About OMICS Group

OMICS Group International is an amalgamation of Open Access publications and worldwide international science conferences and events. Established in the year 2007 with the sole aim of making the information on Sciences and technology 'Open Access', OMICS Group publishes 500 online open access scholarly journals in all aspects of Science, Engineering, Management and Technology journals. OMICS Group has been instrumental in taking the knowledge on Science & technology to the doorsteps of ordinary men and women. Research Scholars, Students, Libraries, Educational Institutions, Research centers and the industry are main stakeholders that benefitted greatly from this knowledge dissemination. OMICS International also organizes 500 International conferences annually across the globe, where knowledge transfer takes place through debates, round table discussions, poster presentations, workshops, symposia and exhibitions.

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OMICS International is a pioneer and leading science event organizer, which publishes around 500 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS International has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

Brazilian Agricultural Research Corporation – EMBRAPA
National Research and Development Center in Agroenergy - EMBRAPA Agroenergy
4th International Conference and Exhibition on Metabolomics & Systems Biology

Metabolomics of xylose-fermenting yeast for increasing efficiency in the production of second generation ethanol

Dr. Patrícia Verardi Abdelnur
Researcher at Laboratory of Biomass and Biofuel Chemistry
Embrapa Agroenergy

EMBRAPA: BRAZILIAN AGRICULTURAL RESEARCH CORPORATION

Embrapa Agroenergia

MISSION

"To provide feasible solutions for the sustainable development of Brazilian agribusiness through knowledge and technology generation and transfer."

INSTITUTIONAL PROFILE

- Created in **1973**
- **9.795** employees, **2.427** researchers, **74%** PhDs
- **47** RD&I and Service Centers
- **International Agenda**: Americas, Europe, Asia and Africa

Scientific Cooperation

- ***Labex United States:***.....USA
- ***Labex Europe:***..... France, UK, Germany
- ***Labex Korea:***..... South Korea
- ***Labex China:***..... China

Technical Cooperation

- ***Embrapa Africa :*** TT Projects
- ***Embrapa Americas :*** TT Projects

Source: Social Balance 2010 – Embrapa.

EMBRAPA AGROENERGY: National Research and Development Center in Agroenergy

Embrapa Agroenergia

Brazilian Agroenergy Plan 2006 – 2011

To produce and transfer knowledge and technology that contribute to the sustainable production of energy from agriculture

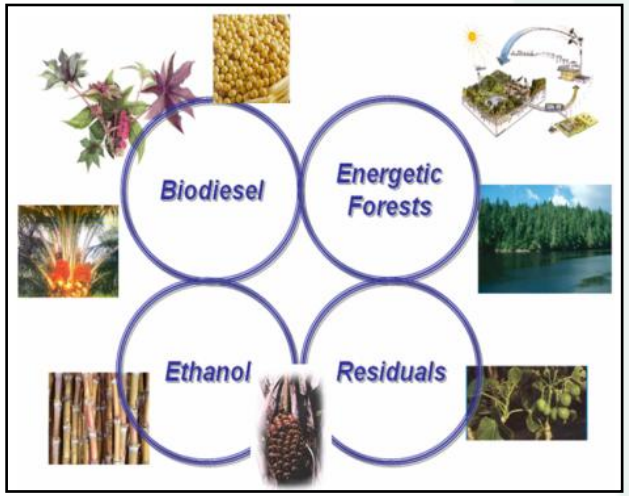
ENVIRONMENT

ECONOMICS

SOCIAL

REGIONAL DIFERENCES

WORLD INSERTION



➢**The guidelines of the Brazilian Agroenergy Plan:**

➢Development of Agricultural Technology (sustainable production systems),

➢Development of Industrial Technology (efficient conversion processes), and

➢Transversal Studies (public policies, environment, socio-economic, markets, etc.)

EMBRAPA AGROENERGY: National Research and Development Center in Agroenergy

Established: May 24 th, 2006

Team:

- Planned: 150 employees
- Actual: 90 employees

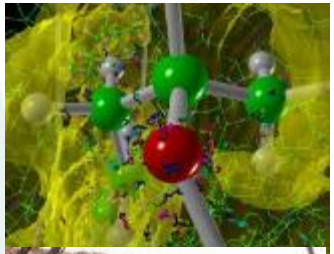
Mission: To enable innovative technological solutions for sustainable development of the agroenergy business in Brazil, for the benefit of the society

Goals:

1) Coordinate Embrapa´s R,D&I actions in Agroenergy
2) Execution of R,D&I projects

https://www.embrapa.br/agroenergia

EMBRAPA AGROENERGY: LABORATORIES




1. Laboratory of Genetics and Biotecnology

Advanced Biology Lab;

Bioinformatics Lab.

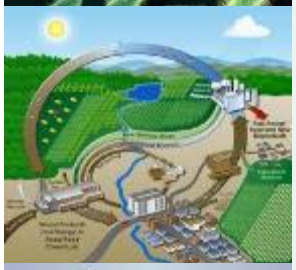
2. Laboratory of Energy Processes

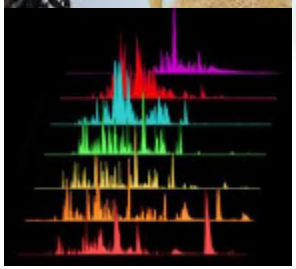
Biochemical Process Lab;

Chemical Process Lab;

3. Laboratory of Biomass and Biofuel Chemistry

4. Pilot Plant in Agroenergy Industrial Operations

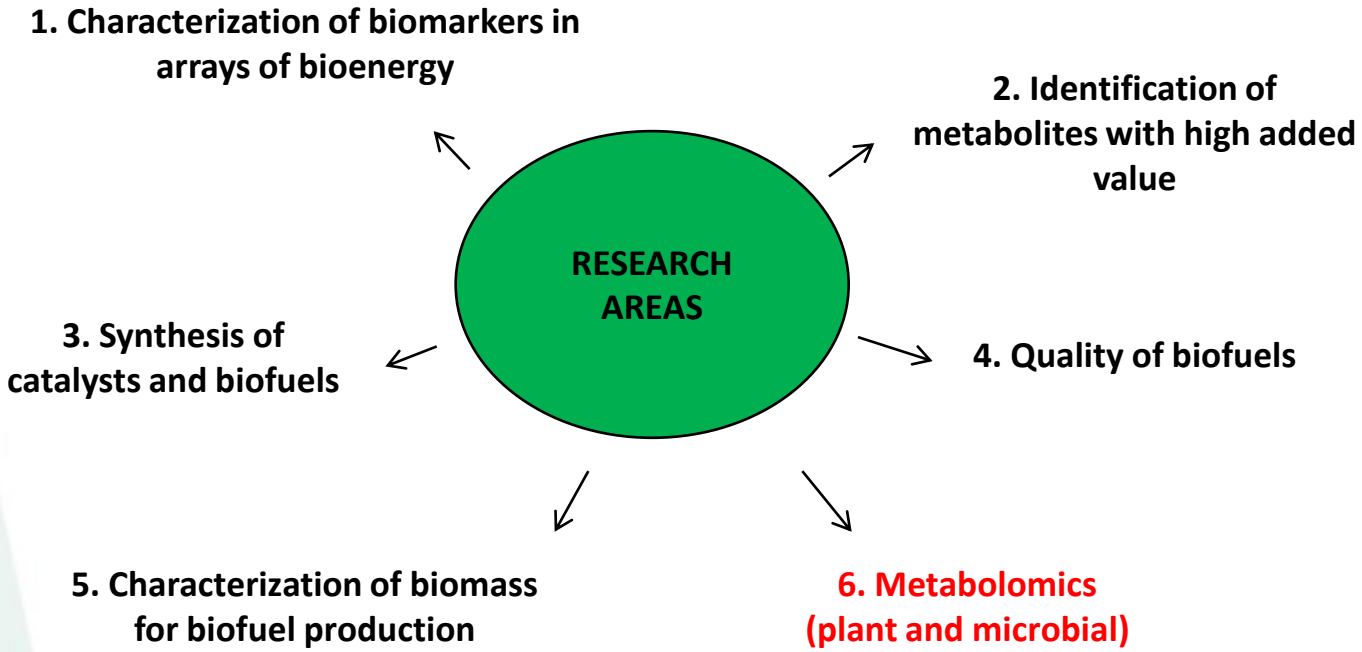





EMBRAPA AGROENERGY: LABORATORY OF BIOMASS AND BIOFUEL CHEMISTRY

Embrapa Agroenergia

Laboratory of Biomass and Biofuel Chemistry



Plant

Sugarcane
Jatropha curcas
Elaeis spp.

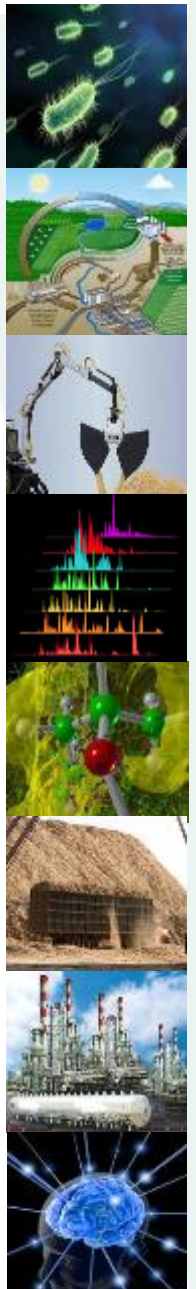
Microorganism

Yeast
Fungi
Bacteria

5 Researchers

4 R&D support

Grad, MSc, PhD, Pos t Doc



Laboratory of Biomass and Biofuel Chemistry

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ICP-OES

GC-FID – Headspace

GC-MS (Pyrolysis probe)
GC-MS

Ion trap

FT-NIRS

UHPLC

HPLC-PDA-RID

UPLC

1 UPLC -PDA-ELSD
1 UPLC -PDA-FLR

Triplo Quadrupolo (QqQ)

Q-ToF

MALDI-TOF/TOF

Embrapa Agroenergia

Metabolomics of xylose-fermenting yeast for increasing efficiency in the production of second generation ethanol

ETHANOL

Embrapa Agroenergia

Ethanol:

-Renewable fuel

-Brazil: Ethanol from sugarcane

Evolution of Brazilian ethanol production from 1948 to 2011

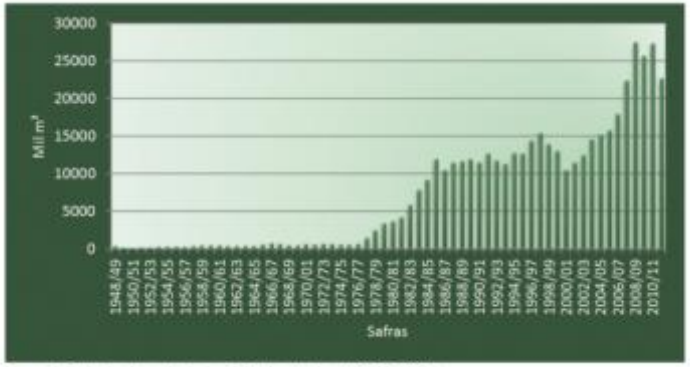


Figura 1. Evolução da produção brasileira de etanol entre 1948 e 2011.
Adaptada de: UNIÃO DA INDÚSTRIA DA CANA-DE-AÇÚCAR, 2012.

Workflow for the Ethanol production from sugarcane

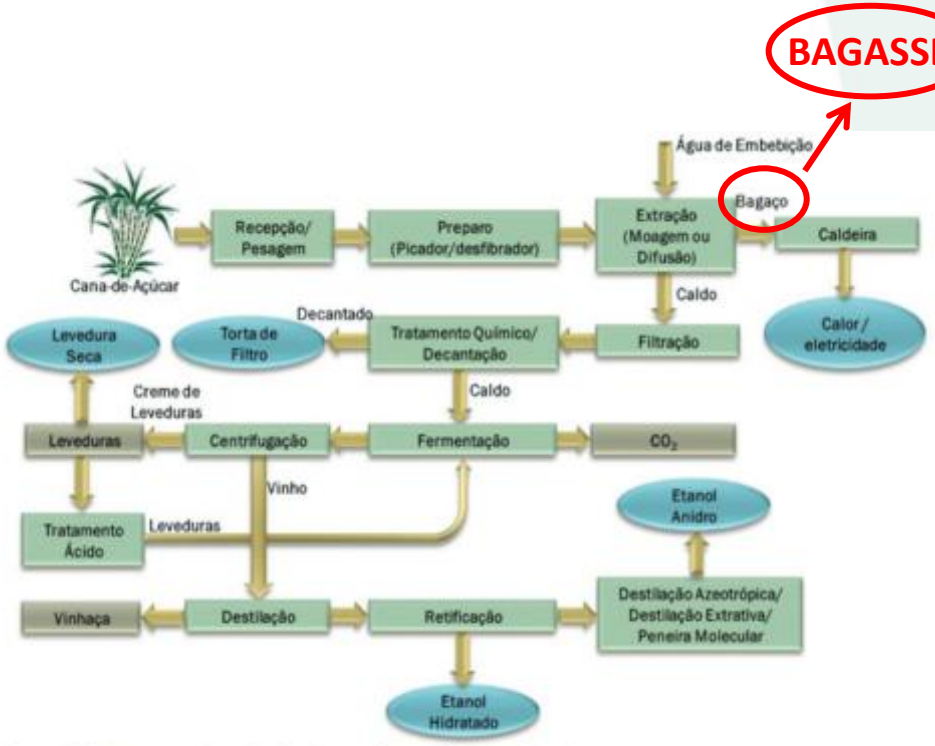


Figura 24. Fluxograma de produção de etanol a partir da cana-de-açúcar.

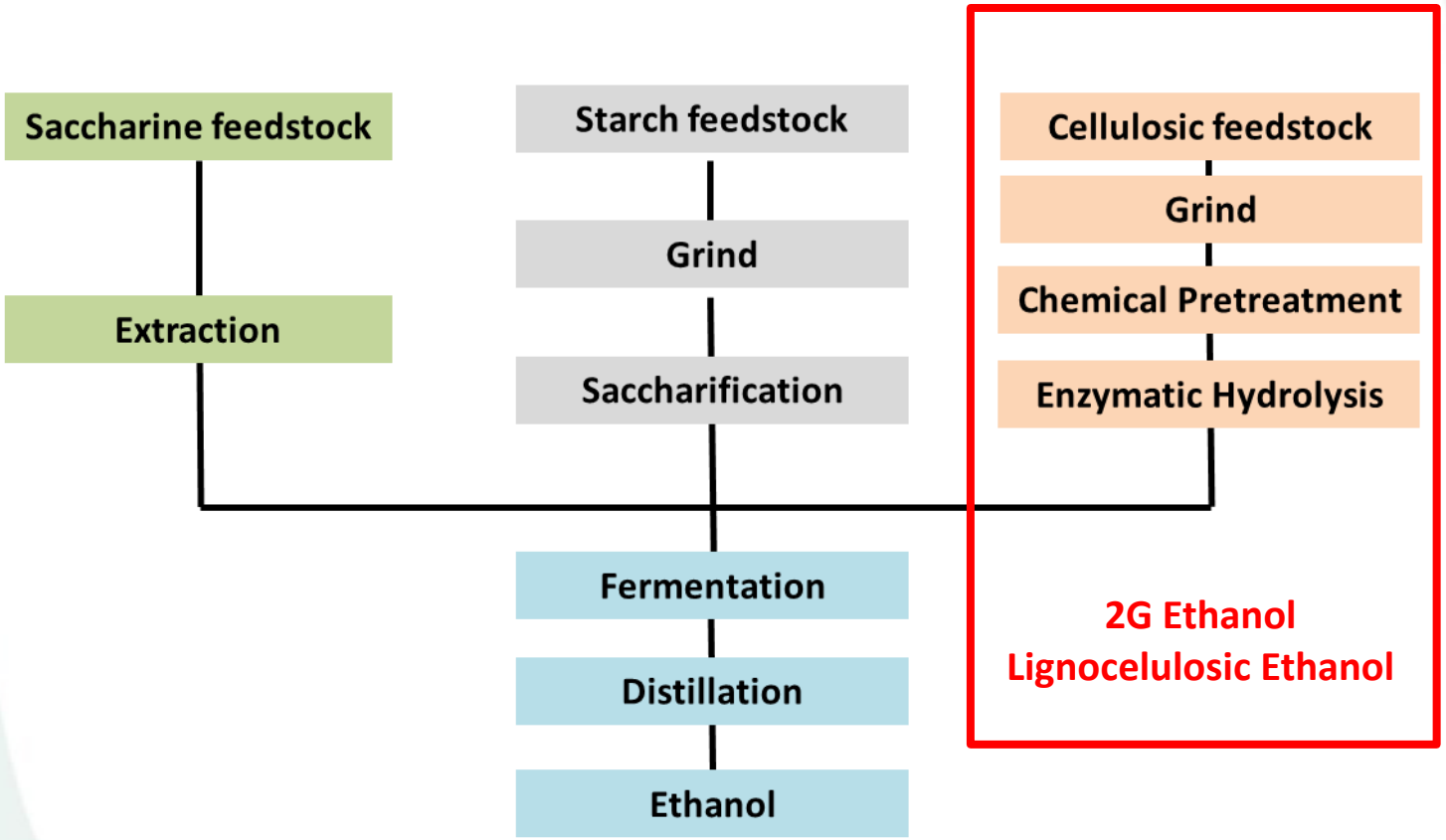
- Bagasse: 2G Ethanol

*Adapted from: Microrganismos na produção de biocombustíveis líquidos / Editora técnica, Cristina Maria Monteiro Machado - Brasilia, DF: Embrapa, 2013, 319 p.

2G ETHANOL

Embrapa Agroenergia

Steps of ethanol production from different feedstocks*

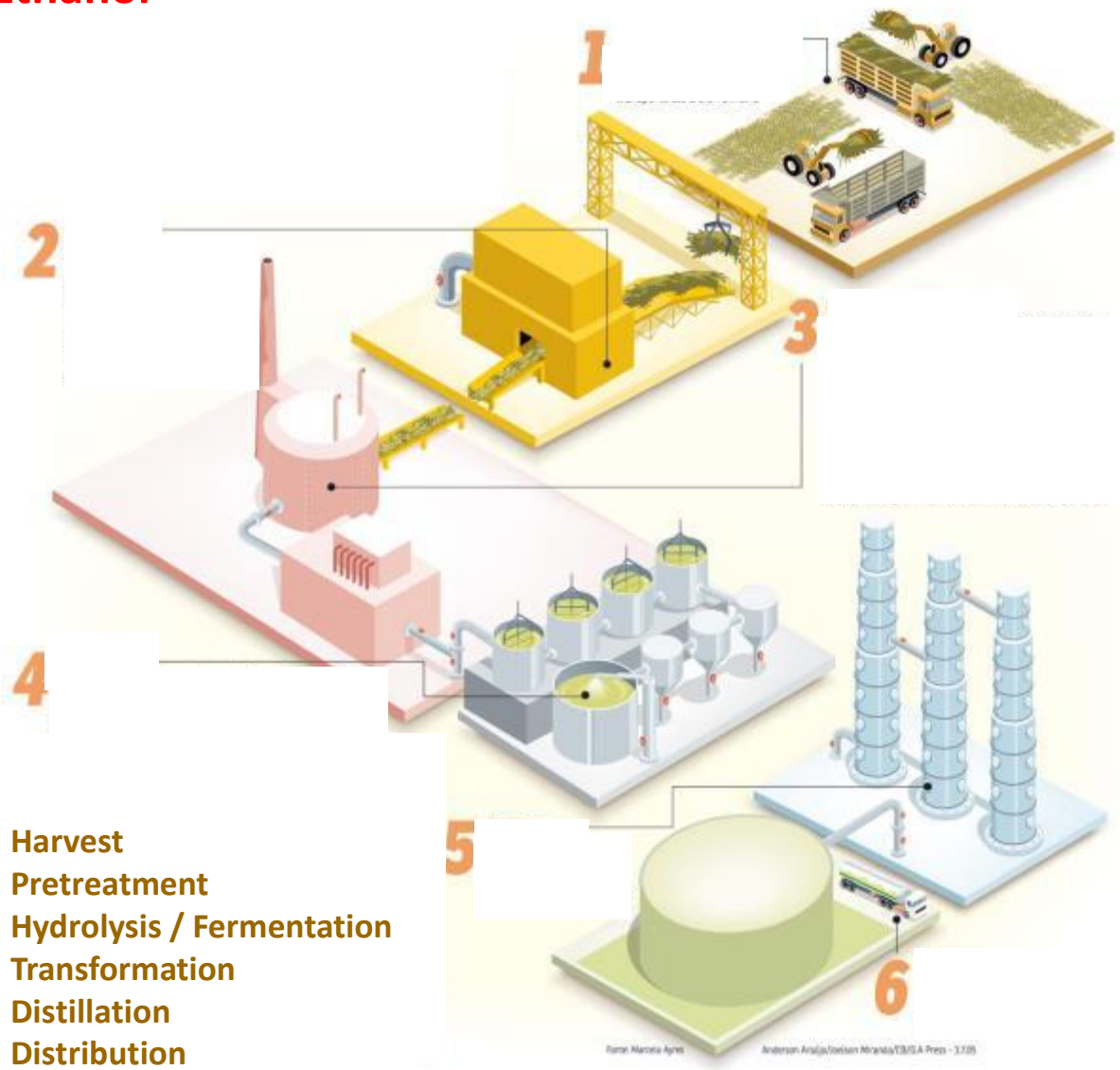


*Adapted from: Microrganismos na produção de biocombustíveis líquidos / Editora técnica, Cristina Maria Monteiro Machado - Brasilia, DF: Embrapa, 2013, 319 p.

2G ETHANOL

Embrapa Agroenergia

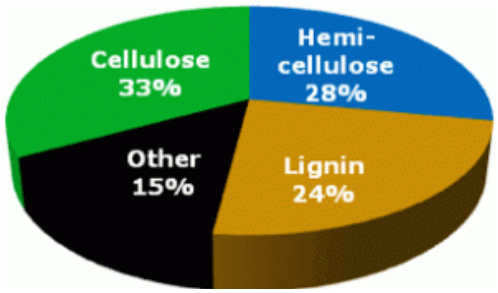
2G Ethanol



1. Harvest
2. Pretreatment
3. Hydrolysis / Fermentation
4. Transformation
5. Distillation
6. Distribution

LIGNOCELULOSIC BIOMASS COMPOSITION

Embrapa Agroenergia



Cellulosic Biomass Composition
http://newenergyandfuel.com/http:/newenergyandfuel/com/2009/01/09/the-leading-cellulosic-ethanol-process

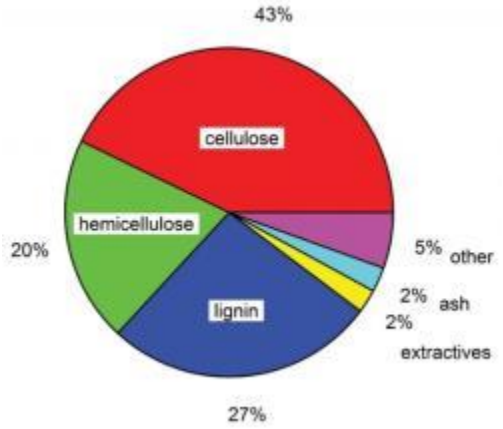


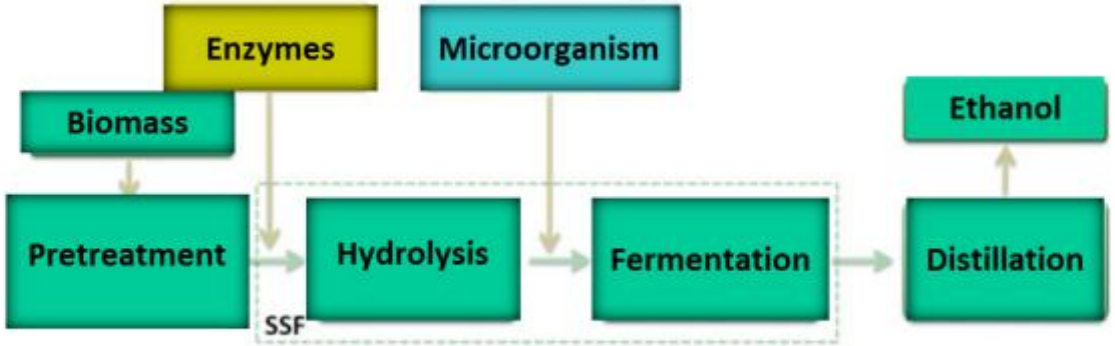
Figure 1. Typical composition of a **softwood tree**, in this case hybrid poplar
http://crf.sandia.gov/index.php/thermochemical-integration-key-to-improving-the-efficiency-of-bio-ethanol-production/#.Ulg8qz_fP0U

Table 4
Composition of various types of lignocellulosic-biomass materials (% dry weight).

Material	Cellulose	Hemicelluloses	Lignin	Ash	Extractives
Algae (green)	20–40	20–50	–	–	–
Cotton, flax, etc.	80–95	5–20	–	–	–
Grasses	25–40	25–50	10–30	–	–
Hardwoods	45 ± 2	30 ± 5	20 ± 4	0.6 ± 0.2	5 ± 3
Hardwood barks	22–40	20–38	30–55	0.8 ± 0.2	6 ± 2
Softwoods	42 ± 2	27 ± 2	28 ± 3	0.5 ± 0.1	3 ± 2
Softwood barks	18–38	15–33	30–60	0.8 ± 0.2	4 ± 2
Cornstalks	39–47	26–31	3–5	12–16	1–3
Wheat straw	37–41	27–32	13–15	11–14	7 ± 2
Newspapers	40–55	25–40	18–30	–	–
Chemical pulps	60–80	20–30	2–10	–	–

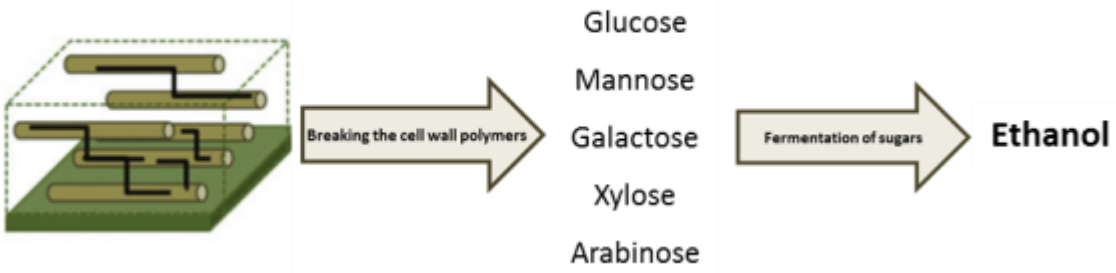
Balat, M. Energy Conversion and Management, 2011, 52, 858-875.

2G ETHANOL PRODUCTION STEPS



SSF: Simultaneous saccharification and fermentation

"Ideal" Microorganism



*Adapted from: Microrganismos na produção de biocombustíveis líquidos / Editora técnica, Cristina Maria Monteiro Machado - Brasilia, DF: Embrapa, 2013, 319 p.

CHALLENGES IN THE PRODUCTION OF 2G ETHANOL

Embrapa Agroenergia

Microorganism Challenges

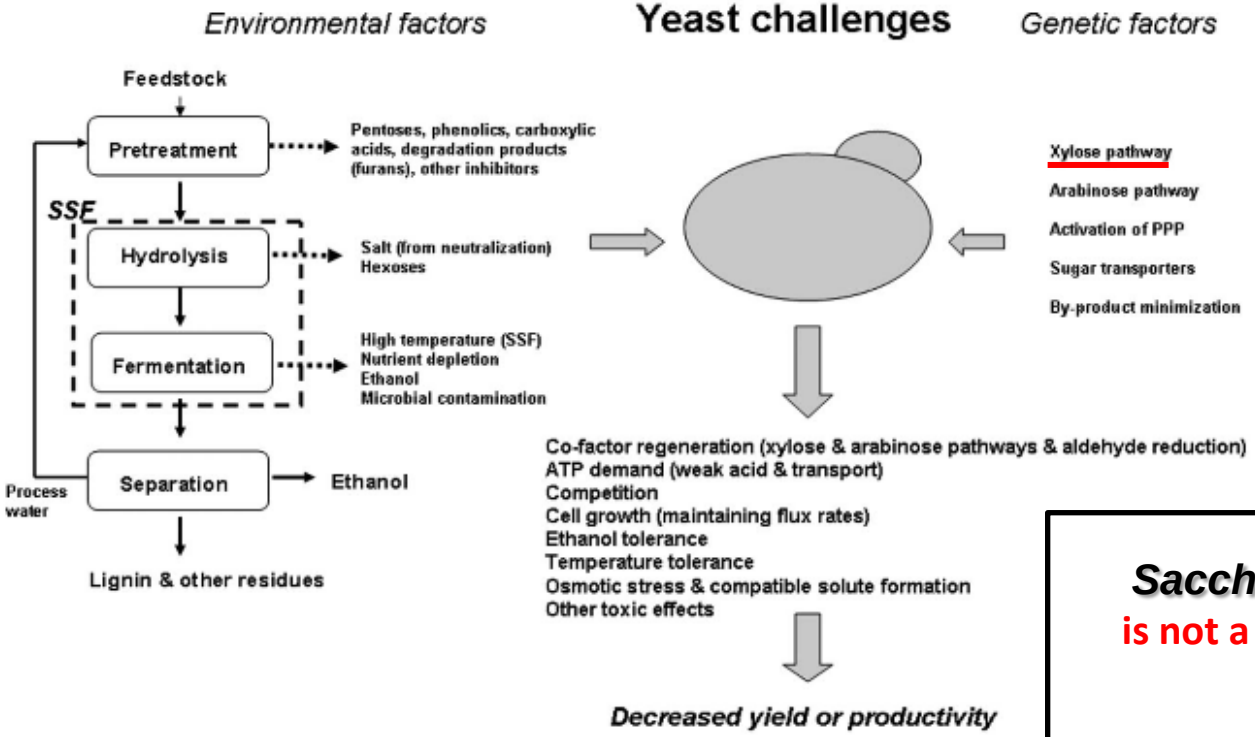


Figure 1. Challenges faced by the yeast *S. cerevisiae* during the production of ethanol from lignocellulosic feedstocks.

Saccharomyces cerevisiae
is not a xylose-fermenting yeast

↓

Xylose ~30% of sugars - sugarcane bagasse

↓

Increase in the ethanol production

Almeida, J. R. M. et al. Biotechnol. J. 2011, 6, 286-299.

XYLOSE CONVERTION TO ETHANOL

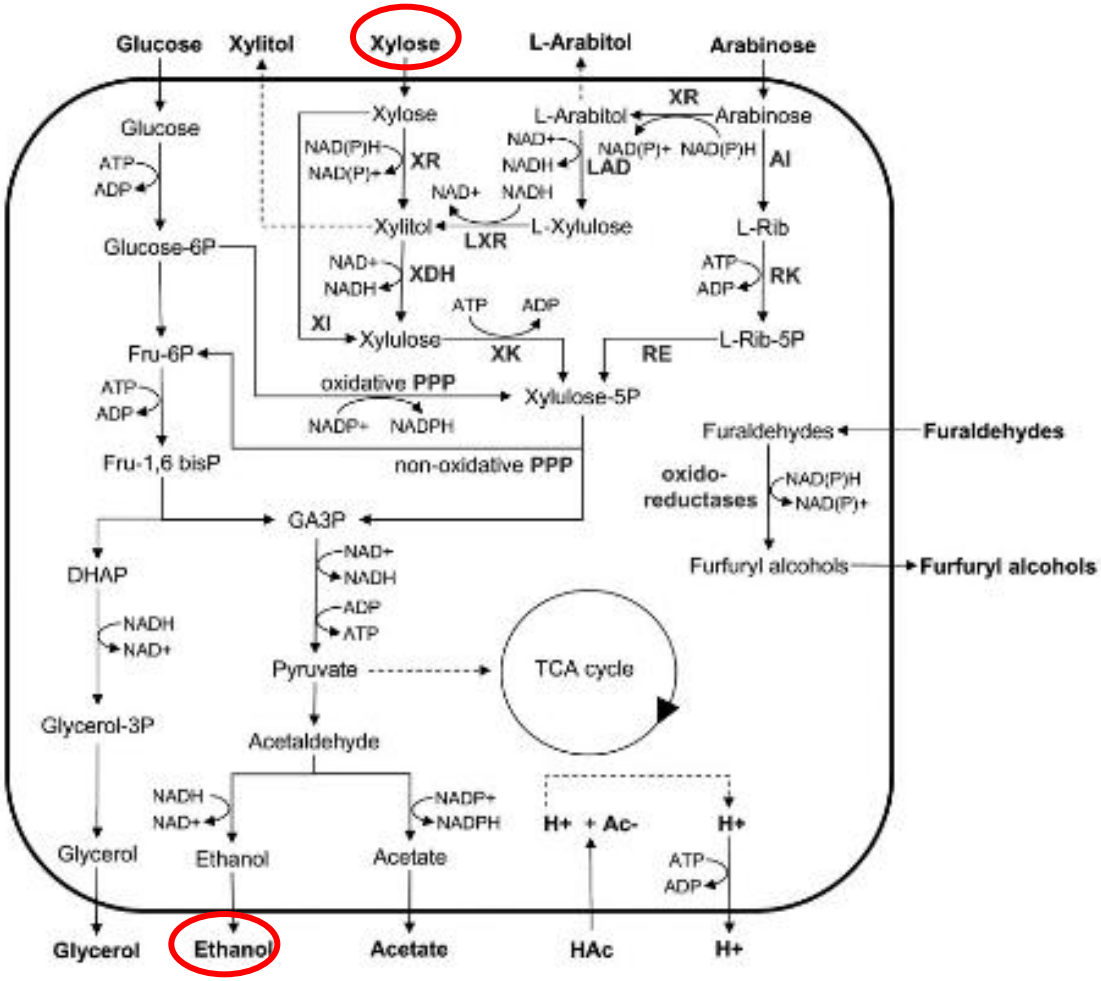


Figure 2. Xylose and arabinose pathways expressed in recombinant *S. cerevisiae*. The utilization of cofactors and ATP in the central carbon metabolism is depicted schematically.

1) Recombinant *S. cerevisiae*
2) MO pentose-fermenting

↓

MO pentose-fermenting

↓

New molecular targets importants to xylose metabolism

↓

Recombinant *S. cerivisae*

↓

INCREASE EFFICIENCY IN THE PRODUCTION OF SECOND GENERATION ETHANOL

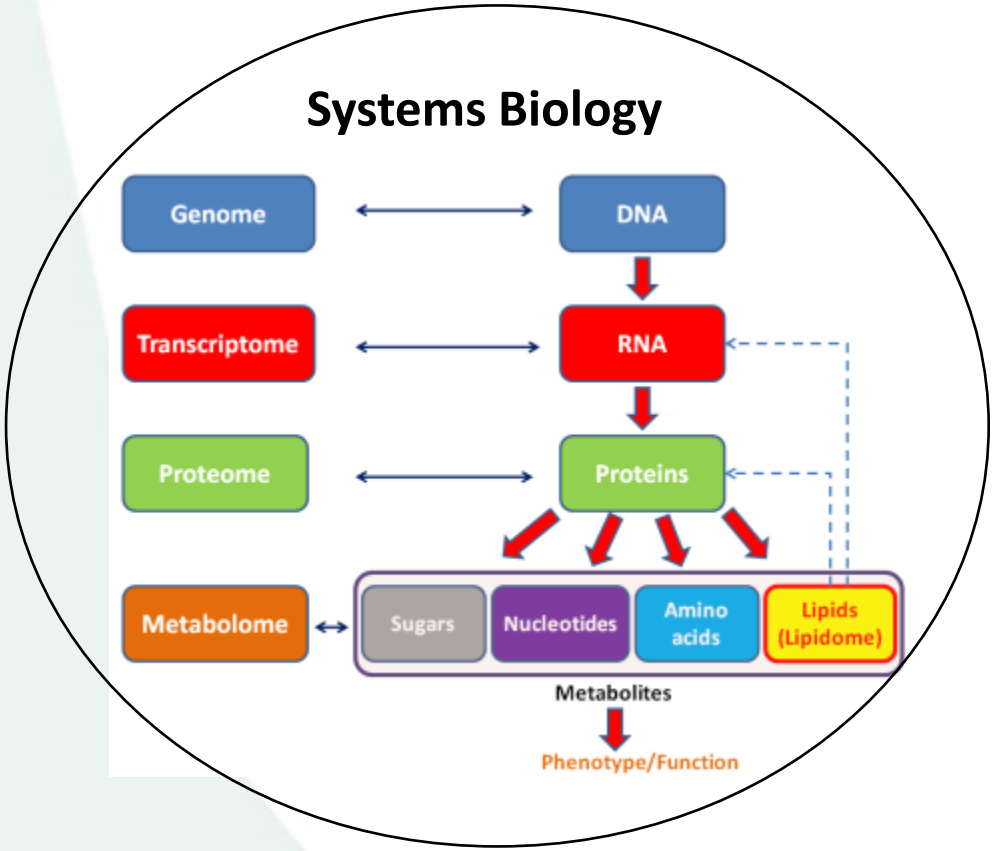
Almeida, J. R. M. et al. Biotechnol. J. 2011, 6, 286-299.

INCREASE EFFICIENCY IN THE PRODUCTION OF 2G ETHANOL

Embrapa Agroenergia

Embrapa Agronergy project:

Functional genomics, transcriptomics and metabolomics, of xylose-fermenting yeast for increasing efficiency in the production of second generation ethanol (Dr. Patrícia V. Abdelnur)



Embrapa

Metabolomics

Transcriptomics

Genomics

METABOLIC ENGINEERING

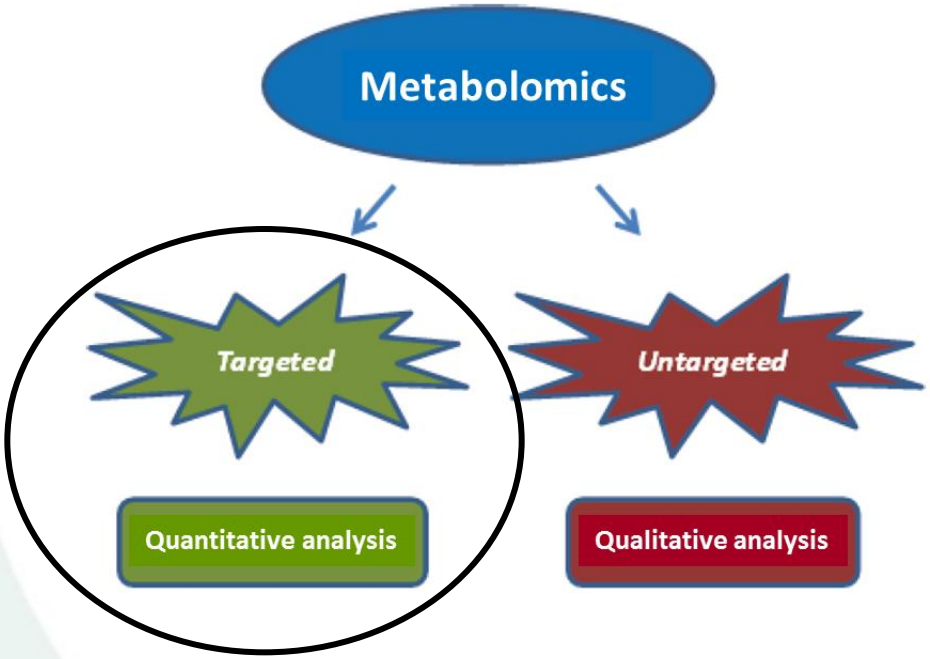
METABOLOMICS APPROACHES

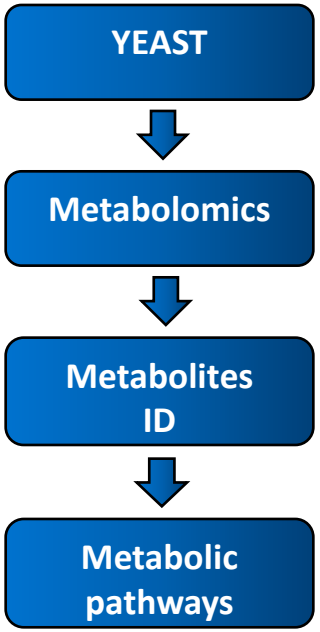
Embrapa Agroenergia

- **METABOLOMICS BASED ON MASS SPECTROMETRY:**

Term	Definition
Metabolome	The complete complement of small molecules present in an organism.
Metabolomics	The technology geared towards providing an essentially unbiased, comprehensive qualitative and quantitative overview of the metabolites present in an organism

Hall , R. D. New Phytologist, 2005.





WORKFLOW

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XYLOSE YEAST FERMENTATION PROCESS

↓

DEVELOPMENT OF METABOLITE ID METHOD LC-MS/MS (MRM)

↓

OPTIMIZATION OF SAMPLE PREP TO YEASTS

↓

METABOLITES ID - YEASTS

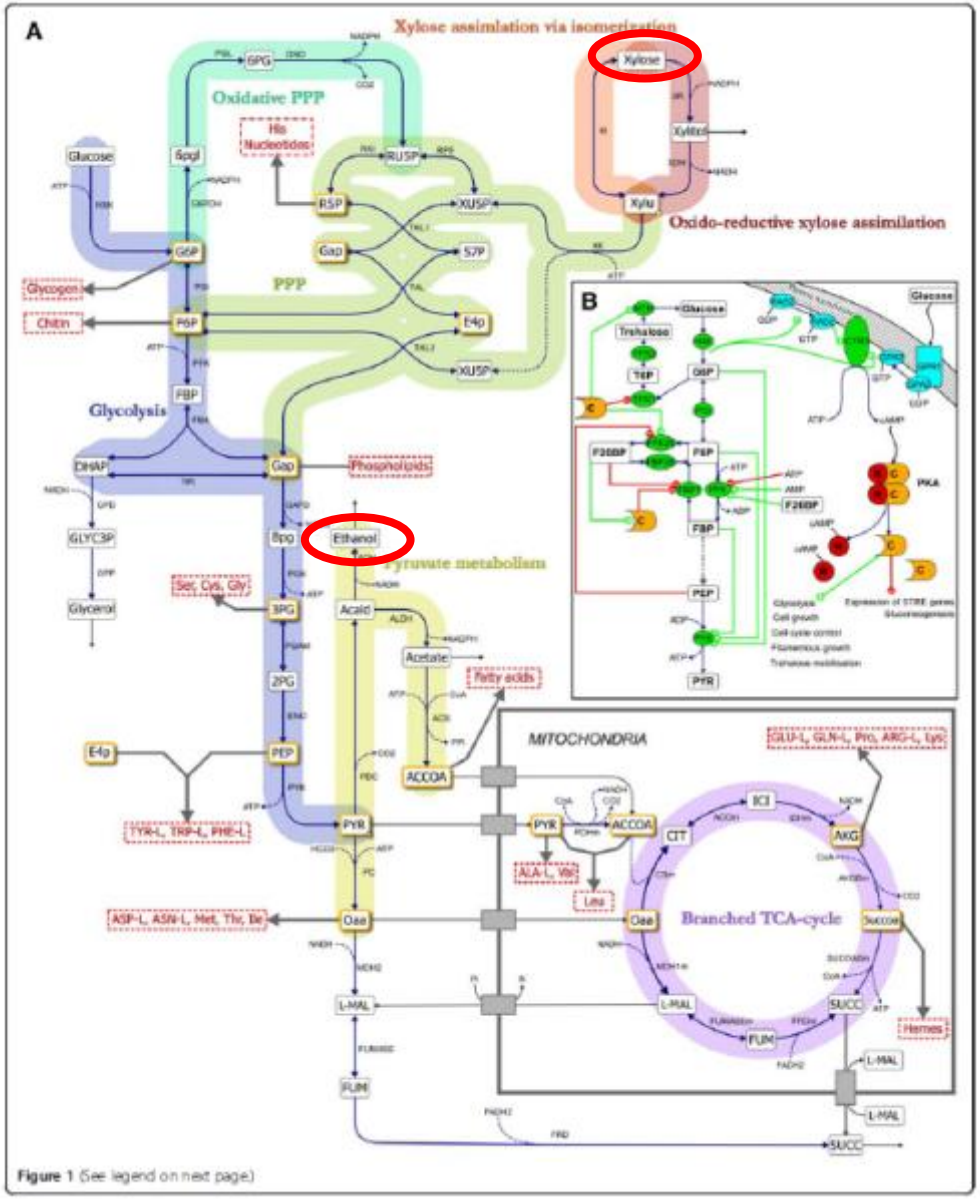

Figure 1 (See legend on next page)

Bergdahl, B. et al. Biotechnol. For Biofuels. 2012,5 ,34.

METABOLITES FROM XYLOSE CONVERSION PATHWAY

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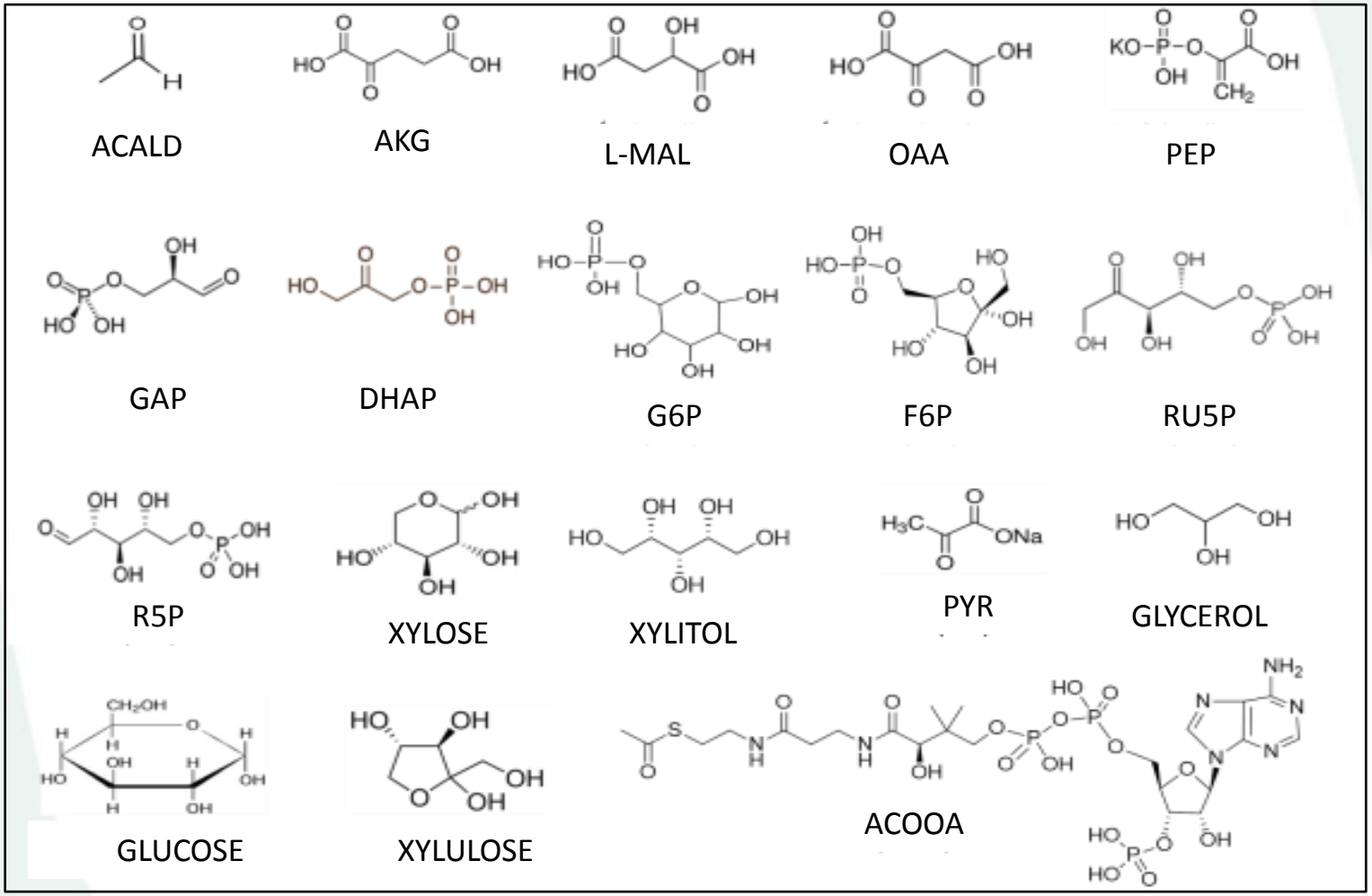

DEVELOPMENT OF ANALYTICAL METHOD: MS

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✓ MASS SPECTROMETRY (MS)

DIMS (*Direct Infusion Mass Spectrometry*)
ESI-(+)-MS and ESI-(-)-MS

↓

Optimization of MS parameters for each metabolite

↓

Metabolite ID (*m/z*)

↓

Tandem MS
ESI-(+)-MS/MS e ESI-(-)-MS/MS

↓

Opmization of cone voltage for each metabolite

↓

MRM (*Multiple Reaction Monitoring*)

MS: TQD Xevo (Waters)

DEVELOPMENT OF ANALYTICAL METHOD: LC

Embrapa Agroenergia

✓ LIQUID CHROMATOGRAPHY (LC)

- ULTRA HIGH PRESSURE LIQUID CROMATOGRAPHY (UHPLC)

- LC Columns

  Ion Pair Chromatography (IPC): BEH-amide (Waters)

  Hydrophilic Interaction Chromatography (HILIC): HSS-T3 (Waters)

  Aminex (Bio-Rad)

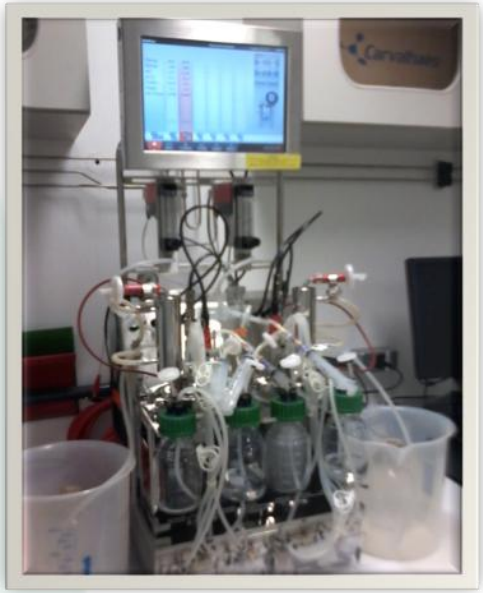
- Mobile Phase
- Temperature
- pH

UHPLC-MS: Acquity Xevo TQD (Waters)

SAMPLE PREPARATION OF YEASTS

Embrapa Agroenergia

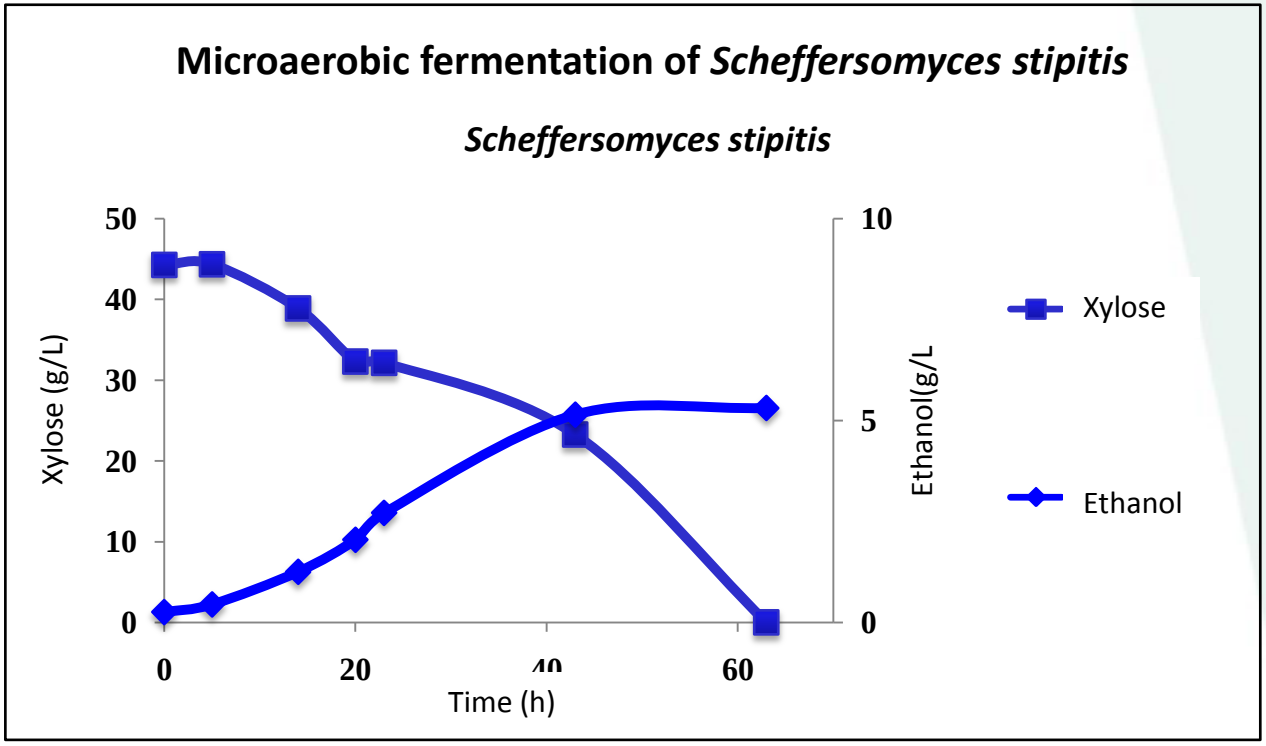
✓ FERMENTATION



» *Scheffersomyces stipitis*

» *Spathaspora passalidarum*

- Samples were collected at 20-40 hours of fermentation
- Different sample volumes were collected: 1 mL, 2 mL e 5 mL



SAMPLE PREPARATION STEPS

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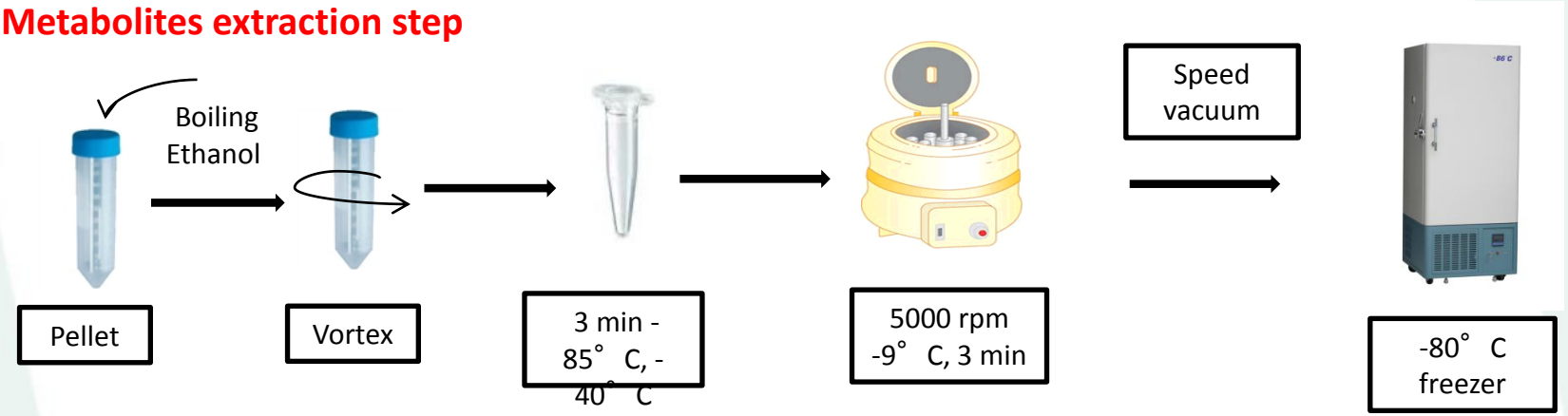
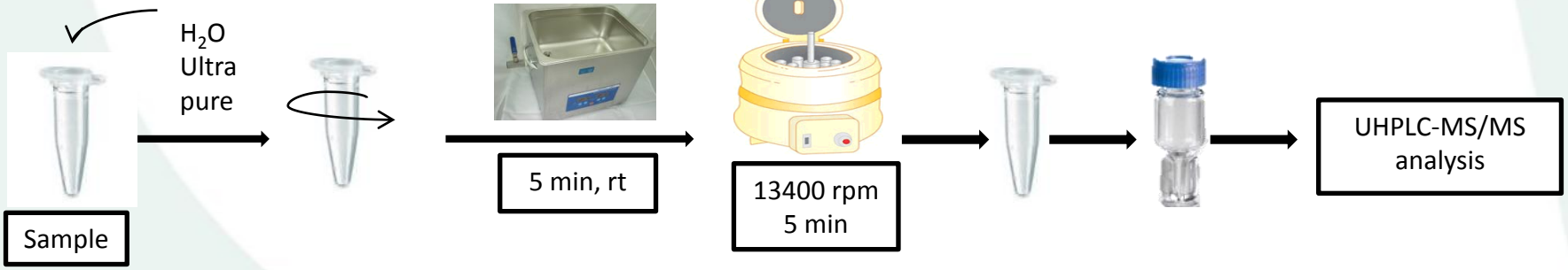
1) Quenching step

2) Metabolites extraction step



3) LC-MS/MS analysis



METABOLITE IDENTIFICATION - DIMS

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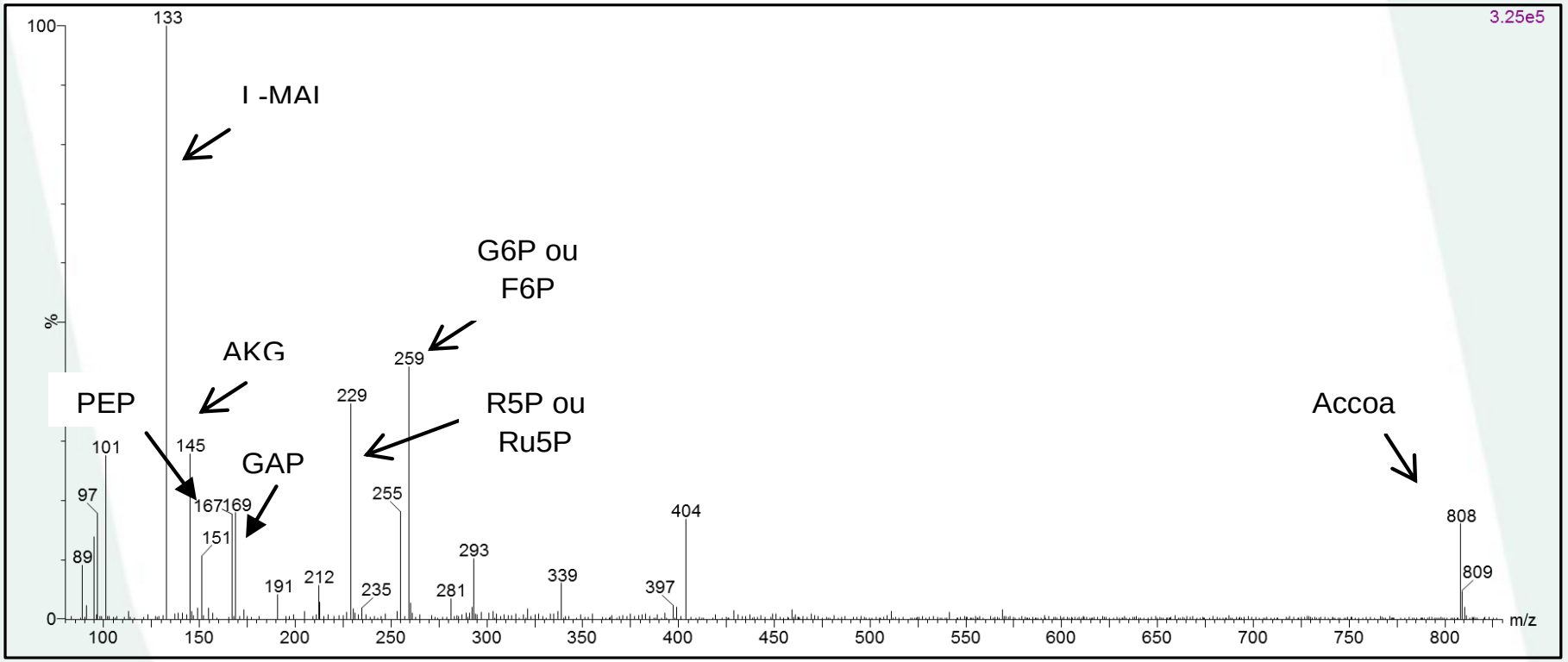
✓ **DEVELOPMENT OF ANALYTICAL METHOD: MASS SPECTROMETRY (MS)**

DIMS

ESI(-)-MS of metabolite standards solution



18 metabolites

Ion suppression

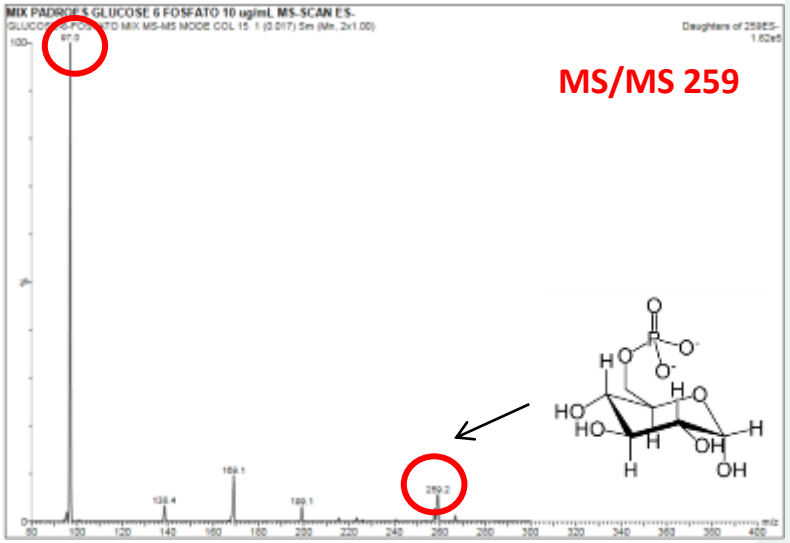
ES[illegible]MS

MS/MS EXPERIMENTS

Embrapa Agroenergia

DIMS

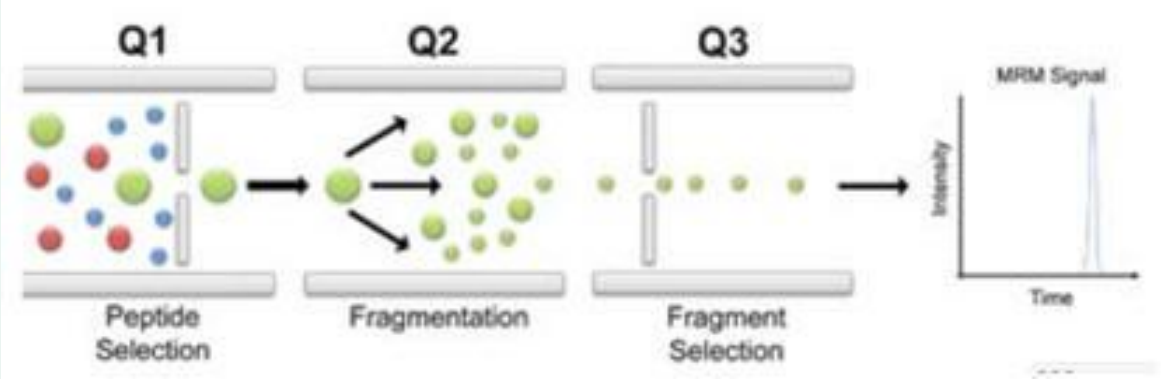
ESI(-)-MS and ESI(-)-MS/MS of standards



LC-MS/MS

MRM experiments – selectivity

MRM 259 - 97



MRM METHODS

Embrapa Agroenergia

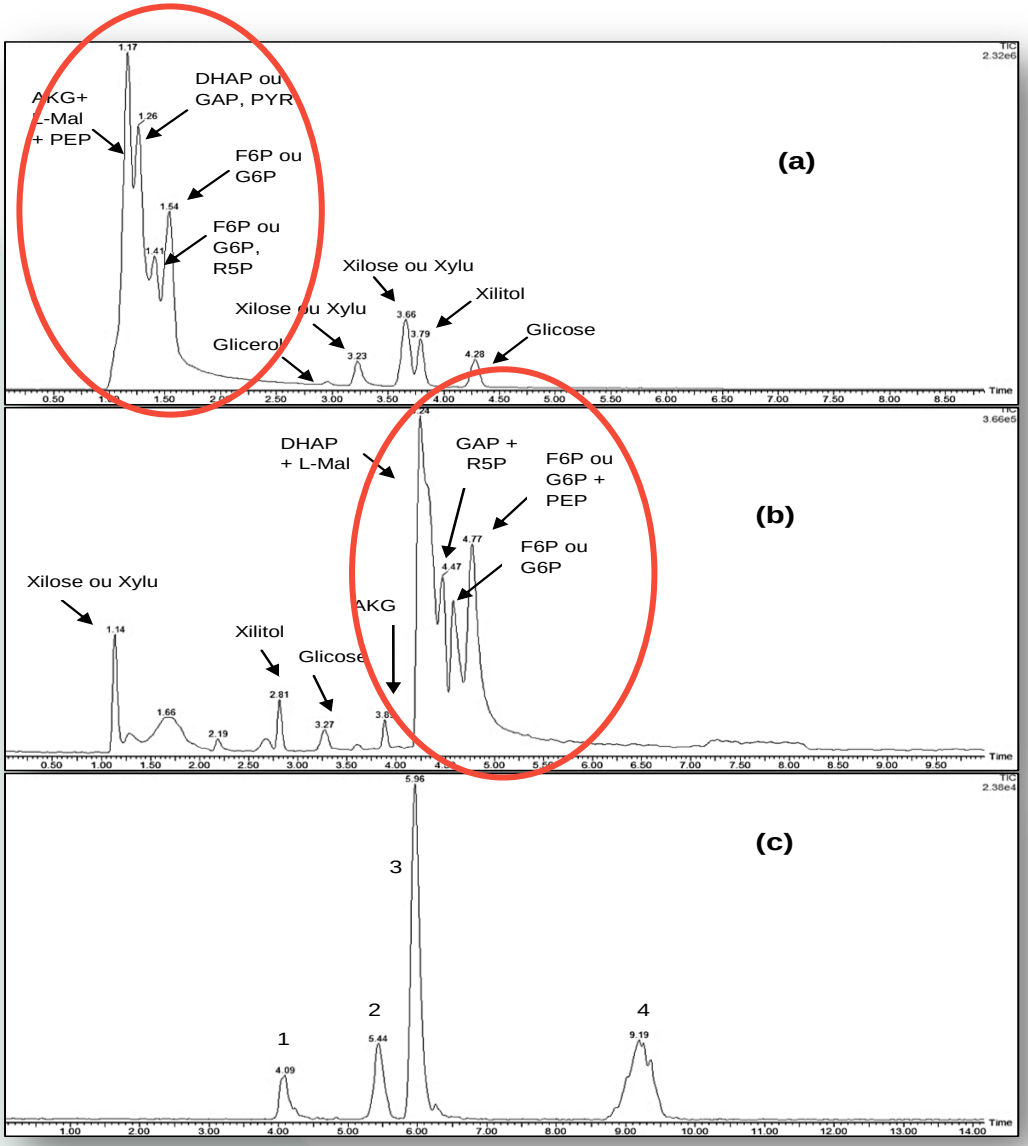
MRM channels and collision energy optimized for each metabolite

Metabolite	Q1 (*m/z*)	Q3 (*m/z*)	Collision energy (eV)
ACCOA	808.1	408	40
AKG	144.6	56.8	15
L-MAL	132.6	114.8	10
Oaa	130.8	86.8	6
Acald	59.7	-	-
DHAP	169	97	18
F6P	258.6	96.8	18
PEP	166.6	78.8	10
G6P	258.7	96.8	20
Glicerol	91.0	59.1	18
GAP	168.8	96.8	18
Glicose	179.1	58.9	18
PYR	86.8	42,8	8
R5P	228.8	96.8	20
RU5P	228.8	79.0	20
Xilose	148.9	59.0	14
Xilitol	151.0	58.9	20
Xylu	149.0	59.0	14

UHPLC-MS/MS USING HILIC

Embrapa Agroenergia

✓ DEVELOPMENT OF ANALYTICAL METHOD: LIQUID CHROMATOGRAPHY (LC)



HILIC
(Hydrophilic Interaction Chromatography)

HSS-T3 (Waters)

14 metabolites

Mobile Phase:

(a) **A:** NH_4OH 0,1%
B: ACN + NH_4OH 0,1%

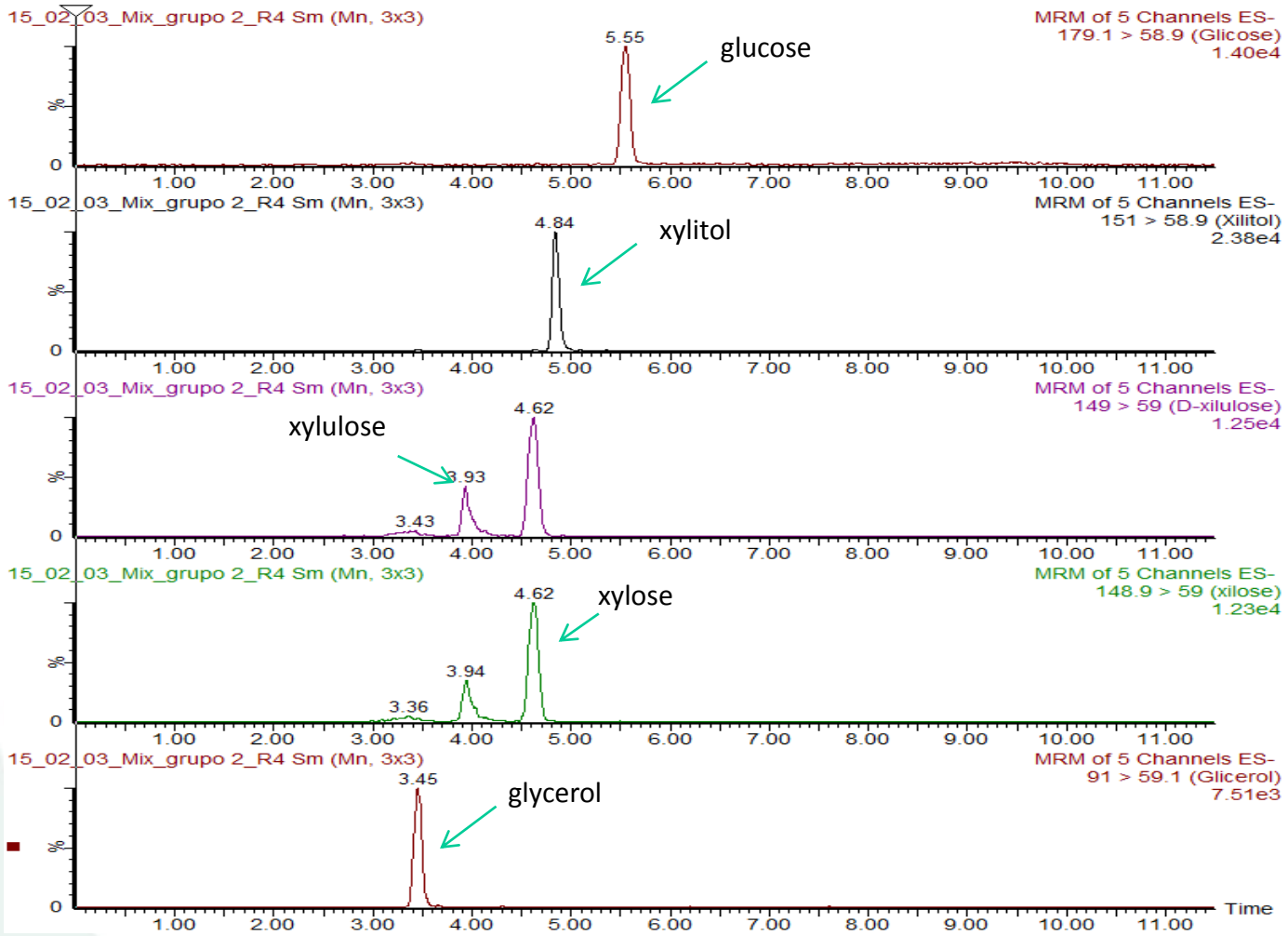
(b) **A:** ACN/H_2O 90/10 + AcNH4 10mM
B:. ACN/H_2O 50/50 + AcNH4 10mM

(c) **A:** ACN/H_2O 90/10 + FormNH_4 5mM + NH_4OH 0.1%
B: ACN/H_2O 10/90 + FormNH4 5mM + NH4OH 0.1%.

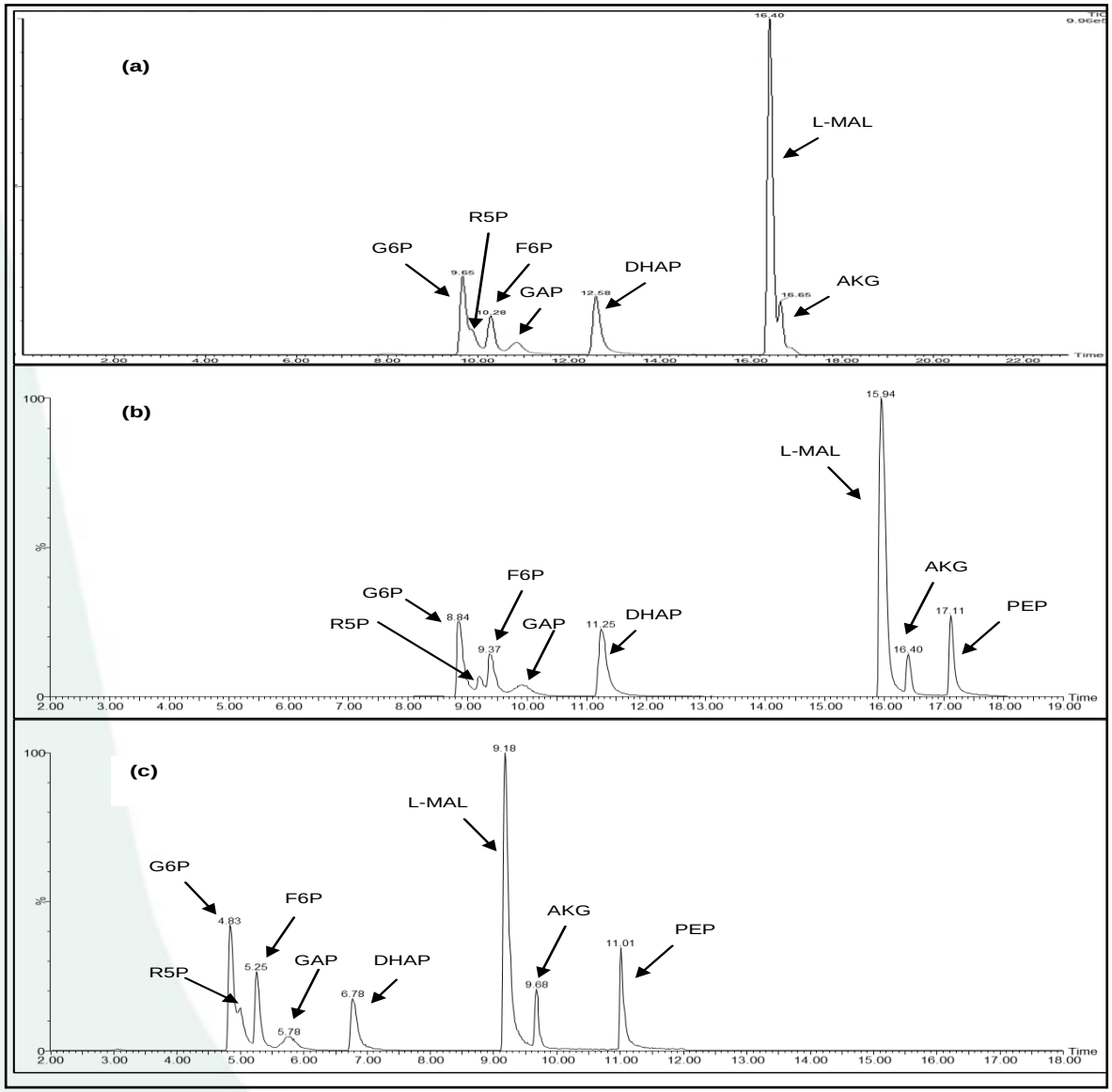
UHPLC-MS/MS USING HILIC

Embrapa Agroenergia

UHPLC-MS: glucose, xylitol, xylulose, xylose and glycerol - HILIC



UHPLC-MS/MS USING IPC



IPC

Ion Pair Chromatography

BEH-Amide (Waters)

8 metabolites

Mobile Phase:

(a) TBA 2mM + acetic acid 3mM, **pH = 5.1**

(b) TBA 2mM + acetic acid 4mM, **pH = 4.8**

(c) TBA 2mM + Ammonium acetate 5 mM, **pH = 6,2**.

UHPLC-MS/MS USING IPC

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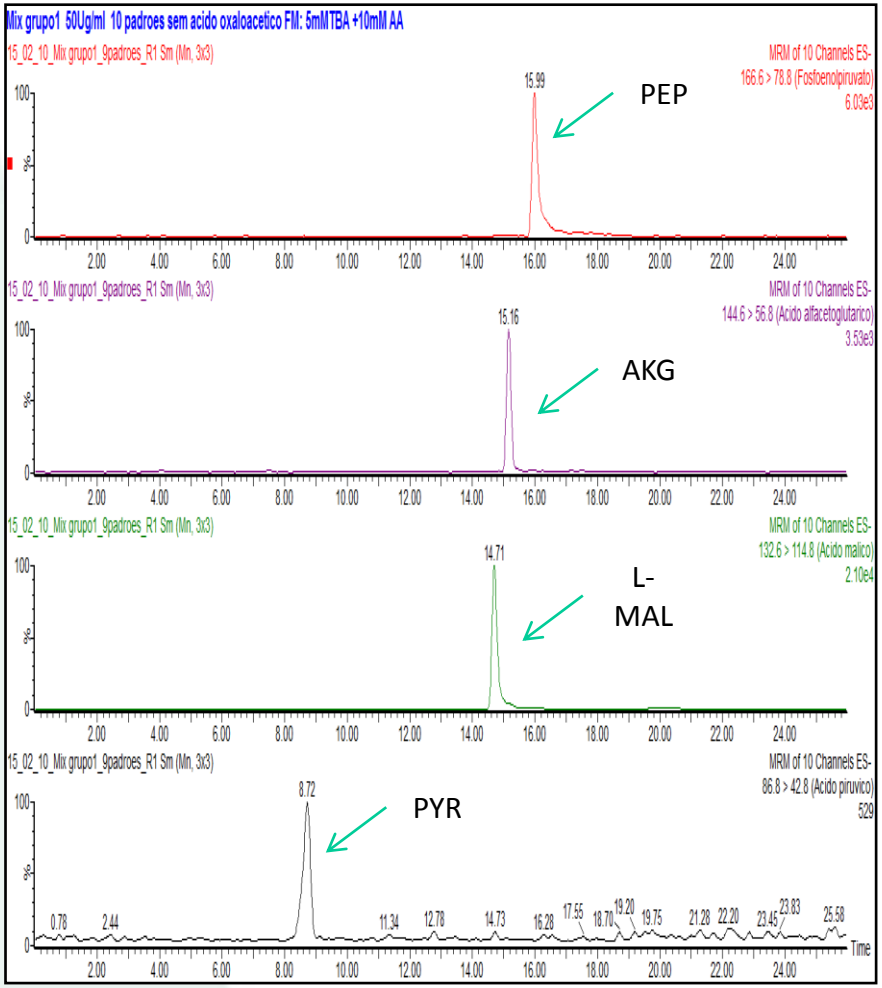
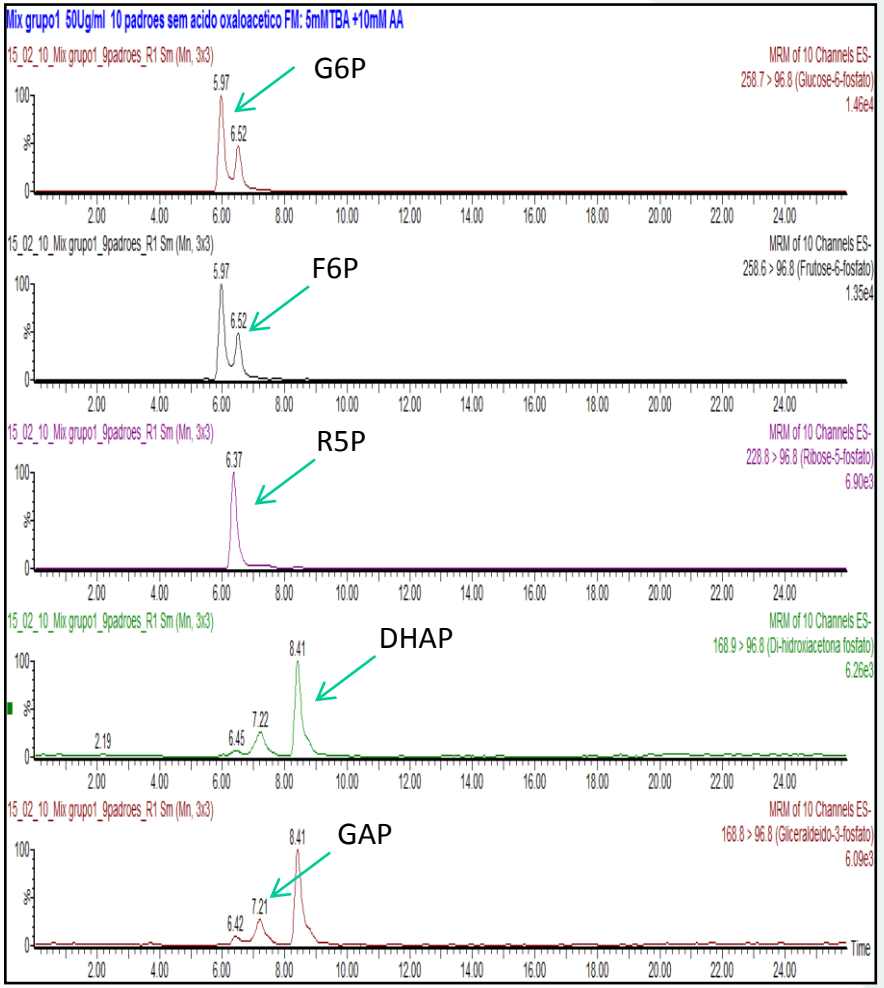
UHPLC-MS: F6P, G6P, R5P, DHAP, GAP, L-MAL, AKG, PEP, PYR - IPC





UHPLC-MS/MS USING AMINEX

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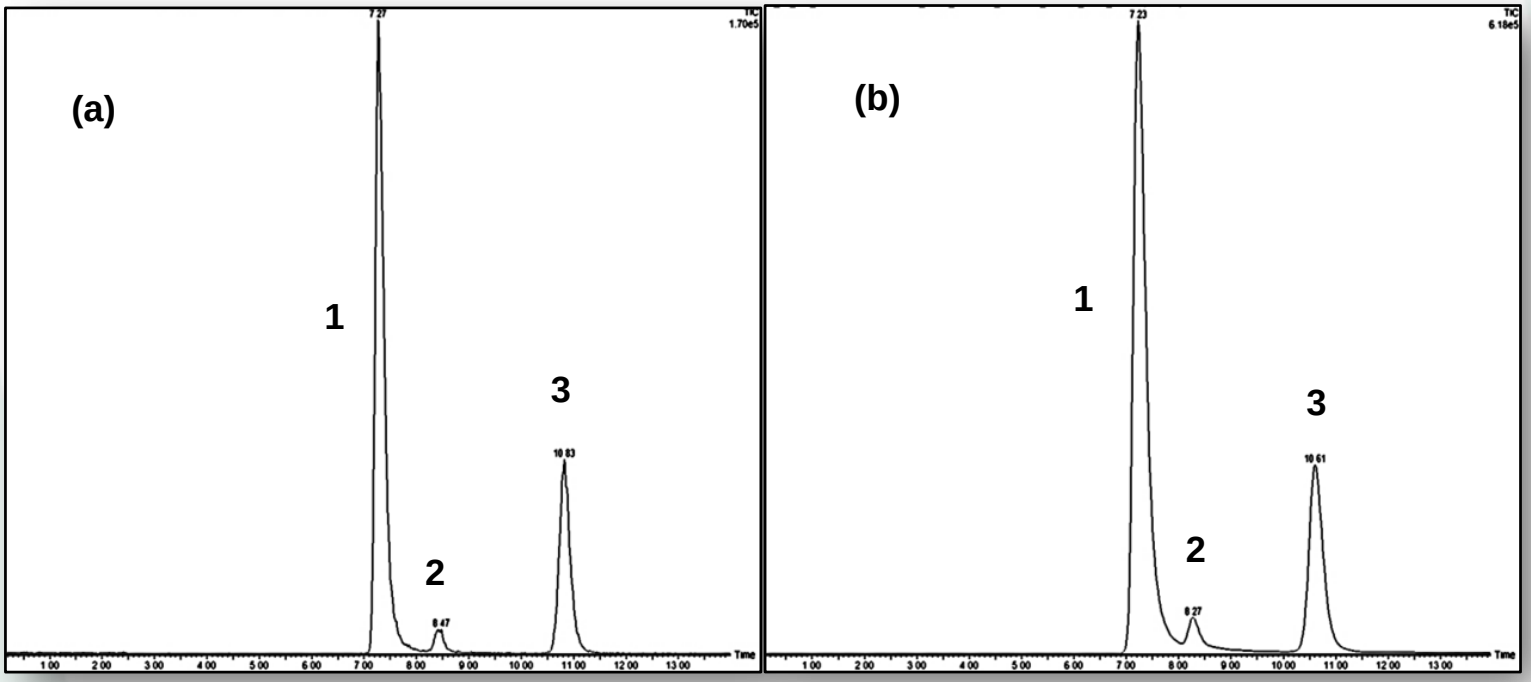
AMINEX (BIORAD)

8 metabolites



MP: Formic acid 0,1%. (a) 45° C (b) 65° C.
Peak 1: DHAP, G6P, F6P, PEP, GAP, R5P
Peak 2: AKG
Peak 3: L-MAL

METABOLITES DETECTED BY UHPLC-MS/MS

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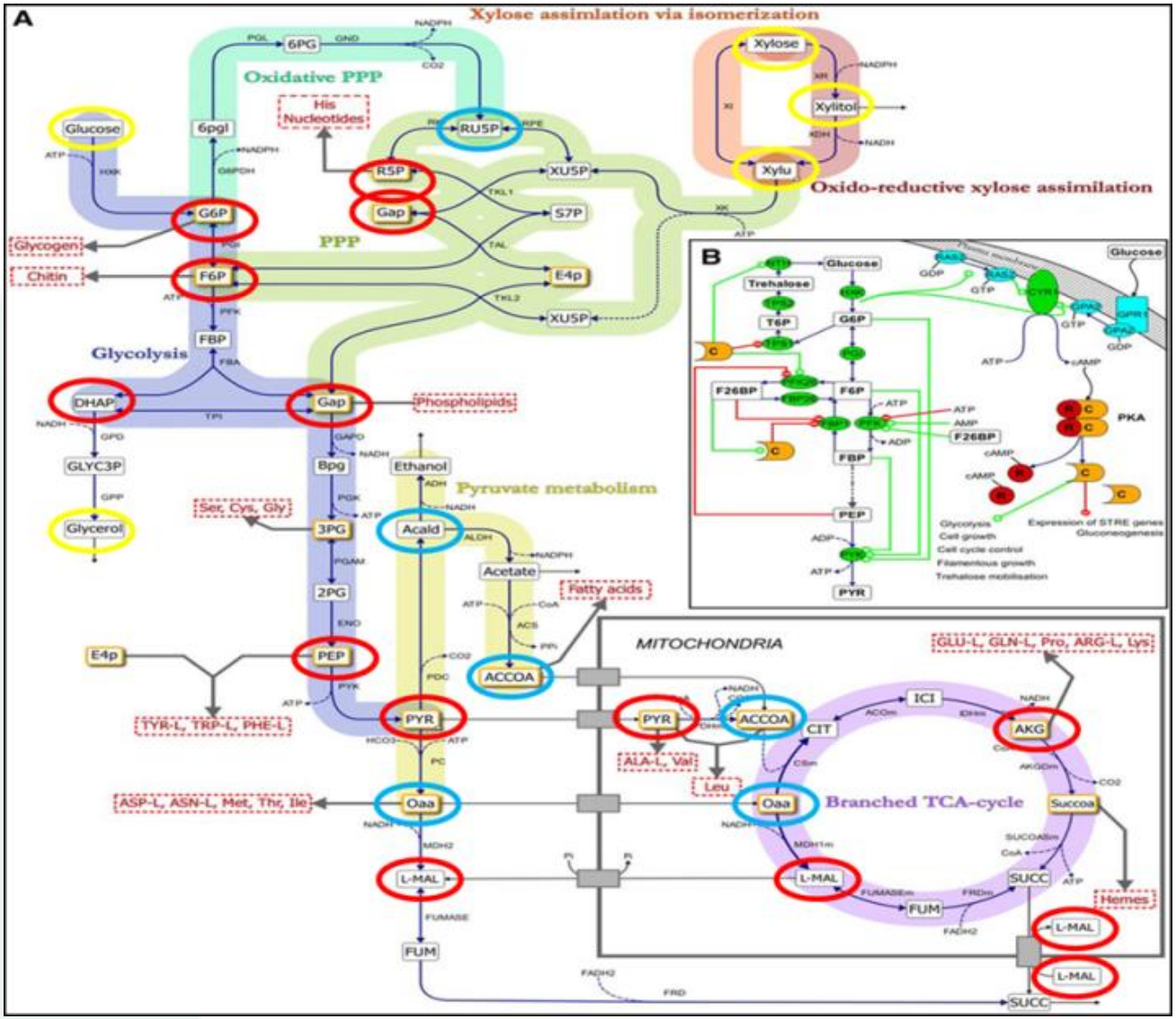

HILIC
(5 METABOLITES)

IPC
(9 METABOLITES)

ND

YEAST ANALYSIS BY UHPLC-MS/MS - IPC

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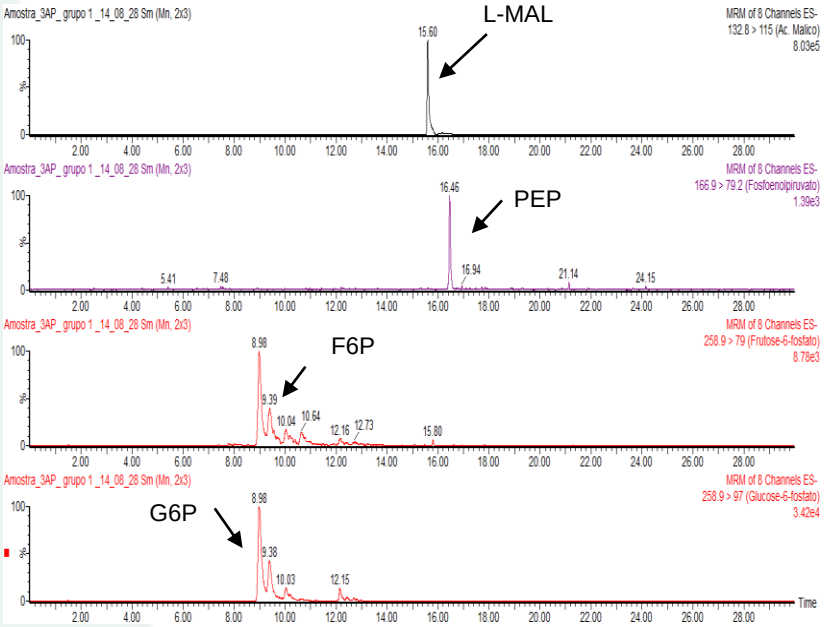
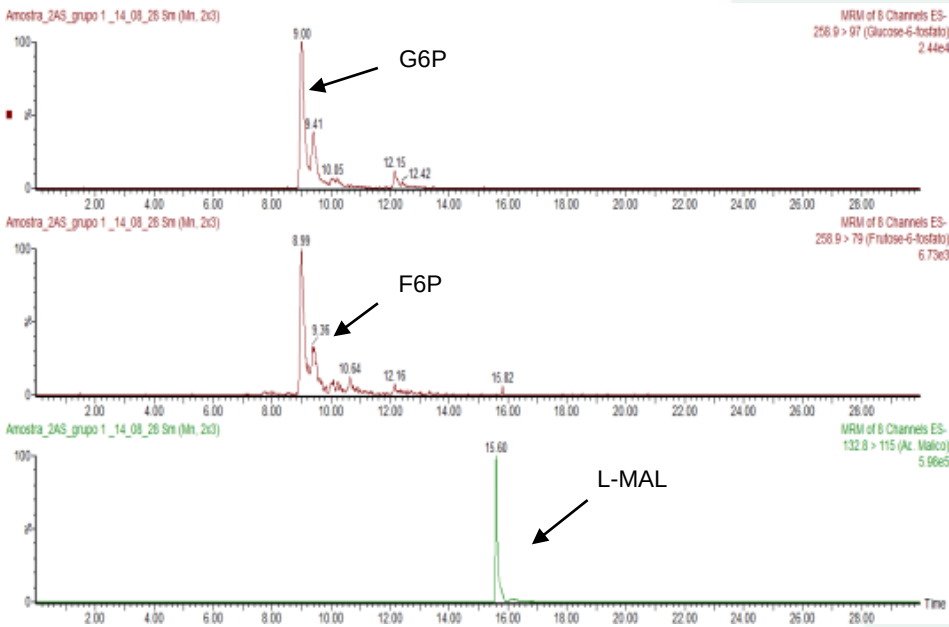
MRM of *Scheffersomyces stipitis*



Metabolites detected: a) malic acid (L-MAL); b) phospho(enol)pyruvate (PEP); c) frutose-6-phosphate (F6P); d) glucose-6-phosphate (G6P).

MRM of *Spathaspora passalidarum*



Metabolites detected: a) glucose-6-phosphate (G6P); b) frutose-6-phosphate (F6P); c) malic acid (L-MAL).

YEAST ANALYSIS BY UHPLC-MS/MS - HILIC

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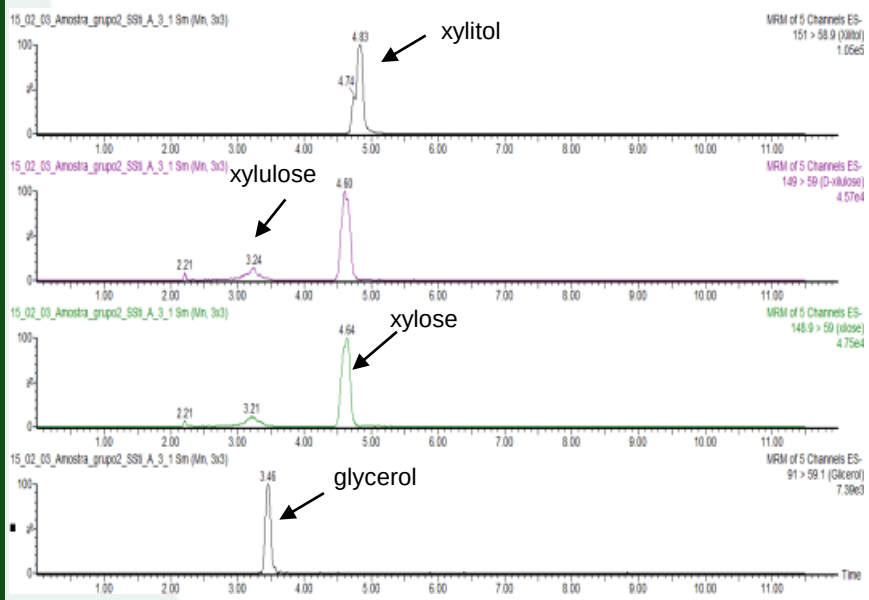
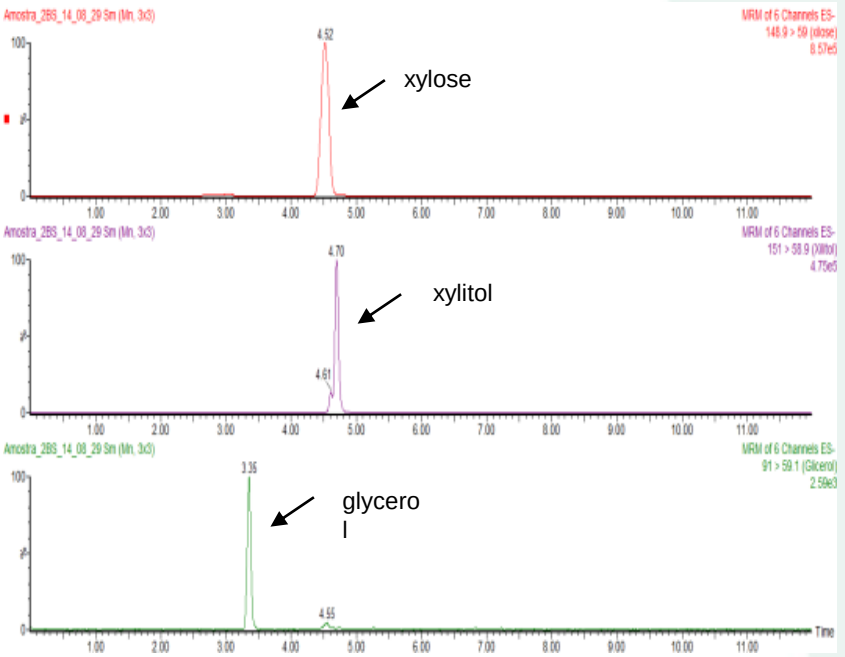
MRM of *Scheffersomyces stipitis*



Metabolites detected: a) xylitol; b) xylulose; c) xylose; d) glycerol.

MRM of *Spathaspora passalidarum*



Metabolites detected: a) xylose; b) xylitol; c) glycerol.

YEAST METABOLITES DETECTION BY UHPLC-MS/MS METHODS

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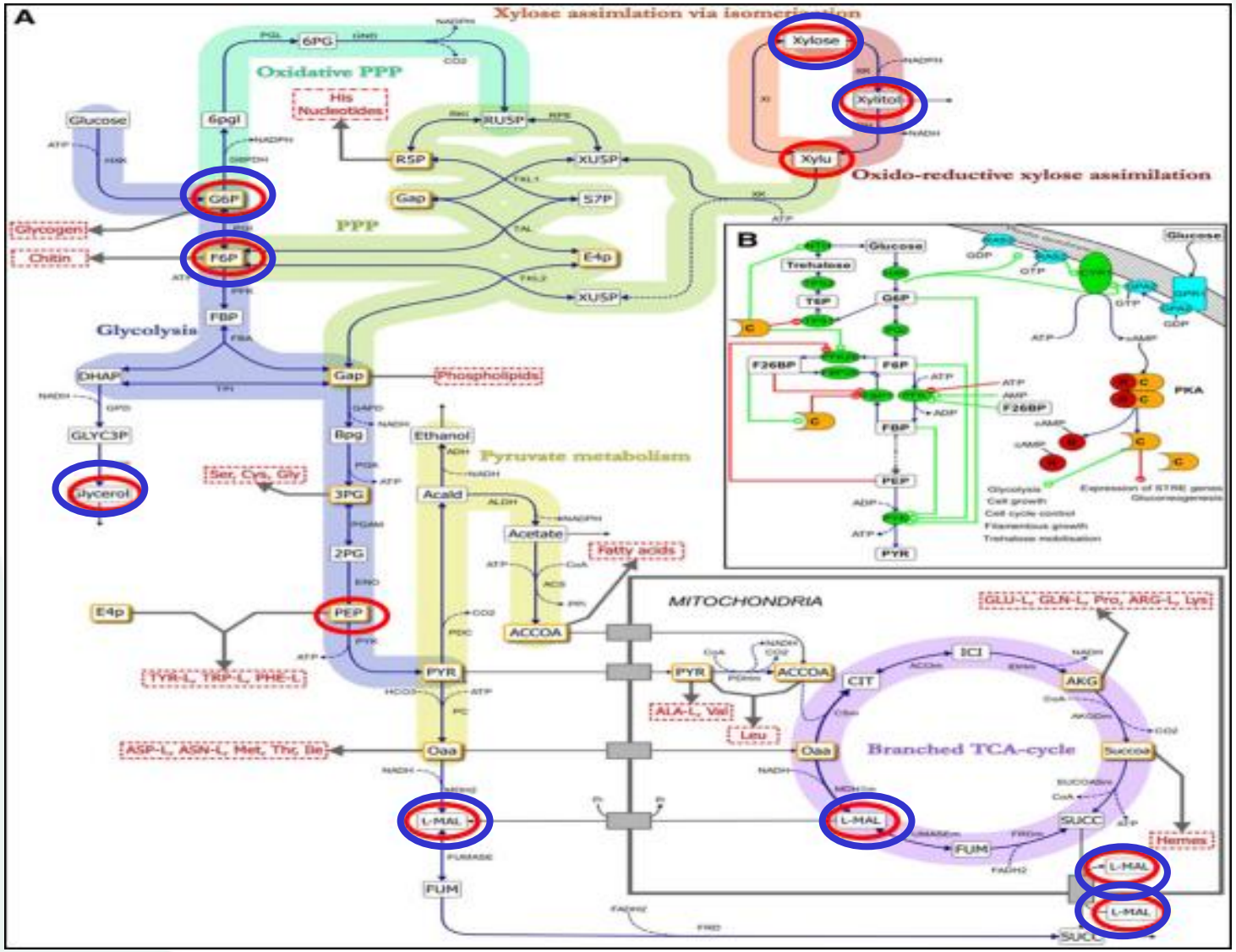
✓ *Scheffersomyces stipitis* and *Spathaspora passalidarum*



CONCLUSIONS

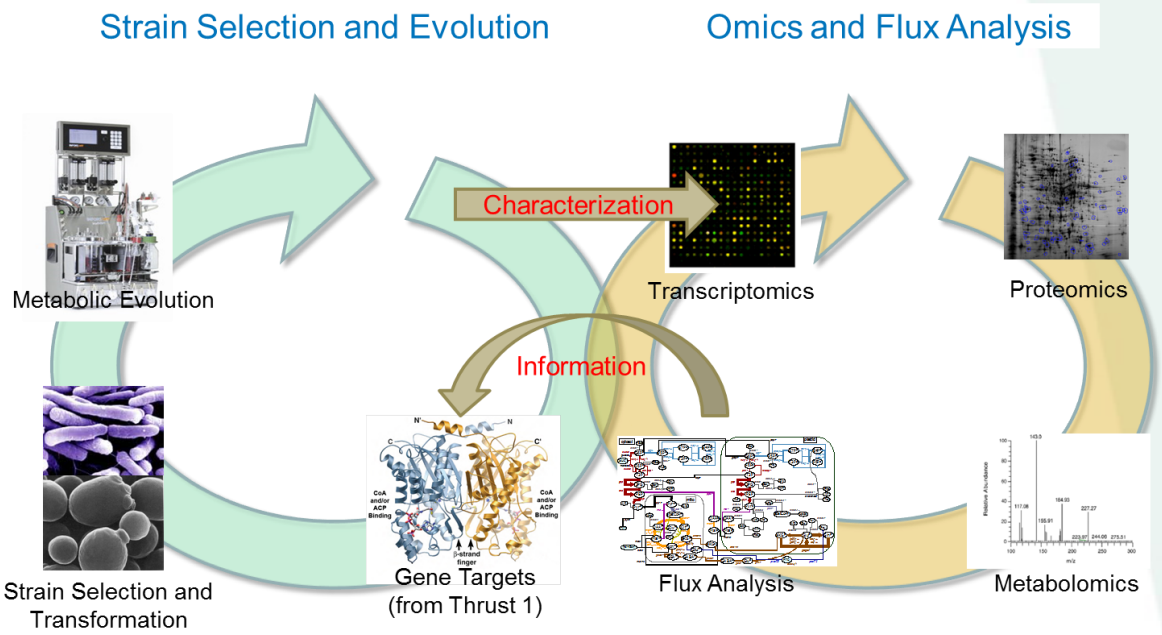
- **Development of LC-MS/MS method – 14 METABOLITES**
- There is no one method for all metabolites – 2 methods
- HILIC: glucose, xylitol, xylulose, xylose, glycerol
- IPC: F6P, G6P, R5P, DHAP, GAP, L-MAL, AKG, PEP, PYR

- **Optimization of sample preparation steps**
- Quenching
- Metabolites extraction

- **Yeast samples analysis**
- *Scheffersomyces stipitis* - 8 metabolites
- *Spathaspora passalidarum* - 6 metabolites
- Microaerobic condition – TCA cycle

PERSPECTIVES

- Fermentation of yeasts using anaerobic condition
- Metabolomics method validation
- Metabolomics of other yeasts
- Transcriptomics
- Genomics
- Flux Analysis
- Targets identification
- Metabolic engineering



- **Powerful yeast strains to convert xylose to ethanol**

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Thank you!!!

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