus asper*, *Bambusa tuldoides* and *Bambusa vulgaris*), and evaluate its physicochemical and technological characteristics, aiming at food applications. The young bamboo culms were harvested at 36 months of age, dried and processed into flour, which exhibited a slightly yellow color and low moisture, protein, lipid and ash contents. Regarding the carbohydrate content, the samples presented potential for fiber extraction (> 64 g/100 g), with an additional potential for starch extraction (> 8 g/100 g) observed for *B. vulgaris* and *D. asper* species. Based on these results, young bamboo culm starch from three species were extracted and evaluated by their morphological, structural, and physicochemical characteristics. All starch granules presented a light white, slight yellow color, with polyhedral shape and diameter < 7 µ. The starches exhibited a higher proportion of amylopectin chains with DP 13-24, A-type polymorphism and gelatinization temperature above 80 ºC. The molecular size distribution of amylopectin and amylose did not vary between the starches from the different parts of the culm and between the bamboo species. The characterization results showed that flour from *D. asper* species was selected for application in cookies formulations, with partial replacement of wheat flour (15%) by the young bamboo culm flour (YBCF). A statistical design (2^2) with two independent variables (0, 25% and 50% reduction of sugar and/or fat contents) and three central points were used. The results were evaluated by response surface methodology ($p \leq 0.10$, $R^2=0.8$). The 50% reduction of sugar and fat contents did not change the volume, color, texture and aw of the cookies, while the 50% sugar reduction led to a decrease in cookies diameter after baking, regardless of fat content. The cookies F2 (reduction of 50% of sugar and 0% of fat) and F3 (reduction of 50% of fat and 0% sugar) were selected to evaluate stability during storage and nutritional composition. A control formulation, without sugar and fat reduction, and without the partial replacement of wheat flour by YBCF was also elaborated. Moisture content (< 4.27 g/100 g) and aw (< 0.39) of the cookies remained within the range recommended for maintenance of the crispness. The results indicated that both YBCF and starches can be used in food processing, improving the nutritional value of the product and corroborating with the increased consumers' demand for healthy products, besides adding economic value to this raw material.

Keywords: starch, bamboo, ingredients, *Poaceae* and sustainability.

SUMÁRIO

– CAPÍTULO 1 –

Introdução Geral

Este capítulo apresenta uma introdução geral sobre o tema que será abordado na tese, bem como apresenta os objetivos da pesquisa, e nele faz-se uma breve explicação sobre a organização da tese.

1. INTRODUÇÃO

Assim como os cereais, o bambu é uma gramínea pertencente à família *Poaceae* e subfamília *Bambusoideae* (LIESE e KOHL, 2015). Do ponto de vista agrícola, é uma cultura economicamente importante por ser perene e produzir colmos assexuadamente, ano após ano, sem necessidade de replantio, com grande produtividade (t/ha) anual por unidade de área. O maior diferencial está relacionado com a velocidade de crescimento de seus colmos, que, diferentemente das madeiras, crescem só em altura (sentido longitudinal), por não apresentarem elementos anatômicos arranjados na direção radial e nem tangencial. Desta forma, podem ter seu primeiro corte efetuado apenas de três a cinco anos após o plantio (AKIRA et al., 2007; LIESE e KOHL, 2015).

A maior ocorrência das espécies de bambus é nas zonas quentes e úmidas, com chuvas abundantes, como as regiões tropicais e subtropicais da Ásia, África e América do Sul (MANHÃES, 2008; PEREIRA e BERALDO, 2016). O Brasil é o país em que ocorre a maior diversidade de bambus, com 34 gêneros e 232 espécies de bambus nativos, das quais 174 espécies são consideradas endêmicas. Os vários gêneros de bambus compreendem desde espécies de pequeno porte, com poucos centímetros de altura, até espécies gigantes (PEREIRA e BERALDO, 2016). Segundo o "International Network for Bamboo and Rattan - INBAR" (2007), o comércio internacional de bambu era estimado em cinco bilhões de dólares, porém no Brasil, não existem dados oficiais sobre o mercado de bambu, e nem sobre sua demanda comercial (DANTAS et al., 2005).

Todavia, este cenário está mudando, e a gramínea vem ganhando maior atenção no mercado nacional, principalmente após a aprovação da Lei nº 12.484 (BRASIL, 2011) em oito de setembro de 2011, que instituiu a Política Nacional de Incentivo ao Manejo Sustentado e ao Cultivo do Bambu. Esta lei tem como objetivo alavancar esta cultura e sua cadeia produtiva, uma vez que os plantios atuais são de pequena escala, feitos por pequenos produtores, resultando em muitas touceiras dispersas e sem a instalação de uma cadeia produtiva sistêmica.

O Brasil abriga uma das maiores reservas de bambus nativos do mundo (180.000 km^2), localizada no sudoeste da Amazônia (DANTAS et al., 2005). Dentre as espécies aqui introduzidas, as mais difundidas e comercializadas informalmente são *Bambusa (*espécies *blumeana, dissimulator, multiplex, tulda, tuldoides, ventricosa,* *vulgaris, beecheyana)*, *Dendrocalamus (*espécies *giganteus, asper, latiflorus, strictus), Phyllostachys (*espécies *aurea, purpuratta, bambusoides, nigra, pubescens)*, *Pseudosasa, Sasa* e *Sinoarundinaria* (MANHÃES, 2008; PEREIRA e BERALDO, 2016).

A maioria dos trabalhos científicos sobre bambu é voltada para a área agronômica (LIESE, 1985; SILVA, PEREIRA e SILVA, 2011), para a aplicação industrial do colmo (LO et al., 2008; HE et al., 2014) ou em aplicação alimentícia no consumo dos brotos, tanto *in natura*, quanto na forma de conserva, ou para extração de fibras (NIRMALA, DAVID e SHARMA, 2007; PARK e JHON, 2009; 2010; NONGDAM e TIKENDRA, 2014). Verifica-se nessa literatura que as condições de processamento dos brotos já se encontram bem estabelecidas, sendo recomendada a retirada de apenas 20 a 30% dos brotos, de modo que a sua extração seja sustentável, e que aumente a longevidade da touceira (RIGUEIRA JUNIOR, 2011).

Os colmos de bambus têm seu uso *in natura* com destaque para a produção de papel, móveis, objetos de decoração e na construção civil, por ser um material rico em hemicelulose. Todavia, um substancial teor de amido presente nas células parenquimáticas, cerca de 25%, acaba reduzindo a sua conversão em fibras celulósicas, durante a fabricação de papel, e também a vida útil dos móveis e objetos de decoração (AZZINI et al., 1987; PEREIRA e BERALDO, 2016). Esses dados foram os pontos de partida para o presente projeto, e que instigou o estudo em maiores detalhes do colmo jovem do bambu, e conduziu à extração e à caracterização do amido das três espécies avaliadas.

2. OBJETIVOS

2.1. Objetivo Geral

O objetivo do trabalho foi avaliar e caracterizar a farinha e o amido do colmo jovem de três espécies de bambus (*Dendrocalamus asper* (Schult. & Schult. f.) Backer ex K. Heyne, *Bambusa tuldoides* Munro e *Bambusa vulgaris* Schrad. ex J. C. Wendl.), e avaliar a aplicação da farinha como ingrediente alimentício em biscoito doce tipo *cookie.*

2.2. Objetivos Específicos

- Avaliar a produção da farinha do colmo jovem de três espécies de bambus e caracterizá-las quanto às suas propriedades físico-químicas, tecnológicas e instrumentais;
- Avaliar a toxicidade da farinha obtida do colmo jovem do bambu
- Avaliar o processo de extração do amido do colmo jovem das três espécies de bambus e caracterizá-los morfológica, físico-química e tecnologicamente;
- Avaliar a aplicação tecnológica da farinha do colmo jovem em biscoito doce tipo *cookies* com redução dos teores de açúcar e/ou gordura.

3. ORGANIZAÇÃO

O presente documento está organizado em capítulos, sendo que alguns deles correspondem a artigos publicados e/ou submetidos a periódicos da área, tal como está especificado na capa de cada um. A disposição dos capítulos não necessariamente corresponde à ordem cronológica segundo a qual as pesquisas foram realizadas, mas corresponde à lógica utilizada para definir o projeto. No **capítulo 1** – **Introdução Geral**, apresenta-se uma visão global do documento, assim como as justificativas para o investimento em tais pesquisas, para que o leitor consiga ter uma visão geral deste documento e um melhor entendimento da interligação entre os capítulos.

No **capítulo 2** – **Bamboo: a new-old culture for construction and food industry**, é apresentada uma revisão sobre o cultivo do bambu, locais de produção, principais espécies encontradas no Brasil e diferentes formas de sua utilização. Foi realizada uma busca das mais variadas publicações que citavam as diferentes utilizações industriais do bambu e sua sustentabilidade, mostrando a diversidade de usos desta gramínea. E ao buscarem-se as aplicações alimentícias, observou-se a utilização milenar dos brotos, e o grande potencial para os colmos, devido à sua composição rica em fibras e amido.

Assim, após estudar-se várias formas de uso do bambu bem como sua composição, percebeu-se seu potencial, e iniciou-se o estudo da farinha do colmo jovem, que está apresentada no **capítulo 3** – **Young bamboo culm flour of**

***Dendrocalamus asper*: Technological properties for food applications.** Nesse estudo, encontrou-se o grande desafio de transformar o colmo jovem de bambu de *D. asper* em farinha, e além do mais, optou-se por obter farinhas de três partes diferentes do colmo (base, meio e topo), e com diferentes granulometrias (F1>0,425 mm e F2≤0,425 mm), pela possibilidade de o amido poder encontrar-se concentrado prioritariamente em uma dessas frações.

Após o desenvolvimento de técnicas e estratégias para a produção da farinha do colmo jovem do *D. asper*, passou-se para a produção das farinhas das duas outras espécies de bambus: *Bambusa tuldoides* e *Bambusa vulgaris*. E tendo em vista que a separação das farinhas em duas granulometrias não apresentou diferença significativa em sua composição, optou-se por manter todo o material obtido da moagem dos colmos, com granulometria característica de farinhas (≤0,150 mm). O **capítulo 4** – **Young bamboo culm: potential food as source of fiber and starch** apresenta a composição química e as propriedades tecnológicas das farinhas obtidas dos colmos jovens das três espécies de bambu avaliadas: *D. asper, B. tuldoides* e *B.vulgaris*.

Considerando-se os resultados obtidos na caracterização das farinhas dos colmos jovens das três espécies de bambus, observou-se a importância da caracterização desses amidos, uma vez que a indústria, de modo geral, mostra crescente interesse na busca por novos ingredientes com características específicas. No **capítulo 5** – **Characterization of young bamboo culm starch from *Dendrocalamus asper,*** apresentam-se os resultados obtidos para a caracterização morfológica, físico-química e das propriedades térmicas do amido extraído do colmo jovem do *D. asper*. No **capítulo 6 – Physicochemical and structural properties of starch from young bamboo culm of *Bambusa tuldoides*,** caracterizou-se o amido extraído do colmo jovem do *B. tuldoides,* e no **capítulo 7 – Young bamboo culm starch from *Bambusa vulgaris* - characterization and evaluation of technological properties,** apresentou-se a caracterização do amido extraído do colmo jovem do *B. vulgaris.*

Devido à sua composição rica em fibras e amido, a farinha do *D. asper* foi selecionada para aplicação em formulações de biscoito doce tipo *cookies*, em substituição parcial ao açúcar e/ou gordura, como apresentado no **capítulo 8** – **Efeito da adição de farinha de colmo jovem de bambu como substituto de**

açúcar e/ou gordura em formulações de *cookies.* Utilizou-se um delineamento com duas variáveis (redução do teor de açúcar e redução do teor de gordura) para selecionarem-se as formulações que passariam pela avaliação nutricional e *shelf-life*.

No **capítulo 9 – Discussão Geral,** apresenta-se uma sinopse dos resultados mais importantes obtidos na caracterização das farinhas e dos amidos extraídos dos colmos jovens das três espécies de bambus avaliadas. Apresenta-se também uma avaliação do efeito da adição da farinha do colmo jovem de *D. asper*, em formulações de *cookies* com reduzidos teores de açúcar e de gordura. E, no **capítulo 10 – Conclusão Geral,** apresentam-se alguns resultados de forma resumida, de modo a condensar as informações de cunho técnico, consideradas de maior importância.

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– CAPÍTULO 2 –

Bamboo: a new-old culture for construction and food industry

Este capítulo apresenta uma revisão sobre o plantio e a produção mundial do bambu, bem como apresenta os diversos usos, indo desde as cercas, artesanatos, e material de construção até o broto em conserva, expandindo as possibilidades de uso desta cultura.

Este capítulo está publicado no livro Agricultural Research Updates, Nova Science Publishers, New York, Volume 12, capítulo 8, páginas 129-141, 2015, e com autorização da Editora para apresentação na tese (Anexo 1).

Bamboo: a new-old culture for construction and food industry

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ABSTRACT

Bamboo is a perennial grass, which is widely distributed throughout the world, with about 50 genera and 1300 species. However, its incidence is higher in humid and warmer areas, such as tropical and subtropical regions of Asia, Africa and South America. In Amazonia (Brazil) there is one of the largest native bamboo reserves in the world. However, the sector of bamboo extraction in Brazil is informal without industrial scale production and without the establishment of a systematic production chain. Bamboo is the most abundant renewable source of natural biological compounds in the world, with several advantages in cultivation, including rapid growth and high renewability. This chapter aims to show the possibilities of the use of bamboo in building material and food. In section of building material, it was presented the use of bamboo in manufacturing panels, flooring, pulp, charcoal, and also scaffolding, because of its mechanical properties. It can be used too on irrigation, house building, kiosks and sheds structure, composites based on cement and particulate bamboo (biokreto), glued laminated bamboo (GLB), and in the production of bamboo sheets or particles for obtaining the bamboo composite resin. In food section, it was reported the use of bamboo shoots in the feed, which can be commercialized as fresh, canned and pickled forms. They are a low-calorie food, rich in fibers, proteins, carbohydrates, amino acids, minerals, fat, sugar and inorganic salts. The only drawback of the use of bamboo shoot is the presence of potentially toxic compounds. Finally, this chapter presents the possibilities of expanding the uses of bamboo in order to improve this important crop for countries whose climate and soil conditions are beneficial to its growth, since bamboo is an easy planting and maintenance plantation.

Keywords: Bamboo, building material, food.

1 INTRODUCTION

Bamboo is a renewable source of natural biological resources which presents some advantages on its cultivation, as quick growth, high resistance to pests and good flexibility. Even young culms can be used for weaving purposes or for the production of baskets (Pereira and Beraldo, 2010).

The bamboo is a grass that belongs to the family *Poaceae* and subfamily *Bambusoideae,* which has approximately 50 genera and 1300 species. It is distributed in the world, predominantly in the tropics and temperate regions, such as Asia, Africa and South America (Terra, 2007; Manhães, 2008; Pereira and Beraldo, 2010; Yuming and Chaomao, 2010).

China is the biggest producer of bamboo, where 6 millions of hectares are planted, generating an economy of approximately US$ 7 billion/year, and the plantations are controlled by government (Scurlock, Dayton and Hames, 2000; He et al., 2014).

India is also a big bamboo producer, with 130 species that occupy around 13% of the total area of forests. Bangladesh, Indonesia and Thailand present also a substantial production (Scurlock et al., 2000), and in Africa, bamboos are distributed in a limited region, from the south to the southeast of Mozambique, and from the north to the east of Sudan, as well as in the island of Madagascar, where it has the bigger floral diversity than the whole continent (Yuming and Chaomao, 2010).

In Brazil, there are 89% of all genera of bamboo and 65% of all species known in America (Pereira and Beraldo, 2010). The biggest forest reservation of native bamboo in the world (180,000 km^2) is located in the southeast of

Amazonia (Brazil), being an economically sustainable solid possibility that is still not explored (Judziewicz et al., 1999; Manhães, 2008).

According to INBAR, in 2007, the international commerce of bamboo was estimated in 5 billion of dollars. Only in Taiwan, the exports of comestible bamboo shoots accounted almost 50 million of dollars, and in the Philippines the furniture industry billed 1.2 million of dollars. In India, around 2.2 millions of tons of bamboo were used only to produce paper, and in the Philippines the furniture industry scored 1.2 millions of dollars (Manhães, 2008).

However, in Brazil there is no establishment of a systematic productive chain, due to the following facts:

- The plantations are small scale, made by small producers;
- Due to the many scattered clumps, there is no concern to invest in a systematic planting; and
- There is no official data about bamboo market neither about its current demand (Dantas, 2005).

In Brazil, the law nº 12.484 (Brazil, 2011), sanctioned on September 8th, 2011, instituted the National Policy to Encourage Sustainable Management and the Cultivation of Bamboo. Since then, increasing demands from pharmaceutical and food industries may have conducted to a change in bamboo management. The objective of this law was to stimulate the sustained management of native formations and to the cultivation of bamboo related to the production of culms, shoots extraction and the obtention of environmental services, as well as the appreciation of these environmental assets as means of ensuring regional socioeconomic development. It was an important step to improve this agricultural culture and its productive chain, once it has the potential to grow.

Thus, the purpose of this chapter is to present the main scientific work already done with bamboo, aiming to increase the dissemination of this culture, which has great growth potential at Brazil.

2 BAMBOO SPECIES IN BRAZIL

Brazil is the country with the greatest diversity of bamboo species, with 34 genera and 232 species of native bamboo, from them 174 species are considered endemic. The many types of bamboo range from small species, with few centimeters of height, mainly for ornamental purposes, to giant species, which can reach 30 m (Pereira and Beraldo, 2010). The species are exotic and originally from East, except the genera Guadua, which is originally from America, and it has been used in Colombia and Ecuador (Pereira, 2001; Pereira and Beraldo, 2010).

The Brazilian states of Pernambuco, Paraíba and Maranhão have significant agro-industrial uses of bamboo, concentrating only in this region 40,000 hectares, which are intended for the production of pulp and paper (Nunes, 2005).

The natural bamboo forests are called *tabocais* in Brazil and of *pacales* in Peru. They cover a big area in the Brazilian state of Acre, expanding to Bolivia and Peru (Judziewicz et al., 1999; Filgueiras and Gonçalves, 2004). The word *tabocais* is the plural of *tabocal*, which means a population of *taboca*, the name given by Brazilian native groups, to designate the bamboo from genera *Guadua* and *Chusquea*.

Among the principal species of bamboo, recommended by INBAR, based on criteria for cultivation, processing and products, genetic resources and edaphoclimatic characteristics, are: *Bambusa* (species *bambos*, *blumeana*, *polymorpha*, *textilis*, *tulda*, *vulgaris*), *Cephalostacyium pergracile*, *Dendrocalamus* (species *asper*, *giganteus*, *latiflorus*, *strictus*), *Gigantochloa* (species *apus*, *levis*, *pseudoarundinacea*), *Guadua angustifolia*, *Melocanna baccifera*, *Ochlandra spp*, *Phyllostachys pubescens*, *Thyrsostachys siamensis* (Pereira and Beraldo, 2010).

The most common species of cultivation in Brazil are *Bambusa vulgaris* and *Dendrocalamus asper* (Filgueiras and Gonçalves, 2004), whose main features are described as follows.

A. Bambusa vulgaris

The specie of *Bambusa vulgaris* is from Asia and it was brought to Brazil by Portuguese immigrants, and it is widespread in the country and used in construction, paper production, furniture manufacture and for handcrafts. This specie forms a kind of bamboo clump, medium-sized (15-25 m), which grows well at altitudes up to 1,500 m, supporting a minimum temperature of -2 °C (Spolidoro, 2008; Pereira and Beraldo, 2010).

Commercially it is known from two large plantations in Brazil of this specie: one in Maranhão and the other one in Pernambuco. However, the greatest use of this variety is for paper manufacture, by João Santos Industrial Group, which produces bags for packing Portland cement (Nunes, 2005).

B. Dendrocalamus asper

This specie is kind of giant bamboo that forms a clump, which culms can reach up to 30 m. It grows well in humid and semi-arid regions, with rich soils, supporting up to 1000 m altitude. It is naturally distributed in India, Thailand, Vietnam, Malaysia, Indonesia, the Philippines and Brazil. This species is habitually called "bamboo bucket" due to its large diameter of culm, which can reach up to 25 cm (Akira, Alexandre et al., 2007), and so it is also commonly misidentified as *Dendrocalamus giganteus.*

This variety has been widely used in civil constructions at rural areas, but its greatest use is for the production of edible shoots, which are considered more sweet and tasty (Pereira and Beraldo, 2010).

3 BAMBOO’S INDUSTRIAL APPLICATIONS

The bamboo is acquainted by its industrial use in the manufacture of products like panels, floor, cellulose, coal, scaffolding and canned food. Furthermore, medications can be produced taking advantage of the high content of phytosterols present in bamboo shoots (Xuhe, 2003; Lo et al., 2008; Pereira and Beraldo, 2010; Chongtham, Bisht and Haorongbam, 2011).

Many researchers, as summarized in the Table 1, have related the different uses of bamboo, natural, processed, isolated or combined with other building materials. It can be mentioned its utilization as pipe to conduct water for irrigation, for building houses using as sealing elements in braided panels, for structuring kiosks and sheds in composition with concrete, in the formation of structured ceramic plates with bamboo in cement based composites and particulate bamboo (biokreto), in the form of glued laminated bamboo, in the production of bamboo plate particles, for obtaining the bamboo resin composite and bamboo glass fibers (BRF) or with rubber residues, as cited by Pereira and Beraldo (2010).

The bamboo fibers are the sclerenchyma and they are the main responsible for the mechanical strength of entire culms. They occur in the internodes and protect the vascular bundles, and they constitute 40 to 50% of the total tissue of culms and 60 to 70% of their weight (Figure 1) (Spolidoro, 2008). Due to bamboo fibers being long and narrow, bamboo has been chosen to substitute the raw material commonly used, such as eucalyptus and pine, since it gives the products better physical characteristics, due to the greater intertwining of the fibers (Matos Junior, 2004; Manhães, 2008). Their growth characteristics, especially intertwining system of rhizomes and roots, has high potential in erosion control and rehabilitation of soil, water conservation and carbon sequestration (Ben-Zhi et al., 2005).

There is a growing awareness of Brazilian society about the huge potential of using bamboo. A small but very enthusiastic group emerged in 2003 and is growing rapidly with the creation of Bamboo Institute (http:www.bambubrasileiro.com, accessed on 10/03/2015). This organization is dedicated to the development of new ways to build houses for the poor people, using bamboo as the main raw material.

Table 1. Several applications of industrial bamboo.

Product	Bamboo form	Mixture with other ingredients	Application	References
Paper and cellulose	*in natura*	No	Food, medicine, meat, detergent, seeds and bran packaging.	Manhães (2008)
Charcoal	*in natura*	No	Biomass and to sewage treatment	Presznhuk (2004)
Line	Entire culm *in natura*	No	Irrigation.	Pereira and Beraldo (2010)
“Bambucreto”	Waterproof culm or cut	Concrete	Column and griddle to civil construction.	Pereira and Beraldo (2010)
“Biokreto”	Particle	Cement	Pressed plates, manufacturing vases ornamental pieces, floors, sidewalks, canals, hollow blocks, corrugated roof tiles, among others.	Pereira and Beraldo (2010)
“BLC” - glued laminated bamboo	Cut or lath	Adhesive	Manufacture of tables, chairs, tools, flooring, among others.	Pereira and Beraldo (2010)
Griddle	Particles pieces of the culm	Organic resin or vegetables	Mats, plywood, panels, among others.	Pereira and Beraldo (2010)
Bamboo resin composites and glass fiber (BRF)	Braided bamboo culms and cuts	Composite of glass fiber and resin	Bamboo mats, bamboo culms frames, frameworks and windows.	Pereira and Beraldo (2010)

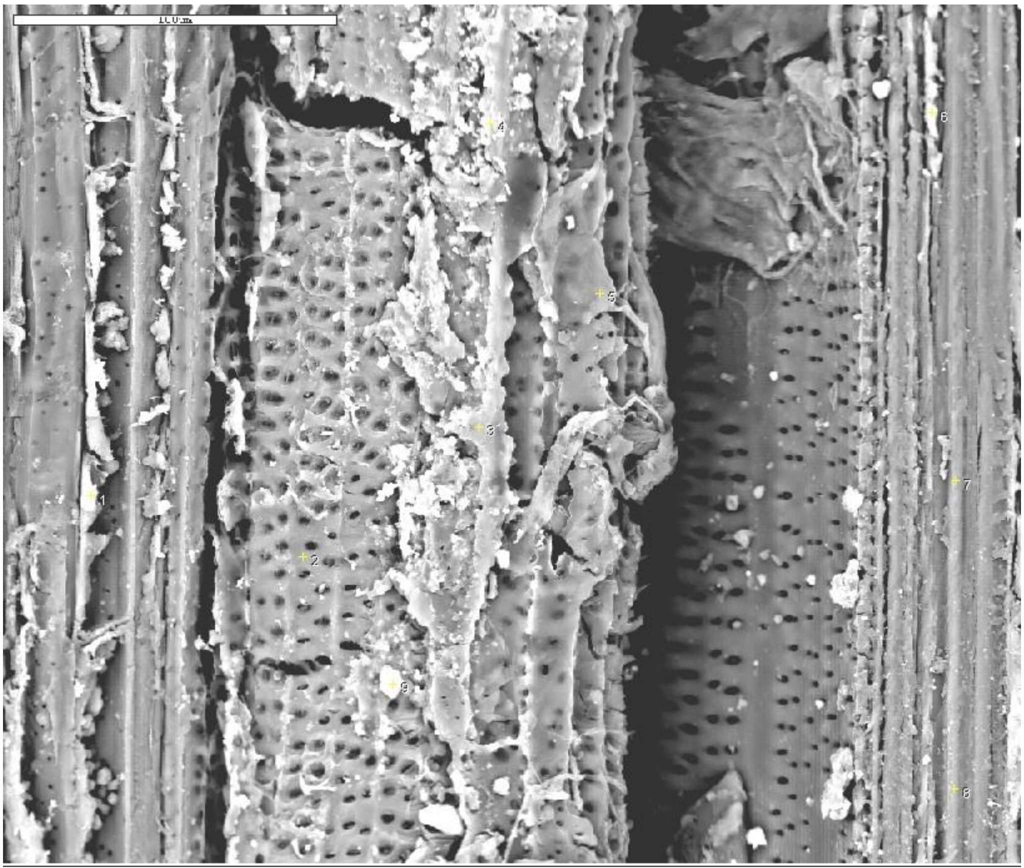

Figure 1. Bamboo culm sectioned shows the parenchyma cell with starch at left, a vessel at the center and fibers on the right.

3.1 Stability of products derived from bamboo

Bamboo is a biological material, and therefore is subject to deteriorate by the action of fungi and insects, with a very variable life, ranging from one to three years, if left untreated, and between 10 to 15 years or more when appropriately treated (Pereira and Beraldo, 2010).

The lack of bamboo stability is related to the presence of starch (Figure 2) and water, being mainly attacked by powderpost beetles, especially *Dinoderus minutus*. It can be said that higher the starch content in bamboo, the greater the likelihood that it will suffer the attack of powderpost beetles (Azzini et al., 1981; Pereira and Beraldo, 2010). The young culms, harvested before issuing the branches and leaves, are not attacked, although they have the same dimensions of mature culms, probably due to the absence of starch in them (Pereira and Beraldo, 2010).

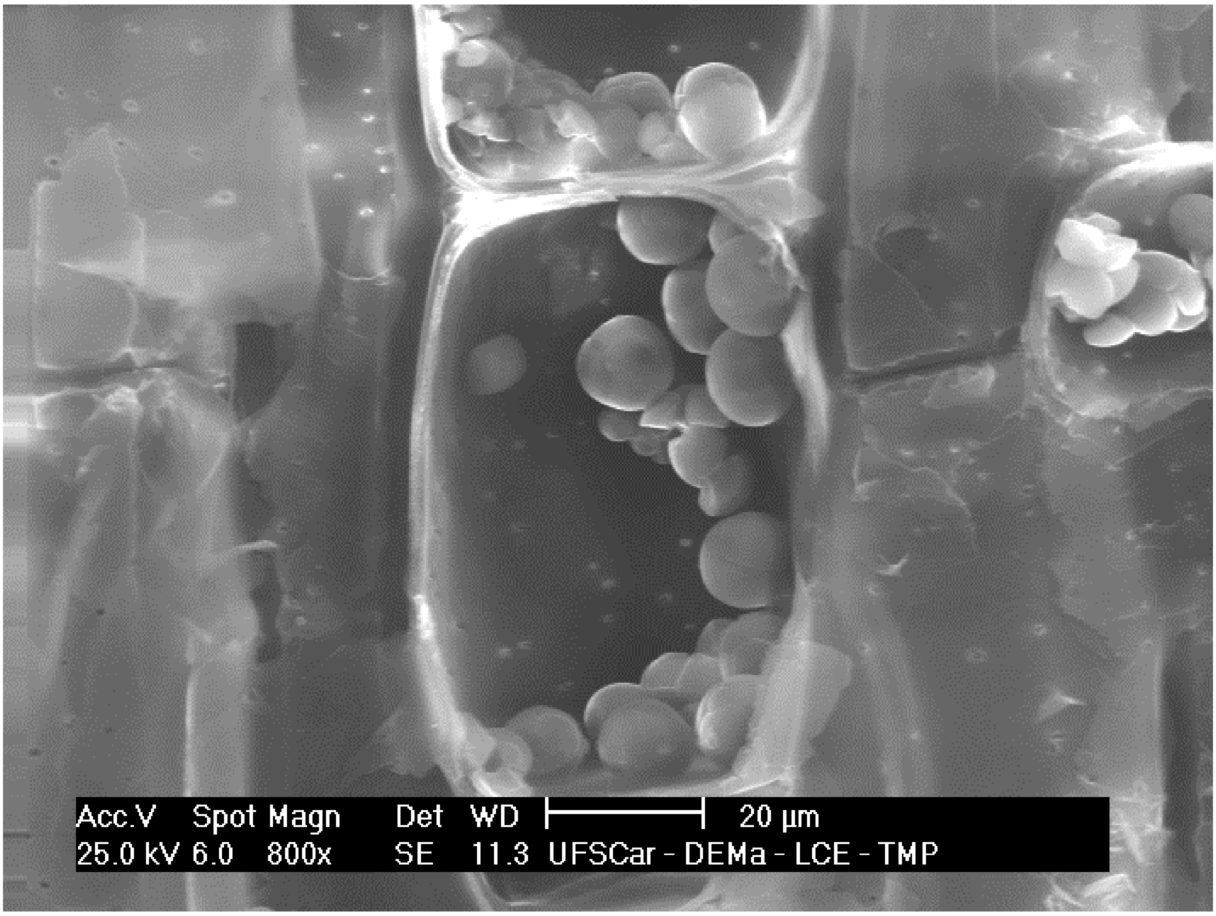

Figure 2. Bamboo starch granules within the parenchyma cells.

The high perishability of bamboo has been an important point, because of its high starch and sugar content, which serve as food for fungi and/or some insects. Some studies have indicated that the content of three sugars, such as glucose, sucrose and fructose, and also the starch content of the bamboo will depend on the time of year it is harvested (Okahisa, Yoshimura and Imamura, 2005).

4 EDIBLE APPLICATIONS OF BAMBOO

The bamboo shoots are rich sources of certain nutrients and act as a nutraceutical ingredient, since they present an additional physiological benefit for the consumer's health. They feature phytosterols on their composition and a high content of fiber, thereby contributing to the reduction of cholesterol levels (Chongtham et al., 2011). Older bamboo culms have very similar composition to wood, being basically composed of cellulose, hemicellulose and lignin, which account for over 90% of the total mass (Youdi et al., 1985). In bamboo leaves, antioxidants have been found with strong inhibitory effects on the transition metal ions and free radicals (He et al., 2014).

4.1 Utilization of bamboo shoots

Worldwide, more than 2 million tons of bamboo shoots are consumed annually, from them about 1.3 million tons are produced only in China (Kleinhenz et al., 2000; Xuhe, 2003; Chongtham et al., 2011). Despite this huge production of bamboo shoots, the use of the culms is still small in food applications, due to a cultural resistance to the acceptance of this material (Beraldo and Azzini, 2004; Manhães, 2008).

The bamboo shoots have in their composition especially potassium, calcium, manganese, zinc, chromium, copper and iron (Nirmala, David and Sharma, 2007). When fresh, they are excellent sources of vitamins A, B1, B3, B6 and E (Visuphaka, 1985). Their fat content is relatively low and the sugar content is lower than other vegetables, while the water content is around 90%. The protein content although low, has 17 amino acids, eight of which are essential to the human body; for these advantages the bamboo shoot is widely used as a traditional food in many cultures (Chongtham et al., 2011; Choudhury, Sahu and Sharma, 2012). Bamboo shoots are very also enjoyed as appetizers, since they are crunchy and soft, and have a sweet taste.

Food products of canned bamboo shoots dominate the international trade, but due to an increased desire of consumers for unprocessed foods, it is expected a significant increase in the consumption of fresh bamboo shoots (Kleinhenz et al., 2000).

The inconvenience of using fresh bamboo shoots is the presence of potentially toxic cyanogenic compounds, as taxiphyllin, which through hydrolysis unfolds in hydroxybenzaldehyde and hydrogen cyanide (HCN) (Ferreira, Marsaioli and Iaderoza, 1991; Azzini et al., 1995). The removal of these compounds can be done through the proper preparation prior to consumption. In the case of bamboo shoots, it should be cut into thin slices followed by boiling in lightly salted water for about 8 to

10 minutes, which leads to the breakage of the cyanogenic glycoside and subsequent removal of the resulting hydrogen cyanide (Fsanz, 2004).

Several studies have revealed that bamboo shoots have a number of benefits for the consumer's health, such as improved appetite and digestion, contributes to weight loss, and healing of some cardiovascular diseases and some cancers. The shoots are recognized for their anticancer, antibacterial, and antiviral activities (Chongtham et al., 2011).

4.2 Utilization of bamboo culm

According to Azzini et al. (1981), the bamboo culm (*Guadua flabellata*) showed approximately 27% of polysaccharides determined as starch, about 50% of crude fiber, 30% soluble and 20% insoluble, which presents potential for use in human food. The term crude fiber is restricted to quantify the fiber content using non-enzymatic analytical methods, underestimating the total fiber content, since this measurement includes only a portion of the insoluble fiber fraction.

Bamboo culm presents cellulose and lignin contents higher than the ones found in corn husk, some grass and aquatic plants. Sun et al. (2011) and Li et al. (2014) observed cellulose content of 47% in bamboo, which is 9% and 10% more than corn husk and the grasses, respectively. According to He et al. (2014), bamboo culm has a lignocellulose total content of around 83%, indicating that bamboo has the highest sugar content and can therefore be considered as potential raw material for ethanol production.

Literature has reported different values of fibers present in bamboo culm depending on the variety and the site of assessment (base, middle or top of the culm). Azzini and Beraldo (2000) observed that fibrous fractions culms were around 608-742 g/kg and the residual fiber 127 to 182 g/kg, which variation is a function of the region of extraction. For species *Dendrocalamus giganteus* and *Gigantochloa verticillata* the highest levels were observed in the middle regions of the stems.

The fibers are the sclerenchyma and occur in internodes of the culms, serving as protection of the vascular bundles. They constitute 40 to 50% of the total tissue of the culm and 60 to 70% of its mass. In the transverse direction (perpendicular to the bamboo culm), the fiber bundles are concentrated on the outside wall of the stems, in contrast to the inside, which concentrates the parenchymal tissue. In the vertical direction, the fiber content increases from the bottom to the top of the culm, where the amount of parenchyma cells decreases (Spolidoro, 2008). Greater use of bamboo culm is required, since in the production of shoots only about 20 - 30% of the culms can be removed, and the rest should be left for promoting the renewal of the clump, allowing its expansion. Thus, the partial cut of bamboo grove makes sustainable extraction and makes each plantation to last more than one hundred years (Rigueira Junior, 2011).

The dietary fibers have all the characteristics necessary to be considered as an important ingredient in the formulation of a functional product, due to their beneficial health effects. Several studies have observed the effect of fiber intake on reducing the glycemic index and cholesterol level, increased fecal matter, reducing the intestinal transit time and also the reduction in the time of formation and action of carcinogens, reducing the incidence of various diseases (Beecher, 1999; Dhingra et al., 2012).

Several researches were reported about the chemical composition and structural characterization of fiber type hemicellulose that comprise the bamboo. Hemicelluloses are complex polysaccharides present in the cell walls of plants and are considered to be the second most abundant polysaccharide in nature, after cellulose.

Hemicelluloses have low molecular weight branched chains, with degree of polymerization ranging from 80 to 200, and that consist mainly of molecules of xylose, arabinose, galactose, mannose and 4-O-methyl-D-glucuronic acid. Usually hemicellulose is linked to cellulose microfibrils by hydrogen bonds and/or interlocking connections, and they are linked to lignin by ferulic acid. This acid esterifies its carboxyl group with hydroxyl from arabinose and etherifies its hydroxyl group with phenyl hydroxyl lignin (Jeffries, 1991; Peng, Peng, Bian, Xu, Sun, et al., 2011a; Peng, Peng, Bian, Xu and Sun, 2011b). Because of this complex structure, studies for use and evaluation of its properties are not as simple and special attention has been paid to the chemical components and their structural characterization (Peng and She, 2014).

Recently some studies have showed that bamboo can be considered as a great candidate for biomass production. Other value-added applications have also been evaluated as bioethanol production, bioflavonoid extraction and functional fibers such as xylooligosaccharides (Tsuda, Aoyama and Cho, 1998; Scurlock et al., 2000; Kobayashi et al., 2004; Littlewood et al., 2013; He et al., 2014). Some of these fibers can be converted into other compounds such as xylooligosaccharides for xylitol production (Aoyama and Seki, 1994; Miura et al., 2013; Xiao et al., 2013).

Potential applications for bamboo hemicellulose have been evaluated, attracting a growing volume of research in recent years due to its biodegradability, excellent biocompatibility and physicochemical properties. Several research groups have reviewed studies on hemicellulose biorefinery and bioconversion to ethanol, however, their focus has been mainly in the conversion of the bamboo hemicellulose into biomaterials, biofuels and food, opening future directions for the use of whole bamboo (Peng and She, 2014).

Biomass production can occur in two ways: by enzymatic or non-enzymatic process, and each one of these methods has its advantages and disadvantages, making a challenge for large scale production. The production of sugars, such as xylose, mannose, and arabinose, from readily available agricultural residues, as bamboo culm, offers an excellent opportunity to improve human health and the global economy. According to Otieno and Ahring (2012), these compounds are of high value and have become increasingly important for the food and pharmaceutical industry, and therefore, knowledge of the molecular structure of the hemicelluloses favors new possibilities for production in commercial scale.

The medium chain hemicelluloses (3 to 10 molecules of xylose, mannose and arabinose, in varying concentrations) play an important role in gastrointestinal health due to their prebiotic effect, as they are non-digestible. Their physiological benefits, especially stimulating the proliferation of lactic acid bacteria and bifidobacteria of colon, corroborate their nutraceutical role, of high added value in the food and pharmaceutical industries (Otieno and Ahring, 2012).

Some studies have noted that the bamboo hemicellulose is decomposed preferably into xylose and xylooligosaccharides (Ogaki et al., 2009; Bian et al., 2013; He et al., 2014). Due to the different characteristics of bamboo and hemicellulose complex structure, the xylan can be extracted with different concentrations of KOH: the yield obtained with 5% KOH solution is 50% hemicellulose, whereas a solution with 18 % KOH can extract almost all of the hemicellulose (Ma et al., 2014).

An important biomaterial is film for flexible packages made with hemicellulose. It presents excellent barrier properties to oxygen, making it a potential candidate for applications for food products. Up to now, a significant number of native and modified hemicelluloses have been developed for use alone or in mixture with other polymers (starch, chitosan, lignin, alginates and plasticizers, for example) to have improved flexibility (Peng and She, 2014).

Thus, both the bamboo sprout and the culm may be considered as an alternative source of dietary fiber for use in food.

5 FUTURE PROSPECTS

Bamboo is already used for food, pharmaceutical and furniture and building applications. The problems of its high perishability should be solved through treatments that prevent the attack of insects and powder post beetles. The outlook is based on better utilization of dietary fiber in an isolated and purified form, for food application, since its high content of hemicellulose points to a beneficial health ingredient.

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– CAPÍTULO 3 –

Young bamboo culm flour of *Dendrocalamus asper*: Technological properties for food applications

Este capítulo apresenta o estudo preliminar do colmo jovem do bambu da espécie Dendrocalamus asper, através da separação do colmo em três partes e da elaboração da farinha, com duas granulometrias diferentes.

Este capítulo foi publicado no periódico LWT – Food Science and Technology, volume 76B, p. 230-235, 2017 (https://doi.org/10.1016/j.lwt.2016.06.015), conforme Anexo 2.

Young bamboo culm flour of *Dendrocalamus asper*: technological properties for food applications

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ABSTRACT

Although bamboo is an agricultural product that moves billions of dollars in international trade, only its shoot has fiber production and use in food application. This study aimed to produce and evaluate the physicochemical and technological characteristics of young bamboo culm flour of *Dendrocalamus asper* for food application. Young culms were collected and divided into three fractions: bottom (B), middle (M) and top (T). The fractions were dried, ground, sieved and separated into two different particle sizes: F1 (d>0.425 mm) and F2 (d≤0.425 mm), totaling six samples: BF1, BF2, MF1, MF2, TF1 and TF2. The flours presented significant differences in proximate composition, whereas ash, protein and lipid contents were below 2%, and these flours can be considered as sources of fiber (67-79 g/100g) and starch (from 6 to 16 g/100g). Water absorption index (WAI) and water solubility index (WSI) were significantly higher for the bottom samples (BF1 and BF2), compared to the other flours. Color parameters indicated white yellow color for all flours, being all the samples F1 (BF1, MF1 and TF1) clearer than flour F2. The results of this study open new perspectives for the food application of young bamboo culm flour.

Keywords: Fiber, flour, healthy, unexplored ingredient, young bamboo culm.

1. INTRODUCTION

According to International Network for Bamboo and Rattan (INBAR, 2012), on international trade, the domestic market for bamboo and rattan products, in 2012, was estimated at about US$ 34,2 billion. However, official data of bamboo market in Brazil and its commercial demand are not available (Judziewicz et al., 1999; Manhães, 2008).

Bamboo is a grass, belonging to *Poaceae* family, as maize, wheat, rye, oat, sugarcane, barley and rice, and it is spread over 1,250 species under 75 genera. Excepting Europe, bamboo occurs naturally in tropical, subtropical and temperate regions of all countries (Liese and Kohl, 2015).

Asia possesses the highest bamboo reservoirs, with a total area over 10 million hectares in southeastern Asia, and about 5 million hectares in China. In the counterpart, in Brazil there are 18 million hectares, located in the southwestern Amazonas, which is one of the biggest reservoirs of native bamboo in the world (Judziewicz et al., 1999; Yuming and Chaomao, 2010). Although the total forest area in many countries has drastically decreased, bamboo forest area has progressively increased (Ben-Zhi et al., 2005).

Dendrocalamus asper (Schult. & Schult.) Backer ex. K. Heyneke is important specie bamboo plantations in Brazil. It is a kind of giant bamboo commonly misidentified as *Dendrocalamus giganteus,* because of its large culm diameter. In India, Thailand, Vietnam, Malaysia, Indonesia and the Philippines, where it has been widely used for housing at many rural areas, and it has been used for handicrafts, daily utensils, furniture and paper (Akira, Alexandre et al., 2007). However, its greatest use is for the production of edible shoots, which are considered sweet and tasty (Pereira and Beraldo, 2010;INBAR, 2012).

In a review article, Chongtham et al. (2011) brought together researches that showed the great potential of using bamboo shoots in the forms fresh, canned or pickled, for human feeding. They explained bamboo shoots are a good source of vitamins, minerals, amino acids, and dietary fiber. They contain vitamin A, B6, C and E, and they have 17 amino acids, 8 of which are essential for the human body, as for example lysine (Visuphaka, 1985; Nirmala et al., 2007; Satya et al., 2010; Chongtham et al., 2011; Choudhury et al., 2012; Nongdam and Tikendra, 2014).

According to data compiled by Chongtham et al. (2011) fresh shoots (90% moisture) contain as the major nutrient fibers (6-8 g/100g) and proteins (1.49-4.04 g/100g) and a content lower than 2% is from other nutrients. The fresh bamboo shoots are being used for fiber extraction, which has been sold in the international market, by several companies, for example, the already commercialized Jelucel®BF (Jelu-Werk, 2016), Nutriloid® Bamboo Fiber (Tic-Gums, 2016) and CreaFibe. And the advertisement used by these companies is their several uses for food applications, to enhance the dietary fiber content of various products, like breads, waffles, pasta, meat products, cheese, yogurt and confectionary.

The increased production of bamboo shoots fiber for food applications may change the sustainability of bamboo clumps, since only 20-30% of shoots can be annually removed (Pereira and Beraldo, 2010). Therefore, the use of young bamboo culm could be an alternative to this demand in the market, because of its higher yield in fibers production.

There is some time that bamboo has been studied, but only recently its culm has gained special attention. Many researchers observed that it has a high content of crude fiber and other polysaccharides, as starch and sugar (Sun et al., 2011; Li et al., 2014). According to Banik (2015) the young bamboo culms have a peak of starch during their growth. The rhizomes of 2-3-years-old culms (young culms) are rich in reserve nutrients (starch and nitrogen) and these culms, because of their plentiful leaves, play a functional role in the plant's metabolism. However, these contents depend not only on the age of bamboo culm, but also on the variety and the culm position, if it is in the bottom, middle or top of the culm (Azzini and Beraldo, 2000). Mainly because of its starch content, it is known that untreated bamboo culms (*in natura*) can be attacked by powder-post beetles, like *Dinoderus minutus* Fabricius (Azzini et al., 1981; Pereira and Beraldo, 2010), and this data indicate that besides the fiber source, the young bamboo culm may also be a source of starch, since there is also demand for new sources of starches.

Until now, few studies have analyzed the physical and chemical composition of bamboo culms for their use in food applications (Azzini et al., 1981; Azzini et al., 1995; Peng, Peng, Bian, Xu, Sun, et al., 2011; Peng and She, 2014). Taking this into account, this study aimed to produce and evaluate physicochemical and technological characteristics of young bamboo culm flour of *D. asper*, as a new ingredient for food products.

2. MATERIAL AND METHODS

2.1. Material

Bamboo culms of *D. asper* was harvested at experimental field from FEAGRI/UNICAMP, Campinas/Brazil, on geographic coordinates 22º82' of south latitude and 47º07' of west longitude. Since the starch was the research interest, the culm collection should be of young culms (on average three years old) and performed during summer to rainy period (January to March of 2015), when the culm was with the apex of starch content. The diameter of the culms was not a relevant parameter due to the absence of vascular cambium in bamboo trees, and they do not increase in diameter with age. A random sampling system was respected to collect three culms.

The young culms were divided in three portions: bottom (B), middle (M) and top (T) (Figure 1-A), and for all the samples, 30cm were discarded between the portions (Figure 1). The cut of the young bamboo culms, as well as the division of the three parts were carried out with a chainsaw.

2.2. Methods

2.2.1. Preparation of the young bamboo culm flour

Each one of three portions of young bamboo culm was cut into little cylindrical pieces with an electric circular saw. Each piece was cut with a chainsaw and then manually in smaller pieces (8cm x 3cm). Sodium metabisulphite solution (200 ppm/1h) was used to prevent growth of microorganisms. After that, the small pieces of bamboo culm were drained and dried in an oven with forced air circulation (50 ºC/72h), until they reached a moisture content below 12%. After drying, the bamboo culm pieces from each portion were ground in a mill of corn husks, branches, roots and tubers (DPM2–Nogueira, São Paulo, Brazil), and then the samples were sieved to two diameter sizes (d): fraction 1 (d>0.425 mm) and fraction 2 (d≤0.425 mm) (Figure 1-B). Bamboo culm was separated into two fractions of different sizes because part of this material would be subsequently applied in a mixture with other ingredients for use in civil construction. Finally, the samples were ground in a ball mill to obtain homogeneous flour to perform the analyses (Figure 1-C).

Therefore, six different samples of bamboo culm flour were evaluated: BF1, BF2, MF1, MF2, TF1 and TF2 (bottom, middle and top fractions 1 and 2, respectively). Afterwards, all the samples were packed in plastic containers, hermetically sealed and stored under refrigeration until analysis.

2.2.2. Physicochemical characteristics of young bamboo culm flour

The bamboo culm flours were evaluated by the following analyses according to AOAC (1998): moisture (method 925.09), ash (method 923.03), protein (method 960.52), lipid (method 920.39), total dietary fibers (methods 992.16), total starch (method 996.11) contents. Other carbohydrates content were calculated by difference. The total sugar content was determined according to AOAC (1984) by Lane-Eynon method and evaluates the contents of reducing and non-reducing sugars (method 31.034-6).

2.2.3. Technological and instrumental characterization of young bamboo culm flour

The pH (method 943.02) of the samples was determined according to AOAC (1998). The water absorption index (WAI) and water solubility index (WSI) were evaluated according to Anderson (1982). The water absorption index is the weight of gel obtained per gram of dry sample, for measuring swelling power of starch, and as an index of water solubility, the amount of dried solids recovered by evaporating the supernatant from the water absorption test was expressed as percentage of dry solids in the weight of sample (2.5 g). Instrumental color analysis was performed on CR-10 colorimeter (Konica Minolta, Tokyo, Japan) with a 50 mm port size, illuminant D65, SCI and a 10º standard observer angle. The tri-stimulus CIELab color space method was used, determining the lightness (L*), a* (+, red, to -, green) and b* (+, yellow, to -, blue) values.

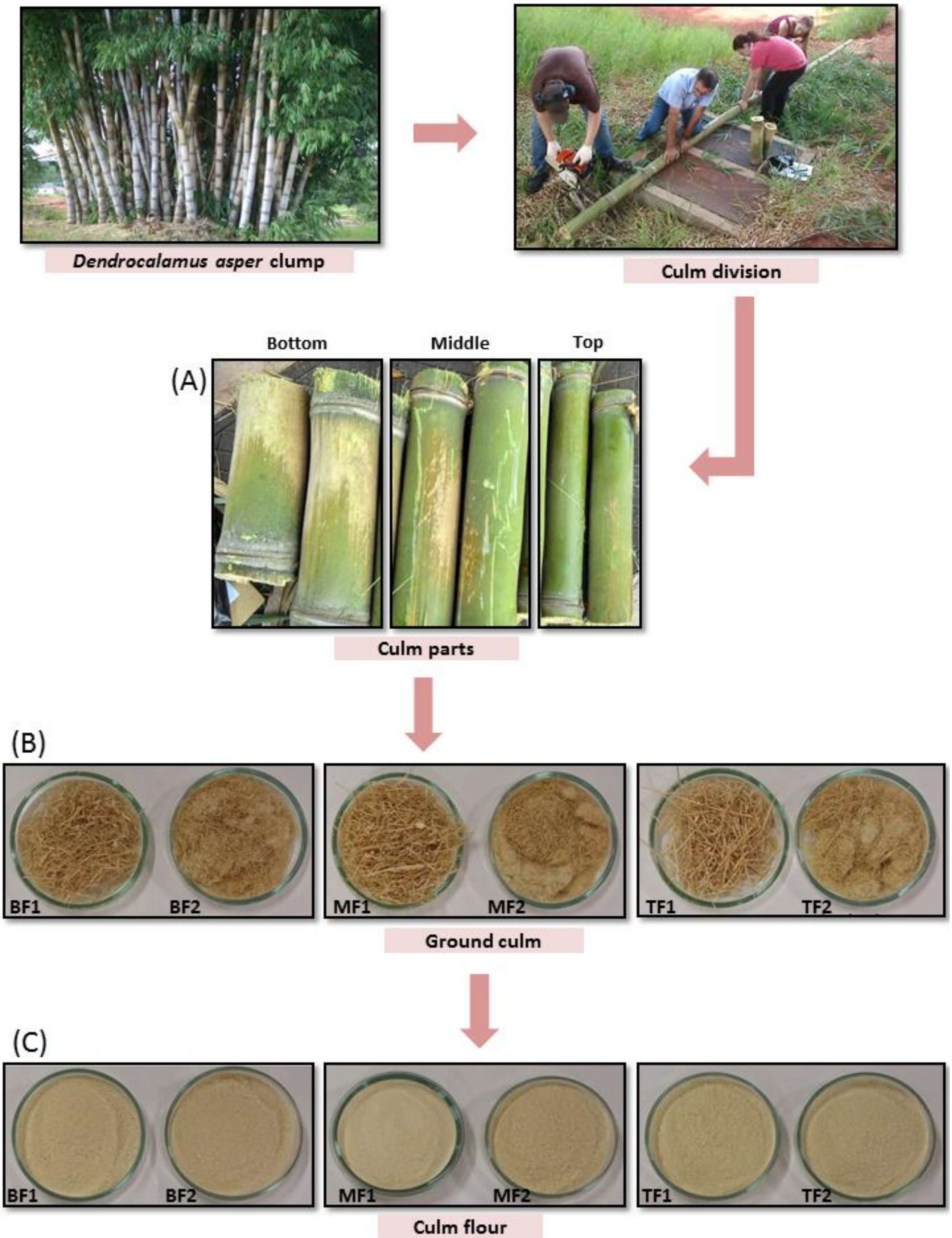

Figure 1 - Schematic illustration of harvesting and grinding of young bamboo culms of *Dendrocalamus asper*. BF1, BF2: Bamboo bottom culm flour from fractions 1, 2; MF1, MF2: Bamboo middle culm flour from fractions 1, 2; TF1, TF2: Bamboo top culm flour from fractions 1, 2.

2.3. Statistical analysis

All the analysis were performed in triplicate and analysis of variance (ANOVA) of the means was performed using the Sisvar software, version 5.6 (Federal University of Lavras, Lavras, MG, Brazil) at a significance level of 95%. When significant, the Scott-Knot test was used to determine statistical differences between means ($p \leq 0.05$) (Ferreira, 2008).

3. RESULTS AND DISCUSSION

3.1. Physicochemical characteristics of young bamboo culm flour

Table 1 shows the proximate composition of young bamboo culm flour, including total fiber, total starch and other carbohydrates contents by difference. The moisture content is consistent with the technical regulation of cereal flours which requires a maximum of 15 g/100g of moisture, for storage and marketing (Brasil, 2005). The moisture content is an important parameter because it is considered one of the main acceleration factors of chemical and enzymatic reactions, and it can influence the quality of the product (Gutkoski and Jacobsen Neto, 2002).

The values obtained for protein content (1.31-1.75 g protein/100g) are very low, similar to the common grass, like *Brachiari*a, *Cynodon*, *Paspalum* and *Panicum*, which are used for hay and forage production (Soares Filho, Rodrigues and Perri, 2002). Similarly, the data obtained for lipids were also very low (0.27-0.90 g/100g). Although the samples showed significant differences between them in relation to protein and fat levels, they are very poor in these two components, being similar to cassava flour (Chisté et al., 2006).

Additionally, the data obtained shows that *D. asper* is rich in fiber (67.27-79.61 g/100g) with significant differences between the samples, where the highest values were observed for BF2 and TF2, and the lowest were found to MF1. The fiber content of these bamboo culm flours was superior to the levels found in other plants flours: some vegetables flours such as eggplant (44 g/100g) (Perez and Germani, 2004), and collards stalk flour and spinach stalk flour (36 and 49 g/100g, respectively) (Mauro, Silva and Freitas, 2010).

According to Pereira and Beraldo (2010), it is a common practice not to use the highest culm parts and throw them as waste. It is important to take into consideration that, depending on the variety, for example *Bambusa vulgaris* and *Bambusa tuldoides*, the culm can grow up to 15-25 m high, or up to 40 meters, like *Dendrocalamus asper* (Graça, 1992; Pereira and Beraldo, 2010). Comparing the diameter of the parts of the culm, the top diameter is the smallest of all, which may weaken the support performed by the material; however, our results indicated that the base and top of young bamboo culm could be indicated for fiber extraction process.

Comparing the results obtained in the literature with our results, we found that white wheat fiber, which is extracted from wheat plant culm and then purified is considered a source of insoluble fiber (up to 97 g/100g dry basis) (JRS, 2016). According to the same authors, white wheat fiber presents several nutritional advantages like reduction of fat and caloric value of food products and besides technological benefits, improving texture, controlling the moisture migration and reducing the weight loss of product. In addition to the high content of insoluble fibers, the similarity between the bamboo culm flour and wheat fiber is the fact that both are extracted and generated from the plant culm, and in both cases, they are also types of grass.

Total fiber content in the young bamboo culm flour is lower than in white wheat fiber (Ishida, 2012) which may be explained by the presence of starch in the young bamboo culm (6-10 g/100g), once it has not been purified yet. If young bamboo culm flour is used for fiber production, it will take advantage of the culm for starch extraction, because it showed amounts greater than 10 g/100g in many parts of the culm. Furthermore, after the extraction of starch and other nutrients, it may be considered an ingredient with fiber content similar to white wheat fiber.

Table 1 – Proximate composition of young bamboo culm flours[1]

Sample[2]	Nutrients (g/100 g)						
	Moisture	**Ash**	**Protein**	**Lipids**	**Total dietary fiber**	**Total starch**	**Other carbohydrates[3]**
BF1	8.17 ± 0.36[a]	1.66 ± 0.02[b]	1.62 ± 0.02[b]	0.90 ± 0.23[a]	72.83 ± 1.56 [c]	11.34 ± 0,10[c]	3.48
BF2	7.48 ± 0.11[b]	2.32 ± 0.01[a]	1.55 ± 0.00[c]	0.29 ± 0.02[b]	79.61 ± 0.25 [a]	6.59 ± 0,30 [e]	2.17
MF1	7.99 ± 0.06[a]	1.21 ± 0.01[b]	1.34 ± 0.01[d]	0.38 ± 0.04[b]	67.27 ± 0.55 [d]	16.59 ± 0,54 [a]	5.23
MF2	7.93 ± 0.34[a]	1.24 ± 0.11[b]	1.31 ± 0.03[e]	0.27 ± 0.08[b]	79.35 ± 0.39 [a]	9.61 ± 0,17 [d]	0.29
TF1	7.13 ± 0.31[b]	1.21 ± 0.05[b]	1.63 ± 0.02[b]	0.36 ± 0.05[b]	76.74 ± 0.99 [b]	12.17 ± 0,83 [b]	0.76
TF2	6.96 ± 0.36[b]	1.17 ± 0.05[b]	1.75 ± 0.01[a]	0.30 ± 0.13[b]	76.74 ± 1.36 [b]	11.19 ± 0,30 [c]	1.91

[1]Means followed by the same lower case letters in the same column did not differ ($p < 0.05$) by Scott-Knot test.[2] BF1, BF2: Bamboo bottom culm flour from fraction 1, 2; MF1, MF2: Bamboo middle culm flour from fraction 1, 2; TF1, TF2: Bamboo top culm flour from fraction 1, 2. [3]Calculated by difference: 100 – (moisture + ash + protein + lipids + total dietary fiber + total starch).

Bamboo shoot fibers are already being commercialized in the international market, with intense dissemination of their use in several food applications (Farris and Piergiovanni, 2008; Farris, Piergiovanni and Limbo, 2008; Choudhury et al., 2015). In Brazil, the bamboo fiber has been used in gluten-free products, such as bread, toast and others, providing products targeted to a select and specific consumer market, which has grown in recent years. Comparing the young bamboo culm with the fiber of bamboo shoots commercially available, this one has greater size and weight, and may have higher yield in fiber extraction and for co-product, as well the starch.

Consumption of low-digestible carbohydrates may have beneficial health effects, including reducing the risk of chronic diseases or treating other health conditions. These benefits are related to their incomplete digestion and absorption, increase of colonic fermentation, and high excretion (Grabitske and Slavin, 2008). Their use is therefore associated with reducing the risk of coronary heart disease and diabetes, promoting beneficial physiological effects (decreased blood cholesterol and triglycerides), preventing cancer, and having a laxation effect (Charalampopoulos et al., 2002; Rodríguez et al., 2006; Tosh and Yada, 2010). The high dietary fiber content in bamboo culm flours allows their use as an alternative and novel ingredient consumed all around the world.

Concerning the starch content, MF1 and TF1 presented the highest values (>10 g/100g), while BF2 and MF2 the lowest values (<10 g/100g) of total starch. It was expected that there would be a higher accumulation of starch in the top than in the bottom because of reserve substances accumulation to culm growth. This clear separation of starch between bottom, middle and top was not observed in the present study, probably because of some climate change during the harvest, or due to the separation into fractions, where there could have happened a greater aggregation of starch particles in the first fraction, since most of the starch granules are within fibrous structures, filling the parenchyma cells (Graça, 1992; Pereira and Beraldo, 2010; Liese and Kohl, 2015).

The reserve of starch in 1-year old bamboo culm is highest just before sprouting occurred, and the lowest level occurred during the growth of shoots. After this period is complete, the amount of starch reserve in the culm decreases again when the growth of the rhizome starts and then gradually increases (Liese and Kohl, 2015). It is observed that the starch reserves of rhizomes and culm is to provide energy for the sprouting and due to it, the shoots have not significant starch content. The bamboo shoot, which develops into a new culm, does not synthesize reserve substances required for its growth. These substances are transported mainly from the oldest bamboo culm that led to it (Azzini and Gondim-Tomaz, 1996).

This means that there is a variation of the fiber, starch and sugars contents with aging, due to the physiological and anatomical characteristics of bamboo culms. Moreover, it is precisely in the inner layers of the culm where the highest concentration of parenchymal cells rich in starch can be found, being them the most vulnerable to powder-post beetles attack.

The high content of starch and fibers in young bamboo culm opens prospects for extraction processes of these two nutrients, which can increase food applications and consequently, the value of this raw material. The young bamboo culm flour can be used directly in food applications such as for the extraction of fiber and/or starch.

Table 2 shows the composition of total sugar of the evaluated samples, and BF1 and BF2 presented the highest values, while TF2 contained the lowest values of total sugar. Consequently, this fact must be taken into account when using bamboo culm flour because in formulations where the white sugar will be used, it may be totally or partially replaced by this flour, allowing its use in yeast-leavened products, too.

Table 2 – Total sugar composition of young bamboo culm flours[1]

Samples[2]	**Total sugar (g/100 g)**
BF1	13.57 ± 1.27[a]
BF2	11.43 ± 0.39[b]
MF1	11.00 ± 0.06[b]
MF2	6.99 ± 0.10[c]
TF1	6.01 ± 0.21[c]
TF2	2.27 ± 0.02[d]

[1]Means followed by same lower case letters in the same column did not differ ($p < 0.05$) by Scott-Knot test. [2]BF1, BF2: Bamboo bottom culm flour from fractions 1, 2; MF1, MF2: Bamboo middle culm flour from fractions 1, 2; TF1, TF2: Bamboo top culm flour from fractions 1, 2.

3.2. Technological characteristics of young bamboo culm flours

When assessing the technological characteristics, it was found that the pH (5.53-5.79) of the flours may be considered slightly acidic and the highest values were obtained for the samples BF1 and MF1 (Table 3). These values of pH allow a wide range application in food products and increase their potential uses. Values obtained indicate that the growth of microorganisms should be avoided by the low moisture content (Jay, 2005).

WAI did not vary with the two particle sizes (F1 and F2) of all the flour samples evaluated. WAI represents the ability of a product, under limited conditions of water, of associating to it. The values obtained vary among the evaluated culm parts, with BF1 presenting higher absorption rates (4.73), and the values found in our study were much higher than those reported for melon flour (0.7ml/g) and fluted pumpkin flour (3.4g/g) (Akobundu, Cherry and Simmons, 1982; Giami and Bekebain, 1992), but lower than those obtained by eggplant flour (11.63g/g) (Perez and Germani, 2004). The results obtained suggest that replacement of fibers now used by the bamboo fiber, may not cause major changes in the formulations and it could maintain the same level of water addition, depending on the amount used in the formulation.

Comparing the results of WSI for both fractions, F1 and F2, we observed that F1 showed the highest values. And between the different parts of the culm, the highest values were observed in the base and decrease significantly to the top. The flours with the highest sugar content (Table 3) presented the highest values for WSI, which ranged from 5.54 (TF2) to 10.63 g/100g (BF1). The bottom of the bamboo culm presented highest significant differences in total sugars content (Table 2) and technological properties, and this part presented the higher WSI values, when compared to the middle and the top.

Regarding the color parameters, all the evaluated samples presented high L* (luminosity) values, indicating that bamboo culm flour has a light color. However, the samples presented significant differences among them, with higher values for F1 (BF1, MF1 and TF1), with L* around 84%. Lower values of a* indicate a flour with less reddish color, while larger values of b* characterize the yellowish color.

There are no references in the literature about the color parameters for bamboo culm flour. However, to have a better reference for the values obtained, it was compared with evaluated color parameters for cassava flour: L*=86.9, a*=1.4, b*=14.1 (Lustosa, Leonel and Mischan, 2008), and for defatted sesame flour: L*=67.43, a*=5.38, b*=17.2 (Clerici, Oliveira and Nabeshima, 2013), which have been largely used in bakery products. Comparing bamboo flour samples with cassava flour, we observed that bamboo culm flour is less yellowish and darker, as can be seen in Figure 1. In comparison to defatted sesame flour, the young bamboo culm flour has lighter color, less reddish and higher yellowish color.

Although refined wheat flour (L*=90.75, a*=0.27, b*=8.90) (Ishida, 2012) was lighter than young bamboo culm flour, it is expected little influence on the color of baking products when bamboo culm flour is added in pre-mixes or in partial substitution of refined wheat flour. In a recent study, Choudhury et al. (2015) produced whole meal of bamboo shoots through cooking and drying methods for use in cookies formulations. The use of bamboo shoot fiber in cookies has been previously reported by Farris and Piergiovanni (2008) and Farris et al. (2008), which showed the feasibility of using both bamboo flour and bamboo shoots fiber in bakery products. The present article has shown that young bamboo culm flour can also be used either in full or purified form as fiber, because it presents technological and physicochemical properties, similar to other ingredients with high fiber content.

The results of this research showed that the chemical composition and technological properties of the fractions of young bamboo culm were different and may result in novel ingredients with high fiber content, with yellowish color, and properties of WSI and WAI that facilitate their addition to bakery and pasta products.

4. CONCLUSION

This study showed the great potential of employing young bamboo culm flour as a new ingredient for food products, improving the intake of fiber and considering the consumer demand for healthier products.

This widespread and under-utilized crop has economic advantages and nutritional qualities in relation to fiber of shoots. The production process for young bamboo culm flour and their fiber and starch isolates could be developed in association with cooperative, since fairly simple equipment and techniques are required.

Table 3 – Physical and technological characteristics of young bamboo culm flours[1]

Samples[2]	pH	WAI (g gel/g dry matter)	WSI (%)	Color		
				L*	a*	b*
BF1	5.79 ± 0.02 a	4.73 ± 0.17 a	10.63 ± 0.10 a	85.54 ± 0.12 a	1.32 ± 0.04 c	20.59 ± 0.25 c
BF2	5.68 ± 0.11 b	4.65 ± 0.14 a	8.52 ± 0.18 b	83.62 ± 0.14 d	1.86 ± 0.04 b	21.18 ± 0.05 b
MF1	5.62 ± 0.02 a	4.10 ± 0.04 b	7.54 ± 0.13 c	85.74 ± 0.18 a	1.21 ± 0.06 d	20.42 ± 0.21 c
MF2	5.53 ± 0.03 b	3.91 ± 0.13 b	5.96 ± 0.10 e	83.42 ± 0.17 d	2.02 ± 0.05 a	21.81 ± 0.19 a
TF1	5.68 ± 0.06 b	3.74 ± 0.11 c	6.34 ± 0.04 d	84.71 ± 0.23 b	0.68 ± 0.07 e	22.16 ± 0.27 a
TF2	5.63 ± 0.09 b	3.68 ± 0.03 c	5.54 ± 0.03 f	84.42 ± 0.16 c	1.20 ± 0.04 d	21.85 ± 0.07 a

[1]Means followed by same lower case letters in the same column did not differ ($p < 0.05$) by Scott-Knot test.[2]BF1, BF2: Bamboo bottom culm flour from fractions 1, 2; MF1, MF2: Bamboo middle culm flour from fractions 1, 2; TF1, TF2: Bamboo top culm flour from fractions 1, 2.

5. ACKNOWLEDGEMENTS

The authors are grateful to CAPES for the scholarship for Mária Herminia Ferrari Felisberto, and to FAEPEX for the financial support for participation in events (SLACA 2015).

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– CAPÍTULO 4 –

Young bamboo culm: potential food as source of fiber and starch

Este capítulo apresenta os resultados obtidos para a caracterização tecnológica das farinhas obtidas do colmo jovem de três espécies de bambu: Dendrocalamus asper, Bambusa tuldoides e Bambusa vulgaris, bem como descreve o potencial de cada uma delas para extração de amido e/ou fibras.

Este capítulo está publicado no periódico Food Research International, volume 101, p. 96-102, 2017 (https://doi.org/10.1016/j.foodres.2017.08.058), conforme Anexo 3.

Young bamboo culm: potential food as source of fiber and starch

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ABSTRACT

With the objective of widening the use of bamboo in the food industry, the present work aimed to produce and characterize the young bamboo culm flours from *Dendrocalamus asper, Bambusa tuldoides* and *Bambusa vulgaris* species as potential sources of fiber and starch. The young culms were collected, cut in three sections (bottom, middle, top), processed into flour, and they were physically, chemically and technologically analyzed. The data were obtained in triplicate and evaluated by means of average differences, using analysis of variance (ANOVA) and Scott-Knott test ($p < 0.05$). The young bamboo culms flours presented low values for moisture content (< 10 g/100g), protein, lipids and ash contents (< 3 g/100g). Regarding the carbohydrates profile, the flours were significantly different in their sugar, starch and total fiber contents. All flour samples presented a potential for fiber extraction (> 60 g/100 g), and the species *B. vulgaris* and *D. asper*, presented an additional potential for starch extraction (16 and 10 g/100 g, respectively). Regarding technological characteristics, all flours presented bright yellow color, lightly acidic pH (> 5.0), water solubility index (WSI) lower to 2.5%, excepting *D. asper*, which presented a WSI superior to 7.5%. In this way, the evaluated young bamboo culms present potential application in the food industry as flours and as source of fibers; in addition, the species *D. asper* and *B. vulgaris* can also be used for starch extraction.

Keywords: Bamboo, young culm, healthiness, sustainability.

1 INTRODUCTION

International trade of bamboo and rattan increased constantly in the last decades, and in 2012 accounted 2.5 billion dollars, according to INBAR (International Network for Bamboo and Rattan) (INBAR, 2012). It is a sector that has gained global attention, due to its fast development and its potential for supporting a green sustainable growth. Bamboo is a crop that does not require pesticides and has no history of plagues propagation like soy, wheat or sugar cane crops (Silva and Costa, 2012). From the agricultural point of view, it is an economically viable crop due to its perennial and asexual culms, without the need of replanting. Another advantage is related with the culms growth speed, given that they do not present tangential or radial anatomic elements, they only grow in height, contrary to wood; therefore, they can be cut for industrial use after three to five years of planting (Akira, Alexandre et al., 2007). The bamboo has a sustainable extraction and the its plantation can last for more than one hundred years because it is not necessary to cut it totally but only partially (Rigueira Junior, 2011), being only recommended the clump cleaning with removal of the mature culms, being them in average 30% in an stabilized clump (Pereira and Beraldo, 2016).The highest occurrence of bamboo plantations is in the zones with hot weather and with abundant rain, as the tropical and subtropical regions in Asia, Africa and South America (Manhães, 2008; Pereira and Beraldo, 2016), where Brazil presents the biggest diversity, with 34 genera and 232 native bamboo species, with several bamboo types, ranging from small to giant sizes (Pereira and Beraldo, 2016). However, in spite of the richness in varieties, most of them have not been adequately studied, opening a gap in technical information regarding its potential use.The bamboo is recognized as nutraceutical (Xuhe, 2003; Lo et al., 2008; Chongtham et al., 2011; Pereira and Beraldo, 2016) because of its rich composition in phytosterols and high fiber content, and due to this, it has been used as food and bamboo shoots have had their use expanded from crafts and canned food, to the extraction of dietary fiber. Some commercial labels of bamboo shoot fiber, as Jelucel®BF (Jelu-Werk, 2016), Nutriloid® Bamboo Fiber (Tic-Gums, 2016) and CreaFibe (Nutrassim, 2017) present great acceptance in the international market, given that they can be applied in several products as breads, pasta, meat products, cheese and yogurt, increasing their fiber content and giving them a healthy claim.The bamboo shoot fiber market for food applications is already established, and it presents a wide variety of applications. As for example, Mustafa et al. (2016) and Thomas et al. (2016), who observed good sensory acceptance, when they added bamboo shoot fiber in cookies and pork nuggets, respectively. On pork nugget, it was also observed an improvement of microbiological characteristics. Staffolo et al. (2004) evaluated sensory, rheological and technological properties of yogurt fortified with this fiber, and they also observed good sensory acceptance, besides maintaining stable pH, water activity and color parameters. Zeng et al. (2016) observed significant reductions of fat absorption in deep-fat fried battered and breaded fish balls added with bamboo shoot dietary fiber to the batter.It can be seen that the wide use of the bamboo shoots increases the need for alternatives for keeping the balance in the clump maintenance, due to the recommendation of removal of only 20 to 30% of the culms. In the present work, we propose the study of young bamboo culm for fiber and starch production, given that it presents a high yield and will be beneficial to keep the balance of the clumps.Some species with special highlight for the industrial processing are: *Bambusa*, *Dendrocalamus, Phyllostachys*, *Pseudosasa, Sasa* and *Sinoarundinaria* (Manhães, 2008; Pereira and Beraldo, 2016), and among them, three can be easily recognized: 1) *Bambusa vulgaris* is a specie that forms a clump, reaches medium height (15 to 25 m), grows in places located up to 1500 m over the sea level, and resists a minimal temperature of -2°C (Graça, 1988; Pereira and Beraldo, 2016). In Brazil, it is specially found in the states of Maranhão and Pernambuco, which plantation is for paper production. 2) *Bambusa tuldoides* can grow forming a clump of medium height up to 15 m, well adapted to various climates, in locations up to 1500 m over the sea level. Its culms and leaves are lightly blue when young, and dark green when mature (De Oliveira, 2012; Pereira and Beraldo, 2016). In Brazil it is known in some regions as bamboo “crioulo”, and is commonly used as stake in tomato plantation (De Oliveira, 2012). 3) *Dendrocalamus asper* is the most cultivated species for canned bamboo shoot production. It forms clumps and reaches up to 40 m height, and its culms can have up to 20 cm diameter (Pereira and Beraldo, 2016).Studies about young bamboo culms composition are few and Azzini et al. (1981) presented composition data where bamboo contained 50% of gross fiber and 26% parenchymal residue in its insoluble fraction, and the rest were polysaccharides, like pentosane, starch and glucose. However, the authors did not identify the bamboo specie nor the evaluated fraction of the culm. Therefore, the aim of our study was the production and evaluation of flours from young bamboo culms from *D. asper*, *B. tuldoides* and *B. vulgaris* species, in the sections bottom, middle and top, regarding chemical composition, including starch and dietary fiber content, and technological characteristics, aiming for future food applications.

2 MATERIAL AND METHODS

2.1 Raw material

Young bamboo culms from *D. asper*, *B. tuldoides* and *B. vulgaris* species were harvested at experimental field from FEAGRI/UNICAMP, Campinas/Brazil, on geographical coordinates 22º82' south latitude and 47º07' west longitude, taking care of collecting only culms of approximately 36 months old.

2.2 Collection and division of young bamboo culms

Each young culm was cut in three equal parts, from bottom to top of the culm, taking into account the different heights of the culms from each specie. For all samples, the first 30 cm from the soil were discarded and the first fraction cut ("bottom - B"). After this, 30 cm were discarded again, and the next fraction was cut ("middle - M"), and after discarding the following 30 cm, the last fraction was cut ("top - T"). In total, nine samples were taken: DABF, DAMF, DATF (*Dendrocalamus asper* bottom, middle and top flour, respectively), BTBF, BTMF, BTTF (*B. tuldoides* bottom, middle and top flour, respectively), BVBF, BVMF, BVTF (*B. vulgaris* bottom, middle and top flour, respectively).

2.3 Obtainment of the flours from young bamboo culms

The young bamboo culms were cut, treated and dried, according Felisberto, Beraldo and Clerici (2017). After drying, the material was passed through a hammer mill (DPM 2 - Nogueira, São Paulo, Brasil) for reduction of the size. Afterwards, a knife mill was used (EQ-FH-203-TREU S.A., Rio de Janeiro, Brasil), and all obtained flours were sieved and granulometry was standardized for size inferior to 0.150 mm. Finally, the material was packed in plastic packages hermetically sealed and kept under refrigeration before further analyses were done.

2.4 Color parameters from young bamboo culm flours

Color parameters were obtained in the CIELab space (L, a* and b*), using a colorimeter CR-10 (Konica Minolta, Tokyo, Japan), with illuminant D65, 50 mm target mask, with Specular Component Included (SCI), 10º observer. For obtaining an average value, three light flashes were used.

2.5 Scanning electron microscopy (SEM) from young bamboo culm flours

Images from flour samples from each bamboo section were obtained by SEM (JEOL JSM 5800 LV, Tokyo, Japan), with magnification of 100X at 10 kV. A small flour sample was placed on carbon tape, adhered to a stubb, sputter-coated with a thin layer of gold (20 nm) (SCD 050 Sputter Coater, Balzers).

2.6 Physicochemical characterization of the young bamboo culm flours

Young bamboo culm flours were evaluated following AOAC (1998) for moisture contents (method 44-15.02), proteins (method 46-13.01), lipids (method 920.39), ash content (method 08-01.01), total starch (method 996.11), total fiber (method 992.16), insoluble fiber (method 991.42) and total carbohydrates were calculated by difference, and the results were presented on wet basis (wb). Total sugar content was also evaluated (for both reducing and non-reducing sugars) following AOAC (1984) with modified method by Layne-Eynon (method 31.043-6).

2.7 Evaluation of technological properties from young bamboo culm flours

The following technological properties were evaluated: (1) pH evaluation, following AOAC (1998); (2) Water absorption index (WAI) and water solubility index (WSI) following Anderson (1982).

2.8 Statistical analysis

The data were obtained at least in triplicate and analysis of variance (ANOVA) of the means was performed using the Sisvar software, version 5.6 (Federal University of Lavras, Lavras, MG, Brazil) at a significance level of 95%. When significant, the Scott- Knot test was used to determine statistical differences between means ($p \leq 0.05$) (Ferreira, 2008).

3 RESULTS AND DISCUSSION

3.1 Color parameters of young bamboo culm flours

All obtained flours, shown in Figure 1, presented color parameters located in the light-yellow region (Table 1). Comparing these values with those from cassava flour, which is commonly used as starch source in Brazil (Lustosa et al., 2008) we observed similarity in the L and a* values (86.90 and 1.4, respectively).

The slight variations observed between the flours can be explained by the presence of sugars, which can promote browning reactions during the drying step. In the case of these flours being used for extractive processes, the sugars will be easily removed with water, allowing the obtainment of lighter fiber/starches, and similar to the results obtained by Rosell, Santos and Collar (2009), which presented L: 87.52 ± 0,15, a*: -0.73 ± 0.23 and b*: 3.45 ± 0.02, for commercial bamboo culm fibers.

The variation between light and dark yellow color benefits the use of these flours in food products, such as bread, cakes and cookies, given that the addition of whole grain flours, which are rich in fiber, generates darker and lighter yellow products, for example, as observed by Gómez et al. (2010) who added wheat, rye, triticale, barley and tritordeum whole grain flours to cake formulations. However, this did not happen when bamboo shoot flour was added in cookie formulations, and compared to a control formulation, as observed by Mustafa et al. (2016).

Figure 1 - Illustrative image of young bamboo culm flour obtained from *D. asper*, *B. tuldoides* and *B. vulgaris* species.

3.2 SEM from young bamboo culm flours

Figure 2 presents the SEM micrographs of the flours, where is possible to see a great fibrous fraction (letter a), with some small starch granules (letter b) disperse in them. The fiber presence could favor the use of these flours, due to the fact that when they are cooked, the film formed by the gelatinized starch may surround the fibers. This behavior will physically interfere in the link between the enzyme and the substrate, by Lehmann and Robin (2007), the interaction of starch with fiber, protein or other food components could prevent the efficient diffusion and adsorption of the enzyme.

Table 1 – Color parameters of young bamboo culm flour from *D. asper, B. tuldoides* and *B. vulgaris*[1]

Sample [2]	Color parameters		
	L	a*	b*
DABF	85.55 ± 0.16[a]	1.45 ± 0.07[c]	21.01 ± 0.42[b]
DAMF	83.23 ± 0.02[d]	2.39 ± 0.01[b]	22.95 ± 0.34[a]
DATF	85.32 ± 0.19[b]	1.18 ± 0.06[d]	20.20 ± 0.18[c]
BTBF	85.13 ± 0.16[b]	1.00 ± 0.04[f]	16.53 ± 0.36[e]
BTMF	85.20 ± 0.02[b]	1.07 ± 0.01[e]	16.71 ± 0.18[e]
BTTF	84.12 ± 0.08[c]	0.89 ± 0.03[g]	16.42 ± 0.13[e]
BVBF	81.04 ± 0.06[f]	2.61 ± 0.03[a]	17.69 ± 0.10[d]
BVMF	82.23 ± 0.03[e]	1.44 ± 0.07[c]	17.83 ± 0.10[d]
BVTF	78.09 ± 0.08[g]	2.36 ± 0.13[b]	17.48 ± 0.14[d]

[1] Results expressed as means ± standard deviation. Means followed by the same letters in the same column did not differ significantly ($p<0.05$) by the Scott-Knott test. [2]Young bamboo culm flour, respectively from the bottom, middle and top portions of *D. asper* (DABF, DAMF e DATF), B. *tuldoides* (BTBF, BTMF e BTTF) and *B. vulgaris* (BVBF, BVMF e BVTF) species.

3.3 Physicochemical characterization of the young bamboo culm flours

3.3.1 Proximate composition

Regarding the physicochemical composition (Table 2), we observe that all flours presented moisture values inferior to 10 g/100g, which was expected, due to the drying process, which is ideal for guaranteeing a better product preservation. Similar results of moisture contents were obtained by Rosell et al. (2009) when evaluating commercial bamboo culm fiber (7.06 g/100g) and by Mustafa et al. (2016) when assessing powdered bamboo culm.

The protein contents ranged from 1.31 to 2.03 g/100 g and were similar to those observed in the grasses *Brachiaria*, *Cynodon*, *Paspalum* and *Panicum* (Soares Filho et al., 2002) and in the wheat white fiber (Ishida, 2012). However, these values were inferior to the one obtained for the powdered bamboo culm (19.32 g/100g) (Mustafa et al., 2016), and superior to those of the commercial bamboo fiber (0.09 g/100g) (Rosell et al., 2009). The low protein value is an ally for the extraction process of fiber and starch; for example, for the corn processing, whose protein content is approximately 10%, is required the use of chemical reagents for increasing the starch extraction, while for tubers, like cassava, the extraction process requires only water (Bemiller and Whistler, 2009).

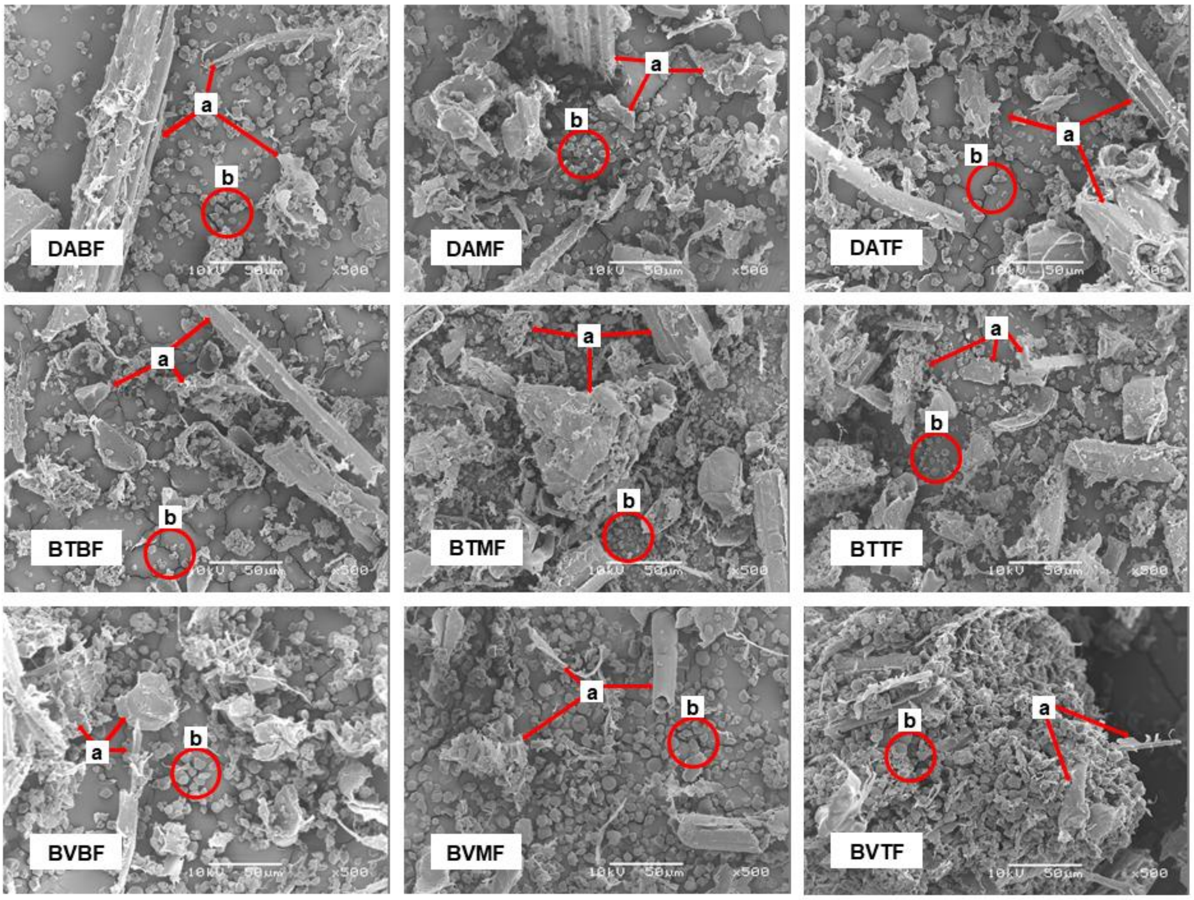

Figure 2 Scanning electron micrographs of young bamboo culm flour from bottom (B), middle (M) and top (T) portions of *D. asper* (DAB, DAM e DAT), *B. tuldoides* (BTB, BTM e BTT) e *B. vulgaris* (BVB, BVM e BVT).

The lipid contents were inferior to 1 g/100 g, and similar to those of the powdered bamboo culm (Mustafa et al., 2016) and of the commercial bamboo fiber (Rosell et al., 2009). This relationship between the lipid and protein contents (both low) was similar to the observed in fresh bamboo shoot by Thomas et al. (2016) and Mustafa et al. (2016). The lipids in vegetable products are removed before extraction process and nutrients purification, as happens with the removal of corn germ before starch extraction (Eckhoff and Watson, 2009), for avoiding the oxidative or hydrolytic rancidity at further processing steps, that may confer unpleasant odor to the starch. Therefore, the low lipid content is beneficial for the use of young bamboo culm as source of fiber and starch.

Table 2 – Chemical composition of young bamboo culm flour (wb) from *D. asper, B. tuldoides* and *B. vulgaris*[1].

Sample [2]	Nutrients (g/100 g)				
	Moisture	**Protein**	**Lipid**	**Ash**	**Carbohydrates**
DABF	7.34 ± 0.31^a	1.43 ± 0.05^e	0.16 ± 0.02^b	1.66 ± 0.06^c	89.41
DAMF	7.16 ± 0.09^a	1.31 ± 0.04^f	0.31 ± 0.06^b	0.87 ± 0.04^e	90.35
DATF	6.79 ± 0.35^b	1.54 ± 0.01^d	0.22 ± 0.03^b	0.89 ± 0.01^e	90.56
BTBF	3.20 ± 0.16^e	1.57 ± 0.08^d	0.12 ± 0.04^b	0.80 ± 0.03^e	94.31
BTMF	3.34 ± 0.30^e	1.43 ± 0.03^e	0.20 ± 0.04^b	0.94 ± 0.06^e	94.09
BTTF	3.19 ± 0.15^e	1.72 ± 0.02^c	0.39 ± 0.11^b	2.38 ± 0.14^b	92.32
BVBF	5.02 ± 0.18^c	1.74 ± 0.01^c	0.34 ± 0.13^b	1.33 ± 0.12^d	91.57
BVMF	4.12 ± 0.21^d	1.85 ± 0.01^b	0.35 ± 0.04^b	1.58 ± 0.12^c	92.10
BVTF	4.85 ± 0.11^c	2.03 ± 0.01^a	0.76 ± 0.48^a	2.77 ± 0.05^a	89.59

[1] Results expressed as means ± standard deviation, on wet basis (wb). Means followed by the same letters in the same column did not differ significantly ($p<0.05$) by the Scott-Knott test. [2]Young bamboo culm flour, respectively from the bottom, middle and top portions of *D. asper* (DABF, DAMF e DATF), B. *tuldoides* (BTBF, BTMF e BTTF) and *B. vulgaris* (BVBF, BVMF e BVTF) species.

Regarding the ash contents (0.80 to 2.77 g/100g), BVTF presented the highest content, significantly different to the other evaluated flours. Similar results were observed for the wheat white fiber (Ishida, 2012)**,** the bamboo shoot extract from specie *B. polymorpha* (Thomas et al., 2016) and for the powdered bamboo shoot (Mustafa et al., 2016). It should be highlighted that the protein, lipid and mineral salts contents of all studied species and in the analyzed sections (bottom, middle, top) may benefit the fiber extraction processes.

3.3.2 Carbohydrates profile

In Table 3 are shown the carbohydrates including sugars, starch, and dietary fiber. We observe that the total sugar contents differed significantly between the flours, ranging from 0.94 to 13.40 g/100 g, being the flours from *D. asper* specie the ones that presented the highest values for the bottom, middle and top sections, 13.40, 7.19 and 7.63 g/100g, respectively. These flours also presented a slightly sweet flavor, which could benefit their use in bakery products. In case of applying them in fermented products, this natural sugar could be used for the initial fermentation step, with no required addition of sugar (sucrose).

Table 3 – Carbohydrate profile of young bamboo culm flour from *D. asper*, *B. tuldoides* e *B. vulgaris* [1].

Sample [2]	**Carbohydrates**			
	Total sugar	**Total starch**	**Total fiber**	**Insoluble fiber**
DABF	13.40 ± 0.51[a]	8.17 ± 0.32[d]	72.40 ± 1.00[c]	71.27 ± 0.60[d]
DAMF	7.19 ± 0.15[c]	10.99 ± 0.53[c]	72.55 ± 0.21[c]	68.64 ± 0.55[e]
DATF	7.63 ± 0.59[b]	10.16 ± 0.60[c]	71.74 ± 0.89[c]	70.73 ± 0.24[d]
BTBF	1.29 ± 0.01[d]	2.23 ± 0.40[e]	89.46 ± 0.49[a]	89.79 ± 0.44[b]
BTMF	1.47 ± 0.05[d]	2.65 ± 0.27[e]	90.21 ± 0.53[a]	87.97 ± 0.21[a]
BTTF	0.94 ± 0.03[e]	1.75 ± 0.12[e]	86.21 ± 0.31[b]	82.91 ± 0.42[c]
BVBF	1.03 ± 0.03[e]	15.30 ± 0.76[b]	70.86 ± 0.61[d]	68.75 ± 0.89[e]
BVMF	1.06 ± 0.02[e]	16.31 ± 0.68[a]	64.70 ± 0.05[e]	62.57 ± 0.41[f]
BVTF	1.38 ± 0.10[d]	16.89 ± 0.81[a]	64.12 ± 0.06[e]	62.54 ± 0.12[f]

[1] Results expressed as means ± standard deviation. Means followed by the same letters in the same column did not differ significantly ($p<0.05$) by the Scott-Knott test. [2]Young bamboo culm flour, respectively from the bottom, middle and top portions of *D. asper* (DABF, DAMF e DATF), B. *tuldoides* (BTBF, BTMF e BTTF) and *B. vulgaris* (BVBF, BVMF e BVTF) specie.

Considering that this sugar is mostly soluble in water, the culms with the lowest sugar contents (*B. tuldoides* and *B. vulgaris*) could be more stable to processing, both for flour production and fiber and starch extraction, given that they have more storage time. An opposite behavior was presented by the young culm from *D. asper*, due to the fact that a higher sugar content increases the material instability, that may undergo a fermentation in few hours during storage, even at room temperature; therefore, its processing must be as fast as possible (authors observation), for avoiding natural fermentation processes, and according to (Pereira and Beraldo, 2016) the culm can remain submerged in water and be added with chemical reagents, for a higher stability.

Regarding the total starch contents (Table 3), a big variation was observed between the samples (1.84 to 17.75 g/100 g), being the flour samples from *B. vulgaris* (BVBF, BVMF and BVTF) the ones that presented the highest significant values, followed by the *D. asper* specie. In this way, the young culms from *B. vulgaris* and *D. asper* present huge potential for starch extraction (higher than 10%), being it a by-product from fiber extraction. This separation might be facilitated by the density difference between starch and fiber in aqueous solution, making it similar to starch extraction from tubers, such as cassava and potato (Breuninger, Piyachomkwan and Sriroth, 2009; Grommers and Krogt, 2009).

All flours presented total and insoluble fiber contents higher than 62 g/100g (Table 3), showing the low soluble fiber contents in all evaluated samples. All fiber values obtained in this study were superior to those obtained for the powdered bamboo shoot (24.44 g/100g) (Mustafa et al., 2016) and inferior to those of the commercial bamboo fiber, whose content was 97 g/100g (Rosell et al., 2009), given that it is a purified ingredient. In this way, we can consider the flours from the three different bamboo species, obtained in this work, to have great potential for fiber extraction, which confirms the results obtained by Felisberto et al. (2017), for the flour from *D. asper.*

The variation of starch and fiber content among the species and different parts of bamboo culm was already expected, because, according to Liese and Kohl (2015) the chemical composition of the bamboo culm varies with the species, the conditions of growth, the age of the bamboo, the part of the culm and harvest season. So, considering that within a year the bamboo culm completes the full maturation, its total lignin content tends to remain constant. However, the reserve starch varies along the length of the culm, as explained by Azzini and Gondim-Tomaz (1996), since it is used as energy source for the growth of shoots.

The flours from young bamboo culms, from the nutritional point of view, are a source of carbohydrates, as dietary fiber, containing starch and sugars in quantities lower than 17 and 13%, respectively. This fact will be beneficial given that the flour will present a complex carbohydrate profile, with slow, fast, and also non-digestible molecules, or that may extend satiety and promote the slow starch release. It is important to highlight that the process of fiber and starch extraction from young bamboo culms can be done only physically and using water as a solvent, resulting in a clean, environmentally-friendly process.

Due to the possibility of direct use of the flours from young bamboo culms from the three studied species, we evaluated their technological properties, aiming their use in bakery products, as they are among the products that most present possibilities of insertion of new ingredients, as shown in the work by Felisberto et al. (2015) who substituted fat by chia mucilage in pound cake formulations, Belghith Fendri et al. (2016) who added pea and bean pod fibers in mold breads, Bonnand-Ducasse et al. (2010) who added wheat fiber in breads, or Kaur, Singh and Kaur (2017) that substituted wheat flour by flaxseed flour in cookie formulations, among others.

3.4 Technological properties

In Table 4 can be seen the results of the flours technological characterization. We observe that pH values range from 5.43 to 5.84, corresponding the highest value to BTTF (5.84 ± 0.02), and the lowest value to DATF (5.43 ± 0.01). This pH range is similar to the pH values found by Thomas et al. (2016), who evaluated a bamboo shoot extract.

All flours can be classified as lightly acidic (pH > 4.5), which favors its use in several bakery products, fermented or not, due to the fact that most of them have a pH in the range of 5, according to Cauvain and Young (2008) and Magan, Arroyo and Aldred (2003). In relation to WAI (Table 4), we observed that the values differed significantly between the flours, variating from 3.72 to 4.88, corresponding the lowest values to DAMF and DATF, while the highest values were presented by all bottom sections from the evaluated species (DABF, BTBF and BVBF), presenting BVBF the highest value. This index is directly related to the presence of gelatinized starch, soluble fiber and other components which behave as hydrocolloids, as observed by in eggplant flour, which has a high soluble fiber content, and presented an index of 11.63. This observation justifies the low WAI values obtained in the present study, given that the flours presented high insoluble fiber and starch contents. In bakery products, where main ingredients are wheat flour and water, due to the gluten network formation, the addition of new ingredients must be carefully observed. These ingredients must be chosen with low WAI values, for avoiding the need of water addition, and as a consequence, for not interfering in the tridimensional gluten network formation, which occurs at the mixing step.

Table 4 – Technological characterization of young bamboo culm flour from *D. asper, B. tuldoides* and *B. vulgaris*[1]

Sample [2]	pH	WAI	WSI (%)
DABF	5.62 ± 0.04[c]	4.49 ± 0.06[c]	9.47 ± 0.18[a]
DAMF	5.60 ± 0.06[c]	3.85 ± 0.09[f]	7.46 ± 0.80[b]
DATF	5.43 ± 0.01[e]	3.72 ± 0.04[f]	7.91 ± 0.17[b]
BTBF	5.50 ± 0.01[d]	4.70 ± 0.12[b]	2.29 ± 0.17[c]
BTMF	5.52 ± 0.01[d]	4.56 ± 0.03[c]	2.18 ± 0.04[c]
BTTF	5.84 ± 0.02[a]	4.02 ± 0.01[e]	2.00 ± 0.06[c]
BVBF	5.78 ± 0.02[b]	4.88 ± 0.09[a]	2.17 ± 0.05[c]
BVMF	5.59 ± 0.03[c]	4.26 ± 0.12[d]	1.88 ± 0.05[c]
BVTF	5.48 ± 0.01[d]	4.33 ± 0.15[d]	2.44 ± 0.09[c]

[1] Results expressed as means ± standard deviation. Means followed by the same letters in the same column did not differ significantly ($p<0.05$) by the Scott-Knott test. [2]Young bamboo culm flour, respectively from the bottom, middle and top portions of *D. asper* (DABF, DAMF e DATF), B. *tuldoides* (BTBF, BTMF e BTTF) and *B. vulgaris* (BVBF, BVMF e BVTF) species.

Regarding the WSI values (Table 4), these variated from 1.88 to 9.47% among the evaluated flours, presenting DABF the highest value. Flours from *D. asper* specie presented the highest indices (average 8.28%), significantly differing from the other samples, and this result may be related to the fact that this bamboo specie presents the highest sugar content. The flours comparative study showed that there exists a great potential for the use and exploitation of the young bamboo culms, with application of their flours in food products, and specifically in bakery products, given that they presented appropriate technological characteristics, such as bright coloration and low WAI and WSI values.

4 CONCLUSION

This study showed that the three bamboo species presented different compositions, and *D. asper* and *B. vulgaris* species presented potential for starch extraction, with fiber as by-product, which opens up the doors for their use in the food and non-food industry. The obtained flours would be an alternative for the traditionally whole flours used in cakes, cookies and breads formulations, with the advantage of not altering the coloration of the final product, in addition to the fiber supply. Bamboo starch could be an alternative source to starches traditionally used as thickeners, fat replacers or body agents in both food and cosmetics formulations.

The culm division in bottom, middle and top sections was made with the aim of evaluating the possible differences between them, in relation to the starch and fiber contents, and the possibility of choosing one of the sections. However, the results showed that it is factible to use entirely the young bamboo culm for the nutrients extraction process, thus avoiding the fractionating step, for the studied species.

It is important to emphasize that a sustainable management from bamboo clumps can be achieved in three stages: sprout, young culm, or mature bamboo extraction, where the young culm could have application in the food industry, both as flour as well as for starch and fiber extraction.

5 ACKNOWLEDGEMENTS

The authors are thankful to CAPES, for the doctorate scholarship of student Mária Herminia Ferrari Felisberto; to FAEPEX and FAPESP (2015/196374), for their financial support; and to CNPq and PIBIC/Unicamp, for the scholarship for scientific initiation of student Patricia Satie Endo Miyake.

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– CAPÍTULO 5 –

Characterization of young bamboo culm starch from *Dendrocalamus asper*

Este capítulo apresenta a caracterização do amido extraído do colmo jovem de Dendrocalamus asper e, consequentemente, seu potencial como fonte alternativa de amido para aplicações industriais.

Este capítulo foi aceito para publicação no periódico Food Research International, em 28/03/2018, (https://doi.org/10.1016/j.foodres.2018.03.074) conforme Anexos 4 e 5.

Characterization of young bamboo culm starch from *Dendrocalamus asper*

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ABSTRACT

The search for new and natural ingredients has been stimulated by the food and non-food industries, and the fresh young bamboo culm of *Dendrocalamus asper* emerges as promising for industrial production due to its composition with more than 10% of starch. So, this study aimed to characterize starch, extracted in aqueous solution, from three different parts (bottom, middle and top) of the young bamboo culm of *D. asper* (SB, SM and ST, respectively). Morphological and physicochemical characteristics of the young bamboo culm starches were evaluated, besides thermal properties, and the obtained data were evaluated by ANOVA and Scott-Knot test ($p<0.05$). The starches presented pale yellow coloration, with high luminosity ($L^*>89$), and lower index in the red region. SEM images showed compound granules, which under polarized light exhibit a Maltese cross. The starches presented polyhedral shape and small size with an average diameter of 5.4 µm. All the samples presented low moisture (7.0 g/100 g), protein (2.0 g/100 g), lipid (0.3 g/100 g) and ash (1.0 g/100 g) contents. ST and SB showed apparent amylose content similar to starches from cereals and isolated from bamboo seeds. This agrees to molecular size distribution of starch chains, since the SB, SM and ST presented amylopectin levels higher than those of amylose, as well as normal starches. The chain length of amylopectin presented the main peak at DP 12-13 and the second on at DP 43, similar to cereals like wheat, rice and barley. Its chain has higher proportion of short chains, which corroborates with the A-type polymorph presented. Concerning about

thermal properties, all the samples presented high gelatinization temperature (> 78 ºC) and low enthalpies values (< 6.35 $J.g^{-1}$), which indicates the greater molecular organization. The gelatinization temperatures of gelatinized starches were lower than the native ones. The physicochemical and thermal characteristics of the obtained starches corroborate with the success of the extraction, which keep the starch granule native, and were similar to those of other starches already used in food and non-food products.

Keywords: carbohydrate, grass, industrial application, natural ingredient, sustainability environment.

1. INTRODUCTION

Unmodified starches have very limited use in the food industry, because of their properties: corn, waxy corn, and cassava granules swell with relative ease and rupture with minimum abuse to produce weak-bodied and cohesive pastes. Through chemical and/or physical treatments, starches can be altered so their resultant pastes will withstand chemical and physical abuses which normally would cause breakdown or gelation. However, the search for new sources of starches with different technological properties of those already used commercially, has been stimulated, aiming to decrease the use of chemically modified starches.

The starch industry has grown quickly, and starch production has expanded in several countries. Primary starch sources production (in millions of tons) in 2006 was in the following order: corn (46.1), cassava (9.1), wheat (5.15), and potato (2.45) (Daniel, Whistler, Roper, & Elvers, 2008), and the demands for commercially produced starch have increased significantly, since starch can be used in a wide range of products, such as foods and beverages, paper, textiles, building materials and alcohol for fuel (BeMiller & Whistler, 2009).

Because of a negative perception regarding the use of chemically modified starch products in food applications, much attention has been focused on genetic, and more recently, physical modifications. The search for new sources of starch, with properties that would meet current demand, would be an alternative. For industrial applications, there is significant interest in replacement or substitution of synthetic polymers with starch and other biopolymers, due to their biodegradable and sustainable natures (Huber & BeMiller, 2010).

In previous work, Felisberto, Beraldo, and Clerici (2015) showed that the young bamboo culm of *D. asper* emerges as promising for industrial production, and in 2017, Felisberto, Beraldo, and Clerici (2017) observed the potential for production of more than 10% of starch. The use of *D. asper* young culm would still have the additional advantage of sustainable extraction of the clumps, because it is recommended to remove only 20-30% of the bamboo shoots and 30% of culm, to make the cleaning of the clump (Pereira & Beraldo, 2016; Rigueira Junior, 2011).

D. asper is a bamboo species whose food use can be of different forms: the bamboo shoots are marketed in the form of canned bamboo shoot, or vacuum packed in nature, for different food preparations, since they are sweet and tasty (Liese & Kohl, 2015; Vasconcellos, 2006). Bamboo shoots has also been used for fiber extraction in the international market, aiming to increase the fiber supply of various food products, as previously reported by Felisberto, Beraldo, et al. (2017). Thus, the extraction of starch from the young culm could enhance the productive chain of bamboo, once the shoots are already widely used.

So, this study aimed to investigate a new and viable source of starch for industrial applications, through the characterization of morphological, structure and physicochemical properties of starch extracted from young bamboo culm of *D. asper*.

2. MATERIAL AND METHODS

2.1. Obtainment of young bamboo culm chips

Young bamboo culms of *D. asper* were harvested in 2015 (from January to March), on average three years old, at experimental field from School of Agricultural Engineering (FEAGRI), at University of Campinas (UNICAMP), Campinas, São Paulo, Brazil, on geographic coordinates 22º82’ of south latitude and 47º07’ of west longitude. The collected culm was divided in three parts (B - bottom, M - middle and T - top) and the drying of the young culms was performed as described by Felisberto, Beraldo, et al. (2017). Thus, the small pieces of bamboo culm from each portion were ground in a hammer mill (DPM2–Nogueira, São Paulo, Brazil) until the bamboo chips were obtained, which were used for the starch extraction.

2.2. Starch extraction

The starch was extracted from three different parts of the young bamboo culm chips in aqueous solution, according to Azzini, Salgado, Teixeira, and Moraes (1981) and Toledo, Azzini, and Reyes (1987) with some modifications: 60 g of bamboo chips were weighed and disintegrated in a OBL10/2 blender (OXY, Santana de Parnaíba, Brazil) with 500 mL of 200 ppm sodium metabisulphite solution, at 5 °C for 60 sec.

After, the material was left to rest for 16 h/ 5 ºC, so the suspension was passed through a sieve (80 µm) and the material retained was triturated two more times. The filtrate was centrifuged at 17,000 g/10 min/10 °C. The supernatant was discarded, and the precipitate was resuspended with distilled water, three times. The extracted starch was then vacuum filtered, washed with 99.5% ethanol (twice) and then acetone, and dried at 45 °C/16 h, in a vacuum oven TE 395 (TECNAL®, Piracicaba, Brazil). The starch bottom (SB), starch middle (SM) and starch top (ST) were packed in plastic hermetically sealed. The obtained material was homogenized and weighed to evaluate the yield of the extraction process, which was calculated in relation to the initial weight of each sample.

2.3. Instrumental color of young bamboo culm starch

Instrumental color analysis was performed on CR-10 colorimeter (Konica Minolta, Tokyo, Japan) with a 50 mm port size, illuminant D65, SCI and a 10º standard observer angle. Tristimulus CIELab color space method was used, determining L*, to represent luminosity (0=black; 100=white), a* (+a=redness; -a=greenness), and b* (+b=yellowness; -b=blueness).

2.4. Morphological evaluation of starch granules

Optical Microscopy (OM) – The starch sample was placed on a glass slide and mixed with 1-2 drops of water. The slide was then covered with a glass cover slip and the granule morphology was observed in an optical microscopy (BX51, Olympos, Tokyo, Japan), coupled with a camera (E-330-ADU1.2X, Olympus, Tokyo, Japan). The micrographs were captured at 100X magnifications, with and without polarized light.

Scanning Electron Microscopy (SEM) – The young bamboo culm chips and starch were placed on carbon adhesive tape attached to the stub (one type of metal bracket) and sputter-coated with 20 nm of gold (SCD 050 Sputter Coater, Balzers). The morphology of starch granules was observed in a SEM (JEOL JSM 5800 LV, Tokyo, Japan) at 10 kV acceleration, and a large number of micrographs were acquired to select the most representative ones. Diameters of starch granules were measured, at least 45 starch granules, on SEM micrographs (1000 and 3000x) using ImageJ software, according to Abramoff, Magalhães, and Ram (2004).

2.5. Physicochemical characterization of young bamboo culm starch

2.5.1. Chemical composition

The obtained starches were evaluated by the following analyses according to AACCI (2010): moisture (method 44-15.02), protein (method 46-13.01, 6.25 as conversion factor), lipid (method 30-25.01) and ash (method 08-01.01) contents. Total carbohydrates content was calculated by difference [100 – (moisture + protein + lipid + ash)].

2.5.2. Apparent amylose content

The apparent amylose content was determined according to ISO (2007) methodology, and calculated from standard amylose from potato (A-0512) (Sigma Chemical Co., St. Louis, EUA) curves.

2.5.3. Molecular size distribution

Starch molecular weight distribution profiles were determined according to Franco, Wong, Yoo, and Jane (2002), with some modifications: it was used a gel-permeation chromatography (GPC) column (1.5 cm x 70 cm - Pharmacia Biotech) packed with Sepharose CL-2B gel. Samples (100 mg) were previously dispersed in 10 mL of 90% dimethyl sulfoxide solution (DMSO) in a bath of boiling water under stirring for 1 h and then stirred for more 16 h at 25 °C. An aliquot (1.5 mL) of the starch solution was mixed with anhydrous ethanol (6 mL) and centrifuged at 12,000 g for 10 min. The precipitated starch was resuspended with 5 mL of eluent and 0.5 mg of anhydrous glucose were added. This mixture was again kept in a bath of boiling water for 30 min, cooled to room temperature, and centrifuged at 12,000 g for 10 min. An aliquot (3.5 mL) of the supernatant (containing 9 mg of starch and 0.35 mg of anhydrous glucose) was injected into the base of the column and eluted upward. Fractions of the sample (2.5 mL) were collected every 5 minutes and analyzed, according to Dubois, Gilles, Hamilton, Rebers, and Smith (1956), for total sugars (CHO) (sulfuric phenol methodology), and according to Juliano (1971) blue value method (BV) (iodine staining) was performed to determine the elution profile of the starches, at 492 and 630 nm, respectively.

2.5.4. Branch chain length distribution of amylopectin

The branch chain length distributions of amylopectin from young bamboo culm were analyzed according Costa, Volanti, Grossmann, and Franco (2017). The enzyme isoamylase used was from *Pseudomonas* sp. (EC 3.2.1.68) (Megazyme International, Wicklow, Ireland).

2.5.5. X-ray diffraction

The X-ray diffraction pattern was determined using a benchtop X-ray diffractometer (MiniFlex 300, Rigaku, Tokyo, Japan) equipped with Cu-Kα monochromatic radiation (λ=0.1542 nm). The scan was performed at 30 kV and 10 mA for a 2θ range of 3-40º with a step size of 0.01. Prior the analysis, the samples were previously stored in a desiccator containing saturated $BaCl_2$ solution (25 °C, Aw=0.9) for 10 days. The relative crystallinity was quantitatively estimated based on the relationship between the peak area and the total area of the diffractogram according to the method described by Nara and Komiya (1983). The analysis was performed in triplicate, and the results expressed in percentage of crystallinity.

2.6. Thermal properties of young bamboo culm starch

The thermal properties were analyzed using a Differential Scanning Calorimeter (DSC-Pyris 1, Perkin Elmer, Norwalk, USA) according to Franco et al. (2002) with modifications: the weighed samples were kept for 12 h at room temperature to equilibrate and scanned at a rate of 5 °C/min over a temperature range of 25-125 °C. Each gelatinized starch sample was stored for 7 days at 4 °C, and then analyzed for the thermal properties of the retrograded starch using the same apparatus and parameters. Transition temperatures (Onset-T_0, Peak-T_p, and

End-T_e) and enthalpy change (ΔH) of the starches were determined using Pyris 1 software (Perkin Elmer, Norwalk, USA), and the percentage retrogradation (%R) was calculated by the ratio of $\Delta H_{retrogradation}/\Delta H_{gelatinization}$. The apparatus was calibrated with indium. An empty aluminum vessel was used as a reference. All analyzes were performed in triplicate.

2.7. Statistical analysis

Analysis of variance (ANOVA) of the means was performed using the Sisvar software, version 5.6 (Federal University of Lavras, Lavras, MG, Brazil) at a significance level of 95%, and when it was significant, the Scott-Knot test was used to determine statistical differences between means ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1. Starch extraction from young bamboo culm

The starch granules are located inside the parenchyma cells (Figure 1) in the bamboo culm wall, and we do not use any chemical/enzymatic treatment to improve the extraction, because our objective was to prioritize the release of the starch that is inside the vegetal cells, without modifications.

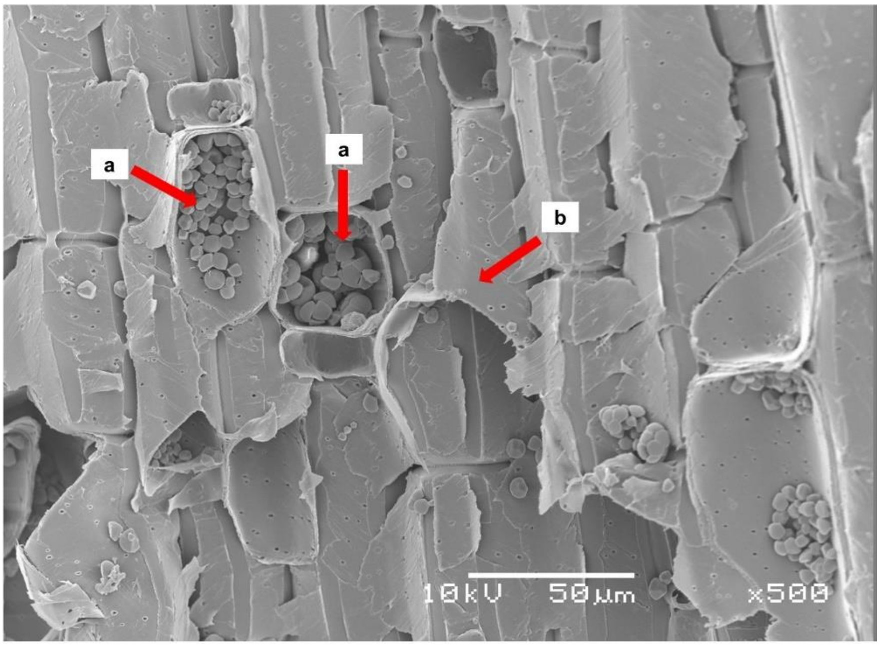

Figure 1 – Scanning electronic micrographs of young bamboo culm chips, with starch granules (a) filling the parenchyma cells (b) of *D. asper*

Extraction yields (Table 1) was higher than 10%, with samples SM and ST having the highest values. Felisberto, Miyake, Beraldo, and Clerici (2017) observed 9.4% of starch content, on average, for young bamboo culm flour. So, the yield obtained in this work can be optimized. Although the literature does not have parameters for yield of starch extraction in culms (in the case of bamboo) on industrial scale, it can be said that many factors interfere in the extraction process, such as: the location of the starch granule, the use of chemical or enzymatic reagents to break down the fibrous and vascular structure surrounding this tissue, in addition to losses during the filtration and centrifugation stages, which can be used to increase the extraction of starch. For example, BeMiller and Whistler (2009) that reported some industrial processes are well-established, among which we can mention the standard starch extraction of corn or rice grains, which requires the use of chemical reagents due to the high protein content (10%), while the extraction of cassava and potato starches requires only water, and both of them require filtration and centrifugation. Moreover, the isolation of starches may result in an incorrect granule size distribution due to the loss of certain granule fractions, particularly the smaller granules, or to granule damage experienced during isolation (Lindeboom, Chang, & Tyler, 2004).

Table 1 – Extraction yield, color parameters and size of starch granules isolated from different portions of the young culm of bamboo *Dendrocalamus asper*[1].

Starch [2]	Extraction yield[3] (w/w)	Color parameters			Diameter (μm)
		L*	a*	b*	
SB	11.06 ± 1.90^{b}	89.41 ± 0.04^{b}	0.72 ± 0.02^{a}	15.49 ± 0.15^{b}	5.28 ± 0.23^{ns}
SM	15.19 ± 1.47^{a}	89.66 ± 0.04^{a}	0.66 ± 0.01^{b}	14.99 ± 0.17^{c}	5.40 ± 0.51^{ns}
ST	14.62 ± 2.03^{a}	89.18 ± 0.04^{c}	0.18 ± 0.01^{c}	16.10 ± 0.11^{a}	5.66 ± 0.48^{ns}

[1]Results expressed as means ± standard deviation. Means followed by different letters in the same column differ significantly ($p < 0.05$) by the Scott-Knott test and ns = not significant. [2]Starches isolated from young bamboo culm, respectively from the bottom (SB), middle (SM) and top (ST) portions. [3]db = dry base.

3.2. Morphological characteristics of young bamboo culm starch

3.2.1. Instrumental color

It was observed that all the starches differed statistically from each other and characterize yellowish coloration and luminosity (L*) above 89 (Table 1), and ST presented the lowest color index in the red region (index a*) and highest color index in the yellow region (index b*). The total color difference (ΔE) between starches were 0.55 and 0.85, for SB and SM, and for SB and ST, respectively, and between SM and ST it was 1.30, however they are not distinguishable by naked eye, because according to Moritz (2011) this difference must be greater than 2.

The values of L* for the starches obtained in the present work are lower than those observed for cassava (L*=95.33) (Benesi, Labuschagne, Dixon, & Mahungu, 2004), amaranth (L*=96.64) and corn (L*=98.13) starches (Chandla, Saxena, & Singh, 2017). Our results are very close to the whiteness of 90, which Boudries et al. (2009) classifies as acceptable for starch purity.

Industrially the extracted starches undergo a bleaching process, aiming to remove compounds formed by the enzymatic darkening of the vegetables during the process (Eliasson, 2004), but in this research, we used only the sodium metabisulphite inhibitor and no oxidizing agent, such as hydrogen peroxide and/or hypochlorite.

3.2.2. Morphological evaluation of starch granules

Optical Microscopy (OM) – The obtained starches observed by OM, without polarized light, present small sizes, and polyhedral shape (Figure 2 A). If observed under polarized light, they exhibit a Maltese cross (Figure 2 B). This positive birefringence indicates a radial orientation of the macromolecules, which means normal to the growth rings and the surface of the granule, confirming that the extraction process did not affect the structure of the granule, because, according to BeMiller and Whistler (2009), the loss of birefringence on heating is an indicative of disordering processes.

Scanning Electron Microscopy (SEM) – It was observed by SEM (Figure 2 C) that the obtained starches present a smooth, nonporous surface and polyhedral shape, and some of them have rounded shape. And they also contain compound granules. According to Jane (2009) and Pérez and Bertoft (2010), this formation of agglomerates may be due to the granules being produced simultaneously in a single amyloplast, which restricted the space for development of the granule, giving it a polyhedral shape. Similar shape can be observed in granules of cereal starches, such as rice, waxy rice, oats (Jane, 2009), which belong to the grass family *Poaceae*, as well as bamboo.

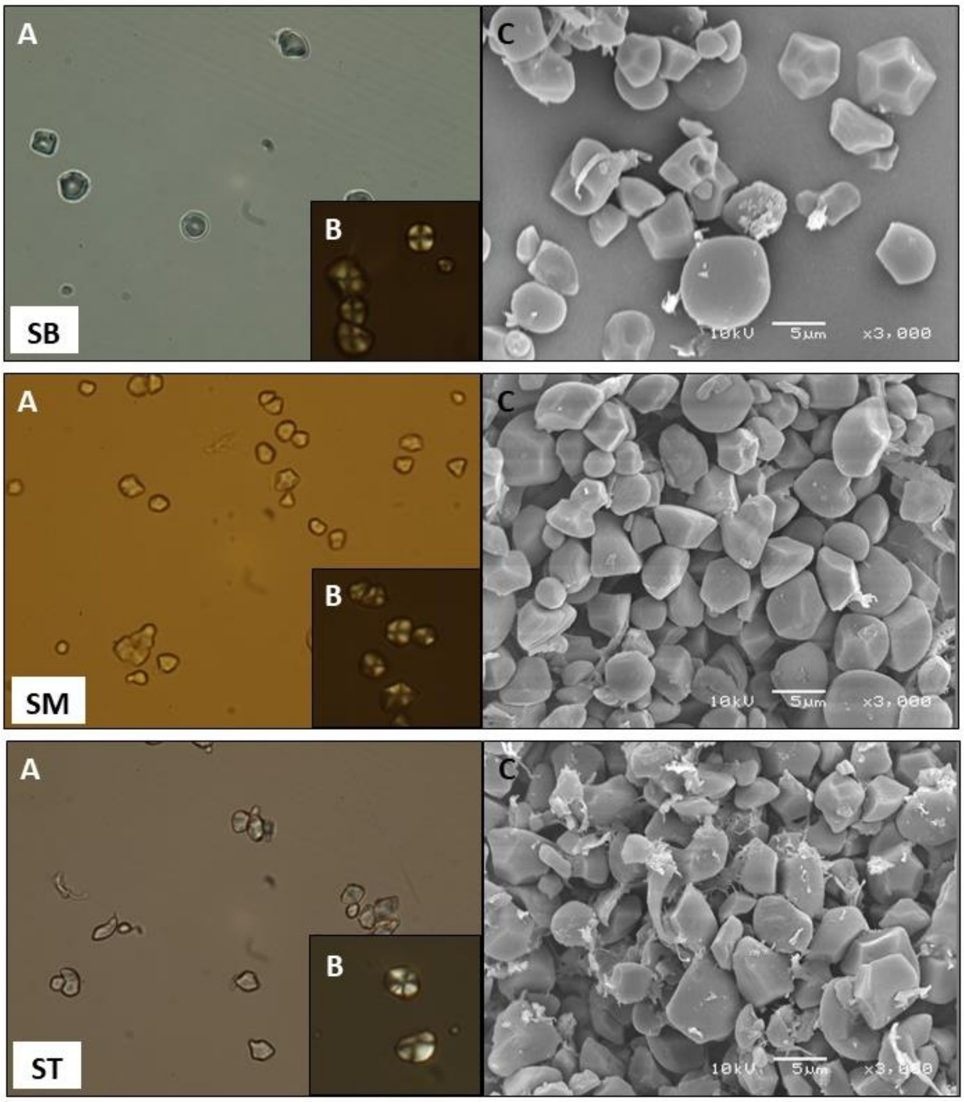

Figure 2 – Micrographs of isolated starches granules respectively from bottom (SB), middle (SM) and top (ST) portions of young culm of bamboo *D. asper* observed in: optical microscope without polarized light (A - 100 x magnification); optical microscope with polarized light (B - 100X magnification); scanning electron microscope (C - 3000X magnification).

Table 1 shows that there was no significant difference on average diameter of the starches extracted from different parts of the young bamboo culm. The granules displayed a diameter of less than 6 µm, which may be classified as small, according to Lindeboom et al. (2004). However, the granules presented a large variation of diameter and shape, so we grouped them into spherical (1-3 µm) and polyhedral (3-9 µm). This range of size is similar to that observed for rice starch granules (3-8 µm), as described by Pérez and Bertoft (2010), and for wild rice starch granules (2-8 µm), as evaluated by Hoover, Sailaja, and Sosulski (1996). Similar results for starches extracted from *Guadua flabellata* culm and *Phyllostachys heterocycla* seeds were obtained by Toledo et al. (1987) and to Ai et al. (2016), respectively. Although, there is a peculiarity that would interfere with the use of bamboo seed starch is that, according to Liese and Kohl (2015) depending on the species, the culms of one population flower after 40-80 years, mostly with a subsequent dying of the entire population across large regions.

3.3. Physicochemical characteristics of young bamboo culm starch

3.3.1. Chemical composition

Table 2 shows that all the obtained starches presented low values of protein, lipid and ash, resulting in a starch with high purity.

Table 2 – Chemical composition of obtained starches from different portions of the young culm of the bamboo *Dendrocalamus asper*.

Starches [2]	**Nutrients (g/100 g)** [1]					
	Moisture	**Protein**	**Lipid**	**Ash**	**Total Carbohydrates** [3]	**Apparent amylose (%)**
SB	5.88 ± 0.41 [ns]	2.17 ± 0.03[a]	0.21 ± 0.04[b]	1.13 ± 0.02[a]	90.61	29.71 ± 0.96[a]
SM	6.07 ± 0.63 [ns]	1.76 ± 0.02[b]	0.23 ± 0.02[b]	0.96 ± 0.02[b]	90.99	12.03 ± 0.44[c]
ST	6.88 ± 0.65 [ns]	2.12 ± 0.02[a]	0.46 ± 0.08[a]	0.90 ± 0.01[c]	89.64	24.21 ± 0.82[b]

[1]Results expressed as means ± standard deviation. Means followed by different letters in the same column differ significantly ($p < 0.05$) by the Scott-Knott test and ns = not significant. [2]Starches isolated from young bamboo culm, respectively from the bottom (SB), middle (SM) and top (ST) portions. [3]Total carbohydrates = 100 – (moisture + lipid + protein + ash).

3.3.2. Apparent amylose content

Starches can be classified according to amylose and amylopectin contents in waxy starch (> 99% amylopectin), normal starch (contain 70-80% amylopectin and 20-30% amylose) and high-amylose starch (> 50% amylose). Amylose content in normal starches may range, as follows: tuber and root starches ranging from 15 to 38% (Hoover, 2001) and cereals from 20 to 30% (BeMiller & Whistler, 2009).

The ST and SB showed apparent amylose content (Table 2) similar to normal cereal starches, which was already expected since the bamboo belongs to the same family. These result were similar to that observed by Ai et al. (2016), in starch isolated from bamboo seeds (24%), and by Hoover et al. (1996), in wild rice (21.1%) and long grain brown rice (22.8%) starches. This amylose content also indicates that the starch present in the middle of the culm (SM) is more easily hydrolyzed by amylases, into simple sugars, that according to Liese and Kohl (2015) will be used during sprouting and elongation, when the older culm release the sugar to the growth of the shoots, and the culm growth in height. This fact corroborates with that reported by Felisberto, Miyake, et al. (2017), where the flour with the highest starch content (middle portion) also presented the lowest sugar content, when compared to the other samples.

3.3.3. Molecular size distribution

Molecular weight distributions of SB, SM and ST starches exhibit similar elution profiles (Figure 3). Amylopectin of all samples was eluted at the void volume and shown as the first peak (at the volume of 65 mL), which has a large molecular mass. The second peak (at the volume of 132.5 mL) correspond to amylose, and the last one was glucose (G), added as a marker of the end of the elution.

Ratios of blue value (BV) to total sugars (BV/CHO) at amylopectin peak were different between samples (SB=0.46; SM=0.37; ST=0.32), suggesting that amylopectin of SB and SM consisted of higher proportions of longer branched chains, even though no difference was evidenced for branched chains on item 3.3.4.

In relation to amylopectin peak, both for blue value (BV) and total sugar (CHO) response, we observed that the results are opposite to those of apparent amylose content: SB and ST presented the highest peak for amylopectin; however, they presented also the highest values for amylose content (Table 2). These results may be due to the quantification of the apparent amylose content, since in a recent study Fitzgerald et al. (2009) highlighted the need to standardize the way amylose is measured. They evaluated the reproducibility of amylose quantification between the quality evaluation laboratories of the world and they observed a variation of 4 to 40%

in the amylose content of the same sample analyzed by different laboratories. The explanation of this difference is based on the way the standard curve is constructed and the iodine binding capacity of potato amylose used as standard.

So, although quantifying the actual proportion of amylose in young bamboo culm starch still has not suitable conditions, the size exclusion of the polymers gives a real proportion of the ratio between amylose and amylopectin chains.

3.3.4. Amylopectin branch-chain length distribution

Normalized chain length of amylopectin from the three different parts of young bamboo culm exhibited bimodal distribution (Figure 3), with the main peak at 12-13 degree polymerization (DP) and the second peak at 43 DP, without difference between them in the distribution of side chains. Similar results to those obtained in the present study were observed in some cereal starches, as observed by Jane et al. (1999), who evaluated wheat (DP 12 and 41), normal rice (DP 12 and 46) and barley (DP 12 and 43) starches.

Table 3 shows that amylopectin from the starches of the young bamboo culm have a large number of short chains (DP 6-12) and long branch chains (DP≥37) and the highest proportion of chains with DP 13-24. According to Genkina, Wikman, Bertoft, and Yuryev (2007) the chains with DP 13-24 present the ideal size to form stable double helices. The SB, SM and ST starches did not have a shoulder on their branch chain length distributions, suggesting that these starches do not present imperfections in the crystalline structure (Jane et al., 1999). As observed in the molecular size distribution, the starches did not present structural differences independent of the extracted part of the bamboo culm.

For all the evaluated samples we obtained average DP 23, which were longer than that observed by Ai et al. (2016) for bamboo seed (DP 19.1), indica (DP 19.7) and japonica (DP 17.1) rice starches, and closer to that observed by Jane et al. (1999) for normal rice (DP 22.7) and wheat (DP 22.7) starches. This fact can be explained by the heterogeneity of the samples that were compared, although they belong to the same botanical family, *Poaceae*, they are of different ages, and starch was extracted from different parts of the plant. Once bamboo starch was extracted from the culm, that was collected at the age of three years, rice and wheat starches were extracted from the grain, which were collected at least 6 months of age.

According to Jane (2009), the maturity, origin and the location of the molecules in the granule makes the average branch chain length of amylopectin varies between samples. Because of this, according to Pérez and Bertoft (2010), the average branch-chain length of amylopectin give a brief description of the true structure of amylopectin, because all samples possess short and long chains, and this is useful for the structural characterization of the starches.

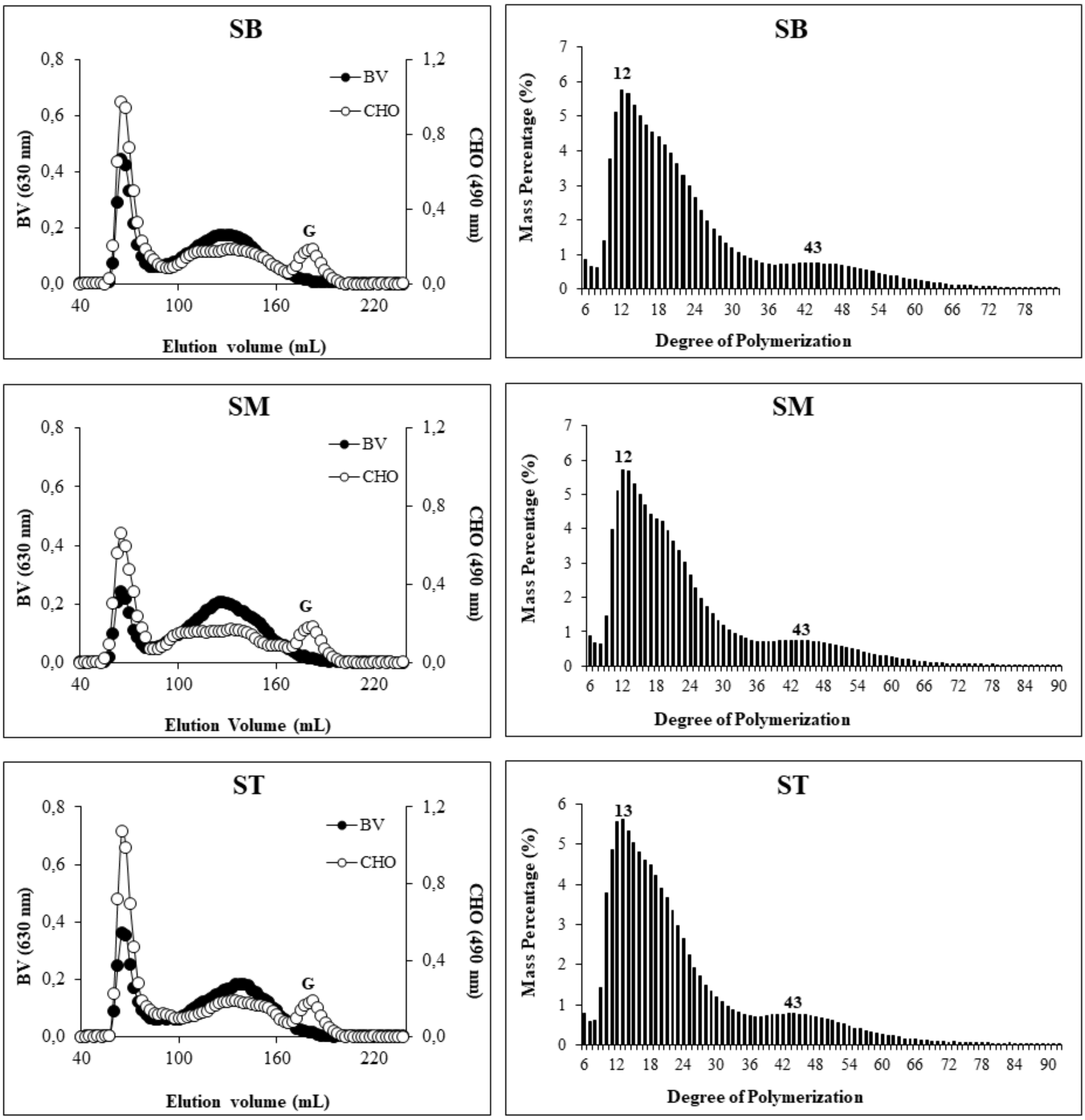

Figure 3 – Elution profile on Sepharose CL-2B and amylopectin branch chain length distributions of the isolated starches granules respectively from bottom (SB), middle (SM) and top (ST) portions of young culm of bamboo *D. asper*, where ● = elution profile according blue value (BV) method and ○ = elution profile according total sugars (CHO) method.

Table 3 – Amylopectin branch chain length distribution of isolated starches from different portions of the young culm of bamboo *Dendrocalamus asper*.

Starches[2]	Branch Chain Length Distribution (%)[1]					Highest detectable DP
	DP 6-12	DP 13-24	DP 25-36	DP > 37	$\overline{DP}$	
SB	18.41 ± 0.32 ns	50.45 ± 0.20 ns	15.12 ± 0.23 ns	16.02 ± 0.29 ns	23.24 ± 0.15ns	83
SM	18.41 ± 0.10 ns	50.29 ± 0.16 ns	15.09 ± 0.07 ns	16.22 ± 0.01 ns	23.33 ± 0.01 ns	90
ST	17.89 ± 0.41 ns	50.68 ± 0.04 ns	14.97 ± 0.14 ns	16.45 ± 0.31 ns	23.46 ± 0.18 ns	92

[1]Results expressed as means ± standard deviation. Means followed by different letters in the same column differ significantly ($p < 0.05$) by the Scott-Knott test and ns = not significant. [2]Starches isolated from young bamboo culm, respectively from the bottom (SB), middle (SM) and top (ST) portions. $\overline{DP}$ – average Degree of Polymerization.

3.3.5. X-ray diffraction

Figure 4 presents the obtained diffractograms, and all of samples exhibited peaks at 15.1°, 16.8°, 18.0° and 22.3° at 2θ, characteristics of the A-type crystalline pattern. This type of profile observed for young bamboo culm starch presents similarity with cereal starches, according to Gallant et al. (1982) as these present 2 peaks at 2θ between 16° and 18º and another one nearby 23°. The difference between them is that the starch of bamboo was extracted from the culm, while the starch of cereals is extracted from the grain.

The results observed at Figure 4 are in agreement with those observed for the amylopectin branch chain length distribution (Table 3) because, according to Hizukuri (1986), Jane (2006) and BeMiller and Whistler (2009) starch granule that display the A-type polymorph has a lower proportion of long chains and a larger proportion of short chains, as it was observed in the present study.

It was observed that SM presented higher relative crystallinity (RC) (Figure 4), and also presented lower amylose content (Table 2), once amylopectin is responsible for the crystalline structure of starch granules. In the same way, as expected, SB and ST, which presented higher levels of amylose, also presented lower RC. According to Hoover (2001), the RC of starch is directly linked to the amylopectin content, and inversely to the amylose content, and for native starches, it is ranged from 15 to 45% (Cheetham & Tao, 1998). Although, bamboo seeds starches, evaluated by Ai et al. (2016), presented A-type polymorph and amylose content similar to ST, the RC were higher than the observed in the present work (32.1%).

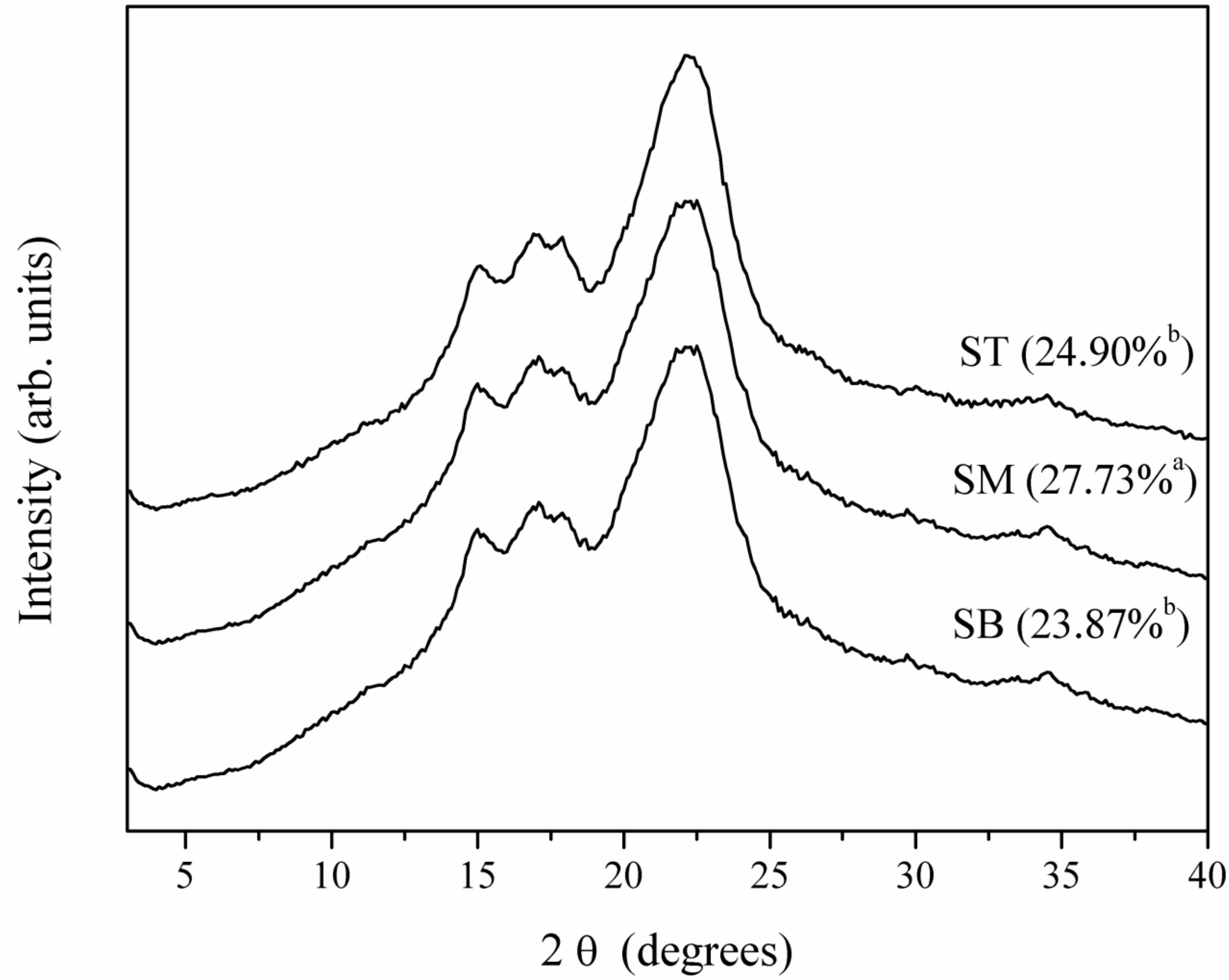

Figure 4 – X-ray diffraction patterns of isolated starches granules respectively from bottom (SB), middle (SM) and top (ST) portions of young culm of bamboo *D. asper* and relative crystallinity is given in parentheses. Means followed by different letters differ significantly ($p<0.05$) by the Scott-Knott test.

3.3.6. Thermal properties of young bamboo culm starch

The isolated starches presented high gelatinization temperatures (Table 4), with peak temperature close to 83.8 °C, which is related to the higher proportion of chains with DP 13-24 (Table 3). According to Srichuwong, Sunarti, Mishima, Isono, and Hisamatsu (2005), Genkina et al. (2007) and Costa et al. (2017), these chains of double stable helices favor the dense packing of amylopectin chains in crystalline regions making these structures thermally stable.

The studied starches presented significant differences in gelatinization enthalpy change (ΔH). The SB starch presented lower ΔH followed by ST and SM starches, confirming that the greater ΔH is related to the higher RC. The high enthalpies of gelatinization are related to high crystallinity, due to a high energy required for the melting the crystalline regions, as cited by Franco et al. (2002) and Ai et al. (2016). The results of this study show that the SM starch had higher RC and higher ΔH.

After storing at 4 °C for 7 days, the thermal transition temperatures of the retrograded starches were lower than the gelatinization temperatures of native ones. This reduction in the transition temperature was explained by Karim, Norziah, and Seow (2000) and Singh, Singh, Kaur, Singh Sodhi, and Singh Gill (2003), due to the crystallization involving amylose molecules and the long branch chain of amylopectin, which were inadequate realigned, forming a less ordered and less stable crystalline structures than those in the native starch.

The endothermic peaks of retrograded starches were smaller than those of gelatinization. According to Srichuwong et al. (2005), the reduction of $\Delta H_{retrogradation}$ in relation to the $\Delta H_{gelatinization}$ occurs due to the improper alignment of the amylopectin chains during the recrystallization, which results in a lower energy to melting the restructured crystals. In general, the isolated starches displayed high retrogradation rates (%R) as observed in cereal starches, due to the high amount of amylose and lipids (Table 2) and the high proportion of short chain (DP 6-12) (Table 3). ST starch displayed the highest %R, which is due to the high lipid content since lipids restrict swelling and dispersion of starch granules, but accelerate retrogradation of starch, according to Jane et al. (1999).

The high tendency to retrograde may affect most starchy foods which are cooked and not immediately dried or eaten. And, according to Blennow (2004), this kind of starches may be ideal to achieve gelled, short and chewy bite of gelled products . Furthermore, it may be employed in combination with others food starches and hydrocolloids to take advantage of the bulk and body imparted by the starch product and the low cost and improvements in stability and texture imparted by the hydrocolloid (Huber & BeMiller, 2010).

Table 4 - Thermal properties of isolated starches from different portions of the young culm of the bamboo *Dendrocalamus asper*[1].

Starches [2]	Gelatinization				Retrogradation				Retrogradation (%)
	T_0 (ºC)	T_p (ºC)	T_e (ºC)	ΔH ($J.g^{-1}$)	T_0 (ºC)	T_p (ºC)	T_e (ºC)	ΔH ($J.g^{-1}$)	
SB	78.58 ± 0.02^c	82.76 ± 0.08^c	86.16 ± 0.10^c	4.25 ± 0.16^c	43.70 ± 0.84^{ns}	56.52 ± 0.09^b	66.19 ± 0.02^a	2.32 ± 0.11^b	54.57^c
SM	80.16 ± 0.09^b	84.07 ± 0.10^b	87.07 ± 0.23^b	6.35 ± 0.14^a	41.61 ± 0.78^{ns}	55.66 ± 0.19^a	65.24 ± 0.50^b	3.89 ± 0.12^a	61.30^b
ST	80.88 ± 0.05^a	84.67 ± 0.17^a	87.84 ± 0.30^a	5.19 ± 0.18^b	43.07 ± 1.42^{ns}	55.38 ± 0.51^a	66.46 ± 0.29^a	3.89 ± 0.05^a	74.90^a

[1]Results expressed as means ± standard deviation. Means followed by different letters in the same column differ significantly ($p < 0.05$) by the Scott-Knott test and ns = not significant. [2]Starches isolated from young bamboo culm, respectively from the bottom (SB), middle (SM) and top (ST) portions of the *Dendrocalamus asper* specie. T_0, T_p and T_e – Onset, peak and end temperatures, ΔH - enthalpy change, and Retrogradation (%) = ($\Delta H_{retrogradation}/\Delta H_{gelatinization}$) * 100.

4. CONCLUSION

The extraction of young bamboo culm starch from *D. asper,* in laboratory scale, assured the obtainment of a native starch with acceptable purity content. The young bamboo culm starch presented characteristics of small diameter and A-type polymorph. Furthermore, they presented high gelatinization temperatures and high tendency to retrograde, which are strongly influenced by the amylose content, amylopectin short chains (DP 6-12) and by the lipid content. Due to this, the evaluated starches present potential to be applied in industrial processes, where greater stability to temperature and mechanical friction is required.

The young bamboo culm starches can be an alternative source of starches to those traditionally used, such as corn, rice or wheat starches. Since rice starch has a well-established market, the use of this grain for the extraction of starch is not enough to supply the market demand. So, the obtained results indicate that adjustments for industrial scale processing can be performed, in order to optimize the extraction of starch from the young culm of bamboo, as a new ingredient for industrial applications.

5. ACKNOWLEDGEMENTS

The authors are grateful to CAPES for the scholarship for Mária Herminia Ferrari Felisberto, to FAPESP (2015/196374) and FAEPEX (519292-2425/16) for the financial support, and to CNPq, PIBIC and SAE/Unicamp, for the scholarship of the students who supported along the project.

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− CAPÍTULO 6 –

Physicochemical and structural properties of starch from young bamboo culm of *Bambusa tuldoides*

Este capítulo apresenta a caracterização do amido extraído do colmo jovem de Bambusa tuldoides e, consequentemente, seu potencial como fonte alternativa de amido para aplicações industriais.

Este capítulo será submetido ao periódico internacional Food Hydrocolloids, de alto impacto para a área.

Physicochemical and structural properties of starch from young bamboo culm of *Bambusa tuldoides*

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ABSTRACT

Starch is used in a wide variety of food and non-food industrial applications. However, in some cases it is necessary to apply physical and/or chemical treatments to modify some of its properties, besides being an expensive process, it is environmentally unfriendly. However, the development of genetic engineering technologies could be an alternative in the production of modified starches in the future, using less exploited crops, like bamboo. So, the objective of this study was to characterize the morphology, structure, physicochemical and thermal properties of starch extracted from three different parts of the young culm (bottom-SB, middle-SM and top-ST) of *B. tuldoides*. The obtained data were evaluated by ANOVA and Scott-Knot test ($p<0.05$). The starches presented pale yellow coloration (on average $a^*=0.65$ and $b^*=11.26$) and high luminosity (on average L=89.39). Polyhedral shape and small size (diameter=5.4 µm) were observed for all the starches through optical microscope (OM) and by scanning electron microscopy (SEM). SB, SM and ST showed apparent amylose content similar to starches from cereals and isolated from bamboo seeds. The molecular size distribution of amylose chains corroborates with SM (33.35%) and ST (24.48%) amylose content, since they presented the highest values. The highest proportion of chains of amylopectin were observed for short chains, with DP 13-24, without significant difference among the samples, regardless of the part of the culm from which the starch was extracted. This result corroborates with the A-type polymorph presented for all the samples, SM presented the lowest value of relative crystallinity (RC), which agrees with the highest amylose content. Gelatinization temperature above 81 ºC was observed for all the samples, corroborating with the higher proportions of amylopectin short chains. The obtained starches presented characteristics of native starches and they were very similar to the starches traditionally used. Thus, starch from the young culm of bamboo would be an alternative source to traditional ones, such as rice, wheat, or corn, corroborating with its potential for industrial applications.

Keywords: carbohydrate, grass, industrial application, natural ingredient, sustainability environment.

1. INTRODUCTION

Starch, which is stored in the seeds and tubers of various agricultural crops, is the major reserve of carbohydrate in plants. The production of corn, cassava, wheat and potatoes has expanded in several countries, because their starches has been used in the production of food and beverages, manufacture of adhesives, cosmetics, detergents, paper, textiles, biodegradable packing materials and biodegradable plastics (Ellis et al., 1998; Davis et al., 2003; Bemiller and Whistler, 2009).

This increased demand for starch on the market and has expanded the search for new sources of starches, and for chemically or physically modified starches. Great attention has been given to the advances made by genetic engineering technologies, which could allow changes in the synthesis of starch *in planta*, reducing or eliminating post-harvest modifications, as cited by Davis et al. (2003).

Some crops, such as bamboo, have great potential for the application of genetic engineering, since despite their low commercial value, their starch could present properties of commercial interest, as has already been done in the production of starches with high amylose content and waxy starches using maize, wheat, rice and potato starches (Murata, Sugiyama and Akazawa, 1965; Hovenkamp-Hermelink et al., 1987; Nakamura et al., 1995; Gao et al., 1998).

The genetic engineering technique is very versatile and could be applied in various sources for the production of different types of starch, reducing not only the processing costs of the industry, but also the damages to the environment, by reducing the need for the chemical treatments. Thus, the greater the types and sources of known starches, the greater the production of modified starches by transgene technology in the future, which can present the most varied possibilities of industrial application.

The use of the culm of *Bambusa tuldoides* would be a great option for the application of transgene technology to modify starches. It is a perennial crop that grows fast, without the need of replanting, and that does not require pesticides (Silva and Costa, 2012). It is a high-yield renewable resource, cheap and abundant across the globe (Akinlabi, Anane-Fenin and Akwada, 2017). Although the young bamboo culm has a higher content of starch, which is more easily extracted (Felisberto et al., 2017) when compared to bamboo seed starch (Ai et al., 2016), and the young bamboo culm starch from this specie, *B. tuldoides*, has not yet been characterized with respect to its morphological and physico-chemical properties.

So, the objective of this study was to characterize morphology, structure, physicochemical and thermal properties of starch extracted from young bamboo culm of *B. tuldoides*, to subsidize new industrial applications.

2. MATERIAL AND METHODS

2.1. Obtainment of young bamboo culm chips

Young bamboo culms of *B. tuldoides* were harvested in 2016 (from January to March), on average three years old, at experimental field from School of Agricultural Engineering (FEAGRI), at University of Campinas (UNICAMP), Campinas, São Paulo, Brazil, on geographic coordinates 22º82’ of south latitude and 47º07’ of west longitude. The collected culm was divided in three parts (B - bottom, M - middle and T - top) and the drying of the young culms was performed as described by Felisberto, Beraldo and Clerici (2017). After that, the small pieces of bamboo culm from each portion were ground in a hammer mill (DPM2–Nogueira, São Paulo, Brazil) until the bamboo chips were obtained, which were used for the extraction of the starch.

2.2. Starch extraction

The starch was extracted from three different parts of the young bamboo culm chips in aqueous solution, according to Azzini et al. (1981) and Toledo, Azzini and Reyes (1987) with some modifications: 60 g of bamboo chips were weighed and disintegrated in a OBL10/2 blender (OXY, Santana de Parnaíba, Brazil) with 500 mL of 200 ppm sodium metabisulphite solution, at 5 °C for 60 s.

After, the material was left to rest for 16 h/5 ºC, so the suspension was passed through a sieve (80 µm) and the material retained was triturated two more times. The filtrate was centrifuged at 17,000 g/10 min/10 °C. The supernatant was discarded, and the precipitate was resuspended with distilled water, three times. The extracted starch was then vacuum filtered, washed with 99.5% ethanol (twice) and then acetone, and dried at 45 °C/16 h, in a vacuum oven TE 395 (TECNAL®, Piracicaba, Brazil). The starch bottom (SB), starch middle (SM) and starch top (ST) were packed in plastic hermetically sealed. The obtained material was homogenized and weighted to evaluate the yield of the extraction process, which was calculated in relation to the initial weight of each sample.

2.3. Morphological characterization of young bamboo culm starch

2.3.1. Instrumental color

Instrumental color analysis was performed with a 50 mm port size, illuminant D65, SCI and a 10º standard observer angle, on CR-10 colorimeter (Konica Minolta, Tokyo, Japan). Tristimulus CIELab color space method was used, to determine L* (luminosity: 0=black; 100=white), a* (+a=redness; -a=greenness), and b* (+b=yellowness; -b=blueness).

2.3.2. Morphological evaluation of starch granules

Optical Microscopy (OM) – The starch sample was placed on a glass slide and mixed with 1-2 drops of water, and then covered with a glass cover slip. The granule morphology was observed in an optical microscopy (BX51, Olympos, Tokyo, Japan), coupled with a camera (E-330-ADU1.2X, Olympus, Tokyo, Japan), and the micrographs were captured at 100X magnifications, without polarized light.

Scanning Electron Microscopy (SEM) – The starch was placed on carbon adhesive tape attached to the stub (one type of metal bracket) and sputter-coated with 20 nm of gold (SCD 050 Sputter Coater, Balzers). SEM (JEOL JSM 5800 LV, Tokyo, Japan) at 10 kV acceleration, was used to observe he morphology of starch granules and a large number of micrographs were acquired to select the most representative ones.

Diameters of starch granules were measured, at least 65 starch granules were employed on SEM micrographs (1000 and 3000X) using ImageJ software, according to Abramoff, Magalhães and Ram (2004).

2.4. Physicochemical characterization of young bamboo culm starch

2.4.1. Chemical composition

The obtained starches were evaluated according to AACCI (2010), to moisture (method 44-15.02), protein (method 46-13.01, 6.25 as conversion factor), lipid (method 30-25.01) and ash (method 08-01.01) contents. Total carbohydrates content was calculated by difference [100 – (moisture + protein + lipid + ash)].

2.4.2. Apparent amylose content

The apparent amylose content was determined according to ISO (2007) methodology, and calculated from standard potato amylose (A-0512) (Sigma Chemical Co., St. Louis, EUA) curves.

2.4.3. Molecular size distribution

Starch molecular weight distribution profiles were determined according to Franco et al. (2002), with some modifications: it was used a gel-permeation chromatography (GPC) column (1.5 cm x 70 cm - Pharmacia Biotech) packed with Sepharose CL-2B gel. Samples (100 mg) were previously dispersed in 10 mL of 90% dimethyl sulfoxide solution (DMSO) in a bath of boiling water under stirring for 1 h and then stirred for more 16 h at 25 °C. An aliquot (1.5 mL) of the starch solution was mixed with anhydrous ethanol (6 mL) and centrifuged at 12,000 g for 10 min. The precipitated starch was resuspended with 5 mL of eluent and 0.5 mg of anhydrous glucose were added. This mixture was again kept in a bath of boiling water for 30 min, cooled to room temperature, and centrifuged at 12,000 g for 10 min. An aliquot (3.5 mL) of the supernatant (containing 9 mg of starch and 0.35 mg of anhydrous glucose) was injected into the base of the column and eluted upward. Fractions of the sample (2.5 mL) were collected every 5 min and analyzed, according to Dubois et al. (1956), for total sugars (CHO) (sulfuric phenol methodology), and according to Juliano (1971) blue value method (BV) (iodine staining) was performed to determine the elution profile of the starches, at 492 and 630 nm, respectively.

2.4.4. Branch chain length distribution of amylopectin

The branch chain length distributions of amylopectin from young bamboo culm were analyzed according Costa et al. (2017). The enzyme isoamylase used was from *Pseudomonas* sp. (EC 3.2.1.68) (Megazyme International, Wicklow, Ireland).

2.4.5. X-ray diffraction

The X-ray diffraction pattern was determined using a benchtop X-ray diffractometer (MiniFlex 300, Rigaku, Tokyo, Japan) equipped with Cu-Kα monochromatic radiation (λ=0.1542 nm). The scan was performed at 30 kV and 10 mA for a 2θ range of 3-40º with a step size of 0.01. Prior the analysis, the samples were previously stored in a desiccator containing saturated $BaCl_2$ solution (25 °C, Aw=0.9) for 10 days. The relative crystallinity was quantitatively estimated based on the relationship between the peak area and the total area of the diffractogram according to the method described by Nara and Komiya (1983). The analysis was performed in triplicate, and the results expressed in percentage of crystallinity.

2.5. Thermal properties of young bamboo culm starch

The thermal properties were analyzed using a Differential Scanning Calorimeter (DSC-Pyris 1, Perkin Elmer, Norwalk, USA) according to Franco et al. (2002) with modifications: the weighed samples were kept for 12 h at room temperature to equilibrate and scanned at a rate of 5 °C/min over a temperature range of 25-125 °C. Each gelatinized starch sample was stored for 7 days at 4 °C, and then analyzed for the thermal properties of the retrograded starch using the same apparatus and parameters. Transition temperatures (Onset-T_0, Peak-T_p, and

End-T_e) and enthalpy change (ΔH) of the starches were determined using Pyris 1 software (Perkin Elmer, Norwalk, USA), and the percentage retrogradation (%R) was calculated by the ratio of $\Delta H_{retrogradation}/\Delta H_{gelatinization}$. The apparatus was calibrated with indium. An empty aluminum vessel was used as a reference. All analyzes were performed in triplicate.

2.6. Statistical analysis

Data obtained were evaluated by analysis of variance (ANOVA) of the means, using the Sisvar software, version 5.6 (Federal University of Lavras, Lavras, MG, Brazil) at a significance level of 95%, and when it was significant, the Scott-Knot test was used to determine statistical differences between means (p≤0.05).

3. RESULTS AND DISCUSSION

3.1. Starch extraction from young bamboo culm

Since our objective was to prioritize the release of the starch that is inside the vegetal cells, without modifications, we do not use any chemical/enzymatic treatment to improve the extraction of the starch from the young bamboo culm, obtaining low extraction yields (Table 1).

Table 1 – Extraction yield, color parameters and size of starch granules isolated from different portions of the young culm of bamboo *Bambusa tuldoides*[1].

Starch [2]	Extraction yield (w/w)	Color parameters			Diameter (µm)
		L*	a*	b*	
SB	3.55 ± 0.50^{ns}	89.12 ± 0.07^{b}	0.53 ± 0.01^{c}	11.66 ± 0.05^{b}	4.44 ± 0.47^{ns}
SM	3.31 ± 0.38^{ns}	91.07 ± 0.05^{a}	0.69 ± 0.01^{b}	10.14 ± 0.06^{c}	4.39 ± 0.50^{ns}
ST	2.51 ± 0.66^{ns}	87.98 ± 0.04^{c}	0.72 ± 0.00^{a}	11.98 ± 0.03^{a}	5.08 ± 0.67^{ns}

[1]Results expressed as means ± standard deviation. Means followed by different letters in the same column differ significantly (p < 0.05) by the Scott-Knott test and ns = not significant. [2]Starches isolated from young bamboo culm, respectively from the bottom (SB), middle (SM) and top (ST) portions.

Although the literature does not reports parameters for yield of starch extraction in culms (in the case of bamboo) on industrial scale, it can be considered that many factors interfere in the extraction process, such as: the location of the starch granule, the use of chemical or enzymatic reagents to break down the fibrous and vascular structure surrounding this tissue, in addition to losses during the filtration and centrifugation stages, which can be used to increase the extraction of starch.

3.2. Morphological characteristics of young bamboo culm starch

3.2.1. Instrumental color

We observed that although the starches from the young bamboo culm of *B. tuldoides* differed statistically from each other, all of them presented yellowish coloration and luminosity (L*) above 87 (Table 1), and SB presented the lowest color index in the red region (index a*) and ST presented the highest color index in the yellow region (index b*). Our results are very close to the whiteness of 90, which Boudries et al. (2009) classifies as acceptable for starch purity. Our results are lower than those observed for cassava (L*=95.33), amaranth (L*=96.64) and corn (L*=98.13) starches, as observed by Benesi et al. (2004) and Chandla, Saxena and Singh (2017), respectively.

The total color difference (ΔE) between starches were 2.48 and 1.20, for SB and SM, and for SB and ST, respectively, and between SM and ST it was 3.59. According to Moritz (2011), these difference are distinguishable by naked eye, because ΔE is greater than 2, as can be observed on Figure 1 B.

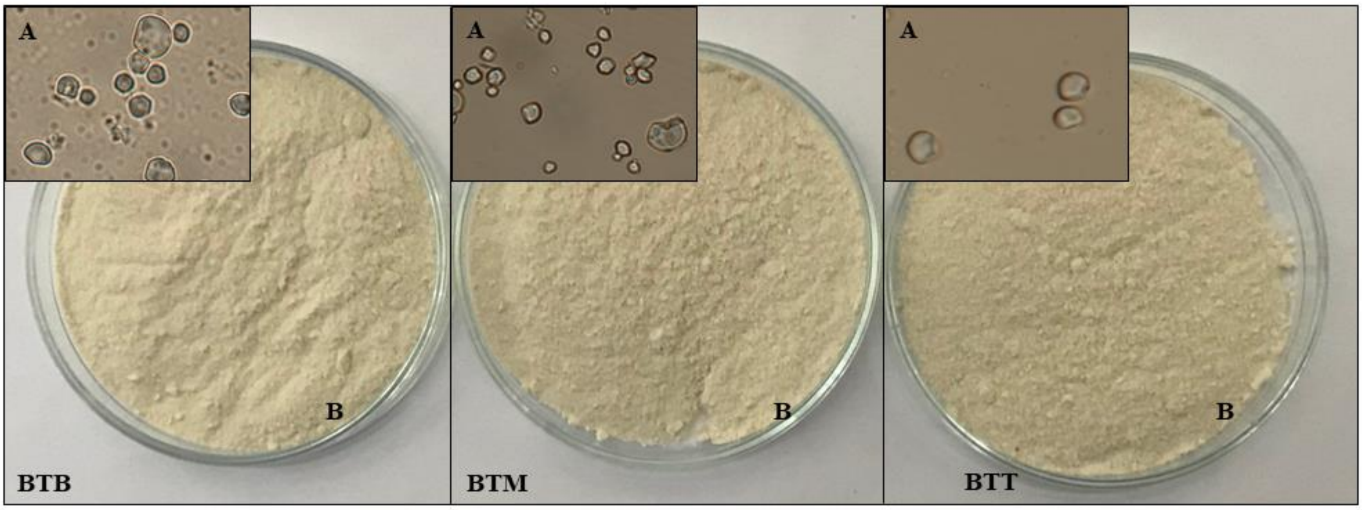

Figure 1 – Micrographs in optical microscope (100 x magnification) (A) and illustrative image (B) of starches granules extracted respectively from bottom (SB), middle (SM) and top (ST) portions of young culm of bamboo *Bambusa tuldoides.*

Otherwise what happens in the industry, in this research, we used only the sodium metabisulphite inhibitor and no oxidizing agent or bleaching process to remove compounds formed by the enzymatic darkening of the vegetables during the process (Eliasson, 2004).

3.2.2. Morphological evaluation of starch granules

Optical Microscopy (OM) – The starches from young bamboo culm, observed by OM, without polarized light, present small sizes, and polyhedral shape (Figure 1 A) and some of them have rounded shape.

Scanning Electron Microscopy (SEM) – We observe by SEM (Figure 2) that the obtained starches contain compound granules, which may be due to the granules being produced simultaneously in a single amyloplast, according to Jane (2009) and Pérez and Bertoft (2010). This type of starch production restricts the space for development of the granule, giving it a polyhedral shape, similar to cereal starches, such as rice, waxy rice, oats (Jane, 2009).

Concerning about starch granules diameter, no significant difference can be observed (Table 1), and they presented on average a diameter of 4.6 µm being classified as small, according to Lindeboom, Chang and Tyler (2004). However, the granules presented a large variation of diameter and shape, being grouped into spherical (1-5 µm) and polyhedral (5-9 µm), which is similar to starches extracted from *Guadua flabellata* culm and *Phyllostachys heterocycla* seeds as observed by Toledo, Azzini and Reyes (1987) and by Ai et al. (2016), respectively.

Nevertheless, the use of bamboo seed starch is not a continuous source of starch production, since the culms of one population flower after 40-80 years, mostly with a subsequent dying of the entire population across large regions, according to Liese and Kohl (2015).

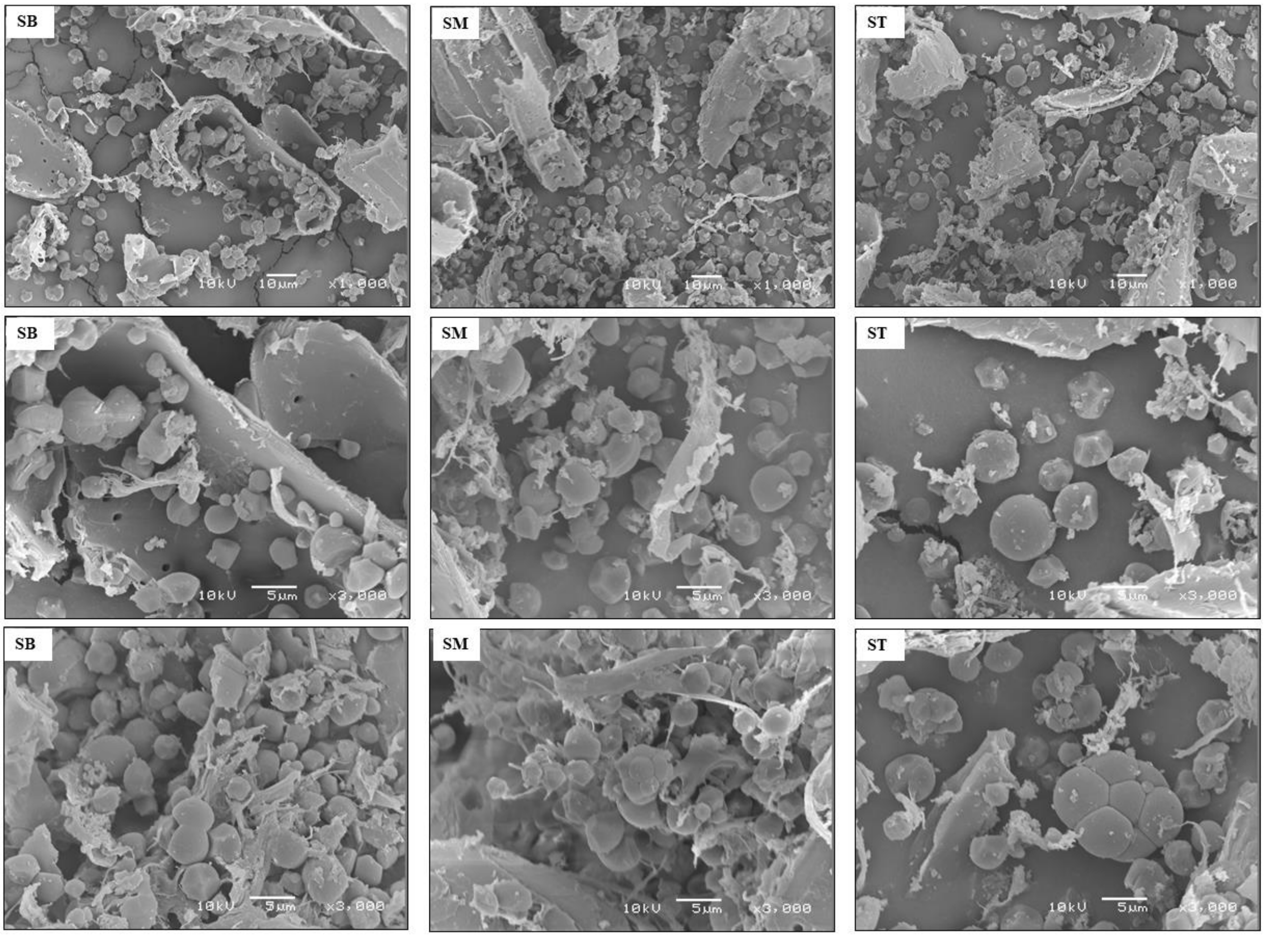

Figure 2 – Micrographs of starches granules extracted respectively from bottom (SB), middle (SM) and top (ST) portions of young culm of bamboo *Bambusa tuldoides* observed by SEM (1000 and 3000X magnification).

3.3. Physicochemical characteristics of young bamboo culm starch

3.3.1. Chemical composition

Table 2 shows that starches from the young culm of *B. tuldoides* presented values of protein, lipid and ash lower than 5 g/100 g, 1 g/100 g and 6 g/100 g, respectively. Total carbohydrates contents were higher than 80 g/100 g for all the samples, resulting in a starch with enough purity.

Table 2 – Chemical composition of obtained starches from different portions of the young culm of the bamboo *Bambusa tuldoides*.

Starches [2]	**Nutrients (g/100 g) [1]**					**Apparent amylose (%)**
	Moisture	**Protein**	**Lipid**	**Ash**	**Total Carbohydrates [3]**	
SB	7.89 ± 0.25[ns]	3.12 ± 0.10[c]	0.23 ± 0.02[b]	1.47 ± 0.00[c]	87.28	19.26 ± 1.26[c]
SM	7.25 ± 0.75[ns]	3.67 ± 0.06[b]	0.35 ± 0.02[b]	1.75 ± 0.02[b]	86.99	33.35 ± 2.11[a]
ST	7.56 ± 0.08[ns]	4.66 ± 0.05[a]	0.61 ± 0.10[a]	5.96 ± 0.15[a]	81.22	24.48 ± 0.89[b]

[1]Results expressed as means ± standard deviation. Means followed by different letters in the same column differ significantly ($p < 0.05$) by the Scott-Knott test and ns = not significant. [2]Starches isolated from young bamboo culm, respectively from the bottom (SB), middle (SM) and top (ST) portions. [3]Total carbohydrates = 100 – (moisture + lipid + protein + ash).

3.3.2. Apparent amylose content

Starches can be classified according to amylose and amylopectin contents in waxy starch (> 99% amylopectin), normal starch (contain 70-80% amylopectin and 20-30% amylose) and high-amylose starch (> 50% amylose). Amylose content in normal starches may range, as follows: tuber and root starches ranging from 15 to 38% (Hoover, 2001) and cereals from 20 to 30% (Bemiller and Whistler, 2009).

SB presented lower amylose content (Table 2) in relation to the other starches extracted from young culm of *B. tuldoides*. However, all the starches presented apparent amylose content similar to normal cereal starches, which was already expected since bamboo belongs to the same family (*Poaceae*).

These result were similar to those observed by Ai et al. (2016), in starch isolated from bamboo seeds (24%), and by Hoover, Sailaja and Sosulski (1996), in wild rice starch (21.1%) and long grain brown rice starch (22.8%). This amylose content also indicates that the starch present in the bottom of the culm is more easily hydrolyzed by amylases, into simple sugars, that according to Liese and Kohl (2015) will be used during sprouting and elongation, when the culm growth in height.

3.3.3. Molecular size distribution

Similar elution profiles (Figure 3) was exhibited on molecular weight distributions of young bamboo culm starches of *B. tuldoides*: Amylopectin was eluted at the void volume and shown as the first peak (volume of 65 mL), which has a large molecular mass; Amylose was then eluted, and is shown in the second peak (volume of 132.5 mL); Glucose (G) was the last one to be eluted, as a marker of the end of the elution.

In relation to blue value response (BV), it is observed that the second BV peak of SM and ST agrees with amylose content presented on the Table 2, since these samples presented the highest values. Concerning to total sugars (CHO) response, the amylose peak of ST is more polydisperse, which suggests a greater heterogeneity in the molecular mass of the amylose molecules.

Even the samples presenting different ratios of BV to total sugars (BV/CHO) at amylopectin peak (SB=0.43; SM=0.41; ST=0.50), no evidence of different proportions of branched chains among the samples could be observed, as shown in item 3.3.4.

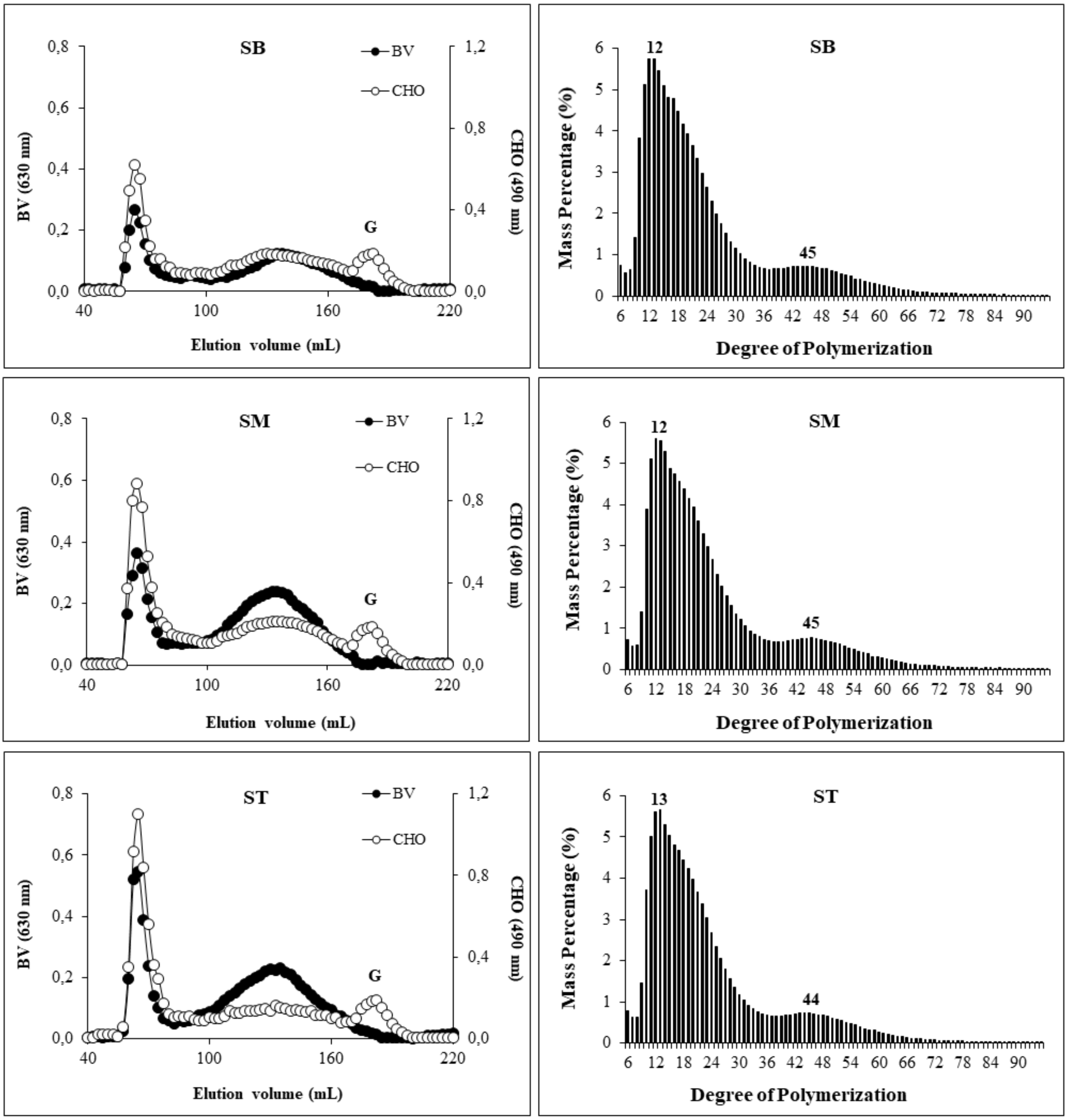

Figure 3 – Elution profile on Sepharose CL-2B and amylopectin branch chain length distributions of the isolated starches granules respectively from bottom (SB), middle (SM) and top (ST) portions of young culm of bamboo *Bambusa tuldoides*, where ● = elution profile according blue value (BV) method and ○ = elution profile according total sugars (CHO) method.

3.3.4. Amylopectin branch-chain length distribution

Anion-exchange chromatograms of the amylopectin from the three different parts of young bamboo culm of *B. tuldoides* are shown in Figure 3. In general, all the starches exhibited bimodal distribution, with the main peak at 12-13 degree polymerization (DP) and the second peak at 44-45 DP. Similar results were observed for wheat (DP 12 and 41), normal rice (DP 12 and 46) and barley (DP 12 and 43) starches, by Jane et al. (1999).

A large proportion of short chains (DP 6-12) can be observed on Table 3. Although, the highest proportion of chains were observed for DP 13-24, which present the ideal size to form stable double helices, according to Genkina et al. (2007). And similarly to the observed in the molecular size distribution, the starches did not present structural differences with respect to the extracted part of the bamboo culm.

Table 3 – Amylopectin branch chain length distribution of isolated starches from different portions of the young culm of bamboo *Bambusa tuldoides*.

Starches[2]	Branch Chain Length Distribution (%)[1]					Highest detectable DP
	DP 6-12	DP 13-24	DP 25-36	DP > 37	$\overline{DP}$	
SB	17.83 ± 0.33[ns]	49.25 ± 2.52[ns]	15.61 ± 1.03[ns]	17.30 ± 1.81[ns]	23.94 ± 0.84[ns]	95
SM	18.10 ± 0.34[ns]	49.90 ± 0.19[ns]	15.29 ± 0.02[ns]	16.71 ± 0.13[ns]	23.65 ± 0.10[ns]	95
ST	17.73 ± 0.13[ns]	50.73 ± 0.19[ns]	15.19 ± 0.02[ns]	16.35 ± 0.30[ns]	23.55 ± 0.12[ns]	95

[1]Results expressed as means ± standard deviation. Means followed by different letters in the same column differ significantly ($p < 0.05$) by the Scott-Knott test and ns = not significant. [2]Starches isolated from young bamboo culm, respectively from the bottom (SB), middle (SM) and top (ST) portions. $\overline{DP}$ – average Degree of Polymerization.

Furthermore SB, SM and ST starches did not have a shoulder on their branch chain length distributions, which suggests absence of imperfections in the crystalline structure (Jane et al., 1999).

On average DP 23 was observed for all the evaluated samples. Lower DP was observed for bamboo seed (DP 19.1), indica (DP 19.7) and japonica (DP 17.1) rice starches (Ai et al., 2016), and higher values were observed for normal rice (DP 22.7) and wheat (DP 22.7) starches (Jane et al., 1999). Despite they belong to the same botanical family (*Poaceae)*, they are of different ages, and the starch was extracted from different parts of the plant: bamboo starch was extracted from three years old culm, while rice and wheat starches were extracted from six months old grain. According to Jane (2009), it happens because of the maturity, origin and the location of the molecules in the granule.

3.3.5. X-ray diffraction

We can observe that all the starches extracted from young bamboo culm exhibited A-type crystalline pattern (Figure 4), which is similar to cereal starches, according to Gallant et al. (1982). But, while bamboo starch is extracted from the culm, starch of cereals is extracted from the grain.

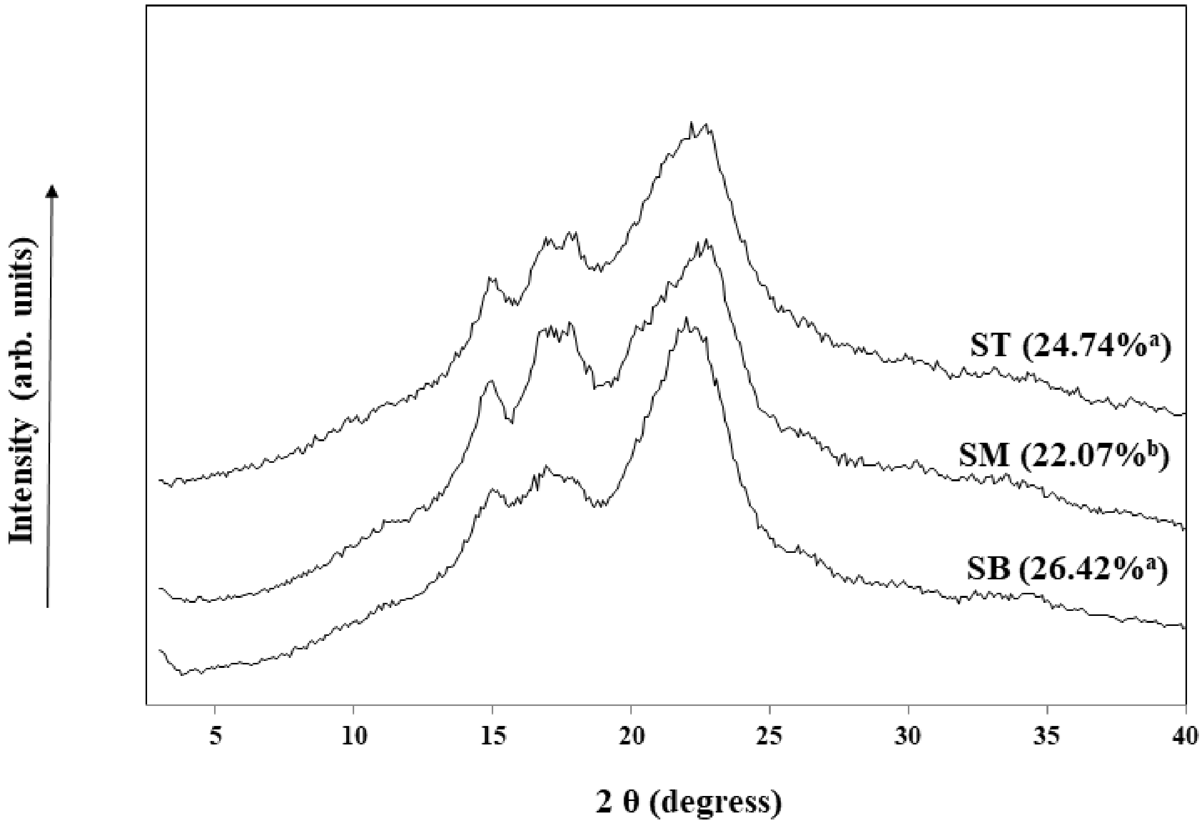

Figure 4 – X-ray diffraction patterns of isolated starches granules respectively from bottom (SB), middle (SM) and top (ST) portions of young culm of bamboo *Bambusa tuldoides* and relative crystallinity is given in parentheses. Means followed by different letters differ significantly ($p < 0.05$) by the Scott-Knott test.

These results are in agreement with those observed for the amylopectin branch chain length distribution (item 3.3.4) since starch granule that display A-type polymorph has a lower proportion of long chains and a larger proportion of short chains, according to Hizukuri (1986), Jane (2006) and Bemiller and Whistler (2009).

Concerning about relative crystallinity (RC), we can observe that SM presented the lowest value of RC (Figure 4), and also presented the highest amylose content (Table 2), compared to SB and ST. It happens, because the RC of starch is directly linked to the amylopectin content, and inversely to the amylose content (Hoover, 2001).

These results were contrary to that observed for starch extracted from bamboo seed by Ai et al. (2016). They observed for bamboo seed starch A-type polymorph and amylose content similar to ST, although the RC was higher (32.1%) than that observed in the present study.

3.4. Thermal properties of young bamboo culm starch

The onset gelatinization temperature (T_0) (Table 4) varied from 81.47 to 82.20 ºC in the starches extracted from the young culm of *B. tuldoides*. The peak gelatinization temperature was close to 85.2 °C, which is related to the higher proportion of chains with DP 13-24 (Table 3). According to Srichuwong et al. (2005), Genkina et al. (2007) and Costa et al. (2017), these chains of double stable helices favor the dense packing of amylopectin chains in crystalline regions making these structures thermally stable, i.e. high values for gelatinization temperatures.

The gelatinization enthalpy change (ΔH) ranged from 4 $J.g^{-1}$ for SB to 8 $J.g^{-1}$ to SM. The great ΔH are related to crystallinity, due to a high energy required for melting the crystalline regions, as cited by Franco et al. (2002) and Ai et al. (2016). However, contrary results were observed in the present study, where despite the fact that SM presented high ΔH, it presented lower RC.

After storing the gelatinized starches at 4 °C for 7 days, we observed lower values for gelatinization temperatures of native starches, due to the inadequate realigned of amylose molecules and the long branch chain of amylopectin, forming a less ordered and less stable crystalline structures, as is explained by Karim, Norziah and Seow (2000) and Singh et al. (2003). The ΔH of gelatinized starches were also smaller than those of native ones, because of the improper alignment of the amylopectin chains during the recrystallization, which results in a lower energy to melting the restructured crystals, according to Srichuwong et al. (2005).

Concerning about retrogradation rates (%R), in general, it was observed high values, similar to cereal starches, due to the high amount of amylose and lipids (Table 2) and the high proportion of short chain (DP 6-12) (Table 3).

4. CONCLUSION

Young bamboo culm starch from *B. tuldoides* presented characteristics of small diameter, A-type polymorph, high gelatinization temperatures and high tendency to retrograde, which are influenced by the amylose content, amylopectin short chains (DP 6-12) and lipid content. Because of this, the young bamboo culm starch present potential for industrial applications, and as an alternative source of starches to those traditionally used.

The presented results indicate new possibilities of applying the starch extracted from the young bamboo culm, and in view of the longevity of the clumps as well as their sustainability, a conscious planting of bamboo forests would be an investment opportunity.

5. ACKNOWLEDGEMENTS

The authors are grateful to CAPES for the scholarship for Mária Herminia Ferrari Felisberto, to FAPESP (2015/196374) and FAEPEX (519292-2425/16) for the financial support, and to CNPq, PIBIC and SAE/Unicamp, for the scholarship of the students who supported along the project.

Table 4 - Thermal properties of isolated starches from different portions of the young culm of the bamboo *Bambusa tuldoides*[1].

Starches [2]	Gelatinization				Retrogradation				Retrogradation (%)
	T_0 (ºC)	T_p (ºC)	T_e (ºC)	ΔH ($J.g^{-1}$)	T_0 (ºC)	T_p (ºC)	T_e (ºC)	ΔH ($J.g^{-1}$)	
SB	81.96 ± 0.45[ns]	85.22 ± 0.27[ns]	87.80 ± 0.36[ns]	4.07 ± 0.06[c]	42.24 ± 1.49[a]	55.28 ± 0.49[ns]	64.90 ± 2.77[ns]	3.18 ± 0.07[c]	78.18[b]
SM	82.20 ± 0.22[ns]	85.44 ± 0.25[ns]	88.33 ± 0.33[ns]	8.06 ± 0.16[a]	40.20 ± 0.71[b]	55.51 ± 0.01[ns]	66.90 ± 0.28[ns]	6.68 ± 0.11[a]	82.99[a]
ST	81.47 ± 0.12[ns]	84.89 ± 0.17[ns]	88.08 ± 0.14[ns]	6.06 ± 0.11[b]	40.18 ± 0.37[b]	54.73 ± 0.26[ns]	64.94 ± 1.70[ns]	4.01 ± 0.15[b]	66.23[c]

[1]Results expressed as means ± standard deviation. Means followed by different letters in the same column differ significantly ($p < 0.05$) by the Scott-Knott test and ns = not significant.
[2]Starches isolated from young bamboo culm, respectively from the bottom (SB), middle (SM) and top (ST) portions of the *Bambusa tuldoides* specie. T_0, T_p and T_e – Onset, peak and end temperatures, ΔH - enthalpy change, and Retrogradation (%) = ($\Delta H_{retrogradation}/\Delta H_{gelatinization}$) * 100.

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– CAPÍTULO 7 –

Bambusa vulgaris starch - characterization and evaluation of technological properties

Este capítulo apresenta a caracterização do amido extraído do colmo jovem de Bambusa vulgaris e, consequentemente, seu potencial como fonte alternativa de amido para aplicações industriais.

Este capítulo será submetido ao periódico internacional Starch, de alto impacto para a área.

***Bambusa vulgaris* starch - characterization and evaluation of technological properties**

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ABSTRACT

Starch industry has grown quickly, and starch production has expanded in several countries because it is a very versatile ingredient and raw material of numerous products, despite limitations of use due to its properties. So, this study aimed to obtain and characterize morphologically and physicochemically the properties of the starch from *B. vulgaris* extracted from three different parts (bottom, middle and top) of the young bamboo culm (SB, SM and ST). The obtained data were evaluated by ANOVA and Scott-Knot test ($p<0.05$). The evaluated starches presented pale yellow coloration ($L^* > 88$), lower index in the red region, and SEM images showed compound granules with polyhedral shapes and small size (average diameter of 6.5 µm). The starches presented low moisture (8.0 g/100 g), protein (1.6 g/100 g), lipid (0.4 g/100 g) and ash (1.5 g/100 g) contents. The samples presented gelatinization temperature above 80 ºC, due to the larger proportion of long branch-chains of amylopectin, with main peak at DP 12 and the second on at DP 43-44. Although the amylose content of the samples (on average 37.45%) is close to that of the native tubers starches, all of the other characteristics evaluated are close to those of the native cereals starches, without difference among the parts of the culm, indicating its potential for industrial application, replacing already used starches, like corn wheat, rice and barley.

Keywords: carbohydrate, *Poaceae*, industrial application, natural ingredient, sustainability environment.

1. INTRODUCTION

Starch is a very versatile ingredient is employed as raw material of numerous products, like foods and beverages, paper, textiles, building materials and alcohol for fuel. Starch industry has grown quickly, and starch production has expanded in several countries (BeMiller & Whistler, 2009). Nevertheless, native starches have very limited properties, and thus are directly suitable for relatively few specific end uses. For the majority of industrial uses, enzymatic, chemical and/or physical treatments are necessary to improve its functionality (Davis, Supatcharee, Khandelwal, & Chibbar, 2003).

Recent advances have been made in studies of new starch modification techniques, because of a negative perception regarding the use of chemically modified starch products in food applications. So, attention has been focused on genetic modifications, which allows the modification of the starch even when in biosynthesis in the plant (Davis et al., 2003; Huber & BeMiller, 2009).

The genetic engineering technique is very versatile and could be applied in various sources of starches to modify starch synthesis and produce novel starches that will reduce or eliminate the need for costly and environmentally unfriendly post-harvest modifications. According to Davis et al. (2003), this technique has been applied in the production of starches from main crops, with high amylose content, waxy starches, high phytoglycogen content, intermediate amylose level, altered chain lengths and branching patterns, starches with β-linkages, phosphorylated starches and altered size of the starch granule. Thus, the greater the quantity and variety of characterized starches, the greater the starch options to be modified by this method and used for industrial application.

Bamboo starch may be an option for genetic engineering, since it is also a grass, like cereals. The difference is that bamboo starch reserve is in the culm, while cereals starch reserve is in the grains. Moreover, bamboo culm presents the maximum peak of starch production in 36 months (Pereira & Beraldo, 2016), being a very profitable crop from the agricultural point of view. Bamboo crop is perennial and produces shoots asexually, year after year, without the need of replanting, with great annual yield per unit area. Another advantage is related to the speed of growth of its culms, which, because they do not present anatomical elements in the radial and tangential directions, only grow in height, unlike the wood, being able to be harvested after three to five years after planting (Akira, Sakuma, Dambiski, & Moretti, 2007). The starch from bamboo seeds has been characterized by Ai et al. (2016), although the flowering of bamboo can occur every 40-80 years, mostly with a subsequent dying of the entire population across large regions, depending on the species (Liese & Kohl, 2015). Thus, the characterization of the starch from the young culm of bamboo could be an advance in providing another option of starch that can be used by this technique.

So, this study aimed to investigate a new and viable source of starch for industrial applications, through the characterization of morphological, structure and physicochemical properties of starch extracted from young bamboo culm of *B. vulgaris*.

2. MATERIAL AND METHODS

2.1. Obtainment of young bamboo culm chips

Young bamboo culms of *B. vulgaris* were harvested in 2016 (from January to March), on average three years old, at experimental field from School of Agricultural Engineering (FEAGRI), at University of Campinas (UNICAMP), Campinas, São Paulo, Brazil, on geographic coordinates 22º82’ of south latitude and 47º07’ of west longitude. The collected culm was divided in three parts (B - bottom, M - middle and T - top) and the drying of the young culms was performed as described by Felisberto, Beraldo, and Clerici (2017). After that, the small pieces of bamboo culm from each portion were ground in a hammer mill (DPM2–Nogueira, São Paulo, Brazil) until the bamboo chips were obtained, and were used for the extraction of the starch.

2.2. Starch extraction

The starch was extracted from three different parts of the young bamboo culm chips in aqueous solution, according to Azzini, Salgado, Teixeira, and Moraes (1981) and Toledo, Azzini, and Reyes (1987) with some modifications: 60 g of bamboo chips were weighed and disintegrated in a OBL10/2 blender (OXY, Santana de Parnaíba, Brazil) with 500 mL of 200 ppm sodium metabisulphite solution, at 5 °C for 60 s.

After, the material was left to rest for 16 h/5 ºC, so the suspension was passed through a sieve (80 µm) and the material retained was triturated two more times. The filtrate was centrifuged at 17,000 g/10 min/10 °C. The supernatant was discarded, and the precipitate was resuspended with distilled water, three times. The extracted starch was then vacuum filtered, washed with 99.5% ethanol (twice) and then acetone, and dried at 45 °C/16 h, in a vacuum oven TE 395 (TECNAL®, Piracicaba, Brazil). The starch bottom (SB), starch middle (SM) and starch top (ST) were packed in plastic hermetically sealed. The obtained material was homogenized and weighed to evaluate the yield of the extraction process, which was calculated in relation to the initial weight of each sample.

2.3. Morphological characterization of young bamboo culm starch

2.3.1. Instrumental color

Instrumental color analysis was performed on CR-10 colorimeter (Konica Minolta, Tokyo, Japan) with a 50 mm port size, illuminant D65, SCI and a 10º standard observer angle. Tristimulus CIELab color space method was used, determining L*, to represent luminosity (0=black; 100=white), a* (+a=redness; -a=greenness), and b* (+b=yellowness; -b=blueness).

2.3.2. Morphological evaluation of starch granules

Optical Microscopy (OM) – The starch sample was placed on a glass slide and mixed with 1-2 drops of water. The slide was then covered with a glass cover slip and the granule morphology was observed in an optical microscopy (BX51, Olympos, Tokyo, Japan), coupled with a camera (E-330-ADU1.2X, Olympus, Tokyo, Japan). The micrographs were captured at 100X magnifications, with and without polarized light.

Scanning Electron Microscopy (SEM) – The starch was placed on carbon adhesive tape attached to the stub (one type of metal bracket) and sputter-coated with 20 nm of gold (SCD 050 Sputter Coater, Balzers). The morphology of starch granules was observed in a SEM (JEOL JSM 5800 LV, Tokyo, Japan) at 10 kV acceleration, and a large number of micrographs were acquired to select the most representative ones.

Diameters of starch granules were measured, at least 45 starch granules were taken on SEM micrographs (1000 and 3000X) using ImageJ software, according to Abramoff, Magalhães, and Ram (2004).´

2.4. Physicochemical characterization of young bamboo culm starch

2.4.1. Chemical composition

The obtained starches were evaluated by the following analyses according to AACCI (2010): moisture (method 44-15.02), protein (method 46-13.01, 6.25 as conversion factor), lipid (method 30-25.01) and ash (method 08-01.01) contents. Total carbohydrates content was calculated by difference [100 – (moisture + protein + lipid + ash)].

2.4.2. Apparent amylose content

The apparent amylose content was determined according to ISO (2007) methodology, and calculated from standard amylose from potato (A-0512) (Sigma Chemical Co., St. Louis, EUA) curves.

2.4.3. Molecular size distribution

Starch molecular weight distribution profiles were determined according to Franco, Wong, Yoo, and Jane (2002), with some modifications: it was used a gel-permeation chromatography (GPC) column (1.5 cm x 70 cm - Pharmacia Biotech) packed with Sepharose CL-2B gel. Samples (100 mg) were previously dispersed in 10 mL of 90% dimethyl sulfoxide solution (DMSO) in a bath of boiling water under stirring for 1 h and then stirred for more 16 h at 25 °C. An aliquot (1.5 mL) of the starch solution was mixed with anhydrous ethanol (6 mL) and centrifuged at 12,000 g for 10 min. The precipitated starch was resuspended with 5 mL of eluent and 0.5 mg of anhydrous glucose were added. This mixture was again kept in a bath of boiling water for 30 min, cooled to room temperature, and centrifuged at 12,000 g for 10 min. An aliquot (3.5 mL) of the supernatant (containing 9 mg of starch and 0.35 mg of anhydrous glucose) was injected into the base of the column and eluted upward. Fractions of the sample (2.5 mL) were collected every 5 minutes and analyzed, according to Dubois, Gilles, Hamilton, Rebers, and Smith (1956), for total sugars

(CHO) (sulfuric phenol methodology), and according to Juliano (1971) blue value method (BV) (iodine staining) was performed to determine the elution profile of the starches, at 492 and 630 nm, respectively.

2.4.4. Branch chain length distribution of amylopectin

The branch chain length distributions of amylopectin from young bamboo culm were analyzed according Costa, Volanti, Grossmann, and Franco (2017). The enzyme isoamylase used was from *Pseudomonas* sp. (EC 3.2.1.68) (Megazyme International, Wicklow, Ireland).

2.4.5. X-ray diffraction

The X-ray diffraction pattern was determined using a benchtop X-ray diffractometer (MiniFlex 300, Rigaku, Tokyo, Japan) equipped with Cu-Kα monochromatic radiation (λ=0.1542 nm). The scan was performed at 30 kV and 10 mA for a 2θ range of 3-40º with a step size of 0.01. Prior the analysis, the samples were previously stored in a desiccator containing saturated $BaCl_2$ solution (25 °C, Aw=0.9) for 10 days. The relative crystallinity was quantitatively estimated based on the relationship between the peak area and the total area of the diffractogram according to the method described by Nara and Komiya (1983). The analysis was performed in triplicate, and the results expressed in percentage of crystallinity.

2.5. Thermal properties of young bamboo culm starch

The thermal properties were analyzed using a Differential Scanning Calorimeter (DSC-Pyris 1, Perkin Elmer, Norwalk, USA) according to Franco et al. (2002) with modifications: the weighed samples were kept for 12 h at room temperature to equilibrate and scanned at a rate of 5 °C/min over a temperature range of 25-125 °C. Each gelatinized starch sample was stored for 7 days at 4 °C, and then analyzed for the thermal properties of the retrograded starch using the same apparatus and parameters. Transition temperatures (Onset-T_0, Peak-T_p, and End-T_e) and enthalpy change (ΔH) of the starches were determined using Pyris 1 software (Perkin Elmer, Norwalk, USA), and the percentage retrogradation (%R) was calculated by the ratio of $\Delta H_{retrogradation}/\Delta H_{gelatinization}$. The apparatus was calibrated with indium. An empty aluminum vessel was used as a reference. All analyzes were performed in triplicate.

2.6. Statistical analysis

Analysis of variance (ANOVA) of the means was performed using the Sisvar software, version 5.6 (Federal University of Lavras, Lavras, MG, Brazil) at a significance level of 95%, and when it was significant, the Scott-Knot test was used to determine statistical differences between means ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1. Starch extraction from young bamboo culm

Extraction yields (Table 1) of the starches extracted from chips of young culm of *B. vulgaris* was higher than 15%, without significant difference between the samples. Although, since we do not use any chemical/enzymatic treatment to improve the starch extraction, the yield obtained can be optimized, since Felisberto, Miyake, Beraldo, and Clerici (2017) observed 9.4% of starch content, on average, for young bamboo culm flour. The literature does not reported parameters for yield of starch extraction in culms of bamboo on industrial scale, while other cultures already have it well-established, like standard starch extraction of corn or rice grains, according to BeMiller and Whistler (2009). For example, it is known that many factors interfere in the starch extraction of young bamboo culm, like the location of the starch granule, the age of the culm and the use of chemical or enzymatic reagents. While cassava and potato starches extraction require only water, corn or rice starch extraction requires the use of chemical reagents due to the high protein content (10%).

Table 1 – Extraction yield, color parameters and size of starch granules isolated from different portions of the young culm of bamboo *Bambusa vulgaris*[1].

Starch [2]	Extraction yield (w/w)	Color parameters			Diameter (µm)
		L*	a*	b*	
SB	16.84 ± 0.88^{ns}	92.83 ± 0.09^{b}	-0.17 ± 0.01^{c}	10.32 ± 0.15^{b}	6.58 ± 1.14^{ns}
SM	19.15 ± 3.23^{ns}	93.36 ± 0.05^{a}	-0.10 ± 0.00^{b}	09.98 ± 0.12^{b}	6.99 ± 0.32^{ns}
ST	15.02 ± 2.09^{ns}	88.21 ± 0.04^{c}	0.02 ± 0.01^{a}	14.03 ± 0.13^{a}	6.07 ± 0.44^{ns}

[1]Results expressed as means ± standard deviation. Means followed by different letters in the same column differ significantly ($p < 0.05$) by the Scott-Knott test and ns = not significant. [2]Starches isolated from young bamboo culm, respectively from the bottom (SB), middle (SM) and top (ST) portions.

3.2. Morphological characteristics of young bamboo culm starch

3.2.1. Instrumental color

It was observed that all the starches extracted from the young bamboo culm differed statistically from each other, however, all of them characterize yellowish coloration and luminosity (L*) above 88 (Table 1). Among the samples, SB presented the lowest color index in the red region (a* = -0.17), and ST the highest color index in the yellow region (b* = 14.03). The total color difference (ΔE) between starches were 0.64 and 5.93, for SB and SM, and for SB and ST, respectively, and between SM and ST it was 6.55, so that they are distinguishable by naked eye, according to Moritz (2011).

The values of L* for the starches obtained in the present work are lower than those observed for cassava (L*=95.33) (Benesi, Labuschagne, Dixon, & Mahungu, 2004), amaranth (L*=96.64) and corn (L*=98.13) starches (Chandla, Saxena, & Singh, 2017). Our results are very close to the whiteness of 90, which Boudries et al. (2009) classifies as acceptable for starch purity.

Industrially the extracted starches undergo a bleaching process, aiming to remove compounds formed by the enzymatic darkening of the vegetables during the process (Eliasson, 2004), but in this research, we used only the sodium metabisulphite inhibitor and no oxidizing agent, such as hydrogen peroxide and/or hypochlorite were employed.

3.2.2. Morphological evaluation of starch granules

Optical Microscopy (OM) – When observing the starches from young culm of bamboo by OM, without polarized light (Figure 1 a), we verified that they present small size, polyhedral shape and some of them have rounded shape. If observed under polarized light (Figure 1 b), they exhibit a Maltese cross, indicating that our objective was achieved since the starch was obtained without modifications on its structure. According to BeMiller and Whistler (2009), the Maltese cross indicates a radial orientation of the macromolecules, and the loss of birefringence on heating is an indicative of disordering processes.

Scanning Electron Microscopy (SEM) – It was observed that the starches contain compound granules (Figure 1 c), and according to Jane (2009) and Pérez and Bertoft (2010) it is due to the granules being produced simultaneously in a single amyloplast, which restricted the space for development of the granule. Similar shape can be observed in granules of starches that belong to the grass family *Poaceae*, such as rice, waxy rice and oats starches (Jane, 2009).

On Table 1 we can observed that the granules of starches displayed a diameter of less than 7 µm, which may be classified as small, according to Lindeboom, Chang, and Tyler (2004), and without significant difference on average diameter among the starches extracted from different parts of the young bamboo culm.

However, the large variation of diameter and shape between the granules caused us to group them into spherical (1-5 µm) and polyhedral (5-9 µm), similar to the starch extracted from young bamboo culm of *Dendrocalamus asper*, as observed previously by our research group. These results are similar to those observed by Ai et al. (2016), who evaluated starches extracted from *Phyllostachys heterocycla* seeds. However, the use of bamboo seed starch would not be industrially feasible, since the culms of one population flower after 40-80 years depending on the species, according to Liese and Kohl (2015)

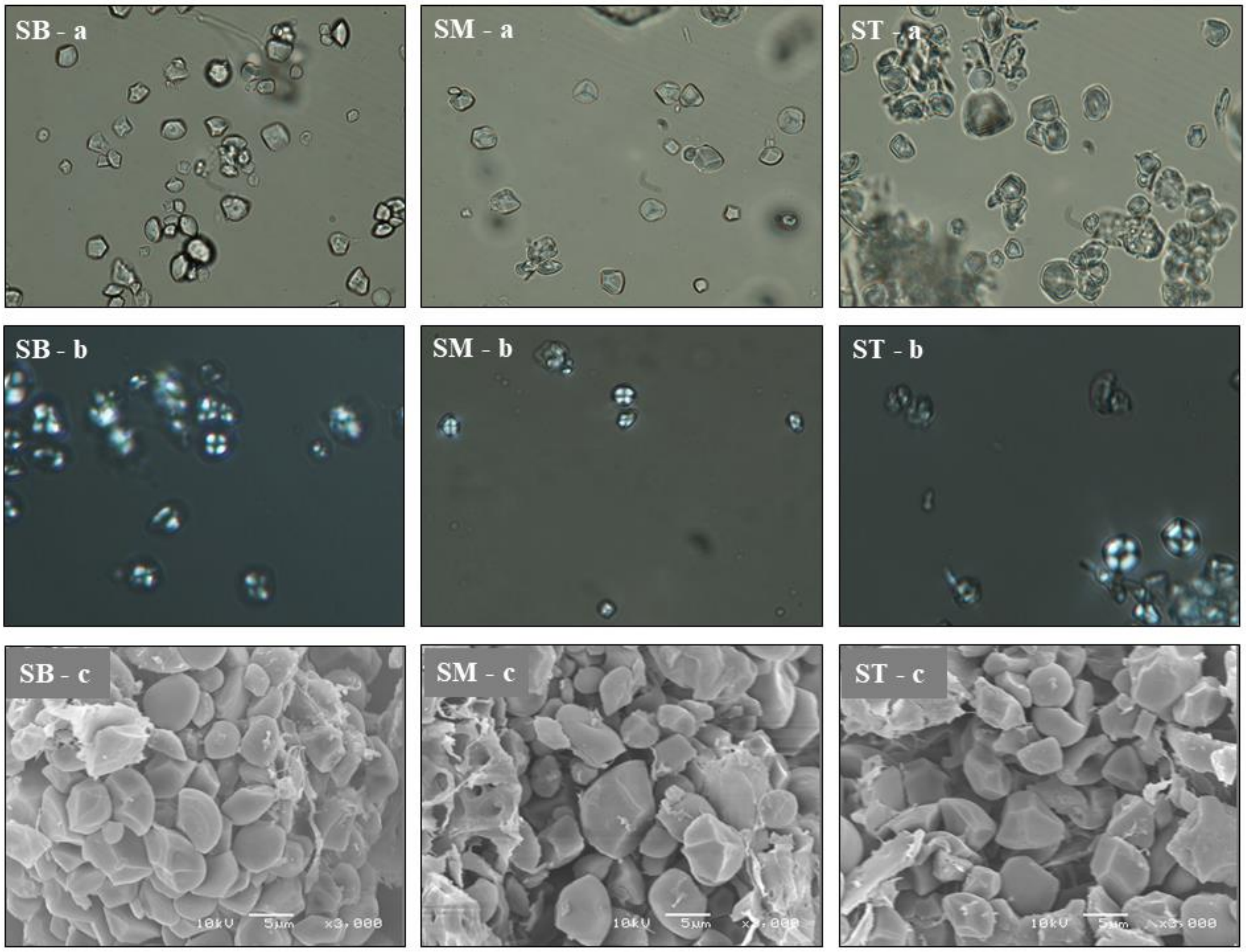

Figure 1 – Micrographs of isolated starches granules respectively from bottom (SB), middle (SM) and top (ST) portions of young culm of bamboo *Bambusa vulgaris* observed in: optical microscope without polarized light (a - 100 x magnification); optical microscope with polarized light (b - 100X magnification); scanning electron microscope (c - 3000X magnification).

3.3. Physicochemical characteristics of young bamboo culm starch

3.3.1. Chemical composition

Table 2 shows that all the obtained starches from the young culm of *B. vulgaris* presented low values of protein, lipid and ash. Taking account that starch granules are located inside the parenchyma cells in the bamboo culm wall (Liese & Kohl, 2015), and we do not use any chemical/enzymatic treatment to improve the extraction, our objective was achieved since the starch was obtained without modifications (section 3.2) and with enough purity, due to the levels of proteins, lipids and ash.

Table 2 – Chemical composition of obtained starches from different portions of the young culm of the bamboo *Bambusa vulgaris*.

Starches [2]	Nutrients (g/100 g) [1]					Apparent amylose (%)
	Moisture	Protein	Lipid	Ash	Total Carbohydrates [3]	
SB	9.25 ± 0.28[a]	1.39 ± 0.02[b]	0.47 ± 0.03 [ns]	0.89 ± 0.01[c]	88.00	40.60 ± 1.28[a]
SM	8.38 ± 0.29[a]	1.27 ± 0.00[c]	0.43 ± 0.12 [ns]	1.19 ± 0.05[b]	88.73	40.20 ± 0.86[a]
ST	6.40 ± 0.63[b]	2.10 ± 0.01[a]	0.29 ± 0.02 [ns]	2.56 ± 0.04[a]	88.65	31.56 ± 1.19[b]

[1]Results expressed as means ± standard deviation. Means followed by different letters in the same column differ significantly ($p < 0.05$) by the Scott-Knott test and ns = not significant. [2]Starches isolated from young bamboo culm, respectively from the bottom (SB), middle (SM) and top (ST) portions. [3]Total carbohydrates = 100 – (moisture + lipid + protein + ash).

3.3.2. Apparent amylose content

Amylose content in normal starches may range from 15 to 38% for tuber and root starches (Hoover, 2001) and from 20 to 30% for cereals starches (BeMiller & Whistler, 2009). And according to amylose and amylopectin contents, they can be classified in waxy, normal, and high-amylose starch.

We observed apparent amylose content (Table 2) above 30% for starches extracted from young bamboo culm. These result were higher than that observed by Ai et al. (2016), in starch isolated from bamboo seeds (24%), by Hoover, Sailaja, and Sosulski (1996), in wild rice starch (21.1%) and long grain brown rice starch (22.8%), and by Franco et al. (2002), in wheat starches (27.2 to 28.7%). However, it was expected that they presented amylose content similar to normal cereal starches since bamboo belongs to the same family. This may have happened due to the fact that we used the colorimetric method without previously defatting or by not taking into account the iodine complexing ability of amylopectin chains, as explained by Hoover (2001).

3.3.3. Molecular size distribution

Molecular weight distributions of SB, SM and ST starches exhibit similar elution profiles (Figure 2). Amylopectin of all samples was eluted at the void volume and shown as the first peak (at the volume of 65 mL), which has a large molecular mass. The second peak (at the volume of 132.5 mL) correspond to amylose, and the last one was glucose (G), added as a marker of the end of the elution.

Despite the ratios of BV to total sugars (BV/CHO) at amylopectin peak differ between SB (0.44) and SM (0.44), compared to ST (0.47), the results from amylopectin branch chain length distributions showed that there was no difference in the proportions of chains among the samples, as presented in item 3.3.4.

3.3.4. Amylopectin branch-chain length distribution

Figure 2 present the normalized chain length of amylopectin from starches of three different parts of young bamboo culm. All the samples exhibited bimodal distribution, with the main peak at 12 degree polymerization (DP) and the second peak at 43-44 DP, without difference among them in the distribution of side chains (Table 3). Similar results to those obtained in the present study were observed in young bamboo culm starch from *Dendrocalamus asper*, in an article submitted for publication, and in wheat (DP 12 and 41), normal rice (DP 12 and 46) and barley (DP 12 and 43) starches, as observed by Jane et al. (1999).

Table 3 shows that SB, SM and ST starches presented the highest proportion of chains with DP 13-24, despite a large number of short chains (DP 6-12). The absence of a shoulder in the branch chain length distributions of evaluated starches suggests that they show a perfect crystalline structure (Jane et al., 1999).

The starches did not present structural differences independent of the extracted part of the bamboo culm, as observed in the molecular size distribution. They presented on average DP 23, which were longer than that observed by Ai et al. (2016) for bamboo seed (DP 19.1), and closer to that observed by Jane et al. (1999) for normal rice (DP 22.7) and wheat (DP 22.7) starches. Although the compared samples belong to the same botanical family, *Poaceae,* they are of different ages (three years old for bamboo culm and at least 6 months for rice and wheat grains). Starch also was extracted from different parts of the plant (bamboo culm, bamboo seed and grain of rice and wheat). Furthermore, according to Jane (2009), the average branch chain length of amylopectin can vary among the samples depending on the maturity, origin and the location of the molecules in the granule.

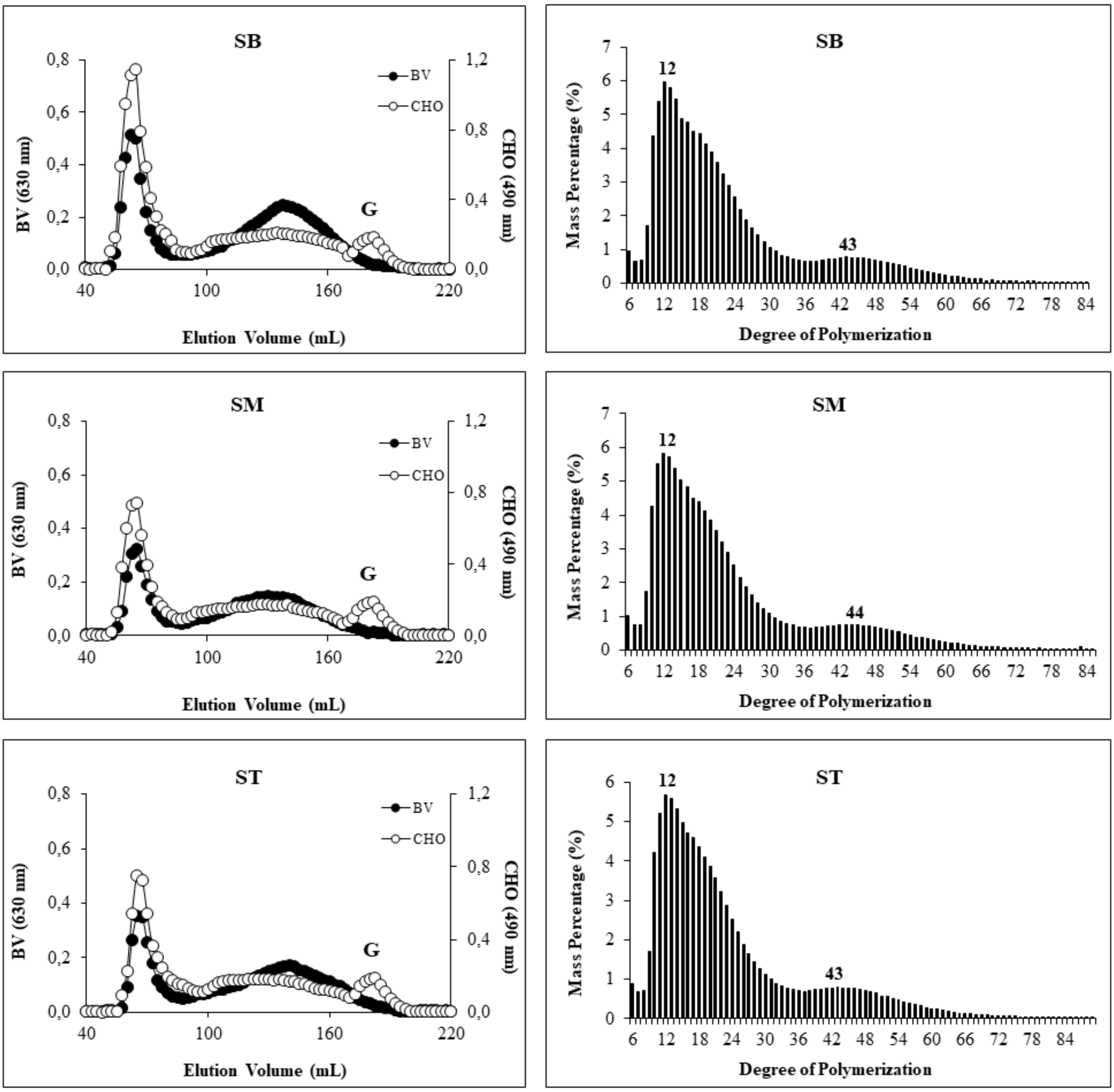

Figure 2 – Elution profile on Sepharose CL-2B and amylopectin branch chain length distributions of the isolated starches granules respectively from bottom (SB), middle (SM) and top (ST) portions of young culm of bamboo *Bambusa vulgaris*, where ● = elution profile according blue value (BV) method, and ○ = elution profile according total sugars (CHO) method.

Table 3 – Amylopectin branch chain length distribution of isolated starches from different portions of the young culm of bamboo *Bambusa vulgaris*.

Starches[2]	Branch Chain Length Distribution (%)[1]					Highest detectable DP
	DP 6-12	DP 13-24	DP 25-36	DP > 37	$\overline{DP}$	
SB	20.88 ± 1.64 ns	47.71 ± 3.39 ns	13.81 ± 0.37 ns	17.60 ± 2.12 ns	23.48 ± 0.64 ns	86
SM	19.81 ± 0.08 ns	50.03 ± 0.05 ns	14.08 ± 0.05 ns	16.08 ± 0.08 ns	23.02 ± 0.02 ns	85
ST	19.24 ± 0.22 ns	49.77 ± 0.16 ns	14.35 ± 0.16 ns	16.64 ± 0.21 ns	23.31 ± 0.10 ns	89

[1]Results expressed as means ± standard deviation. Means followed by different letters in the same column differ significantly ($p < 0.05$) by the Scott-Knott test and ns = not significant. [2]Starches isolated from young bamboo culm, respectively from the bottom (SB), middle (SM) and top (ST) portions. $\overline{DP}$ – average Degree of Polymerization.

The starches did not present structural differences independent of the extracted part of the bamboo culm, as observed in the molecular size distribution. They presented on average DP 23, which were longer than that observed by Ai et al. (2016) for bamboo seed (DP 19.1), and closer to that observed by Jane et al. (1999) for normal rice (DP 22.7) and wheat (DP 22.7) starches. Although the compared samples belong to the same botanical family, *Poaceae,* they are of different ages (three years old for bamboo culm and at least 6 months for rice and wheat grains), and starch was extracted from different parts of the plant (bamboo culm, bamboo seed and grain of rice and wheat). Furthermore, according to Jane (2009), the average branch chain length of amylopectin can vary between samples depends on the maturity, origin and the location of the molecules in the granule.

3.3.5. X-ray diffraction

By the diffractograms (Figure 3) of SB, SM and ST we can observe that all of samples exhibited peaks at 15.1°, 16.8°, 18.0° and 22.3° at 2θ, characteristics of the A-type crystalline pattern, according to Gallant et al. (1982) and it is similar to cereal starch since they present two peaks at 2θ between 16° and 18º and another one nearby 23°. The difference between them is that the starch of bamboo was extracted from the culm, while the starch of cereals is extracted from the grain.

The starch granule that display the A-type polymorph has a lower proportion of long chains and a larger proportion of short chains (Table 3), according to Hizukuri

(1986), Jane (2006) and BeMiller and Whistler (2009), as observed in the present study.

We can observe that SB and SM presented higher relative crystallinity (RC) (Figure 3), a 23.57 and 22.43%, respectively, and also presented higher amylose content (40.60 and 40.20%, respectively) (Table 2), an opposite to what was expected, since according to Hoover (2001), RC of starch is directly linked to the amylopectin content, and inversely to the amylose content. Although, bamboo seeds starches evaluated by Ai et al. (2016), presented A-type polymorph, the amylose was lower and the RC were higher than the observed in the present work (32.1%).

3.4. Thermal properties of young bamboo culm starch

The starches from young culm of *B. vulgaris* presented gelatinization peak temperatures (Table 4) with values higher than 80 ºC, which is related to the higher proportion of chains with DP 13-24 (Table 3) that favor the dense packing of amylopectin chains in crystalline regions, according to Srichuwong, Sunarti, Mishima, Isono, and Hisamatsu (2005), Genkina, Wikman, Bertoft, and Yuryev (2007) and Costa et al. (2017). They presented significant differences in gelatinization enthalpy change (ΔH), and SB starch presented highest ΔH, confirming that the greater ΔH is related to the higher RC, as cited by Franco et al. (2002) and Ai et al. (2016).

The thermal transition temperatures after storing the gelatinized starches for 7 days at 4 °C were lower than the gelatinization temperatures of native starches, due to the inadequate realignment of amylose and amylopectin molecules, which formes a less ordered and less stable crystalline structures, as explained by Karim, Norziah, and Seow (2000) and Singh, Singh, Kaur, Singh Sodhi, and Singh Gill (2003). Endothermic peaks of retrograded starches presented similar behavior. Related to retrogradation rates (%R), we observed high values (above 40%), as observed in cereal starches, due to the high amount of amylose and lipids (Table 2), but mainly due to the high proportion of amylopectin short chain (DP 6-12) (Table 3).

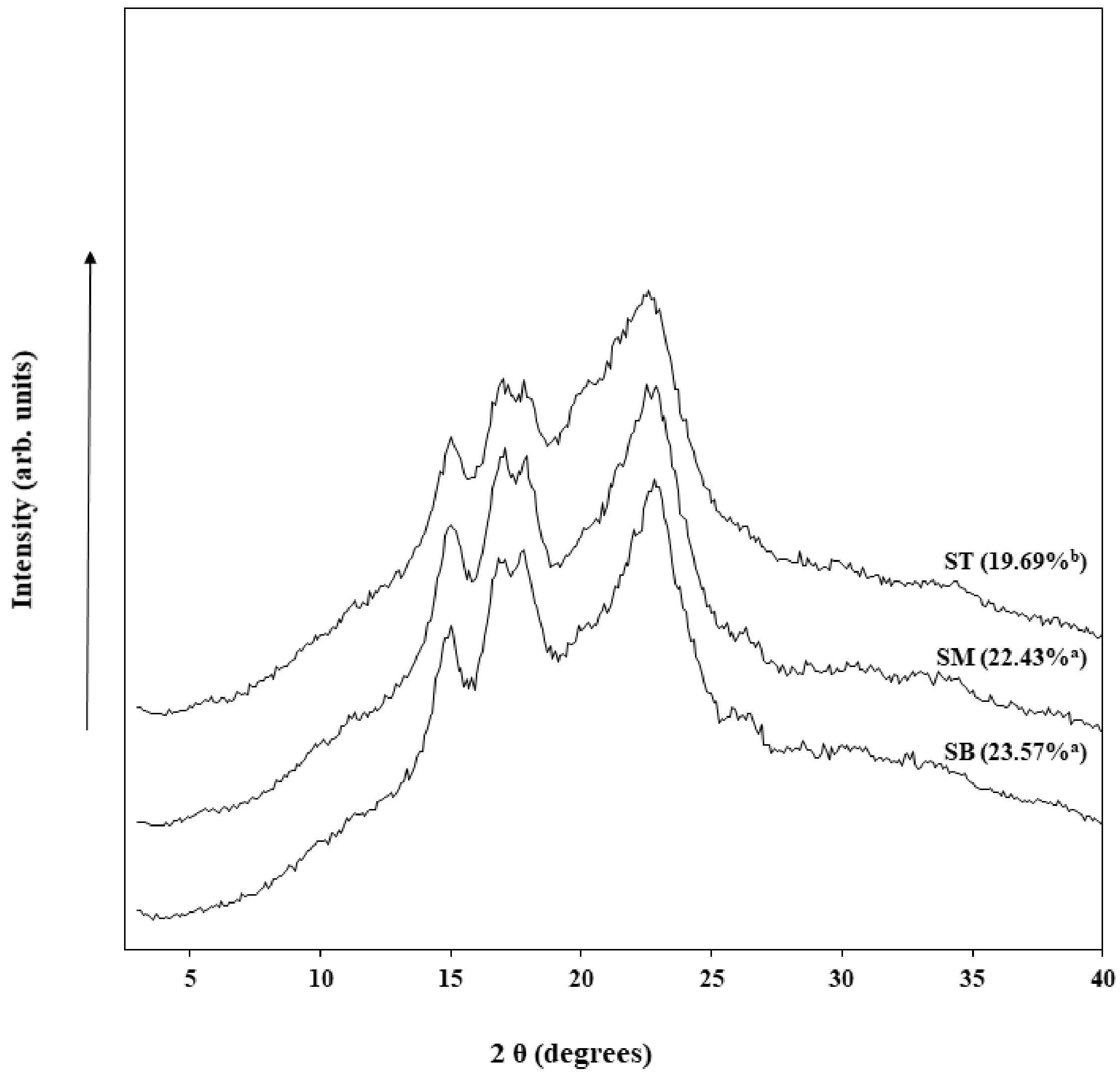

Figure 3 – X-ray diffraction patterns of isolated starches granules respectively from bottom (SB), middle (SM) and top (ST) portions of young culm of bamboo *Bambusa vulgaris* and relative crystallinity is given in parentheses. Means followed by different letters in the same column differ significantly ($p < 0.05$) by the Scott-Knott test.

Table 4 - Thermal properties of isolated starches from different portions of the young culm of the bamboo *Bambusa vulgaris*[1].

Starches [2]	Gelatinization				Retrogradation				Retrogradation (%)
	T_0 (ºC)	T_p (ºC)	T_e (ºC)	ΔH ($J.g^{-1}$)	T_0 (ºC)	T_p (ºC)	T_e (ºC)	ΔH ($J.g^{-1}$)	
SB	76.70 ± 0.13[b]	81.90 ± 0.25[b]	85.05 ± 0.39[b]	10.62 ± 0.21[a]	41.75 ± 0.74 [ns]	55.76 ± 0.14 [ns]	65.91 ± 0.11[a]	5.70 ± 0.31[a]	53.71[b]
SM	76.23 ± 0.33[b]	80.94 ± 0.35[c]	84.68 ± 0.50[b]	9.73 ± 0.32[b]	43.82 ± 1.56 [ns]	55.42 ± 0.17 [ns]	65.57 ± 0.21[a]	4.33 ± 0.31[b]	44.63[c]
ST	79.05 ± 0.25[a]	84.85 ± 0.34[a]	89.48 ± 0.44[a]	4.18 ± 0.02[c]	44.58 ± 1.15 [ns]	55.48 ± 0.43 [ns]	63.98 ± 1.06[b]	2.71 ± 0.04[c]	64.82[a]

[1]Results expressed as means ± standard deviation. Means followed by different letters in the same column differ significantly ($p < 0.05$) by the Scott-Knott test and ns = not significant.
[2]Starches isolated from young bamboo culm, respectively from the bottom (SB), middle (SM) and top (ST) portions of the *Bambusa vulgaris* specie. T_0, T_p and T_e – Onset, peak and end temperatures, ΔH - enthalpy change, and Retrogradation (%) = ($\Delta H_{retrogradation}/\Delta H_{gelatinization}$) * 100.

4. CONCLUSION

The young bamboo culm starch from *B. vulgaris* was extracted and it maintained its native form. The starch presented characteristics of small diameter, A-type polymorph and high gelatinization temperatures, without difference among the parts of the culm. According to its properties, bamboo starch is recommended for use in industrial processes that apply high temperatures and mechanical friction. The starch can also be an alternative to traditional source of starches, such as corn, rice, wheat or barley starches.

5. ACKNOWLEDGEMENTS

The authors are grateful to CAPES for the scholarship for Mária Herminia Ferrari Felisberto, to FAPESP (2015/196374) and FAEPEX (519292-2425/16) for the financial support, and to CNPq, PIBIC and SAE/Unicamp, for the scholarship of the students who supported along the project.

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– CAPÍTULO 8 –

Effect of the addition of young bamboo culm flour as a sugar and/or fat substitute in cookie formulations

Este capítulo apresenta uma avaliação da utilização da farinha de colmo jovem de bambu em substituição parcial à farinha de trigo, em formulações de biscoitos tipo cookies, com reduzidos teores de açúcar e de gordura.

Este capítulo será submetido ao periódico internacional Food Science and Technology, de alto impacto para a área.

Effect of the addition of young bamboo culm flour as a sugar and/or fat substitute in cookie formulations

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ABSTRACT

One of the innovation strategies of the bakery industry is the use of new ingredients capable of increasing the health appeal of the products. The objective of this study was to evaluate the effect of the partial substitution of wheat flour by young bamboo culm flour (YBCF) in cookies formulations with reduced sugar and/or fat contents, using a statistical design with two independent variables (percentages of sugar and fat reduction). Formulations were selected by surface response methodology ($p<0.10$) and evaluated over 28 days of storage for stability and nutritional composition. Only sugar reduction led to significant differences in the diameter of the cookies. Formulations with 50% of reduction of sugar and fat (F2 and F3, respectively) were selected for the study, in addition to a control formulation (CF). Moisture content and aw of the cookies remained low throughout the storage, as recommended for the maintenance of the crispness. Cookies F2 and F3 were light-colored, similar to CF, and presented lower energy value (0.83 and 11.73%, respectively). These results indicate that the YBCF may be a promising ingredient for the bakery industry, once it contains high fiber levels and allows the reduction of up to 50% sugar and fat in cookies.

Keywords: bamboo flour, healthy products, new ingredient.

1. INTRODUCTION

The cookies production in Brazil ranked fifth among the top cookies producing countries, with a production of 1.3 million tons in 2015, and per capita consumption of 6.01 kg per year, surpassed only by Argentina, the United Kingdom, Italy, and United States (ABIMAPI, 2015). This product is highly consumed by different audiences, due to its long shelf-life and ease of consumption, and especially by children, due to the sweet taste and perfect crisp texture (Feddern et al., 2011; Moraes et al., 2010; SIMABESP, 2012).

Cookies can be defined as baked cereal-based products containing high sugar and fat levels, moisture content from 2 to 8%, and water activity ranging from 0.1 to 0.3. Thus, the quality of the ingredients used in cookies formulation has a direct influence on the characteristics of the final product (Gökmen et al., 2008; Pareyt et al., 2009).

The sugar reduction in cookies can affect the texture, flavor, sweetness, color, and the conservation of the product as it assists in the moisture retention (Manley, 2011), while the fat reduction can affect grease lubrication, thus impairing the mixing step, absorption, volume, color, stability, and shelf life of the processed products. Fat is one of the basic components of cookies formulation, found at relatively high levels, up to 60% (Cauvain & Young, 2006; Manley, 2011). It contributes to the production of softer and shorter cookies, while sugar contributes to the increase in cookies diameter and crispness (Cauvain & Young, 2006; Manley, 2011). This is the great challenge of the bakery industry since both cannot be easily replaced, especially in the complex structure of cookies (Pareyt et al., 2009).

Consumers' concern about maintaining health and preventing certain diseases has contributed to increasing the market demand for healthier, low-fat, low-sugar, and fiber-containing products, especially bakery products, which are consumed in large quantities in a daily routine, with an important role in human nutrition (Martins et al., 2017).

Choudhury et al. (2015) reported that replacing up to 10% wheat flour for bamboo shoot fiber in cookies formulations enhanced the nutraceutical and functional properties due to the presence of phenolic compounds, fibers, proteins, and vitamin C. Farris et al. (2008) evaluated the water retention capacity of bamboo shoot fiber in Amaretti biscuits, which contains almond flour rather than wheat flour, and found that the addition of fiber limited the sugar crystallization, maintaining the traditional texture characteristics of the product after 10 days of storage. When evaluating the effect of the addition of bamboo shoot fiber and the processing conditions (time and temperature) of Amaretti biscuits, Farris & Piergiovanni (2008) observed that the addition of fiber, associated with oven time and temperature, increased hardness of the biscuits. However, the authors suggested a greater addition of fiber to improve the mouthfeel as well as the overall quality of the product.

However, it is worth mentioning that increasing the production of bamboo shoot fiber for use in human consumption can alter the sustainability of bamboo clumps, once it is possible to collect only 20% to 30% shoots per year (Pereira & Beraldo, 2016). Thus, alternative sources of fiber are required, and the young bamboo culm can be a raw material of great potential, as it allows a greater fiber yield when compared to bamboo shoots (Felisberto, Miyake, et al., 2017).

Some young bamboo culm species have been highlighted not only by the high potential for extraction of starches and fibers (Felisberto, Miyake, et al., 2017), but also by their sugar content, including the *Dendrocalamus asper* specie*,* which presents on average 9% sugar in the young culms. Although this genus is of Asian origin, it is largely cultivated in Brazil (at Jacareí and Mogi das Cruzes/SP cities) for the production of both canned and *in natura* bamboo shoots. Therefore, it is noticed that the bamboo shoot chain is already well established, while the young culm is still little explored, since its use is restricted to building materials, furniture and handicrafts, with limited shelf life due the attack of powder-post beetles which is attracted by the starch presented in the parenchyma cells.

A young culm from *D. asper* species can reach 40 m in height and up to 20 cm in diameter (Vasconcellos, 2006), and contains high levels of starch and fibers, thus the culm of this species may be an industrial source of young bamboo culm flour, contributing for using the whole plant.

Considering that the search for potential food ingredients has been a strong trend, the use of young bamboo culm flour may be an interesting alternative for the development of healthier products using new ingredients, besides contributing with the sustainability and longevity of bamboo clumps. Thus, the objective of the study was to evaluate the technological and nutritional properties of cookies formulations, with partial substitution of wheat flour for young bamboo culm flour from *D. asper* specie, with reduced sugar and/or fat content.

2. MATERIALS AND METHODS

2.1. Raw materials

The young bamboo culm flour (YBCF) from *D. asper* specie was obtained in a previous study carried out by our research group (Felisberto, Beraldo, et al., 2017). The young culms were collected, cut into smaller pieces, treated with sodium metabisulphite solution (200 ppm/1 h), and dried in an oven (50 ºC/72 h). Then, the material was ground in a knife mill to obtain a homogeneous flour.

All the other ingredients were purchased from the same batch in the local market of Campinas (SP).

2.2. Characterization of wheat flour and young bamboo culm flour

YBCF was evaluated for the presence of total cyanogenic heteroside compounds, according to De Oliveira & Olivera (2010), with adaptations. The method is known as the Konigi method and uses the U.V.-VIS spectrophotometry technique. The method is based on the reduction of sodium picrate by cyanide to form a colored product, which is measured in a colorimeter at 530 nm. In the validation process, this method had a lower detection limit of 5 ppm and a lower limit of quantification of 10 ppm cyanide, with a 2% SD, showing a stable color resistant to other components. The validation was performed using the same parameters proposed by Fukushima et al. (2016), with an adaptation of the matrix effect to young bamboo culm flour. Both wheat flour and YBCF from *D. asper* specie were characterized for proximate composition, according to the methodology of AOAC (1998), for moisture (method 44-15.02), lipids (method 920.39), proteins (method 46-13.01), ash (method 08-01.01) total fiber (method 992-16), while total carbohydrates were calculated by difference [100- (moisture + lipids + proteins + ash + total fiber)].

2.3. Experimental design

A central composite design with two variables (2^2 - sugar and vegetable fat) and three replicates at the central point was used, totaling 7 formulations (Table 1).

In all formulations, a partial substitution (15%) of wheat flour for young bamboo culm flour was performed.

Table 1 – Central composite design 2^2 with three central point of cookies formulations.

Formulations	Coded Variables		Real Variables (%)[1]	
	X_1	X_2	Sugar reduction	Fat reduction
F1	-1	-1	0	0
F2	+1	-1	50	0
F3	-1	+1	0	50
F4	+1	+1	50	50
F5	0	0	25	25
F6	0	0	25	25
F7	0	0	25	25

[1] Percentage reduction of x_1 (sugar) and x_2 (vegetable fat) of each formulation.

2.4. Manufacture of cookies

The formulation of Clerici et al. (2013) was used as a base, with some changes in the amount of ingredients (in relation to 100% wheat flour), as follows: 54.60% sugar, 38% margarine, 9.4% egg yolk, 0.15% vanilla essence, 0.36% condiments (nutmeg, clove powder, and cinnamon powder), 0.61% salt, 2.5% ammonium bicarbonate, and 17.65% maize starch. Two mixing stages were used for the cookies manufacture. First, sugar, fat, egg yolk, and vanilla essence were creamed in a high-speed planetary mixer (Kitchen aid, St Joseph, USA) for 5 min. The other ingredients were then added and homogenized for approximately one min at a low speed. Water was added to the optimal absorption level so that dough reached the optimum development over 3 min of mixing at a medium speed. The dough was then rolled out to 5 mm thick, cut into cylinders of 38 mm in diameter, and baked for about 7 min in the oven at surface and ceiling temperatures of ~200 °C and 220 °C, respectively. Afterwards, the cookies were cooled for 30 min, vacuum-packed and stored at room temperature, protected from light, until analysis.

2.5. Technological characterization of the cookies

2.5.1. Diameter, thickness, mass loss, and specific volume

The diameter and thickness of the cookies were measured using a pachymeter, and the specific volume was calculated by the ratio between the apparent volume and the cookies' weight after baking, according to the methodology of AACCI (2010). The mass loss was calculated as a ratio between raw and baked cookies' weight. The apparent volume was measured by the seed displacement method, according to AACCI (method 10-05.01, 2010). All analyses were performed on 10 cookie samples.

2.5.2. Moisture

Moisture content was determined according to the methodology of the AOAC (1998).

2.5.3. Water activity (aw)

Water activity (aw) was determined by the water activity meter using a dewpoint sensor (Aqualab, 4TEV, Decagon, Pullman, USA), and the readings were performed at room temperature (25 ºC).

2.5.4. Instrumental texture

Texture (N - fracturability) of the cookies was determined using the TA-XT Plus texture meter (Stable Micro Syculms, England) using a 50 kgf load cell, equipped with a three-point bend rig (HDP/3PB) to determine firmness. The test conditions were: pre-test speed of 1 mm/s, test speed of 3 mm/s, post-test speed of 10 mm/s, and penetration distance of 5 mm. The readings were performed on 10 cookie samples.

2.5.5. Color measurements

The color parameters (L*, a*, and b*) were evaluated according to the CIELab syculm, in a colorimeter CR-400 (Konica Minolta, Japan), using illuminant D65 and observer angle 2º, and the readings were performed using three replicates per sample.

2.6. Statistical analysis to select the cookies

Data were evaluated by the Response Surface Methodology to calculate the regression coefficients, followed by analysis of variance (ANOVA), at a significance level of 10% with a minimum coefficient (R^2) of 0.8, using the software Statistica (version 8.0).

2.7. Technological characterization and stability of the cookies

The moisture contents, aw, texture, and color parameters were determined for the selected cookies and the control formulation, as described in Section 2.5, at 1, 7, 14, 21, and 28 days of storage.

2.8. Proximate composition of the cookies

The selected cookies were characterized for the proximate composition, according to the methods described in Section 2.2, and the energy value was calculated considering the energy conversion factors: 9 kcal/g for lipids, 4 kcal/g for proteins, and 4 kcal/g for carbohydrates. A formulation without sugar and/or fat reduction, with no substitution of wheat flour for the young bamboo culm flour was used as control (CF).

2.9. Statistical analysis of the selected cookies

The results of the proximate composition and the technological characterization were obtained at least in triplicate and evaluated by difference between the formulations, through analysis of variance (ANOVA), at a significance level of 5%, followed by the Scott-Knott test, using the statistical program SISVAR, version 5.6.

3. RESULTS AND DISCUSSION

3.1. Characterization of the raw materials

The YBCF presented a cyanogenic content lower than the detection limit (5 ppm) of the method, thus its consumption does not represent health risks.

The wheat flour for home use contains 12.57 ± 0.06 g/100 g moisture; 0.64 ± 0.01 g/100 g lipids; 10.92 ± 0.11 g/100 g protein; 0.42 ± 0.05 g/100 g ash; 3.50 ± 0.29 g/100 g total fiber; and 71.95% comprising other carbohydrates.

Young bamboo culm flour has 5.37 ± 0.05 g/100 g moisture; 0.29 ± 0.00 g/100 g lipids; 1.27 ± 0.05 g/100 g protein; 1.07 ± 0.05 g/100 g ash; 72.23 ± 0.35 g/100 g total fiber; and 19.77 g/100 g comprising other carbohydrates.

It is observed that the wheat flour exhibited a typical chemical composition of wheat flour for home use, with other carbohydrates as the main component (>70 g/100 g). However, according to Manley (2011), flours with protein content around 11% present ideal conditions for the development of a stronger gluten network, which is indicated for the preparation of cream cracker biscuits. Therefore, we chose to use starch (17.65%) in the cookies formulations (Section 2.4), to dilute the flour proteins and allow the development of a weaker and more extensible gluten network, with minimal changes in texture, crispness, and shape of the cookies.

Young bamboo culm flour had fiber as the major component (>70 g/100 g). This result was higher than that observed in flours from other plants, such as eggplant flour (44 g/100 g) (Perez & Germani, 2004) and flours of cabbage stalks and spinach culms (36 and 49 g/100 g, respectively) (Mauro et al., 2010).

Moreover, the young bamboo culm flour culm is light-colored, with a luminosity index (L*) higher than 80, which does not affect the coloring of the final product, according to Felisberto, Beraldo, et al. (2017), thus it can be used as fiber-rich ingredient.

3.2. Technological characterization of the cookies

Table 2 shows all technological parameters of the cookies, and Table 3 presents the coefficients generated in the response surface analysis. A significant difference was observed only for the effect of the independent variable x1 (sugar reduction) on the parameter diameter.

The formulation F3 presented the highest mean diameter (43.45 mm), that is, the cookies exhibited a higher expansion, probably due to the 50% reduction in fat content, which led to an increase in sugar concentration in the final formulation content. It is known that sugar is an important component in cookies formulations, providing sweetness and flavor to the product, as well as affecting color, texture, expansion, and overall appearance. According to Bertolino & Braga (2017), the expansion is expected when using finer-grained sugar, since more water is available in the formulation. According to those authors, variations in cookies' size may lead to adjustments in packaging by the food industry, which is designed with a maximum variation of 2%, and should be avoided due to the increase in costs.

In relation to the thickness (Table 2), it increased after baking, from 5 to 10.78 mm, which was expected due to the gas produced during chemical aeration. The mass loss was, on average, 17%. The cookies presented an average specific volume of 2.54 mL/g, moisture content of 6.09 g/100 g, and aw of 0.48. Regarding the color parameters, the cookies were very light-colored, with values of 73.27, 5.16, 28.20 for the parameters L*, a*, and b*, respectively, corresponding to the region of light yellowish brown.

Moraes et al. (2010) also observed an increase in diameter after baking of cookies containing higher sugar levels. Even without the addition of fibers to the formulations, the authors found an increase in thickness after baking, from 5 to 8.76 mm thick, mass loss of 16%, fracturability around 55 N, and color parameters very close to the values observed in the present study (L*=67.63, a*=8.33, and b*=27.09).

Table 2 – Dimensions, mass loss, specific volume, moisture analysis, water activity, fracturability and color parameters 1 da after the manufacture of the cookies formulations with addition of young bamboo culm flour[a].

Formulations	Dimensions (mm)		Mass loss (%)[b]	Specific volume (mL/g)	Moisture (g/100g)	Water activity	Fracturability (N)	Color parameters		
	Diameter	Thickness						L*	a*	b*
F1	39.41 ± 0.81	7.60 ± 0.22	12.85 ± 0.10	2.53 ± 0.26	5.73 ± 0.05	0.47 ± 0.00	47.73 ± 1.39	69.74 ± 1.17	7.65 ± 0.27	30.89 ± 0.21
F2	36.18 ± 1.17	7.15 ± 0.68	19.36 ± 0.31	2.62 ± 0.10	4.22 ± 0.05	0.38 ± 0.00	37.80 ± 6.91	67.84 ± 1.84	5.61 ± 0.22	28.78 ± 0.57
F3	43.45 ± 1.45	10.78 ± 0.73	20.49 ± 0.27	2.90 ± 0.11	3.54 ± 0.14	0.31 ± 0.01	83.33 ± 6.50	71.78 ± 0.44	5.79 ± 0.08	29.38 ± 0.50
F4	36.01 ± 0.44	9.32 ± 0.58	18.97 ± 0.34	2.00 ± 0.11	8.00 ± 0.14	0.58 ± 0.00	77.05 ± 6.47	76.14 ± 0.62	3.90 ± 0.43	25.51 ± 0.51
F5	39.10 ± 1.28	10.69 ± 0.28	19.21 ± 0.47	2.98 ± 0.17	5.57 ± 0.06	0.45 ± 0.00	44.79 ± 4.51	71.20 ± 0.66	8.38 ± 0.77	30.88 ± 0.72
F6	36.72 ± 0.93	7.89 ± 0.34	14.14 ± 0.54	2.34 ± 0.13	8.33 ± 0.06	0.61 ± 0.00	35.17 ± 1.63	78.08 ± 0.44	2.53 ± 0.05	26.33 ± 0.16
F7	38.07 ± 0.64	9.60 ± 0.48	18.26 ± 0.31	2.39 ± 0.07	7.23 ± 0.03	0.54 ± 0.00	39.81 ± 3.27	78.08 ± 0.39	2.26 ± 0.14	25.63 ± 0.08

[a] Results expressed as mean ± standard deviation. [b] Calculated b difference (raw dough weight – baked dough weight).

Table 3 – Response surface analysis[a].

Parameters		Mathematical model	Mean Value	R^2	p-value
Diameter		$Y = 38.42 - 2.6675\ x_1$	-	70.14	0.018
Thickness		ns	9.00	63.53	0.330
Mass loss		ns	17.61	69.73	0.255
Specific volume		ns	2.54	62.11	0.347
Moisture		ns	6.09	57.56	0.404
Water activity		ns	0.48	58.14	0.397
Fracturability		ns	52.24	63.56	0.330
Color parameters	**L***	ns	73.27	36.94	0.664
	a*	ns	5.16	20.63	0.851
	b*	ns	28.20	46.03	0.551

[a]ns = not significant

Choudhury et al. (2015) elaborated cookies containing 0, 5, 10, and 15% bamboo shoot flour, and also observed an increase in diameter and a reduction of thickness after baking, for the formulations containing higher fiber levels. However, cookies were darker when compared to cookies of the present study, with L* values ranging from 54.10 to 36.31.

3.3. Selection of the cookies

The response surface analysis (Figure 1) indicated significant differences in cookies' diameter only for the variable sugar reduction, once the lower the sugar content (0 to 50% reduction), the smaller the cookies' diameter (35.75 to 41.09 mm). In contrast, no significant differences were observed for the variable fat content. In addition, the results showed that it was also possible to produce cookies with a reduction of up to 50% fat (F3), without affecting the technological characteristics.

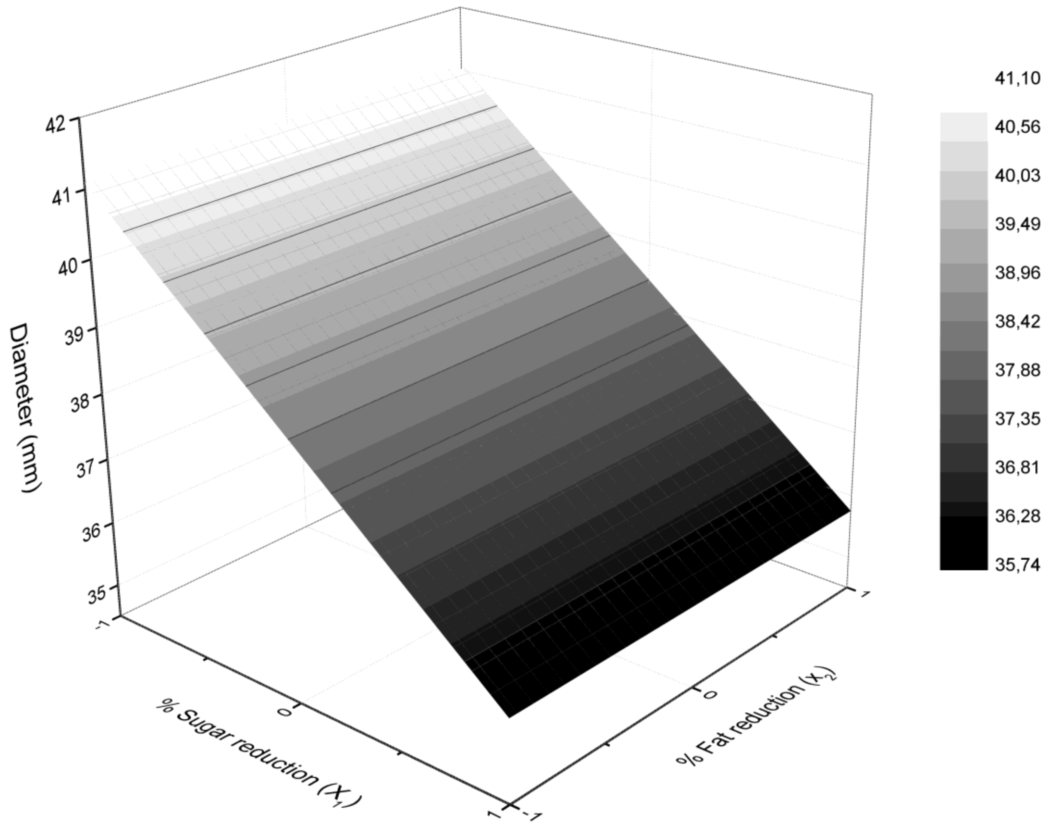

Figure 1 – Response surface analysis of the cookies with sugar and fat reduction (p<0.10).

Thus, two formulations were produced, F2 and F3, with the highest levels of sugar and fat reduction, respectively, together with a control formulation (CF), without the replacement of wheat flour by YBCF and without sugar and fat reduction (as described in Section 2.4), to compare the proximate composition and the technological characteristics.

The control cookies (CF) and those selected in the experimental design (F2 and F3) are shown in Figure 2. The cookies F2 and F3 have a smaller diameter than CF (44.89 mm), which was expected due to the lower amount of ingredients that affect the cookies' expansion, such as sugar and fat.

FC F2 F3

Figure 2 – Control formulation (FC) and cookies with 50% sugar reduction (F2) and 50% of far reduction (F3).

3.4. Technological characterization and stability of the cookies

Table 4 presents the stability of the cookies after 1, 7, 14, 21, and 28 days of storage for moisture, aw, fracturability, and color parameters (L*, a*, and b*).

Although an increase in moisture content was observed at the end of the storage, F2 and F3 showed a significant reduction at days 7 and 14, and 14, respectively. In addition, a higher moisture content was observed in F2 at day 1, which was observed in F3 only on the last day of storage. It is also worth mentioning that both formulations containing YBCF had a higher moisture content at the end of storage when compared to the control.

Similar behavior was observed for aw, with a drop from days 7 to 14, and an increase until the end of the storage, when all cookies exhibited aw < 0.6. As reported by Clerici et al. (2013), a lower aw is required to maintain the cookies' crispness, as observed in the formulations F2, F3, and CF. In addition, F2 presented the highest values during all the storage period, which may be due to its lower fat content. Although the lower moisture and aw values, they are in the optimum range to maintain the crispness (moisture and aw lower than 5% and 0.65, respectively), as reported by Manley (2011).

With respect to the fracturability of the cookies, a small variation was observed during storage, except for F3, and the fat reduction contributed with the significant increase in fracturability, as expected, once according to Pareyt et al. (2009), it enhances the aeration for leavening and volume and makes the cookies more easily breakable.

Similar results were observed by Moraes et al. (2010), who evaluated low-fat cookies and obtained higher tensile strength values (66.65 N) when compared to the cookies of the present study. These results reinforce the role of fat on dough lubrication and aeration, as reported by Pareyt et al. (2009), evidencing the effect of the YBCF associated with fat reduction on the texture of the cookies of the present study.

Regarding the color parameter, the cookies CF, F2, and F3 were in the region of yellowish brown. An increase in L* value was observed over time, which decreased from day 7 to 14 and subsequently increased for all cookies, with no significant differences among the formulations at the end of storage (L*=75.72). Similar results (72.80 to 76.93) were observed by Marangoni (2007), who partially replaced wheat flour for yacon flour and oat flakes in functional Champurrada cookies.

Although some oscillations were observed for the coordinates a* and b* during the storage, a decrease in a* value was observed, while b* values remained or decreased (CF) when compared to day 1. This result shows that although the cookies had lower intensity of red and yellow shades over time, they remained in the slightly yellowish region.

Table 4 – Technological characterization and stability of the control (CF) and selected cookies (F2 and F3) at days 1, 7, 14, 21 and 28 after the manufacture[a].

Analysis		Formulations[b]	Days 1	7	14	21	28
Moisture (g/100 g)		**CF**	2.43 ± 0.04^{Ce}	2.72 ± 0.00^{Cc}	2.89 ± 0.13^{Cb}	2.60 ± 0.07^{Cd}	3.09 ± 0.05^{Ba}
		F2	4.22 ± 0.05^{Aa}	4.27 ± 0.04^{Aa}	3.77 ± 0.03^{Ac}	4.02 ± 0.04^{Ab}	4.13 ± 0.07^{Ab}
		F3	3.54 ± 0.14^{Bb}	3.32 ± 0.03^{Bc}	3.02 ± 0.04^{Bd}	3.38 ± 0.02^{Bc}	4.16 ± 0.02^{Aa}
Water activity (aw)		**CF**	0.20 ± 0.00^{Cd}	0.25 ± 0.00^{Cc}	0.26 ± 0.00^{Cb}	0.28 ± 0.00^{Ca}	0.29 ± 0.00^{Ca}
		F2	0.38 ± 0.00^{Aa}	0.35 ± 0.00^{Ac}	0.37 ± 0.00^{Ab}	0.37 ± 0.00^{Ab}	0.39 ± 0.00^{Aa}
		F3	0.31 ± 0.01^{Bb}	0.29 ± 0.00^{Bc}	0.28 ± 0.00^{Bd}	0.31 ± 0.00^{Bb}	0.34 ± 0.00^{Ba}
Fracturability (N)		**CF**	36.27 ± 2.35^{Ba}	30.20 ± 3.65^{Cb}	28.80 ± 2.15^{Cb}	25.12 ± 2.34^{Cc}	29.93 ± 3.21^{Cb}
		F2	36.03 ± 6.91^{Ba}	38.14 ± 3.53^{Ba}	39.34 ± 7.07^{Ba}	36.37 ± 3.69^{Ba}	42.28 ± 6.11^{Ba}
		F3	85.30 ± 6.50^{Ab}	87.54 ± 7.97^{Ab}	63.06 ± 11.94^{Ac}	110.94 ± 9.11^{Aa}	78.85 ± 10.19^{Ab}
Color parameters	**L***	**CF**	75.08 ± 0.42^{Aa}	73.72 ± 0.71^{Aa}	70.37 ± 0.54^{Ab}	75.67 ± 0.80^{Aa}	76.64 ± 0.75^{Aa}
		F2	67.84 ± 1.84^{Cc}	73.73 ± 0.90^{Aa}	71.01 ± 0.90^{Ab}	73.82 ± 0.48^{Aa}	75.47 ± 0.78^{Aa}
		F3	71.78 ± 0.44^{Bb}	75.56 ± 0.74^{Aa}	72.45 ± 1.33^{Ab}	75.17 ± 0.23^{Aa}	75.05 ± 0.26^{Aa}
	a*	**CF**	3.52 ± 0.15^{Bc}	4.75 ± 0.29^{Ab}	5.47 ± 0.63^{Aa}	2.72 ± 0.13^{Bc}	3.03 ± 0.25^{Cc}
		F2	5.61 ± 0.22^{Aa}	4.50 ± 0.16^{Ab}	4.17 ± 0.20^{Bb}	4.25 ± 0.27^{Ab}	3.97 ± 0.05^{Bb}
		F3	5.79 ± 0.08^{Aa}	4.41 ± 0.12^{Ab}	4.62 ± 0.24^{Bb}	4.62 ± 0.02^{Ab}	5.74 ± 0.05^{Aa}
	b*	**CF**	28.16 ± 1.26^{Ab}	29.45 ± 0.45^{Aa}	29.63 ± 0.79^{Aa}	26.37 ± 0.29^{Bc}	26.12 ± 0.30^{Cc}
		F2	28.78 ± 0.57^{Ab}	29.96 ± 0.19^{Aa}	28.15 ± 0.24^{Bb}	28.84 ± 0.69^{Ab}	28.62 ± 0.32^{Bb}
		F3	29.38 ± 0.50^{Aa}	28.00 ± 0.27^{Bb}	27.70 ± 0.30^{Bb}	28.13 ± 0.28^{Ab}	29.80 ± 0.30^{Aa}

[a]Results expressed as means ± standard deviation. Means followed by different upper-case letters overlapped in the same column for each parameter differ significantly ($p<0.05$) by Scott-Knott test. Means followed by different lowercase letters overlapped in the same line, for each formulation, differ significantly ($p<0.05$) by Scott-Knott test. [b] Control formulation (CF), cookies with 50% of sugar reduction (F2) and 50% of fat reduction (F3).

3.5. Proximate composition of the selected cookies

All cookies (CF, F2, and F3) were evaluated for moisture content, lipids, protein, ash, total fiber, other carbohydrates and energy value, and the results are presented in Table 5. Low moisture values were observed for all cookies (<6 g/100 g), with the lowest values for those with sugar reduction (F2). Despite of the significant changes during the 28 days of storage and between the different cookies (Table 4), the moisture content remained between 2 and 5%, which confers the characteristic crispness to the product, according to Sarantópoulos et al. (2001).

The lower fat content was observed in F3, and differences were observed for the protein content with the addition of YBCF when compared to the CF. Significant differences were also observed for fiber contents among the formulations, with values > 7 g/100 g for both the formulations F2 and F3, which was expected since the YBCF contained > 70 g/100 g. These results corroborate the great potential of YBCF as an alternative raw material, rich in fibers. Regarding the energy value, a significant reduction in F3 was observed, which presented about 10% less calories when compared to CF and F2.

Table 5 – Proximate composition of the control (CF) and selected formulations (F2 and F3)[a].

Formulations[b]	Centesimal composition (g/100 g)						Calories (kcal/100 g)
	Moisture	Lipid	Protein	Ash	Total fiber	Other carbohydrates[c]	
FC	5.39 ± 0.08[a]	14.86 ± 0.40[b]	7.69 ± 0.09[b]	0.42 ± 0.03[b]	1.94 ± 0.32[c]	69.70	443.30
F2	4.97 ± 0.08[b]	18.11 ± 0.01[a]	8.32 ± 0.07[a]	0.63 ± 0.02[a]	7.13 ± 0.08[b]	60.84	439.63
F3	5.50 ± 0.06[a]	9.78 ± 0.14[c]	6.98 ± 0.01[c]	0.42 ± 0.05[b]	8.48 ± 0.12[a]	68.84	391.30

[a] Results expressed as means ± standard deviation. Means followed by different letters in the same column differ significantly (p<0.05) by Scott-Knott test. [b] Control formulation (CF), cookies with 50% of sugar reduction (F2) and 50% of fat reduction (F3). [c] Calculated by difference [100 – (moisture + lipid + protein + ash + total fiber)].

4. CONCLUSION

The results showed that young bamboo culm flour may be an interesting ingredient to reduce sugar in cookies, without compromising the texture characteristics of the product, besides reducing the energy value. The use of this flour also increased the nutritional contribution of fibers when compared to the control formulation, allowing to meet the consumer's demand for healthier fiber-rich products.

5. ACKNOWLEDGEMENTS

The authors are grateful to FAEPEX and FAPESP for the scholarship of Patricia S. E. Miyake (2016/13533-5) and for the financial support (2015/19637-4); CAPES for the scholarship of Mária H. F. Felisberto, CNPq and SAE/UNICAMP for the scholarship of the students who supported along the project.

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– CAPÍTULO 9 –

Discussão Geral

Este capítulo apresenta uma breve discussão dos resultados mais importantes obtidos durante a pesquisa, bem como apresenta-se uma comparação entre os amidos de colmos jovens das três espécies de bambus.

1. DISCUSSÃO GERAL

O bambu é popularmente conhecido por seu uso industrial na fabricação de diversos produtos, tais como, painéis, pisos, celulose, carvão vegetal, e também andaimes, devido às suas propriedades mecânicas, além de poder ser utilizado na produção de medicamentos, devido ao elevado teor de fitoesteróis presentes no broto (Xuhe, 2003; Lo et al., 2008; Chongtham, Bisht e Haorongbam, 2011; Pereira e Beraldo, 2016).

O colmo de bambu apresenta como componentes majoritários celuloses, hemiceluloses e lignina, cujas características variam de acordo com a espécie, a idade e a época da colheita do colmo. Vários estudos avaliaram a utilização dessas fibras para produção de biomassa e bioetanol, para a extração de compostos bioflavonoides (Scurlock, Dayton e Hames, 2000; He et al., 2014) e da fração fibrosa xilooligossacarídica, que pode ser utilizada na produção do xilitol (Aoyama e Seki, 1994; Miura et al., 2013; Xiao et al., 2013). Todavia, o colmo apresenta também significativa composição em amido, que é o responsável pela rápida deterioração de artefatos com ele fabricados, devido ao ataque de carunchos (Pereira e Beraldo, 2016).

O processo de fabricação utilizado no presente trabalho para a elaboração da farinha do colmo jovem de bambu da espécie *D. asper* foi eficiente, uma vez que conseguiu-se separar a farinha em dois materiais de diferentes granulometrias e com baixo teor de umidade, favorecendo a conservação do produto. Outra evidência do sucesso operacional do fluxograma de produção foram os baixos teores de cinzas, proteínas e lipídeos obtidos no produto. Todavia, a separação da farinha do colmo jovem em duas granulometrias diferentes (F1 > 0,425 mm e F2 ≤ 0,425 mm) mostrou-se desnecessária, uma vez que a diferença entre as frações para a composição centesimal foi muito pequena, e com relação à absorção de água não houve diferença significativa, o que facilitaria a incorporação da farinha de *D. asper* em produtos de panificação e na fabricação de massas alimentícias.

Ao comparar as farinhas das três espécies de bambu observou-se um elevado teor de fibras (> 60 g/100 g) em todas as amostras, sendo que as espécies *D. asper* e *B. vulgaris* apresentaram também elevado teor de amido (10 e 16 g/100 g, respectivamente). Desta forma, o colmo jovem de bambu da espécie *B. tuldoides* apresenta potencial para extração de fibras, enquanto que as espécies *D. asper* e *B.*

vulgaris apresentam potencial para extração de amido, aumentando consequentemente as aplicações alimentícias possíveis do colmo jovem de bambu, como matéria-prima.

Levando em consideração a crescente busca das indústrias alimentícias e não-alimentícias por novos ingredientes e que sejam provenientes de fontes naturais, faz-se importante a caracterização dos amidos do colmo jovem de bambu, uma vez que o conhecimento de suas características e propriedades tecnológicas já o direciona para determinado tipo de indústria ou de aplicação. Ao comparar os amidos das três espécies, observou-se que todos apresentaram-se bem claros, com coloração levemente amarelada. Em todas as amostras foram observados amidos com formato poliédrico e de pequeno tamanho, com diâmetro variando, em média, de 4,64 a 6,54 mm. Com relação à composição centesimal foram observados baixos teores de umidade (< 10 g/100 g), de proteínas (média 2,5 g/100 g), de lipídeos (média 0,36 g/100 g) e de cinzas (média 1,87 g/100 g), corroborando com o processo de extração utilizado. Uma vez que nenhum reagente químico foi empregado, o rendimento de extração não foi tão elevado, mas conseguiu-se obter os grânulos de amido em sua forma nativa.

Com exceção das amostras de amido do *B. vulgaris* (31 a 41%), todas as demais apresentaram teor de amilose característico de cereais, variando de 12 a 34% entre as amostras. Com relação à distribuição molecular de tamanho das cadeias, os amidos apresentaram similar comportamento dos picos, tanto para a resposta de *blue value* (BV) quanto para açúcares totais (CHO). Porém, os valores obtidos para os picos de amilose não foram condizentes com o resultado obtido para o teor de amilose aparente, o que já era esperado, uma vez que ainda não existe um padrão de amilose para o amido de bambu.

As amostras não apresentaram diferença significativa entre o comprimento médio das cadeias ramificadas da amilopectina, e todas apresentaram pico principal com grau médio de polimerização (DP) de 12-13, e o segundo pico com DP de 43-45, similar ao comprimento médio das cadeias do trigo, do arroz e da cevada. Todas as amostras possuem grande proporção de cadeias curtas (DP 6-12), mas a maior proporção é de cadeias com DP 13-24, que é o comprimento ideal para a formação de duplas hélices estáveis. Além disso, nenhum dos amidos apresentou um ombro na distribuição do comprimento das cadeias, sugerindo que esses amidos não apresentam imperfeições na estrutura cristalina (Jane et al., 1999).

Os resultados obtidos para distribuição das cadeias de amilopectina, além de serem similares ao padrão dos amidos de cereais, corroboram com o padrão de cristalinidade tipo A, segundo Hizukuri (1986), Jane (2006) e Bemiller e Whistler (2009).

A cristalinidade relativa (CR) varia com o inverso do teor de amilose aparente, como pode ser observado nos resultados obtidos, salvo para o amido de *B. vulgaris* extraído da fração do topo. Entre todas as amostras, CR variou de 19 a 28%, conforme esperado para amidos nativos, de acordo com Cheetham e Tao (1998).

Com relação às propriedades térmicas, os amidos apresentaram elevadas temperaturas de gelatinização (T_p > 80 ºC) e baixa entalpia ($\Delta H < 11$ $J.g^{-1}$), o que indica elevada organização molecular. E ao comparar os amidos nativos com os amidos retrogradados, estes apresentaram menores temperaturas de transição do que os amidos nativos, devido ao realinhamento inadequado das moléculas de amilose e das cadeias laterais da amilopectina, que levam à formação de estruturas cristalinas menos ordenadas e menos estáveis, segundo Karim, Norziah e Seow (2000) e Singh et al. (2003).

Tendo em vista que os amidos das três espécies de bambus apresentaram características similares às de amidos de cereais, e também elevado teor de fibras das farinhas e o potencial para extração de amido de algumas espécies, selecionou-se a farinha de colmo jovem de *D. asper* (FCJB) para a elaboração de biscoitos tipo *cookies,* em substituição parcial (15%) à farinha refinada de trigo. Os biscoitos foram formulados com redução parcial dos teores de açúcar e de gordura (duas variáveis) através de um DCCR com três repetições no ponto central. Observou-se que apenas a variável independente redução do teor de açúcar apresentou efeito significativo sobre o parâmetro diâmetro, e que quanto maior foi o teor de redução do açúcar, menor foi o diâmetro observado nos *cookies*. Mesmo que a redução do teor de gordura não tenha apresentado efeito significativo sobre o diâmetro, a formulação com o maior teor de redução foi selecionada, tendo em vista a crescente preocupação dos consumidores com as questões de saudabilidade e com o teor calórico dos alimentos.

Através da avaliação da composição centesimal das duas formulações selecionadas, com os teores máximos de redução do açúcar e da gordura, foi possível observar a significativa redução do teor de gordura e, consequentemente, a redução calórica. Com relação à estabilidade ao longo de 28 dias de armazenamento, as formulações mostraram-se estáveis, com baixos teores de umidade e atividade de água (aw), parâmetros de cor na região do levemente amarelado, para todas as formulações. Com relação à textura, a formulação com redução do teor de açúcar foi a que mostrou-se mais próxima à da formulação controle evidenciando a FCJB como um novo e promissor ingrediente para a indústria de alimentos.

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– CAPÍTULO 10 –

Conclusão Geral

Os capítulos precedentes são sumarizados e alguns resultados foram resumidos de forma a condensar as informações de cunho técnico, consideradas de maior importância

CONCLUSÃO GERAL

Os resultados deste trabalho demonstraram que, apesar de os brotos do bambu já serem utilizados em uma série de produtos farmacêuticos e para o consumo direto, o colmo jovem ainda não é utilizado para aplicações industriais, sejam elas alimentícias ou não, apesar do potencial apresentado devido ao seu elevado teor de fibras e de amido.

Observou-se que a composição de fibras insolúveis acima de 70% presentes na farinha do colmo jovem de bambu o qualifica como um promissor ingrediente para a indústria de alimentos, uma vez que tem aumentado a demanda dos consumidores por produtos mais saudáveis. De acordo com a espécie de bambu utilizada, o teor de amido (em media, 9,77 g/100 g) e de açúcares (em media, 9,41 g/100 g) pode direcionar a farinha para diferentes aplicações alimentícias, como por exemplo para a substituição parcial da farinha de trigo e redução de até 50% da adição de açúcar e/ou gordura em formulações de biscoito tipo *cookies*, sem alterar a coloração do produto e nem as características de textura, além de aumentar o aporte nutricional, conforme observado no presente trabalho.

Desta forma, o colmo jovem de bambu poderá ser utilizado tanto para a produção de farinha, como para extração de fibras e de amido. A grande vantagem da utilização do colmo jovem para extração de fibras, em comparação com o broto, é o seu tamanho e a manutenção da touceira, uma vez que apenas de 20-30% dos brotos de bambu necessitam ser retirados.

O amido, que pode ser obtido como co-produto da extração das fibras, também é um ingrediente versátil para aplicações alimentícias e para atender a demanda da indústria por novas fontes de amidos e com propriedades específicas. O amido do colmo jovem de bambu, de modo geral, apresenta características similares aos amidos dos cereais, de modo que poderia ser utilizado em substituição àqueles utilizados tradicionalmente, como o amido de milho, arroz e trigo, e com a vantagem de o amido de bambu suportar elevadas temperaturas de processamento e elevado trabalho mecânico.

Acredita-se que este trabalho tenha sido apenas o início de uma longa pesquisa, e que muitos outros resultados promissores certamente virão. Tratou-se de uma experimentação pioneira de uma linha de pesquisa em ingredientes naturais, que está sendo estabelecida no Laboratório de Cereais, Raízes e Tubérculos da

FEA/UNICAMP. Espera-se que estas informações contribuam não só com a comunidade acadêmica, mas também com o desenvolvimento de produto alimentícios mais saudáveis e benéficos ao consumidor. Finalmente, espera-se que, após a regulamentação da Lei Federal nº 12.484 (2011), que as plantações de bambu possam ser consideradas investimentos, com elevado potencial de retorno econômico e não apenas uma simples fonte extrativista.

– CAPÍTULO 11 –

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– CAPÍTULO 12 –

Anexos

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Food Technology MSc - UNICAMP
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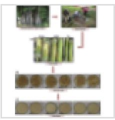

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Publication: Food Research International

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