UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS
Programa de Pós-Graduação em Ciência de Alimentos

PRODUÇÃO DE CERVEJA UTILIZANDO PALHA DE CEVADA PRÉ-TRATADA COM O INIBIDOR DE LIGNINA APLICADO VIA SOLO E FOLIAR

EVANDRO RIBEIRO MACHADO FILHO

Maringá
2022

EVANDRO RIBEIRO MACHADO FILHO

PRODUÇÃO DE CERVEJA UTILIZANDO PALHA DE CEVADA PRÉ-TRATADA COM O INIBIDOR DE LIGNINA APLICADO VIA SOLO E FOLIAR

Dissertação apresentada ao programa de Pós Graduação em Ciência de Alimentos da Universidade Estadual de Maringá, como parte dos requisitos para obtenção do título de mestre em Ciência de Alimentos.

Maringá
2022

Dados Internacionais de Catalogação-na-Publicação (CIP)

(Biblioteca Central - UEM, Maringá - PR, Brasil)

M149p

Machado Filho, Evandro Ribeiro

Produção de cerveja utilizando palha de cevada pré-tratada com o inibidor de lignina aplicado via solo e foliar / Evandro Ribeiro Machado Filho. -- Maringá, PR, 2022.

40 f.: il. color., figs., tabs.

Orientadora: Profa. Dra. Paula Toshimi Matumoto Pintro.

Dissertação (Mestrado) - Universidade Estadual de Maringá, Centro de Ciências Agrárias, Departamento de Agronomia, Programa de Pós-Graduação em Ciência de Alimentos, 2022.

1. Ácido piperonílico. 2. Açúcares fermentescíveis. 3. Cerveja funcional. 4. Compostos bioativos. 5. Parede celular. I. Pintro, Paula Toshimi Matumoto , orient. II. Universidade Estadual de Maringá. Centro de Ciências Agrárias. Departamento de Agronomia. Programa de Pós-Graduação em Ciência de Alimentos. III. Título.

CDD 23.ed. 663.42

Marinalva Aparecida Spolon Almeida - 9/1094

EVANDRO RIBEIRO MACHADO FILHO

"PRODUÇÃO DE CERVEJA UTILIZANDO PALHA DE CEVADA PRÉ-TRATADA COM O INIBIDOR DE LIGNINA APLICADO VIA SOLO E FOLIAR".

Dissertação apresentada à Universidade Estadual de Maringá, como parte das exigências do Programa de Pós-graduação em Ciência de Alimentos, para obtenção do grau de Mestre em Ciência de Alimentos.

Prof. Dr. Wanderley Dantas dos Santos

Prof. Dr. Antonio Roberto Giriboni Monteiro

Profa. Dra. Paula Toshimi Matumoto Pintro
Orientadora

Maringá – 2022

Orientadora

Profª Drª Paula Toshimi Matumoto Pintro

BIOGRAFIA

Evandro Ribeiro Machado Filho nasceu em 10 de março de 1998 na cidade de Maringá, Paraná. Filho de Evandro Ribeiro Machado e Sandra Correia Machado. Possui graduação em Bioquímica pela Universidade Estadual de Maringá, finalizada em 2019. Ingressou no Programa de Pós-Graduação em Ciência de Alimentos em março de 2020. Tem experiência nas áreas de Bioquímica de Plantas e Tecnologia de Alimentos atuando principalmente na alteração estrutural da lignina e aproveitamento de resíduos vegetais do setor agropecuário.

Dedico

A Deus que me capacitou e aos meus pais que sempre me apoiaram.

AGRADECIMENTOS

Agradeço a Deus por sempre me guiar e ouvir minhas orações, cuidando das minhas decisões, me capacitando e me abençoando em cada passo.

Agradeço à minha família, especialmente meus pais, por sempre estar do meu lado, me apoiar e por todo suporte que me deram para chegar até este momento.

Agradeço à minha orientadora Prof.ª Dr.ª Paula Toshimi Matumoto Pintro, por ter me recebido em seu grupo, aceitado realizar o projeto que eu tanto desejava e ainda melhorar e acrescentar ideias excelentes ao trabalho, por todo o ensinamento e principalmente pelo suporte dado em todo este tempo de mestrado, ficará sempre em minhas memórias.

Agradeço à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pela concessão da bolsa de estudos.

Agradeço à Prof.ª Dr.ª Francielle Sato e ao Prof. Dr. Wanderley Dantas dos Santos pela contribuição ao projeto e pela cooperação em análises, ideias e por auxiliar no desenvolvimento do trabalho.

Agradeço a todos do grupo de pesquisa em alimentos funcionais e do laboratório de bioquímica de plantas da Universidade Estadual de Maringá, pelo auxílio e contribuição na realização do projeto. Em especial ao Diego Eduardo Romero Gonzaga, Anderson Lazzari, Bianka Rocha Saraiva, Breno Miguel Joia e Mariana Sversut Gibin.

A todas as pessoas que participaram de forma direta ou indireta no trabalho, gratidão imensa a todos vocês!

APRESENTAÇÃO

Esta dissertação de mestrado está apresentada na forma de um artigo científico:

1 Evandro Ribeiro Machado Filho; Anderson Lazzari; Diego Eduardo Romero Gonzaga; Mariana Sversut Gibin; Wanderley Dantas dos Santos; Francielle Sato; Paula Toshimi Matumoto Pintro. Beer production using barley straw pretreated with lignin inhibitor applied via soil and foliar. Journal of the Science of Food and Agriculture.

GENERAL ABSTRACT

INTRODUCTION. Barley straw is an agroindustrial residue of low added value, being the focus of studies that seek to reuse this material through chemical pretreatments to increase the availability of structural carbohydrates for the production of bioethanol or animal feed. However, these pretreatments are expensive and generate chemical residues. Barley straw is a lignocellulosic material consisting mainly of cellulose, hemicellulose and lignin, with lignin being a barrier that influences the access of enzymes to structural carbohydrates, hindering the release of reducing sugars to be fermented and transformed into alcohol. Beer adjuncts are increasingly used to partially replace malt, aiming to reduce the production cost and incorporate different characteristics to beer, seeking more accessible ingredients that add functional value to the final product.

AIMS. The objective of this study was to evaluate the influence of two methods of lignin inhibitor application in barley field, being via soil or foliar, aiming the use of pretreated straw as a brewing adjunct. The effects of treatments on the structure and application of this straw were analyzed when inserted into the wort with the addition of xylanase and cellulase, in order to increase the release of fermentable sugars and bioactive compounds in beer.

MATERIAL AND METHODS. Two methods (soil and foliar) of field application of the lignin inhibitor in barley cultivation were realized, verifying the parameters of harvest productivity and also analysis of the pretreated barley straw, determining the straw chemical composition, quantification of lignin and enzymatic saccharification with the enzymes xylanase and cellulase. To observe changes in the collected straw structure more specifically, FTIR (Fourier Transform Infrared) and SEM (Scanning Electron Microscopy) experiments were performed. Worts were produced with 2.5%, 5% and 10% proportions of partial malt replacement by barley straw and with different concentrations of xylanase and cellulase enzymes, determining the best concentrations of straw and enzymes in wort through the method of reducing sugars quantification (DNS). Selected worts were analyzed considering parameters of proteins, total polyphenols (TPC), antioxidant activity (DPPH and ABTS), color and pH, being selected those that obtained the best parameters for beer production and evaluation of the total polyphenols and antioxidant activity behavior during storage for 28 days at 4 °C.

RESULTS AND DISCUSSION. Lignin inhibitor applications via soil and foliar in the field did not influence barley productivity, making this pretreatment viable. SEM, FTIR and enzymatic saccharification analyzes showed that treatments with the lignin inhibitor caused structural alterations and changes in the chemical composition of barley straw, resulting in greater enzymatic accessibility to structural carbohydrates, with higher release of reducing sugars using cellulase. Straws obtained with the two application methods showed increases in fermentable sugars release when inserted in wort with 2.5% of barley straw, using cellulase isolated or a xylanase and cellulase mixture in 1:2 proportion,

maintaining the final enzyme concentration of 16 U mL^{-1}. Worts with these concentrations of pretreated straw and enzymes had increases mainly in the quantification of total polyphenols and antioxidant activity. After fermentation of worts, beer showed stability of these bioactive compounds during storage analysis, demonstrating that barley straw pretreated with lignin inhibitor combined with enzymes is capable of increasing the functional value of the beer produced from these worts.

CONCLUSIONS. Barley straw pretreated with lignin inhibitor applied via soil or foliar is capable of being used as a brewing adjunct combined with cell wall hydrolytic enzymes to partially replace malt, acting mainly in increasing the release of fermentable sugars and bioactive compounds in brewer's wort.

Key words: piperonylic acid; fermentable sugars; functional beer; bioactive compounds; cellulase; cell wall

RESUMO GERAL

INTRODUÇÃO. A palha de cevada é um resíduo agroindustrial de baixo valor agregado, sendo foco de estudos que buscam reaproveitar esse material por meio de pré-tratamentos químicos para aumentar a disponibilidade dos carboidratos estruturais visando à produção de bioetanol ou alimentação animal. No entanto, esses pré-tratamentos possuem custos elevados e geram resíduos químicos. A palha de cevada é um material lignocelulósico constituído principalmente de celulose, hemicelulose e lignina, sendo a lignina uma barreira que influencia no acesso das enzimas aos carboidratos estruturais, dificultando a liberação de açúcares redutores para serem fermentados e transformados em álcool. Os adjuntos cervejeiros são cada vez mais utilizados para substituir parcialmente o malte, visando reduzir o custo de produção e incorporar diferentes características para a cerveja, buscando ingredientes mais acessíveis e que agreguem valor funcional ao produto final.

OBJETIVOS. O objetivo deste estudo foi avaliar a influência de dois métodos de aplicação do inibidor de lignina em campo de cevada, sendo estes via solo ou foliar, visando à utilização da palha pretratada como adjunto cervejeiro. Para isto, foram analisados os efeitos dos tratamentos na estrutura e na aplicação desta palha quando inserida no mosto com a adição de xilanase e celulase, a fim de aumentar a liberação de açúcares fermentescíveis e compostos bioativos na cerveja.

MATERIAL E MÉTODOS. Dois métodos (via solo e foliar) de aplicação em campo do inibidor de lignina no cultivo de cevada foram feitos, verificando os parâmetros de produtividade da colheita e também análises da palha de cevada pré-tratada, sendo realizada a composição química da palha, quantificação de lignina e sacarificação enzimática com as enzimas xilanase e celulase. Para observar mudanças na estrutura da palha coletada de forma mais específica, foram feitos os experimentos de FTIR (Infravermelho com Transformada de Fourier) e SEM (Microscopia Eletrônica de Varredura). Os mostos foram produzidos com proporções de 2.5%, 5% e 10% de substituição parcial do malte por palha de cevada e com diferentes concentrações das enzimas xilanase e celulase, determinando as melhores concentrações de palha e enzimas no mosto através do método de quantificação de açúcares redutores (DNS). Os mostos escolhidos foram analisados considerando parâmetros de proteínas, polifenóis totais (TPC), atividade antioxidante (DPPH e ABTS), cor e pH, sendo selecionados os que obtiveram melhores parâmetros para a produção de cerveja e avaliação do comportamento dos polifenóis totais e atividade antioxidante durante o armazenamento destas cervejas por 28 dias à 4 °C.

RESULTADOS E DISCUSSÃO. As aplicações do inibidor de lignina via solo e foliar no campo não influenciaram a produtividade da cevada, tornando este pré-tratamento viável. As análises de SEM, FTIR e sacarificação enzimática mostraram que os tratamentos com o inibidor de lignina causou alterações estruturais e na composição química da palha de cevada, resultando em maior acessibilidade enzimática aos

carboidratos estruturais, apresentando maior liberação de açúcares redutores com a ação da celulase. As palhas obtidas com os dois métodos de aplicação apresentaram aumento na liberação de açúcares fermentescíveis quando inseridas em mosto com 2,5% de palha de cevada, utilizando celulase isolada ou mistura de xilanase e celulase na proporção 1:2, mantendo a concentração enzimática final de 16 U mL^{-1}. Os mostos com estas concentrações de palha pré-tratada e enzimas tiveram aumentos principalmente na quantificação de polifenóis totais e atividade antioxidante. Após fermentação dos mostos, a cerveja apresentou estabilidade destes compostos bioativos durante análise de armazenamento, demonstrando que a palha de cevada pré-tratada com inibidor de lignina combinado com enzimas é capaz de aumentar o valor funcional da cerveja produzida a partir desses mostos.

CONCLUSÕES. A palha de cevada pré-tratada com inibidor de lignina aplicado via solo ou foliar é capaz de ser utilizada como adjunto cervejeiro combinado com enzimas hidrolíticas da parede celular para substituir parcialmente o malte, atuando principalmente no aumento da liberação de açúcares fermentescíveis e compostos bioativos no mosto cervejeiro.

Palavras chaves: ácido piperonílico; açúcares fermentescíveis; cerveja funcional; compostos bioativos; celulase; parede celular

ARTICLE 1

Beer production using barley straw pretreated with lignin inhibitor applied via soil and foliar

Evandro Ribeiro Machado Filho[a], Anderson Lazzari[a], Diego Eduardo Romero Gonzaga[b], Mariana Sversut Gibin[c], Wanderley Dantas dos Santos[b], Francielle Sato[c] and Paula Toshimi Matumoto Pintro[a*].

[a] Departamento de Agronomia, Universidade Estadual de Maringá, Paraná, Brasil;

[b] Departamento de Ciências Biológicas, Universidade Estadual de Maringá, Paraná, Brasil;

[c] Departamento de Física, Universidade Estadual de Maringá, Paraná, Brasil;

*Corresponding author. Departamento de Agronomia, Universidade Estadual de Maringá, Av. Colombo, 5700, Jd. Universitário, 87020-900, Paraná, Brasil. E-mail: ptmpintro@uem.br (Paula T. Matumoto-Pintro). Telephone number: +55 (44) 3011-8946.

Abstract

BACKGROUND: Barley straw is an agricultural residue of low added value and studies search for reuse this straw through chemical pretreatments for alcohol production. However, chemical pretreatments are expensive and generate chemical residues, preventing possible application of this product in food. Beer adjuncts are increasingly used to partially replace malt, aiming to reduce production cost and incorporate different characteristics for beer, increasingly looking for more accessible ingredients that add functional value. This study aims to use a lignin inhibitor applied via soil or foliar in barley field to obtain a straw more accessible to structural carbohydrates, releasing fermentable sugars when enzymatically hydrolyzed and partially replacing malt in beer.

RESULTS: Lignin inhibitor applications did not influence barley productivity. Straws obtained with two application methods showed structural and chemical changes with SEM, FTIR and compositional analysis. These changes resulted in increases on fermentable sugars release when inserted in wort with 2.5% of barley straw, using cellulase isolated or a xylanase and cellulase mixture in 1:2 proportion, maintaining final enzyme concentration of 16 U mL^{-1}. With these same wort parameters, barley straw pretreated with lignin inhibitor combined with enzymes is able to increase the functional value of beer, represented by increase in concentration of total polyphenols and antioxidant activity.

CONCLUSION: Barley straw pretreated with lignin inhibitor applied via soil and foliar can be used as brewing adjunct combined with cell wall hydrolytic enzymes to partially replace malt, acting mainly in increasing the release of fermentable sugars and bioactive compounds in brewer's wort.

Keywords: piperonylic acid; fermentable sugars; functional beer; bioactive compounds; cellulase; cell wall

1 Introduction

New technologies and control techniques has been developed to produce consistent and high quality beers, being possible to predict desirable characteristics for different beer styles. The brewing process and their tradition is maintained by use classic raw materials like barley malt or other malted cereals, water, hops and yeast.[1] Barley (*Hordeum vulgare L.*) is one of the most cultivated cereals in the world, being commonly used as food, animal feed and malting process for beer production. Malt composition and the proper balance of its components is crucial for production of quality beers.[2]

Raw materials, as fruits and unmalted cereals, have been used as adjuncts for beer characteristics improvement, in sensory field and physicochemical aspects of beers.[3] These materials have a specific function in beer production, as a carbohydrates source for fermentation, reduction of production cost and ensure aromatic complexity by phenolic compounds that can increase the antioxidant activity of beer.[4,5] Adjuncts use aims to improve beer aspects and have been used with intention of reducing production cost by malt partial replacement, most popular being maize, rice, sorghum and sucrose-based syrups.[6]

Lignocellulosic material could be used as sugar source, have potential to reduce production cost and can be useful in increasing phenolic compounds in beer, releasing lignin monomers and ferulic acid, enabling a significant increase in the antioxidant activity and health benefits.[7-9]

Barley straw is a residue formed during the grains harvest with low added value. The straw, considered as a lignocellulosic raw material, consists mainly of structural carbohydrates (cellulose and hemicellulose) and lignin.[10] Many carbohydrates present in cell wall can be used in ethanol production when is treated with enzymes or chemical products, providing less recalcitrance lignin and get better access to hidrolysis.[11, 12]

Pretreatment of lignocellulosic biomass is essential when the focus is enzymatic hydrolysis, generally using treatments with high-cost physicochemical conditions and concentrated acid and alkaline solvents.[13] Piperonylic acid is a phenylpropanoid pathway inhibitor and acts directly on the lignin biosynthesis, presenting itself as an environmentally sustainable and economically viable pretreatment.[14]

Novelties in formulations and ingredients from beer production has increased, many

materials have been used as adjuncts to change various aspects of beers as flavor, aroma, and production cost.[15] The aim of this study is use barley straw as beer adjunct, treated with a lignin biosynthesis inhibitor for more accessible structural carbohydrates, and complement with exogenous enzymes to improve fermentable sugars to beer wort.

2 Material and Methods

2.1 Field Barley Experiment

Piperonylic acid solution (0.1 mM, pH 8.0) was prepared for soil application (SA), using activated carbon, and other to foliar application (FA).[16] For SA, activated charcoal was homogenized with piperonylic acid solution for 24h, dried and coated with 1% polyvinyl alcohol solution for one hour to controlled release of the compound and applied at a rate of 57.14 kg ha^{-1}. The solution for FA was applied at 3.32 g ha^{-1} and 0.5% of Aureo® adjuvant (Bayer – Leverkusen, Germany).

The experiment was conducted in South of Brazil (25°33'05.0"S, 51°34'59.9"W), the field area was separated into nine completely randomized plots, with three plots for each treatment and three plots for the control. Piperonylic acid was applied 45 days after plant emergence, when the first node of stem was visible, and the grains and straw were collected in barley harvest. Barley straw was separated from grains and subsequently cleaned and oven dried at 60 °C for 24h. The dried material was milled in a ball mill, standardized in 48 mesh sieve and stored in dry place and protected from light.

2.2 Barley straw with piperonylic acid (BSPA) treatment analysis

2.2.1 Enzymatic activity

Enzyme solutions NS-22083 (xylanase) and NS-22086 (cellulase) from Novozymes® was used. The enzymes activities were analyzed by hydrolysis of 1% carboxymethylcellulose for cellulase and 1% from birchwood xylan for xylanase, in 30 min of reaction at 50 °C in medium containing sodium acetate buffer (50 mM, pH 5.0). Enzymatic activity was quantified by release of reducing sugars in medium and measured by DNS method.[17] Activities were expressed in micromoles of reducing sugar released in hydrolysis per minute of reaction in one milliliter (U mL^{-1}).

Saccharification analysis was realized with xylanase and cellulase separately.

Straws were prewashed with ethanol to remove soluble sugars and weighed 20 mg, placed in a medium containing sodium acetate buffer (1 mL, 50 mM, pH 5.0) and 20 U mL^{-1} of enzyme. After 30 min of reaction at 50 °C, the supernatant was collected and reducing sugars released was evaluated by DNS method.

2.2.2 Barley straw characterization

Chemical composition of dried barley was determined by moisture, crude protein (Kjeldahl method, conversion factor of 6.38), ash, and brute fiber. The total carbohydrates were determined by difference (McCance & Widdowson, 1991) and lignin was analyzed by acetyl bromide method.[18-21]

2.2.3 Fourier Transform Infrared Spectroscopy (FTIR)

Treated and untreated barley straws and grains were evaluated by Fourier Transform Infrared (FTIR) spectroscopy on a Bruker model Vertex 70v spectrometer (Bruker Optik GmbH, Ettilingen, DEU) equipped with an Attenuated Total Reflectance (ATR) accessory with diamond crystal. The spectra were obtained from 4000 to 400 cm^{-1} with a spectral resolution of 4 cm^{-1}, each spectrum being an average of 128 scans, collected in triplicate at room temperature and normalized by the standard.

2.2.4 Scanning Electron Microscopy (SEM)

The barley straw was cut in small pieces, lyophilized and stored in a dry place. For structural analysis, these samples were adhered to aluminum sample stub using double-sided carbon tape and coated with a thin layer of gold. The equipment used for this analysis was a scanning electron microscope (QUANTA 250, FEI) with an accelerating voltage of 15 kV.

2.3 Wort and beer analysis

2.3.1 Wort production

Barley malt was milled in a knife mill, coarsely grinding the malt only to expose the endosperm. Worts were prepared on a small-scale heating bath, using a 1:5 ratio of dry extract and distilled water, with constant stirring at 170 rpm. Total wort extract varied

according to malt replacement proportion by BSPA (2.5%, 5% and 10% of straw in relation to malt used). The wort pH was fixed at 5.2 and enzymes were added at first on mashing. The mashing programmer was: 15 min at 45 °C, 15 min at 50 °C, 20 min at 62 °C, 20 min at 70 °C and 5 min at 78 °C.

2.3.2 Screening of straw and enzymes proportions in wort

The worts were produced by varying the proportion of xylanase, cellulase and barley straw aiming to verify the optimal straw (2.5%, 5% or 10%) and enzymes (4 to 64 U mL^{-1}) concentrations to partially replace malt, being Pure Malt (PM) the control without enzymes and straw for comparison of results. Worts were classified according to reducing sugars release and later by parameters proteins, total polyphenols, antioxidant activity, color and pH. [22, 23]

2.3.3 Total polyphenols content

Total polyphenols content (TPC) were analyzed in worts.[24] Samples was centrifuged (963 × g, 4 °C, 10 min), a supernatant aliquot (125 µL) was mixed with Folin–Ciocalteu reagent (1:1 deionized water; 125 µl) and sodium carbonate (2.25 mL; 3.79 M), homogenized and stored in dark for 30 min and measured at 725 nm (Kasuaki® spectrophotometer, model IL-226). The standard curve was performed with gallic acid (0 to 500 mg L^{-1}) and results were expressed in milligrams of gallic acid equivalent per liter of wort (mg GAE L^{-1}).

2.3.4 Antioxidant activity

The antioxidant activity of the samples were evaluated by 2,2-Diphenyl1-picrylhydrazyl (DPPH) radical scavenging.[25] Wort (150 µL) was mixed with DPPH solution (2.85 mL; 0.06 mM), homogenized and incubated in dark for 30 min and measured at 515 nm. ABTS radical scavenging was evaluated.[26] $ABTS^{+}$ formation occurs by mixing ABTS solution (5 mL; 7 mM) with potassium persulfate (88 µL; 140 mM) and incubated at room temperature in dark for 16h. After, $ABTS^{+}$ was diluted in ethanol until 0.70 ± 0.05 absorbance. $ABTS^{+}$ solution (1.96 mL) was mixed with 40 µL of wort sample and incubated in dark for 6 min, and measured at 734 nm. Antioxidant activities were calculated

using the following equation:

Antioxidant activity (%) = (1- ($A_{sample\ t}/A_{sample\ t=0}$)) x 100

Where: $A_{sample\ t}$ is DPPH samples absorbance at 30 min or ABTS samples absorbance at 6 min, and $A_{sample\ t=0}$ is samples absorbance at time zero.

2.3.5 Beer production and storage

Fermentation was realized in worts with best parameters chosen for seven days at 18°C, with yeast SafAle US-05 (Fermentis Company - Lesaffre, France), and the beer was kept refrigerated in dark for 28 days at 4 °C. Total polyphenols and antioxidant activity were analyzed at 7, 14, 21 and 28 storage days.

2.4 Statistical analysis

The barley straw, wort and beer data were evaluated by analysis of variance using one-way ANOVA (GraphPad Prism 6.0 – San Diego, California), barley treatments were carried out in triplicate and experiments were realized in triplicate of each plot of treatments done in the field. Tukey's test was performed with confidence interval of $p \leq 0.05$ to observe significant differences.

3 Results and Discussion

3.1 Characterization of barley straw pretreated with piperonylic acid

Soil Application (SA) and Foliar Application (FA) pretreatments (Fig. 1) presented similar values when compared to Control (CO) in productivity analysis, showing these applications do not affect the barley cultivation yield. Differences were not observed in lignin concentration (Fig. 2A) in CO, SA and FA pretreatments. Piperonylic acid inhibits synthesis of some compounds in phenylpropanoid pathway and changes lignin monomers concentration. This effect can modify bonds and internal structure of lignin without reduces the concentration of this polymer, maintaining biological action of this barrier.[27] Results showed that SA and FA pretreatments did not affect plant growth, resistance and crop final yield.

Differences in sugar release by BSPA was showed in Fig. 2B and 2C, observing SA and FA barley straw capacity to release more reducing sugars than control when added

cellulase. This demonstrates that lignin pretreatment in barley straw is effective regardless of how it is applied and can be considered more sustainable when compared to chemical straw treatments made after harvest.[28] Cellulase had better hydrolysis yields due to greater cellulose concentration in straw and higher hydrolysis capacity compared to xylanase. Lignin biosynthesis inhibitor treatments can produce cellulose more accessible for enzymes with less aggregation to hemicellulose and can change the internal lignin bonds in these structural carbohydrates, favoring the interaction between cellulase active site and cellulose.[27]

SA and FA pretreatments realized caused changes in straw chemical composition (Table 1). Observing the BSPA results, it was possible to observe a reduction in amount of moisture, ash and proteins. Barley may have rearranged their components between their organs and tissues in response to treatments done with lignin biosynthesis inhibitor application.

Fig. 3 shows the FTIR-ATR spectra averages collected for BSPA and control straw. Region 3600 and 3000 cm^{-1} is characterized by OH stretching vibrations that may be associated with moisture in material or functional groups that contain hydroxyls. This vibrational mode is usually superimposed with NH vibrations, which may come from protein molecules.[29] Bands between 3000 and 2800 cm^{-1} are attributed to the aliphatic chains of CH_2 and CH_3. For lower energies regions, there is the presence of bands associated with carboxylic acids (C=O stretching) centered at 1726 cm^{-1}.[30] The region close to 1635 cm^{-1} is characteristic of amide I vibrational modes and this region may be superimposed with the vibrational mode characteristic of aromatic rings present in phenolic compounds.[31] At approximately 1515 cm^{-1} there is a contribution of lignin. The region from 1290 to 1185 cm^{-1} is related to the presence of cellulosic compounds. Region between 1186 and 947 cm^{-1} associated with C-O stretching modes refers to total carbohydrates present in the samples. Furthermore, there is a contribution of unstructured carbohydrates in the region 946 to 818 cm^{-1}, which comprises bands 896 cm^{-1}.[32]

Individual vibrational mode associated with functional interest group can have its integrated area, indicating the approximate components content in each sampling group (Fig. 3B). SA straws increased concentrations of carbohydrates and cellulosic compounds, showing that piperonylic acid pretreatment can provide better yields in

fermentable sugars release using similar biomass proportions. The FA pretreatment presented alignin concentration reduction in straw, and it is possible that BSPA may have compensated for this change with phenolic compounds increase, production and storage in cell wall.

The SEM analysis showed that CO straw (Fig. 4, A-C) presented more rigid structure appearance, with more intact fibers and cell walls. SA (Fig. 4, D-F) and FA (Fig. 4, G-I) straws pretreatments showed an apparent increase in fiber breakage and detachment, which are important characteristics to obtain biomass with less recalcitrant cell walls and allow more access of enzymes to structural carbohydrates, allowing higher yields in enzymatic hydrolysis (Fig. 2). Piperonylic acid pretreatment can cause structural and chemical changes in plants, which can even affect the barley moisture (Table 1). Cell wall may have become more permeable for water passage from cell to environment or having formed a structure that makes it difficult for water to enter inside barley cells.

3.2 Evaluation of straw and enzymes concentration in wort

CO straw and enzymes proportions analysis (Fig. 5) showed that xylanase and cellulase have a plateau followed by stabilization in rate of reducing sugars release at the point 16 U mL^{-1}, being this concentration chosen to realize further analysis, because increasing enzymes concentration after this point would not obtain proportional yields of reducing sugars. Straw proportions chosen for the following experiments were 2.5% and 10% by similar sugar amounts released and, with this difference between straw proportions, it is possible to evaluate the material influence on some parameters (fermentable sugars, proteins, polyphenols, antioxidant activity, color and pH) when inserted in brewer's wort.

Worts production with BSPA and enzymes (xylanase and cellulase) were evaluated in differences of fermentable sugar release for beer production (Fig. 6). Worts with BSPA obtained higher yields of reducing sugars release compared to Pure Malt (PM) without enzyme. Cellulose from barley straw were more easily hydrolyzed and increase the fermentable sugars in wort, demonstrating potential to partially substitute malt.

Analysis were performed on proteins, total polyphenols, antioxidant activity, color and pH of worts with 2.5% and 10% straw proportions with xylanase (Table 2) and cellulase (Table 3). Worts with 2.5% had increased in parameters proteins, polyphenols and

antioxidant activity (Tables 2 and 3), however with xylanase there were no difference in fermentable sugar release compared to PM (Fig. 6A). This result showed that xylanase increase the bioactive compounds release that are bound to substrate, but does not provide relevant saccharification yields due to its action on hemicellulose, which is present in smaller amounts in barley straw cell wall.

Worts with 10% barley straw had increase in values of polyphenols, antioxidant activity, color and pH. Polyphenols increase may be related to the greater antioxidant activity due to this class of compounds has this characteristic that enables functional beers production with properties relevant to consumers health. Color can be important depending on beer style produced and consuming public preferences, verifying greater values in color using cellulose, capable of break cellulose chains that form cell wall fibers, weakening this structure and releasing more compounds that influence the worts color.[33] The pH decrease in worts with 10% barley straw malt replacement is important for a good fermentation and beer quality control, favoring yeast activity and avoiding unwanted microorganisms contamination. Despite the parameters obtained with 10% straw, this proportion caused a reduction on wort proteins compared to PM, which could influence a number of factors in beer production as impairing yeast nutrition during fermentation, sensory aspects of beer and even foam stability.[6]

Cellulase with 2.5% straw proportion (Table 3) maintains important characteristics of functional beer adjunct as protein concentration and increased antioxidant activity, besides this enzyme is capable of hydrolyze cellulose, which is the main substrate in this material and a fermentable sugars source. Analysis showed in Fig. 7 worts with higher reducing sugars release compared to PM when using enzyme proportion 1:2 (xylanase:cellulase). This is due to xylanase acting on hemicellulose, breaking the pentose bonds and releasing cellulose chains, being easily hydrolyzed by cellulase and producing fermentable sugars more efficiently. Wort with xylanase and cellulase mixture was produced considering this enzymes acts on different substrates and present a synergistic effect when they act together.[34]

Xylanase and cellulase mix in wort has potential comparing with cellulase, seeing that it showed differences in parameters of polyphenols, antioxidant activity, pH and color (Table 4). Even causing 10.65% average decrease on protein concentration compared to

PM, this reduction was smaller than wort with 10% straw with xylanase (Table 2), which obtained 39.61% average protein reduction. This lower protein reduction with xylanase and cellulase mix can provide regular beer production without affecting fermentation and foam retention. Using this enzymes mixture, it is possible to explore the release qualities of bioactive compounds by xylanase with the high fermentable sugars yield by cellulase, being able to extract greater amounts of important compounds from BSPA (Table 4).

3.3 Beer production with barley straw

Polyphenols and antioxidant activity behavior analysis was realized to understand beer functional value (Fig. 8). Worts in 2.5% BSPA proportion with enzymatic mixture and with only cellulase demonstrated potential in release of fermentable sugars and bioactive compounds, being these parameters the differential factors in worts selection for beer production and storage. Results showed that higher polyphenol levels (Fig. 8A) increase antioxidant activity (Fig. 8B and 8C) in beers, leading to better functional product quality. These functional compounds are usually bound to cell wall hemicellulose and are easily released when these structures are weak or distant from other structural carbohydrates (Table 2 and 4).[35] The lignin treatment during barley cultivation provided a more accessible straw for enzymatic activity, influencing the entire beer production process, promoting the fermentable sugars and bioactive compounds release that are able to remain stable during product storage.

4 Conclusions

Barley pretreatment in field with lignin inhibitor applied via soil or foliar made it possible to obtain a more accessible straw for enzymatic hydrolysis, without affecting grain yield at harvest. Best proportion of pretreated straw to partially replace malt in brewer's wort was 2.5% and the best enzymes concentration was 16 U mL^{-1}. Pretreated straws combined with cellulase or with 1:2 proportion of xylanase and cellulase mixture in wort were able to produce a beer with an increase in the release of fermentable sugars and bioactive compounds, adding functional value to this product.

Acknowledgments

The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) by scholarship, thanks the company INBEB (Indústrial Norte Paranaense de Bebidas) for supplying the malt and the owners of Fazenda Lontrão for the area provided for cultivation and treatment of barley in field.

References

1. Bamforth CW. Progress in brewing science and beer production. *Annu Rev Chem Biomol.* **8**:161-176 (2017).
2. Steiner E, Gastl M and Becker T. Protein changes during malting and brewing with focus on haze and foam formation: a review. *Eur Food Res Technol* **232**:191-204 (2011).
3. Habschied K, Živković A, Krstanović V and Mastanjević K. Functional beer—A review on possibilities. *Beverages* **6**:51 (2020).
4. Buiatti S, Bertoli S & Passaghe P. Influence of gluten-free adjuncts on beer colloidal stability. *Eur Food Res Technol* **244**:903-912 (2018).
5. Callemien D, Collin S. Structure, organoleptic properties, quantification methods, and stability of phenolic compounds in beer—A review. *Food Rev Int* **26**:1-84 (2009).
6. Bogdan P, Kordialik-Bogacka E. Alternatives to malt in brewing. *Trends Food Sci Tech* **65**:1-9 (2017).
7. Duque A, Manzanares P, Ballesteros I, Negro MJ, Oliva JM, González A and Ballesteros M. Sugar production from barley straw biomass pretreated by combined alkali and enzymatic extrusion. *Bioresource technol* **158**:262-268 (2014).
8. Sun RC, Sun XF and Zhang SH. Quantitative determination of hydroxycinnamic acids in wheat, rice, rye, and barley straws, maize stems, oil palm frond fiber, and fast-growing poplar wood. *J Agr Food Chem* **49**:5122-5129 (2001).
9. Szwajgier D, Pielecki J and Targoński Z. The release of ferulic acid and feruloylated oligosaccharides during wort and beer production. *J I Brewing* **111**:372-379 (2005).
10. Han M, Kang KE, Kim Y and Choi GW. High efficiency bioethanol production from barley straw using a continuous pretreatment reactor. *Process Biochem* **48**:488-495 (2013).
11. Lara-Serrano M, Sáez Angulo F, Negro MJ, Morales-delaRosa S, Campos-Martin JM and Fierro JL. Second-generation bioethanol production combining simultaneous fermentation and saccharification of IL-pretreated barley straw. *ACS Sustain Chem Eng* **6**:7086-7095 (2018).

12. Buckeridge MS, Dos Santos WD and De Souza AP. Routes for cellulosic ethanol in Brazil. *Sugarcane Bioethanol: R&D for Productivity and Sustainability*, ed. by Cortez LAB. Blucher, São Paulo, pp. 365-380 (2010).
13. Sharma HK, Xu C and Qin W. Biological pretreatment of lignocellulosic biomass for biofuels and bioproducts: an overview. *Waste Biomass Valori* **10**:235-251 (2019).
14. Ferro AP, Marchiosi R, Siqueira-Soares RC, Bonini EA, Ferrarese MDLL and Ferrarese-Filho O. Effects of cinnamic and ferulic acids on growth and lignification of maize roots. *J Allel Int* **1**:29-38 (2015).
15. Poreda A, Czarnik A, Zdaniewicz M, Jakubowski M and Antkiewicz P. Corn grist adjunct–application and influence on the brewing process and beer quality. *J I Brewing* **120**:77-81 (2014).
16. Bortoletto-Santos R and Ribeiro C. Study of the controlled release of ametryn herbicide using PVA: activated carbon composites. *Quim Nova* **37**:1118-1121 (2014).
17. Miller GL. Modified DNS method for reducing sugars. *Anal Chem* **31**:426-428 (1959).
18. Thiex N. Evaluation of analytical methods for the determination of moisture, crude protein, crude fat, and crude fiber in distillers dried grains with solubles. *J AOAC Int* **92**:61-73 (2009).
19. Bligh EG and Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Phys* **37**:911-917 (1959).
20. Holland B, McCance RA, Widdowson EM, Unwin ID and Buss DH (eds). *Vegetables, herbs and spices: Fifth supplement to McCance and Widdowson's The Composition of Foods*, 5th edn. Royal Society of Chemistry, London (1991).
21. Su G, An Z, Zhang W and Liu Y. Light promotes the synthesis of lignin through the production of H2O2 mediated by diamine oxidases in soybean hypocotyls. *J Plant Physiol* **162**:1297-1303 (2005).
22. Shellhammer TH and Bamforth CW, Assessing color quality of beer, in *Color Quality of Fresh and Processed Foods*, ed. Culver CA and Wrolstad RE. American Chemical Society Publications, Washington, pp 192-202 (2008).
23. Kruger NJ. The Bradford method for protein quantitation, in *The protein protocols handbook*, ed. by Walker JM. Humana Press, New Jersey, pp. 17-24 (2009).
24. Singleton VL and Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Viticult* **16**:144-158 (1965).

25. Li DL, Li XM and Wang BG. Natural anthraquinone derivatives from a marine mangrove plant-derived endophytic fungus Eurotium rubrum: structural elucidation and DPPH radical scavenging activity. *J Microbiol Biotechn* **19**:675-680 (2009).
26. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Bio Med* **26**:1231-1237 (1999).
27. Bubna GA, Lima RB, Zanardo DYL, Dos Santos WD, Ferrarese MDLL and Ferrarese-Filho O. Exogenous caffeic acid inhibits the growth and enhances the lignification of the roots of soybean (Glycine max). *J Plant Physiol* **168**:1627-1633 (2011).
28. Sheikh MMI, Kim CH, Park HH, Nam HG, Lee GS, Jo HS, Lee JY and Kim JW. A synergistic effect of pretreatment on cell wall structural changes in barley straw (Hordeum vulgare L.) for efficient bioethanol production. *J Sci Food Agr* **95:**843-850 (2015).
29. Gholizadeh H, Naserian AA, Xin H, Valizadeh R, Tahmasbi AM and Yu P. Detecting carbohydrate molecular structural makeup in different types of cereal grains and different cultivars within each type of grain grown in semi-arid area using FTIR spectroscopy with uni-and multi-variate molecular spectral analyses. *Anim Feed Sci Tech* **194**:136-144 (2014).
30. Hsu TC, Guo GL, Chen WH and Hwang WS. Effect of dilute acid pretreatment of rice straw on structural properties and enzymatic hydrolysis. *Bioresource technol* **101**:4907-4913 (2010).
31. Largo-Gosens A, Hernández-Altamirano M, García-Calvo L, Alonso-Simón A, Álvarez J and Acebes JL. Fourier transform mid infrared spectroscopy applications for monitoring the structural plasticity of plant cell walls. *Front Plant Sci* **5**:303 (2014).
32. Feng X, Sun B and Yu P. Using vibrational molecular spectroscopy to detect moist heating induced carbohydrates structure changes in cool-climate adapted barley grain. *J Cereal Sci* **95**:103007 (2020).
33. Koren D, Hegyesné Vecseri B, Kun-Farkas G, Urbin Á, Nyitrai Á and Sipos L. How to objectively determine the color of beer?. *J Food Sci Tech* **57**:1183-1189 (2020).
34. García-Aparicio MP, Ballesteros M, Manzanares P, Ballesteros I, González A and Negro MJ. Xylanase contribution to the efficiency of cellulose enzymatic hydrolysis of barley straw, in *Applied biochemistry and biotecnology*, ed. by Sachs J. Humana Press, New Jersey, pp. 353-365 (2007).
35. Ruthes AC, Rudjito RC, Rencoret J, Gutiérrez A, del Río JC, Jiménez-Quero A and Vilaplana F. Comparative recalcitrance and extractability of cell wall polysaccharides from

cereal (wheat, rye, and barley) brans using subcritical water. *ACS Sustain Chem Eng* **8**:7192-7204 (2020).

Figure Legends

Fig. 1: Barley crop productivity pretreated with piperonylic acid application. CO – Control; SA – Soil Application; FA – Foliar Application. Different letters above the graphics indicate a significant difference ($p \leq 0.05$).

Fig. 2: Lignin quantification and enzymatic saccharification analysis of barley straw pretreated with piperonylic acid (BSPA). A) Lignin quantification; B) Hydrolysis with xylanase for 4 hours; C) Hydrolysis with cellulase for 4 hours. CO – Control; SA – Soil Application; FA – Foliar Application. Different letters above the graphics indicate a significant difference ($p \leq 0.05$).

Fig. 3: FTIR-ATR spectra (A) and compounds semi-quantification (B) of barley straw pretreated with piperonylic acid. CO – Control; SA – Soil Application; FA – Foliar Application. Different letters above the graphics indicate a significant difference ($p \leq 0.05$).

Fig. 4: Scanning electron microscopy (SEM) images of barley straw pretreated with piperonylic acid (BSPA). First column is straw external surface; second column is straw internal surface; third column is cross section of straw. A - C: Control without piperonylic acid; D - F: Soil application; G - I: Foliar application.

Fig. 5: Reduce sugars in worts with barley straw malt replacement (2.5%, 5% and 10%) and enzymes concentrations.

Fig. 6: Fermentable sugars in worts with barley straw malt replacement and 16 U mL-1 enzymes concentration. A) Wort with 2.5% straw and xylanase; B) Wort with 2.5% straw and cellulase; C) Wort with 10% straw and xylanase; D) Wort with 10% straw and cellulase. PM – Pure Malt; CO – Control; SA – Soil Application; FA – Foliar Application. Different letters above the graphics indicate a significant difference ($p \leq 0.05$).

Fig. 7: Reducing sugar of worts with 2.5% barley straw and xylanase and cellulase mixture. A) Wort with ratio 1:1 or 1:2 xylanase and cellulase; B) Worts with 1:2 ratio of xylanase and cellulase. PM – Pure Malt without enzymes; CO – Control; SA – Soil Application; FA – Foliar Application. Different letters above the graphics indicate a significant difference ($p \leq 0.05$).

Fig. 8: Polyphenols (A) and antioxidant activity (B and C) analysis of beer produced with 2.5% barley straw and storage during 28 days at 4 °C. CO1 – Control straw with cellulase; CO2 – Control straw with 1:2 (xylanase:cellulase); SA1 – Soil Application straw with cellulase; SA2 – Soil Application straw with 1:2 (xylanase:cellulase); FA1 – Foliar Application straw with cellulase; FA2 – Foliar Application straw with 1:2 (xylanase:cellulase); PM – Pure Malt without enzymes.

Figure 1

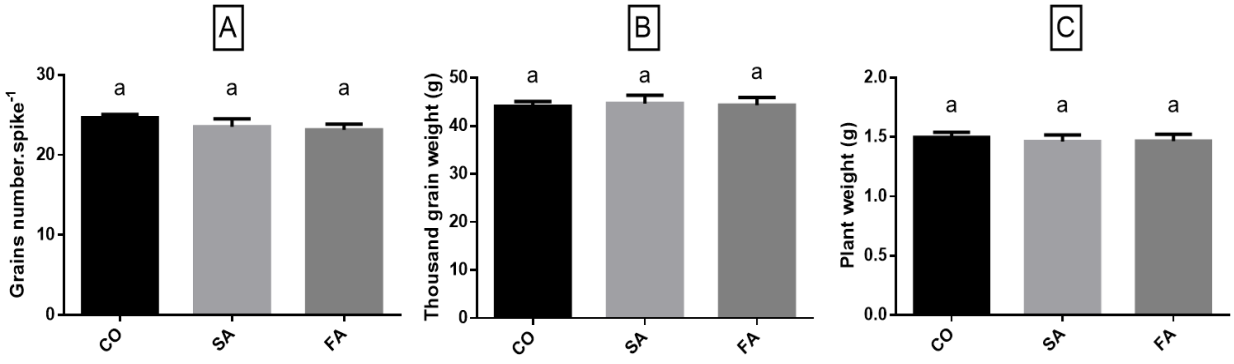
A
B
C
Grains number.spike-1
Thousand grain weight (g)
Plant weight (g)
a
a
a
CO
SA
FA
0
10
20
30
40
50
0.0
0.5
1.0
1.5
2.0

Figure 2

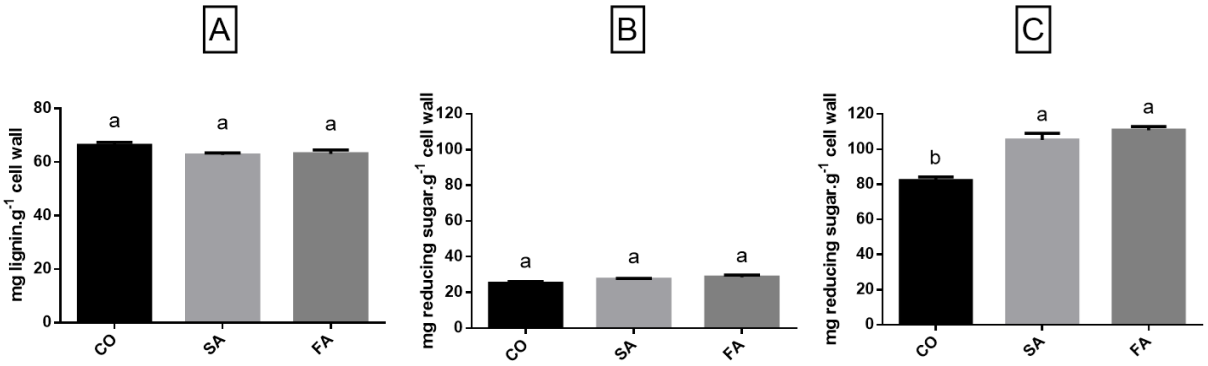
A
B
C
mg lignin.g-1 cell wall
mg reducing sugar.g-1 cell wall
mg reducing sugar.g-1 cell wall
0
20
40
60
80
0
20
40
60
80
100
120
0
20
40
60
80
100
120
a
a
a
a
a
a
b
a
a
CO
SA
FA
CO
SA
FA
CO
SA
FA

Figure 3

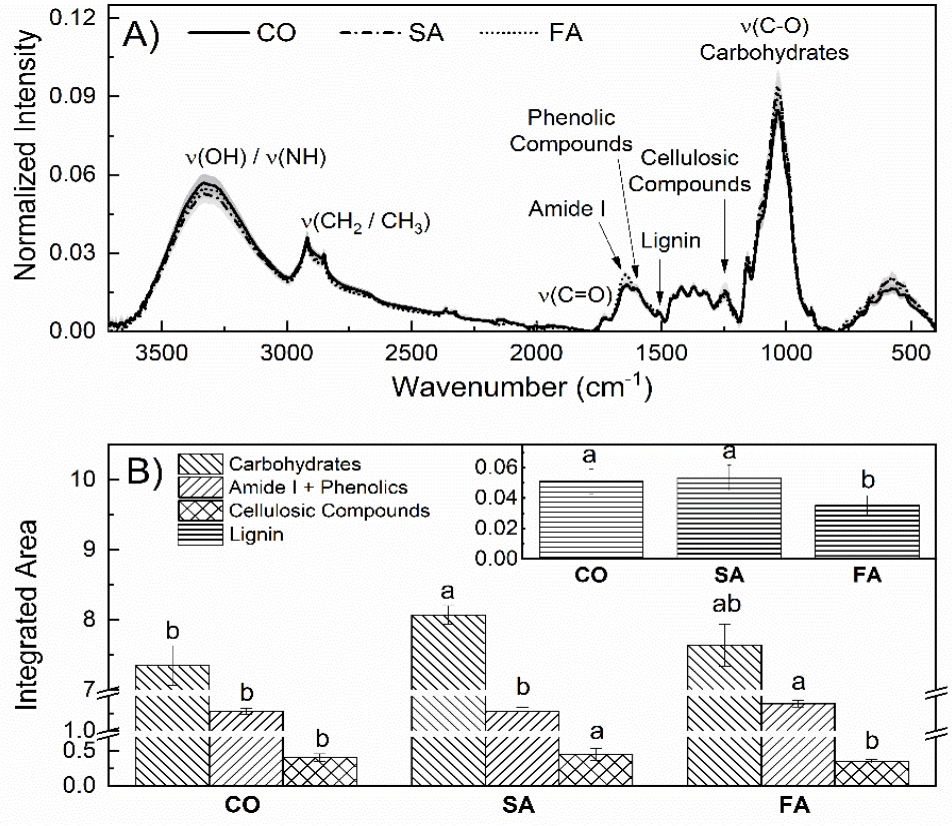
A)
CO
SA
FA
Normalized Intensity
Wavenumber (cm-1)
ν(OH) / ν(NH)
ν(CH2 / CH3)
ν(C=O)
Amide I
Phenolic Compounds
Lignin
Cellulosic Compounds
ν(C-O) Carbohydrates
B)
Carbohydrates
Amide I + Phenolics
Cellulosic Compounds
Lignin
Integrated Area
CO
SA
FA

Figure 4

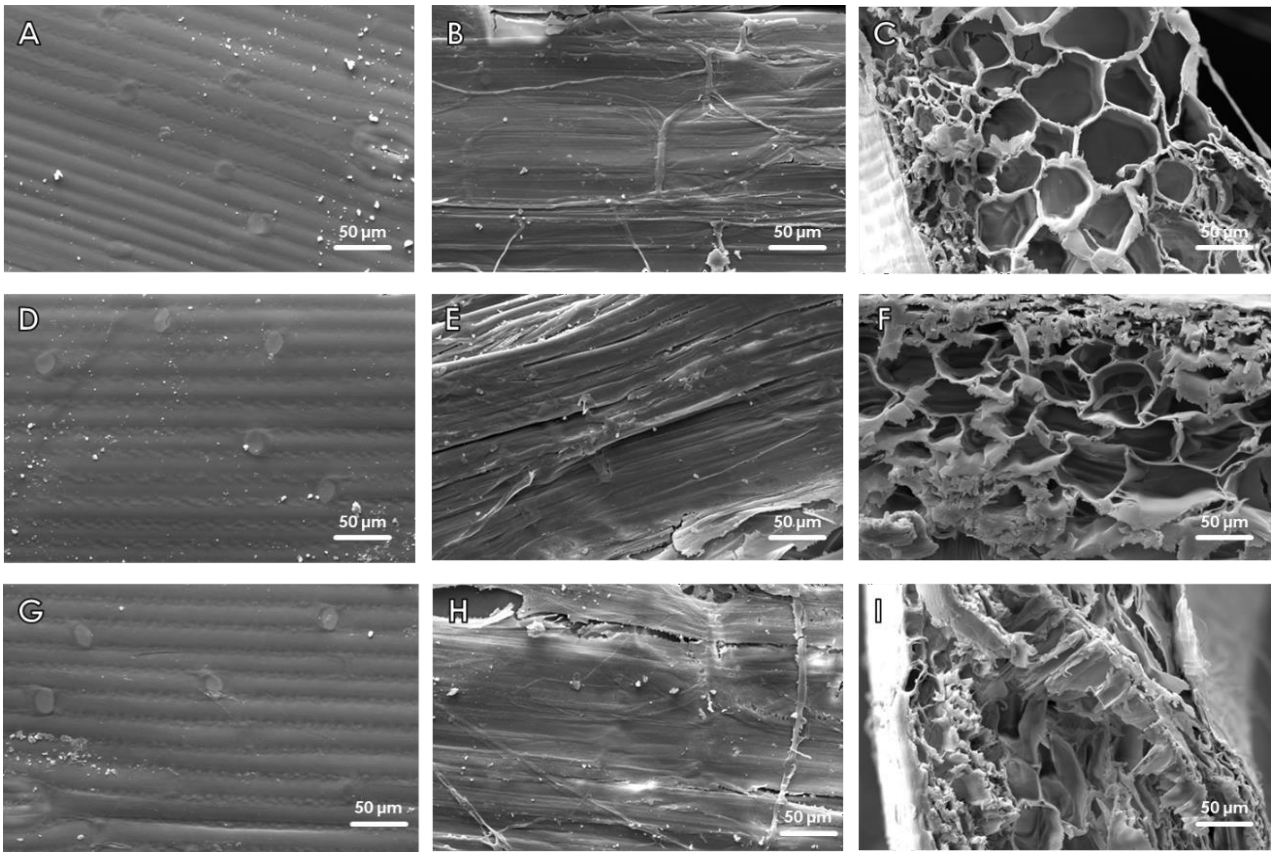

Figure 5

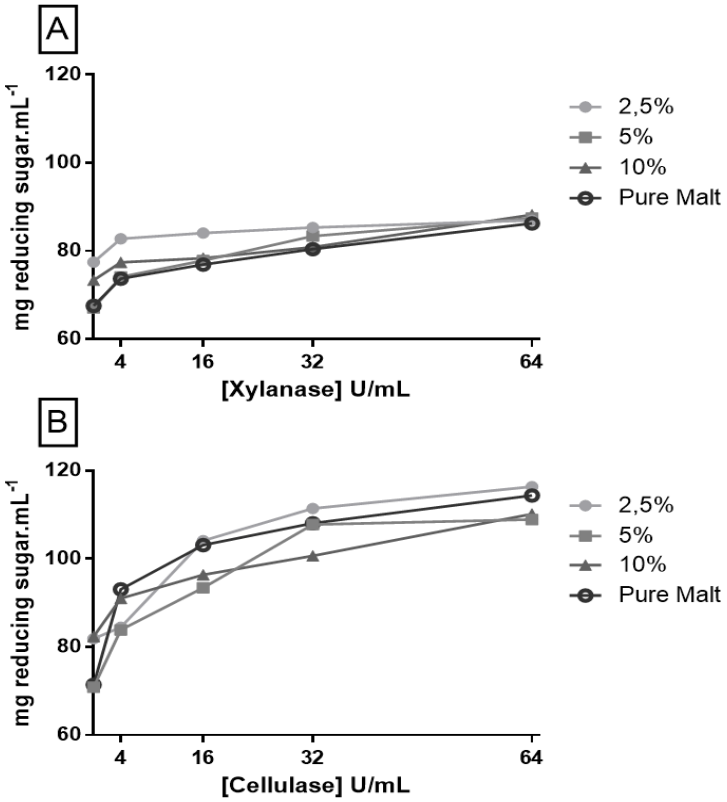
A
120
100
80
60
mg reducing sugar.mL-1
4
16
32
64
[Xylanase] U/mL
2,5%
5%
10%
Pure Malt
B
120
100
80
60
mg reducing sugar.mL-1
4
16
32
64
[Cellulase] U/mL
2,5%
5%
10%
Pure Malt

Figure 6

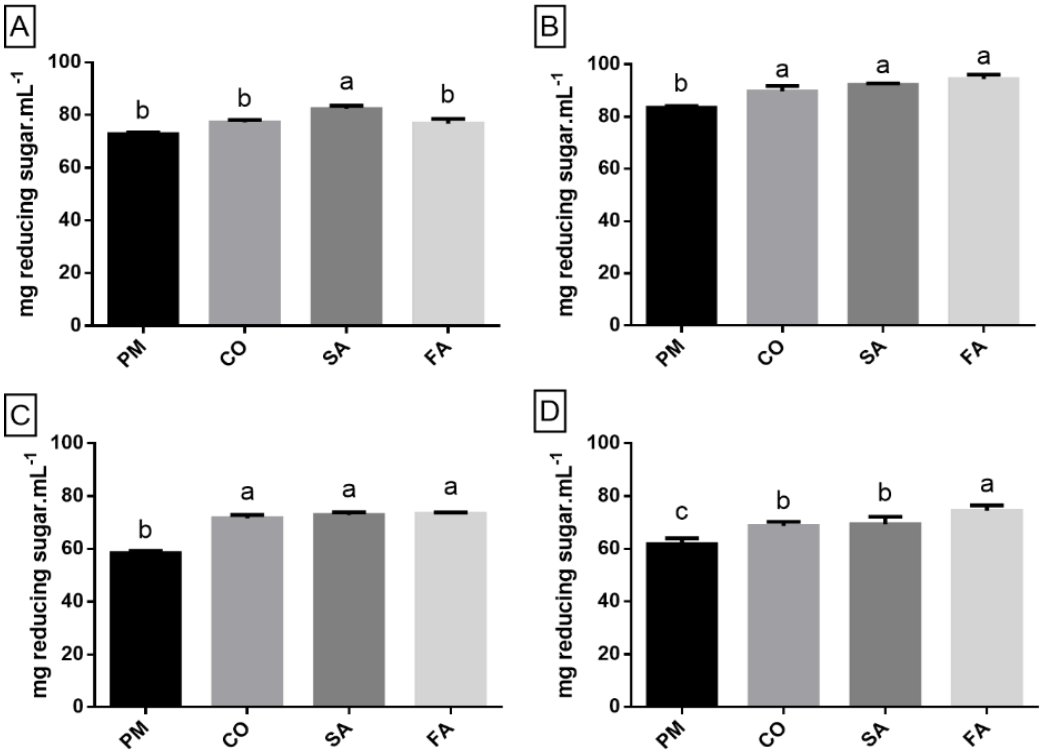
A
B
C
D
mg reducing sugar.mL-1
0
20
40
60
80
100
PM
CO
SA
FA
b
b
a
b
b
a
a
a
b
a
a
a
c
b
b
a

Figure 7

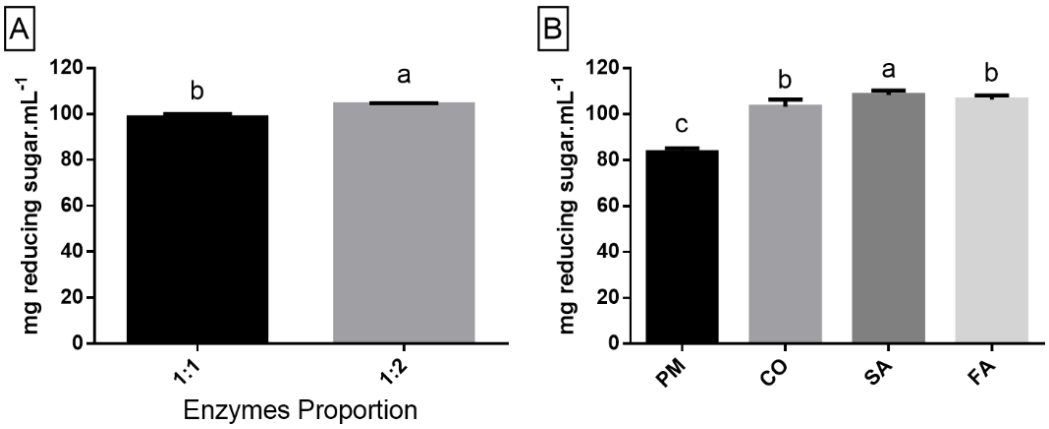
A
B
mg reducing sugar.mL-1
120
100
80
60
40
20
0
b
a
1:1
1:2
Enzymes Proportion
c
b
a
b
PM
CO
SA
FA

Figure 8

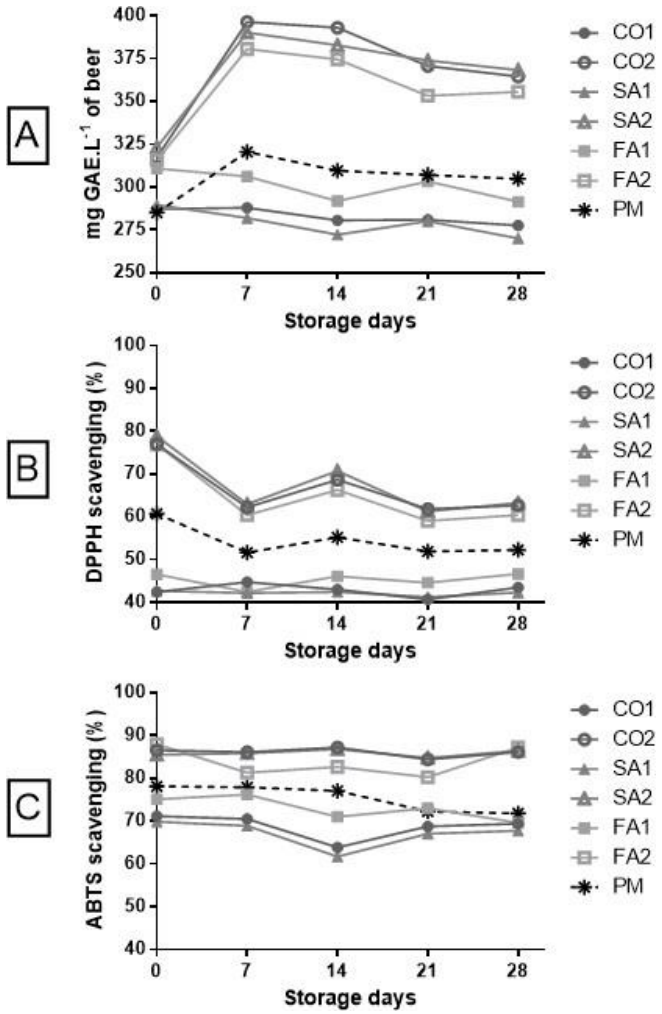
A
mg GAE.L-1 of beer
400
375
350
325
300
275
250
0
7
14
21
28
Storage days
CO1
CO2
SA1
SA2
FA1
FA2
PM
B
DPPH scavenging (%)
100
90
80
70
60
50
40
0
7
14
21
28
Storage days
CO1
CO2
SA1
SA2
FA1
FA2
PM
C
ABTS scavenging (%)
100
90
80
70
60
50
40
0
7
14
21
28
Storage days
CO1
CO2
SA1
SA2
FA1
FA2
PM

Table 1. Chemical composition of barley straw pretreated with piperonylic acid in field.

Treatment	Moisture	Ash	Brute fiber	Protein	Carbohydrates
CO	13,66 ± 0,03^{a}	6,007 ± 0,152^{a}	33,15 ± 1,104^{ab}	3,289 ± 0,039^{a}	43,89 ± 0,78^{b}
SA	12,10 ± 0,04^{b}	5,280 ± 0,025^{b}	32,61 ± 0,141^{b}	2,825 ± 0,061^{b}	47,18 ± 0,08^{a}
FA	11,98 ± 0,08^{b}	5,290 ± 0,020^{b}	34,24 ± 0,306^{a}	2,512 ± 0,037^{b}	45,97 ± 0,23^{b}

Results expressed as mean ± standard deviation. Different letters in columns represent significant differences ($p \leq 0.05$). CO – Control; SA – Soil Application; FA – Foliar Application.

Table 2. Beer worts parameters produced with xylanase (16 U mL^{-1}) and 2.5% or 10% of malt replaced by barley straw pretreated with piperonylic acid.

Treatment	Proteins (mg mL^{-1})	TPC (mg GAE L^{-1})	DPPH (%)	ABTS (%)	Color (EBC)	pH
CO-2.5%	0,939 ± 0,025^{a}	418,0 ± 1,0^{a}	47,69 ± 1,86^{bc}	79,91 ± 0,99^{a}	20,20 ± 0,45^{b}	5,50 ± 0,01^{ab}
SA-2.5%	1,002 ± 0,030^{a}	411,3 ± 5,0^{a}	50,20 ± 2,18^{b}	72,67 ± 3,61^{ab}	22,10 ± 0,20^{a}	5,48 ± 0,01^{b}
FA-2.5%	0,952 ± 0,026^{a}	417,6 ± 3,4^{a}	48,52 ± 3,85^{bc}	78,20 ± 1,30^{a}	21,88 ± 0,40^{a}	5,53 ± 0,01^{a}
CO-10%	0,566 ± 0,018^{b}	427,5 ± 0,3^{a}	57,86 ± 0,86^{a}	77,35 ± 3,13^{a}	18,20 ± 0,20^{c}	4,98 ± 0,01^{c}
SA-10%	0,546 ± 0,013^{b}	421,9 ± 0,9^{a}	53,94 ± 1,25^{ab}	75,65 ± 0,95^{a}	17,92 ± 0,47^{c}	4,96 ± 0,01^{c}
FA-10%	0,589 ± 0,021^{b}	427,6 ± 2,0^{a}	52,93 ± 0,85^{b}	75,65 ± 0,45^{a}	17,12 ± 0,49^{c}	4,97 ± 0,01^{c}
PM	0,939 ± 0,008^{a}	381,6 ± 6,6^{b}	43,68 ± 0,79^{c}	65,20 ± 2,54^{b}	19,56 ± 0,08^{b}	5,49 ± 0,01^{ab}

Results expressed as mean ± standard deviation. Different letters in columns represent significant differences ($p \leq 0.05$). CO – Control straw; SA– Soil Application straw; FA – Foliar Application straw; PM – Pure Malt without enzymes. TPC: Total Polyphenol Content; GAE: Gallic Acid Equivalent; EBC: European Brewery Convention.

Table 3. Beer worts parameters produced with cellulase (16 U mL^{-1}) and 2.5% or 10% of malt replaced by barley straw pretreated with piperonylic acid.

Treatment	Proteins (mg mL^{-1})	TPC (mgGAE L^{-1})	DPPH (%)	ABTS (%)	Color (EBC)	pH
CO-2.5%	1,128 ± 0,002^{a}	367,9 ± 1,5^{b}	53,64 ± 0,72^{a}	68,79 ± 1,85^{a}	25,22 ± 0,10^{a}	5,37 ± 0,01^{c}
SA-2.5%	1,006 ± 0,013^{a}	393,7 ± 5,1^{a}	55,22 ± 1,02^{a}	70,83 ± 2,07^{a}	23,69 ± 0,09^{a}	5,44 ± 0,01^{b}
FA-2.5%	1,115 ± 0,023^{a}	373,0 ± 2,6^{ab}	50,55 ± 1,33^{a}	68,56 ± 0,85^{a}	25,71 ± 0,28^{a}	5,43 ± 0,01^{b}
CO-10%	0,768 ± 0,012^{c}	378,9 ± 4,9^{ab}	50,73 ± 1,54^{a}	70,21 ± 0,29^{a}	25,19 ± 0,35^{a}	4,96 ± 0,01^{d}
SA-10%	0,756 ± 0,009^{c}	372,0 ± 6,2^{ab}	38,62 ± 2,06^{bc}	60,33 ± 1,48^{bc}	23,98 ± 0,77^{a}	4,92 ± 0,01^{d}
FA-10%	0,744 ± 0,014^{c}	367,2 ± 6,2^{b}	37,78 ± 0,30^{c}	59,48 ± 1,30^{c}	23,78 ± 0,76^{a}	4,93 ± 0,01^{d}
PM	0,939 ± 0,008^{b}	381,6 ± 6,6^{ab}	43,68 ± 0,79^{b}	65,20 ± 2,54^{ab}	19,56 ± 0,08^{b}	5,49 ± 0,01^{a}

Results expressed as mean ± standard deviation. Different letters in columns represent significant differences ($p \leq 0.05$). CO – Control straw; SA– Soil Application straw; FA – Foliar Application straw; PM – Pure Malt without enzymes. TPC: Total Polyphenol Content; GAE: Gallic Acid Equivalent; EBC: European Brewery Convention.

Table 4. Beer worts parameters produced with xylanase and cellulase (1:2; 16 U mL^{-1}) and 2.5% malt replaced by barley straw pretreated with piperonylic acid.

Treatment	Proteins (mg mL^{-1})	TPC (mg GAE L^{-1})	DPPH (%)	ABTS (%)	Color (EBC)	pH
CO	$0,818 \pm 0,011^{b}$	$414,3 \pm 4,3^{a}$	$66,71 \pm 2,64^{a}$	$80,86 \pm 0,37^{b}$	$17,21 \pm 0,08^{c}$	$5,38 \pm 0,01^{b}$
SA	$0,848 \pm 0,019^{b}$	$421,7 \pm 3,3^{a}$	$67,41 \pm 0,80^{a}$	$81,83 \pm 1,13^{ab}$	$18,38 \pm 0,31^{b}$	$5,38 \pm 0,01^{b}$
FA	$0,851 \pm 0,005^{b}$	$420,9 \pm 6,9^{a}$	$66,20 \pm 1,27^{a}$	$84,93 \pm 1,21^{a}$	$19,45 \pm 0,39^{ab}$	$5,40 \pm 0,02^{b}$
PM	$0,939 \pm 0,008^{a}$	$381,6 \pm 6,6^{b}$	$43,68 \pm 0,79^{b}$	$65,20 \pm 2,54^{c}$	$19,56 \pm 0,08^{a}$	$5,49 \pm 0,01^{a}$

Results expressed as mean ± standard deviation. Different letters in columns represent significant differences ($p \leq 0.05$). CO – Control straw; SA– Soil Application straw; FA – Foliar Application straw; PM – Pure Malt without enzymes. TPC: Total Polyphenol Content; GAE: Gallic Acid Equivalent; EBC: European Brewery Convention.