Enzymatic pretreatment with laccases from *Lentinus sajor-caju* induces structural modification in lignin and enhances the digestibility of tropical forage grass (*Panicum maximum*) grown under future climate conditions

Emanuelle Neiverth de Freitas[1], Robson Carlos Alnoch[2], Alex Graça Contato[1], Karoline Maria V. Nogueira[1], Eduardo José Crevelin[3], Luiz Alberto B. de Moraes[3], Roberto Nascimento Silva[1], Carlos Alberto Martínez[2], and Maria de Lourdes T. M. Polizeli[1,2]

[1]Departamento de Bioquímica e Imunologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto 14049-900, Brazil; emanuelleneiverthf@gmail.com (E.N.F.); alexgraca.contato@gmail.com (A.G.C.); karolmvnogueira@gmail.com (K.M.V.N); rsilvausp@gmail.com (R.N.S.)

[2] Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto 14050-901, Brazil; robsonalnoch@usp.br (R.C.A.); carlosamh@ffclrp.usp.br (C.A.M.); polizeli@ffclrp.usp.br (M.d.L.T.M.P)

[3] Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto 14050-901, Brazil; ejcrevelin@ffclrp.usp.br (E.J.C); luizmoraes@ffclrp.usp.br (L.A.B.M)

Corresponding author: polizeli@ffclrp.usp.br (M.d.L.T.M.P)

Table S1. Enzymatic activities of crude extract of *Lentinus sajor- caju* CCB 020 cultivated in orange waste for 14 days.

Enzymes	Activities (mU/mL)
Laccase	22,516 ± 627
Manganese peroxidase	178 ± 32
Endo-1,4-xylanase	2,970 ± 300
Endoglucanase	80 ± 20
Mannanase	2,050 ± 56
endo-1,5-α-L-arabinanase	180 ± 10
β-glucanase	130 ± 12
β-D-xylosidase	6.6 ± 0.3
β-D-galactosidase	9.8 ± 0.6
β-D-glucosidase	0.92 ± 0.05
α-L-arabinofuranosidase	1.33 ± 0.4
Cellobiohydrolase	8.5 ± 0.3

Substrates for enzymatic activity: 3-ethylbenzothiazoline-6-sulphonic acid- ABTS (Laccase), 2,6-dimethoxyphenol DMP (Manganese Peroxidase), xylan beechwood (Endo-1,4-xylanase), carboxymethylcellulose—CMC (Endoglucanase), locust bean (Mannanase), Debranched arabinan (Endo-1,5-α-L-arabinanase), β-glucan (β-glucanase), p-nitrophenyl-β-D-xylanopyranoside (β-D-xylosidase), p-nitrophenyl-β-D-galactopyranoside (β-D-galactosidase), p-nitrophenyl-β-D-glycopyranoside (β-D-glucosidase), p-nitrophenyl-α-L-arabinofuranoside (α-L-arabinofuranosidase), and p-nitrophenyl-β-D-cellobioside (Cellobiohydrolase).

Table S2. The amino acid sequence of the putative laccase from *Lentinus sajor-caju*. The peptides corresponding to those identified by mass spectrometry are shown in bold. The mass profiles obtained were used in the TPP server platform (Uniprot database) with the XTandem peptide search engine and ProteinProphet.

>tr|Q9HFT4|Q9HFT4_PLESA Laccase 4 (Fragment) OS= *Lentinus (Pleurotus) sajor-caju* OX=50053 GN=Lac4 PE=2 SV=1

*MFPGARILATLTLALHLLHGTHAAIGPAGN**MYIVNEDVSPDSFARSAVVAR**SVPATDPTP
ATASIPGVLVQGNKGDNFQLNVVNQLSDTTMLRTTSIHWHGFFQAGSSWADGPAFVTQCP
VASGDSFLYNFNVPDQAGTFWYHSHLSTQYCDGLRGPFVVYDPSDPHLSLYDIDNADTVI
TLEDWYHIVAPQNAAIPTPDSTLINGKGRYAGGPTSPLAIINVESNKRYRFRLVSMSCDP
NFTFSIDGHSLLVIEADAVNIVPITVDSIQIFAGQRYSFVLTANQAVDKYWIRANPNLGS
T**GFVGGINSAILRYAGATEDD**PTTTSSTSTPLLETNLVPLENPGAPGPPVPGGADININL
AMAFDFTTFELTINGVPFLPPTAPVLLQILSGASTAASLLPSGSIYELEANKVVEISMPA
LAVGGPHPFHLHGHTFDVIRSAGSTTYNFDTPARRDVVNTGTGAN

Table S3: Analysis of variance (ANOVA) for Central Composite Design (CCD) for sugar yields (g L⁻¹) of laccase pretreated biomass of *P. maximum* control group.

Source of variation	Degree of Freedom (DF)	Sum Square (SS)	Mean Square (MS)	F_{test} $F_{calculated}$	F_{table}
Regression	6	16.22	2.70	10.24	3.09[a]
Residue	11	2.90	0.26		
Lack of fit	8	2.62	0.33	3.48	8.85[b]
Pure error	3	0.28	0.09		
Total	17	19.12			

[a]F 95% = 6;11 ; [b]F 95% = 8;3

Table S4: Analysis of variance (ANOVA) for Central Composite Design (CCD) for sugar yields (g L^{-1}) of laccase pretreated biomass of *P. maximum* eT+eC group.

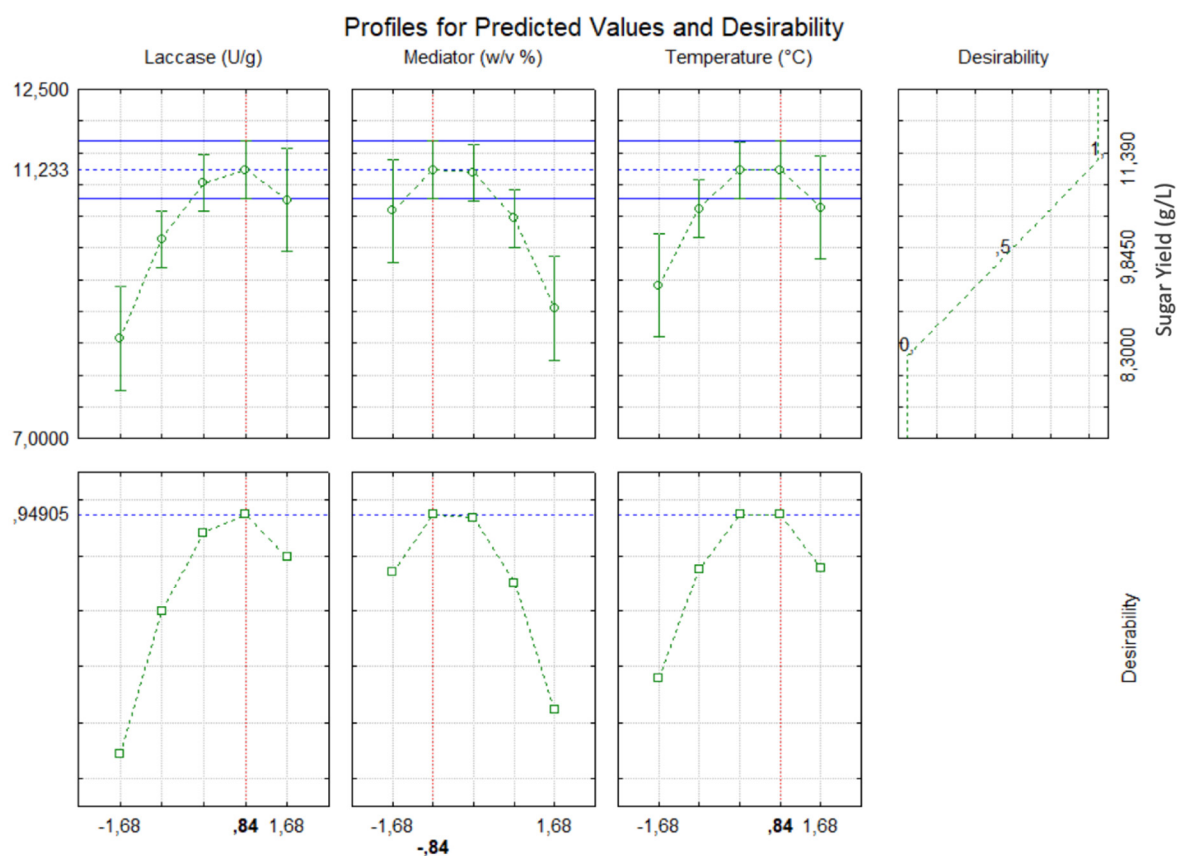| Source of variation	Degree of Freedom (DF)	Sum Square (SS)	Mean Square (MS)	$F_{calculated}$	F_{table}
Regression	7	24.89	3.56	**20.60**	**3.14**[a]
Residue	10	1.73	0.17		
Lack of fit	7	1.61	0.23	**6.17**	**8. 89**[b]
Pure error	3	0.11	0.04		
Total	17	26.1			

[a]F 95% = 7;10 ; [b]F 95% = 7;3

Figure S1. Desirability profile of *P. maximum* control (C) group for Sugar Yield (g L^{-1}). Laccase, mediator, and temperature were the independent variables of CCD 2^3.
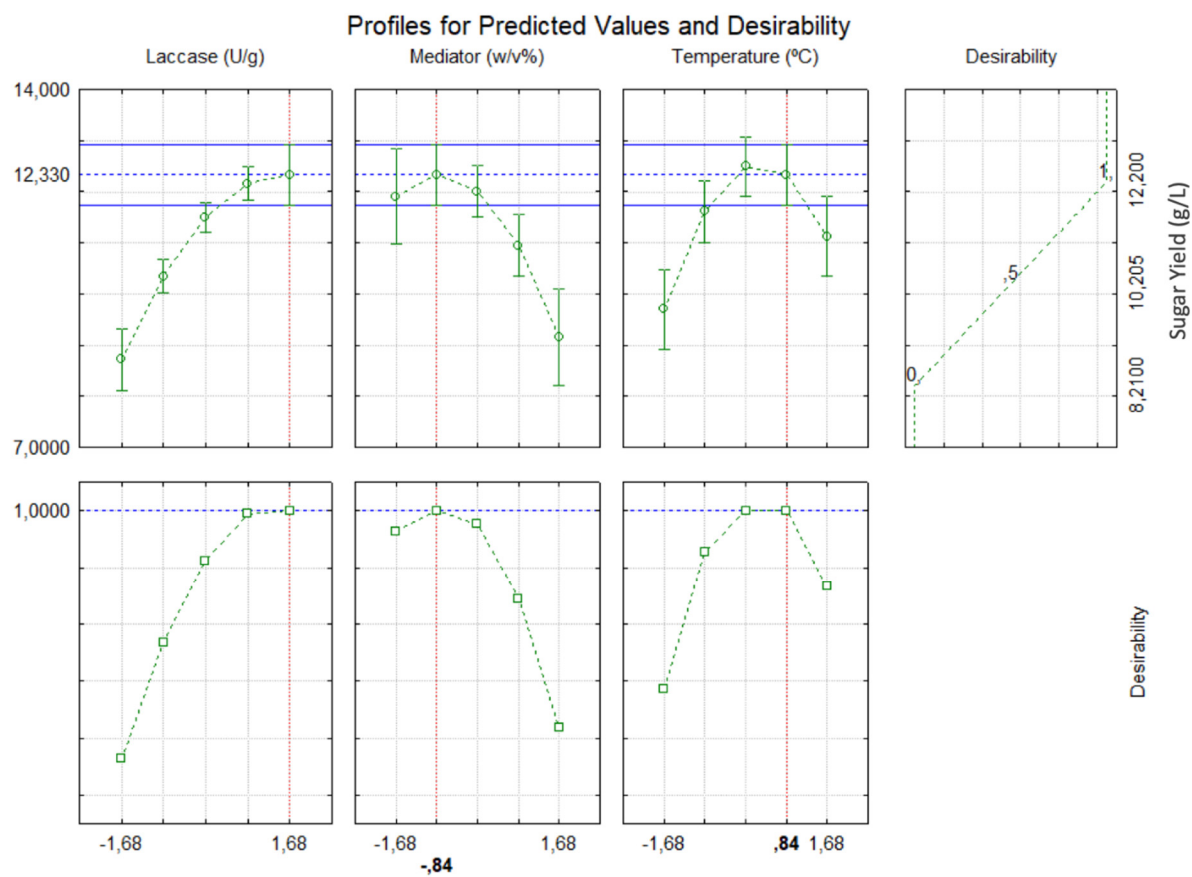
Figure S2. Desirability profile of *P. maximum* elevated temperature+elevated CO_2 (eT+eC) group for Sugar Yield (g L^{-1}). Laccase, mediator, and temperature were the independent variables of CCD 2^3.
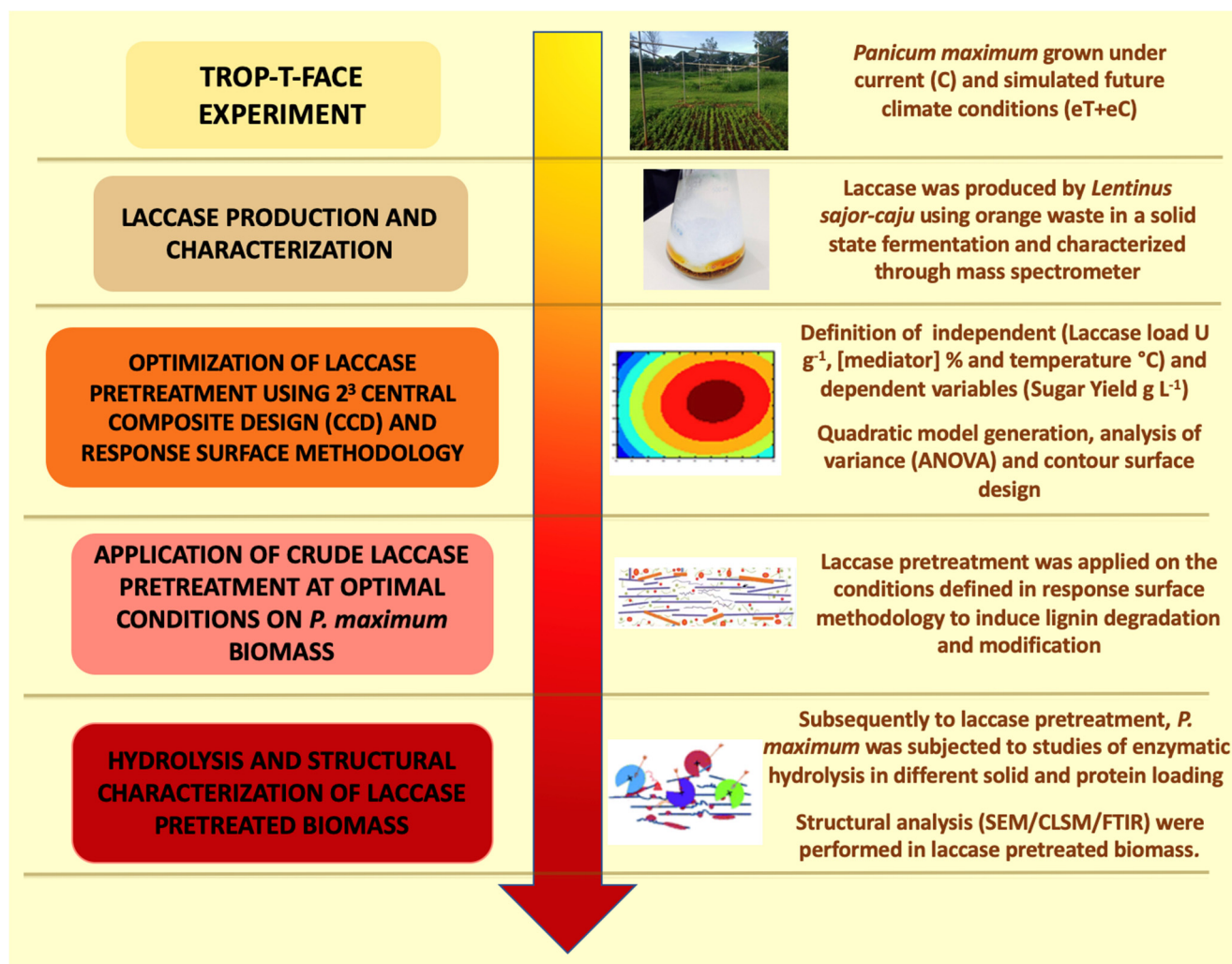
Figure S3. Flow chart of the experimental design with the manly steps performed in the study.