

Saccharomyces ludwigii

Uma levedura sóbria

Ms Marcelo Calide Barga

Dra Bianca Eli Della Bianca

Bio4 Soluções Biotecnológicas Ltda

10º Workshop de Cervejas Especiais

01 de agosto de 2019 – Cooperativa Agrária – Entre Rios-PR



NAB / LAB

No Alcohol Beer (NAB)

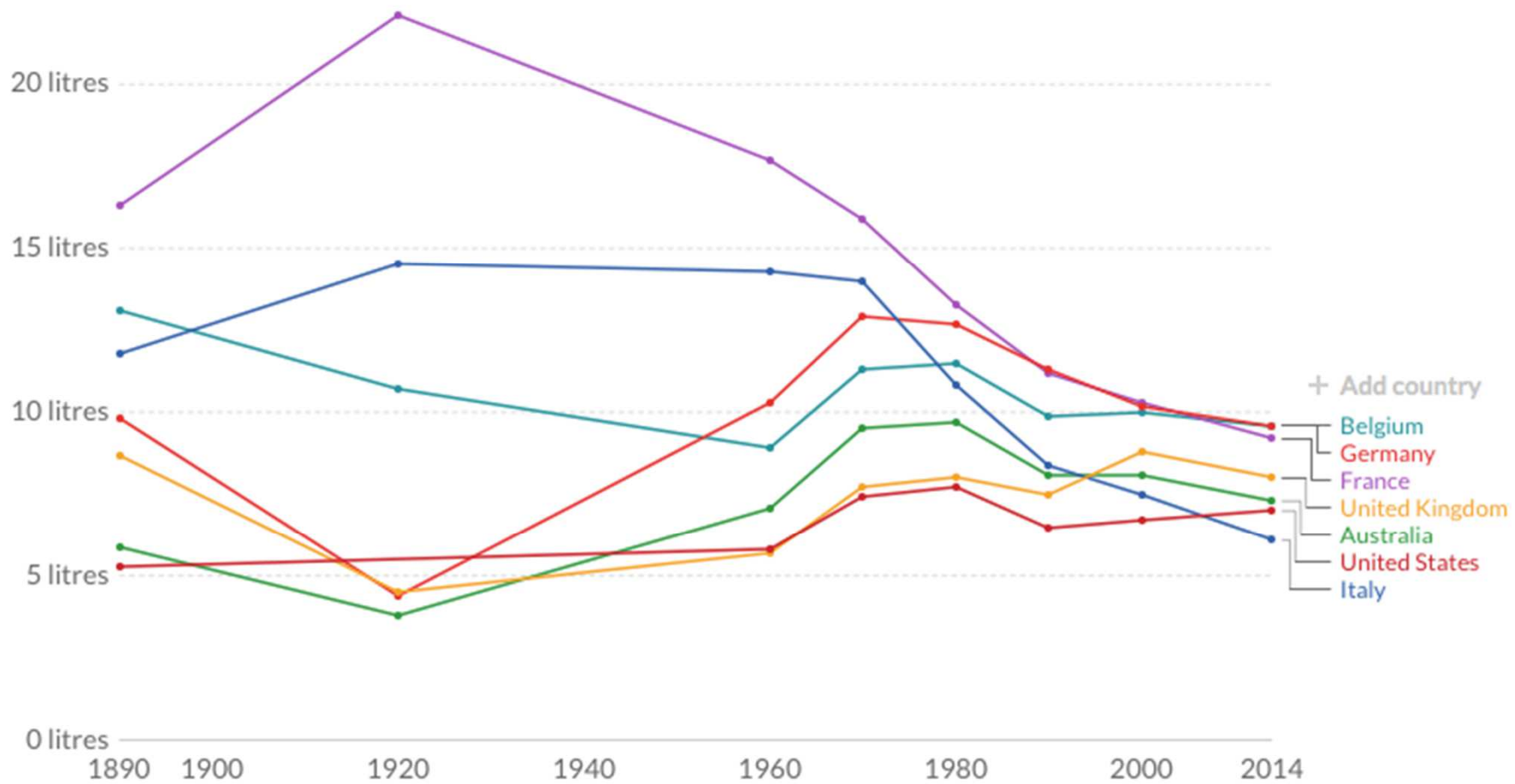
Alcohol-free beer, near beer, small beer,
dealcoholized beer
(0,0% - 0,5% ABV)

Low Alcohol Beer (LAB)

Low alcoholic beer, low alcohol beer,
low-point beer, alcohol-reduced beer, light
beer
(0,6% - 3,5% ABV)



Consumo per capita de álcool



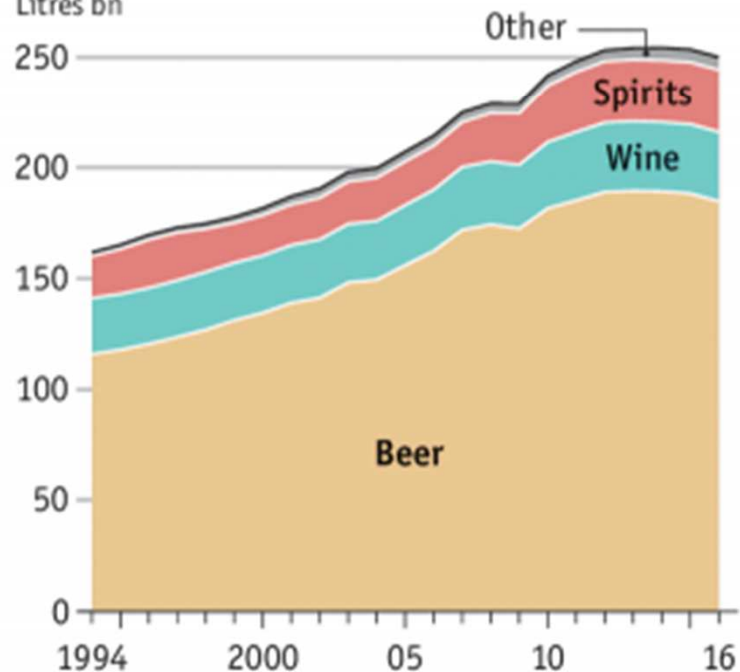
<https://ourworldindata.org/alcohol-consumption>

Mudança de comportamento?

Drying up

Global alcohol consumption

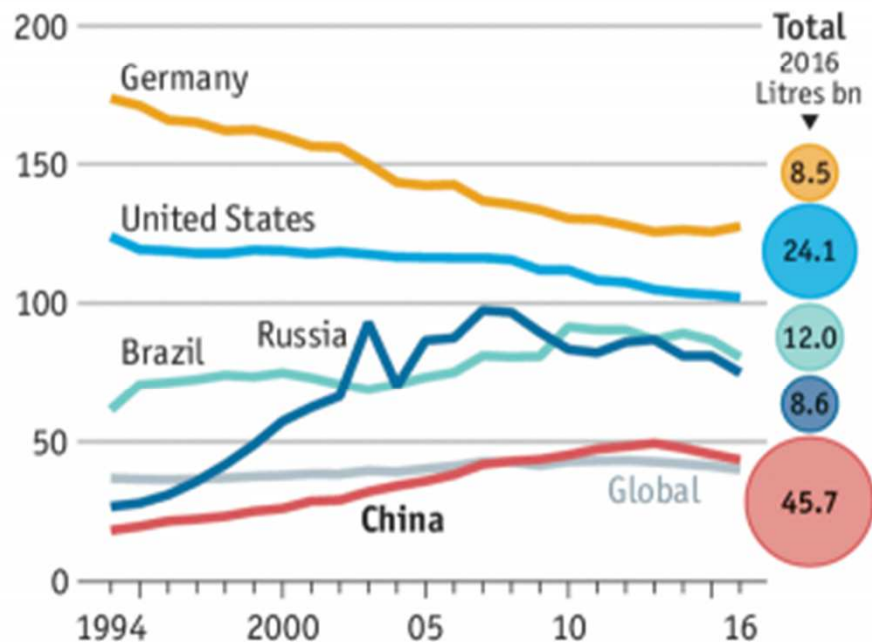
Total
Litres bn



Source: IWSR

Largest beer markets by volume in 2016

Litres consumed per adult



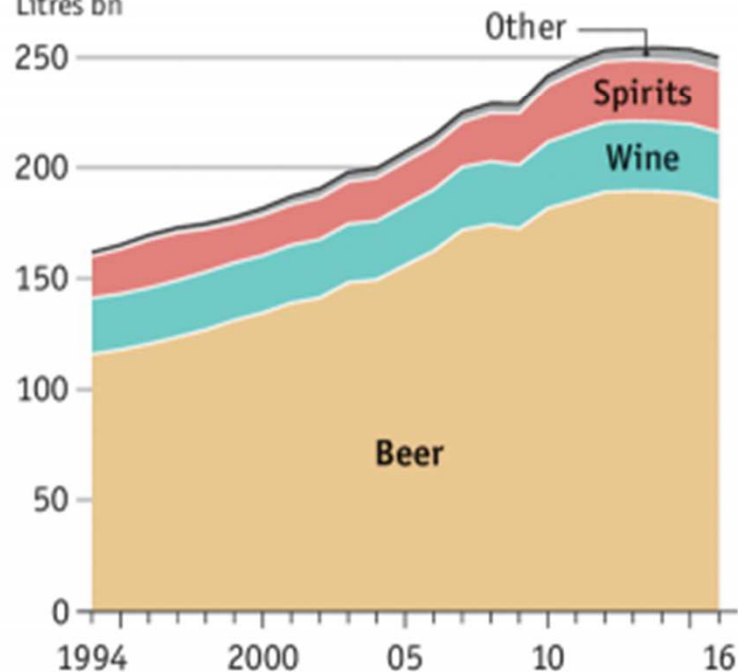
Economist.com

Mudança de comportamento?

Drying up

Global alcohol consumption

Total
Litres bn



Source: IWSR

Aumento da preocupação com saúde e bem-estar

Opção por diminuir o consumo de álcool

Questões religiosas

Leis de trânsito mais restritas (tolerância zero)

Proibição da venda de bebidas alcoólicas em alguns locais

Produtos no mercado



Produtos no mercado



Weiss



Ale

Lager



Panorama do mercado

“The zero alcohol segment in Europe and Russia grew 5% CAGR between 2010 and 2015. We expect this growth to continue (...)”

HEINEKEN

“Cerveja sem álcool ajuda Heineken a ter o melhor desempenho em 10 anos”

Valor Econômico

“We do not expect the category growth to abate. There’s been a dramatic rise in the non-alcoholic beer segment in the market, and there’s no surprise.”

AB InBev

“Garantir que produtos sem ou com baixo teor alcoólico representem pelo menos 20% do volume global de cerveja da AB InBev até o fim de 2025”

Metas Globais de Consumo Inteligente, AB InBev

6.7% of the estimated annual beer consumed in Germany contains no alcohol. Germany’s 1,500 breweries now produce 400 to 500 alcohol-free varieties of beer.

Deutscher Brauer-Bund

Panorama do mercado

Global beer market sizes historical/forecast
total volume million litres

Source: Euromonitor

Category	2015	2016	2017	2018	2019
Beer	195,875.8	194,408.8	196,203.4	198,657.3	200,993.1
Dark beer	8,374.4	8,618.8	8,846.3	9,095.9	9,373.1
Ale	4,600.5	4,767.6	4,884.0	5,020.7	5,156.5
Sorghum	1,271.1	1,302.0	1,312.7	1,324.3	1,338.6
Weissbier/weizen/ Wheat beer	2,502.8	2,549.2	2,649.6	2,750.9	2,878.0
Lager	182,209.3	180,272.8	181,640.2	183,585.4	185,346.5
Non-alcoholic beer	3,557.3	3,718.8	3,868.1	4,052.3	4,261.6
Stout	1,734.7	1,798.4	1,848.9	1,923.6	2,011.9

+2,6
%

+19,8%

<https://www.thedrinksbusiness.com/2018/11/in-focus-top-trends-in-lower-alcohol-beer/>

Estratégias de mercado



Le Tour de France,
1927

@wlc magazine



@life_with_navelski
@harpoonbrewery

Estratégias de mercado

TAP3 Schneider Weisse Mein alkoholfrei

OG 6,5% | 13 IBU | <0,5% ABV

Apoio ao ciclismo



Wheat beer bursting with energy

The refreshing isotonic recovery drink.



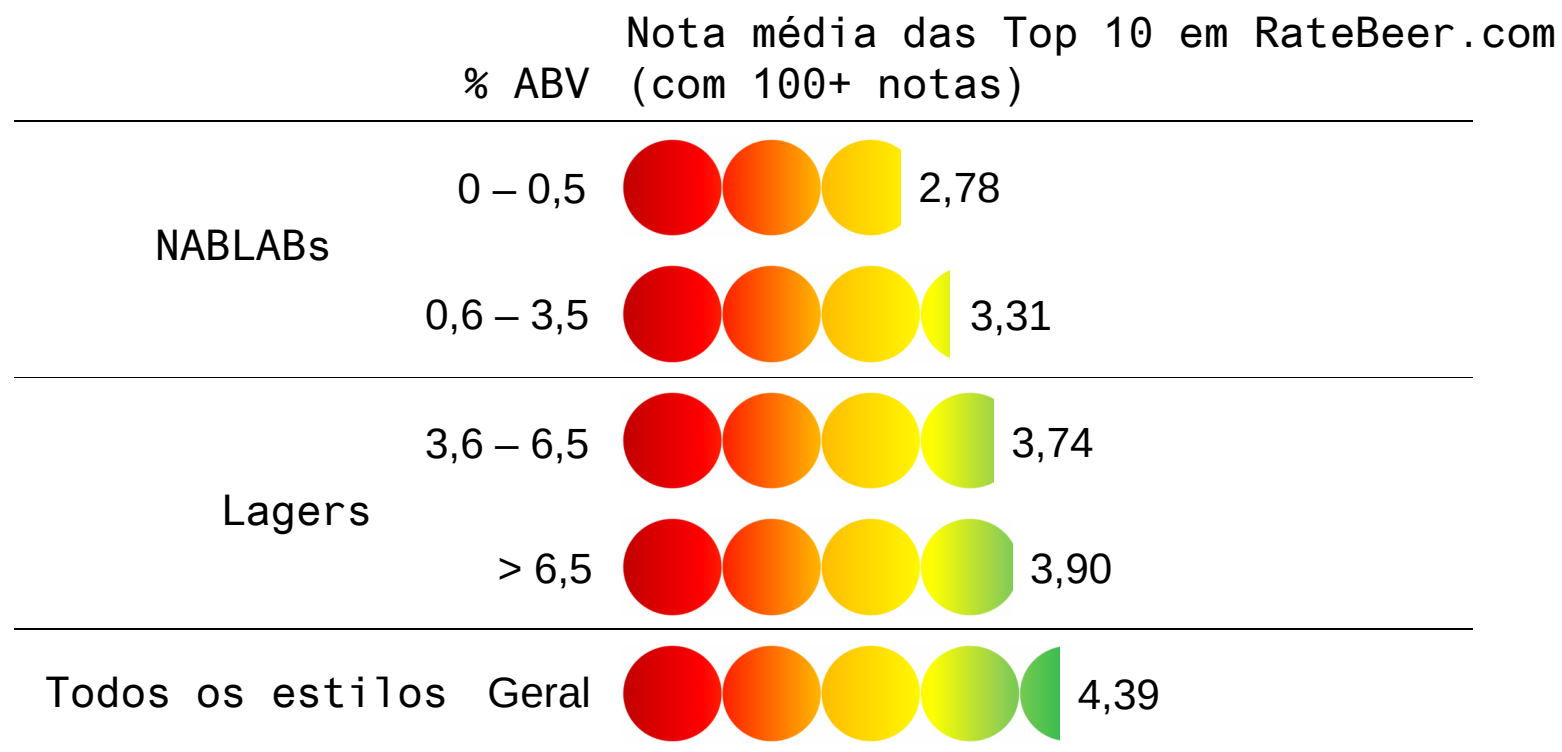
Erdinger Alkoholfrei

OG 7,2 °P | FG 5,3 °P | <0,5%

ABV

*Isotônico fonte de vitamina B,
ácido fólico e minerais*

Desafios sensoriais



Baseado em Bellut & Arendt
(2019)

Desafios da legislação

Lei 8.918/94, regulamentada pelo Decreto 6.871/09 (MAPA)

Artigo 12. As bebidas serão classificadas em:

I – bebidas não alcoólicas: é a bebida com graduação alcoólica até meio por cento em volume, a vinte graus Celsius, de álcool etílico potável, a saber:

- a) bebida não fermentada não alcoólica; ou
- b) bebida fermentada não alcoólica

Artigo 11. O rótulo da bebida deverá conter, em cada unidade, sem prejuízo de outras disposições da lei, em caracteres visíveis e legíveis, os seguintes dizeres:

IX – graduação alcoólica, expressa em porcentagem de volume alcoólico, quando bebida alcoólica

Desafios da legislação

Cerveja com baixo teor alcoólico não pode levar a expressão “sem álcool”

Da Redação [27/10/2016] [12:05]

Gazeta do Povo

PROPAGANDA ENGANOSA

Ambev terá de pagar R\$ 1 milhão por vender Kronenbier como cerveja sem álcool

12 de outubro de 2014, 9h41



[Imprimir](#)



[Enviar](#)



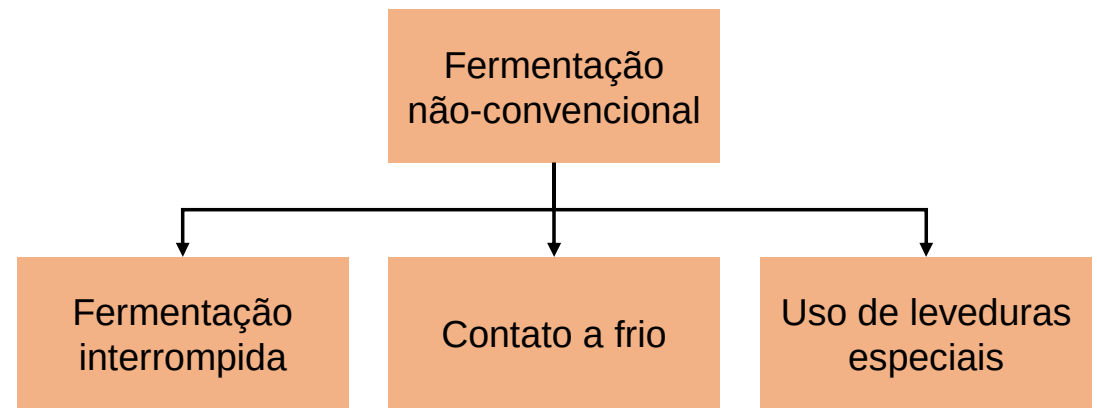
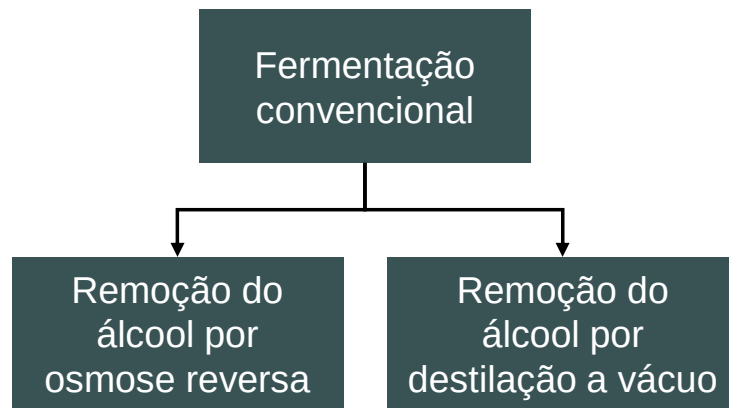
Consultor Jurídico

25/10/2016 às 05h00

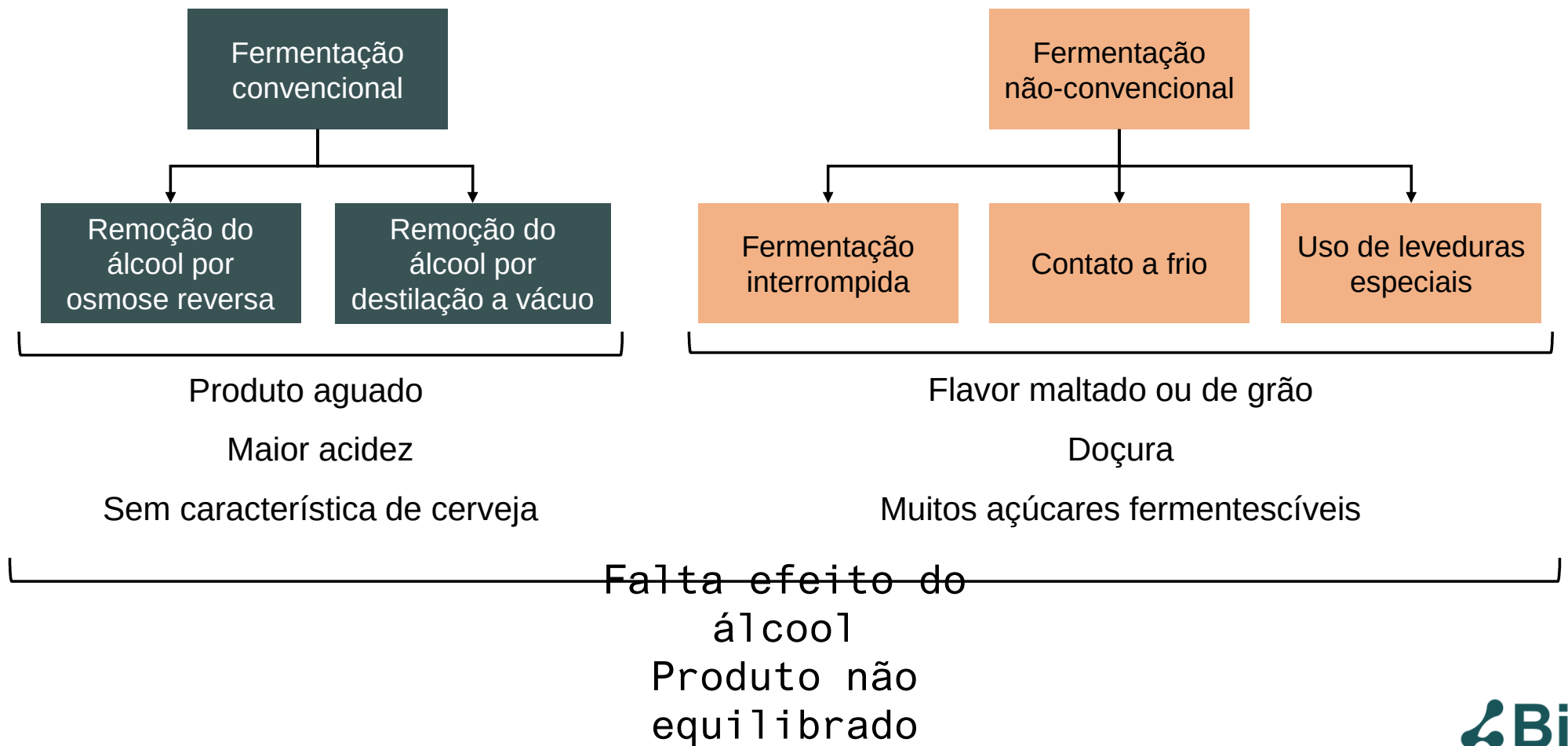
Kaiser perde disputa sobre cerveja 'sem álcool'

Valor Econômico

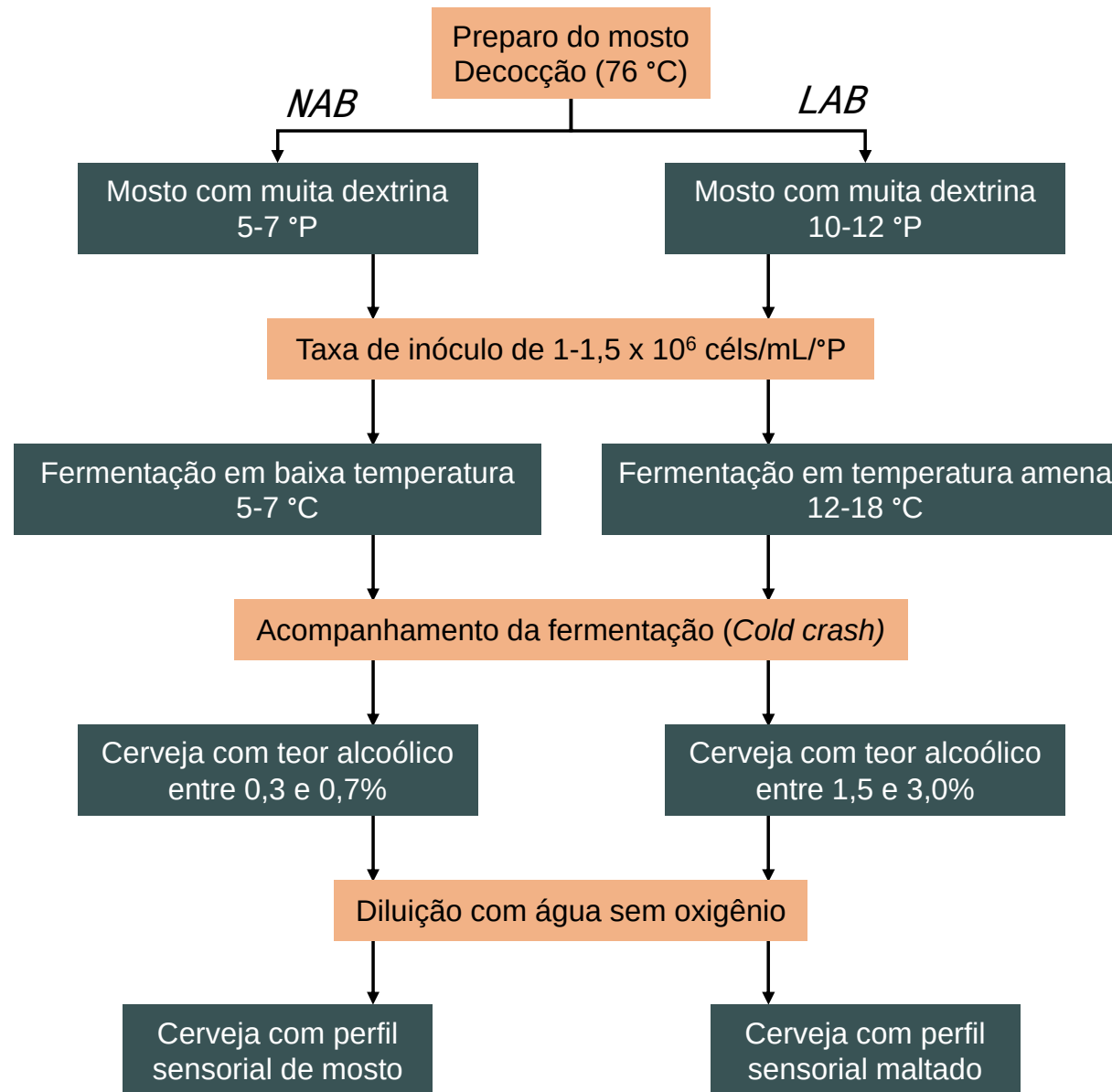
Processos de produção



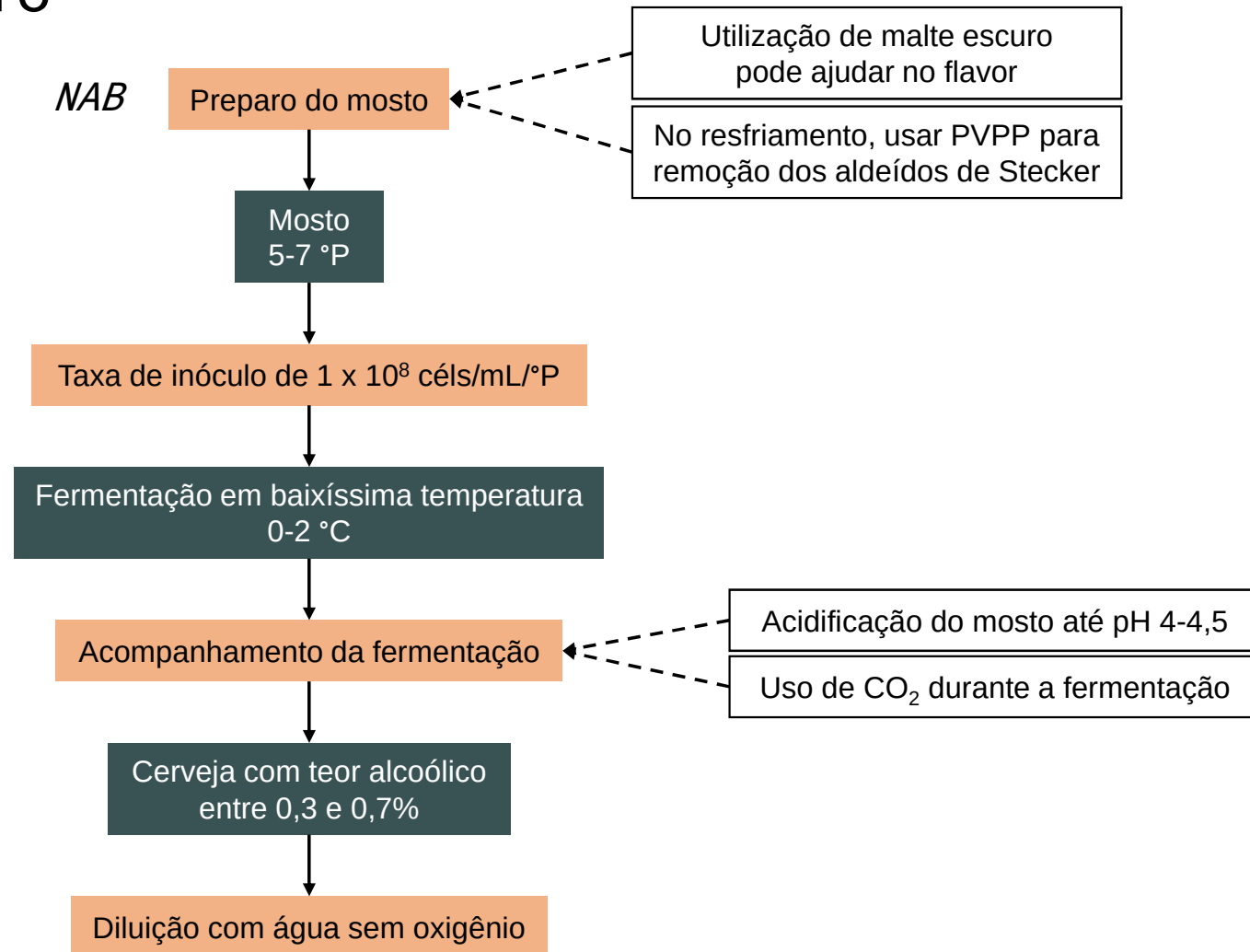
Características não vantajosas



Fermentação interrompida



Contato a frio



Uso de leveduras especiais: não-

Table 2. Non-Saccharomyces strains in wort substrates.

Yeast species	Strain designation	Wort gravity °P	Scale L	Ethanol content % ABV	Fermentation conditions Time (d) / Temperature (°C) / Pitching rate ($\times 10^6$ cells/mL)	Secondary metabolites Σ Esters (mg/L) / Σ Higher alcohols (mg/L)	Sensory	Reference
<i>Candida shehatae</i>	CICC ^a 1766	9	0.3	0.47	NA / 14 / NA	NA	NA	[66]
		9	200	0.37	NA / 14 / NA			
<i>Candida zemplinina</i>	Y.01670 ^b	12 E	0.35	Ca 1.5	8 / 18 / NA	NA	NA	[64]
<i>Candida zemplinina</i>	Y.01670 ^b	12 E	0.35	1.67	8 / 18 / NA	NA	NA	[65]
<i>Cyberlindnera fabianii</i>	9 strains ^c	12 E	0.1	0.6	7 / 20 / NA	40:60 ratio	NA	[69]
<i>Cyberlindnera mrakii</i>	NCYC ^d 500	13.80 E ^e	0.4	1.7	14 / 21 / 0.1	NA	NA	[70]
<i>Hanseniaspora valbyensis</i>	^f	6.6	1.5	0.35	2 / 25 / 8	0.90 / 23.30	Wort-like, honey-like, cereal-like, diacetyl	[62]
<i>Hanseniaspora vineae</i>	^f	6.6	1.5	0.34	2 / 25 / 8	6.00 / 20.2	Wort-like, honey-like, black tea, caramel	[62]
<i>Mrakia gelida</i>	DBVPG ^g 5952	12	25	1.16	22 / 10 / 0.4	0.6 / 25.5	Fruity (apricot, grape, litchi) , malty, hoppy,	[61]
				1.40	15 d bottle-conditioned	3.5 / 27.7		
<i>Pichia kluyveri</i> LAB	PK-KR1 ^h	8.3	1500	0.7	21 / 21 / 5	2.9 / 1.8	NA	[60]
<i>Pichia kluyveri</i> NAB	PK-KR1 ^h	8.3	1000	0.1	21 / 20 / 5	2.5 / 2.0	Preference over commercial unfermented NAB	[60]
	PK-KR2 ^h			0.2		5.4 / 2.0		
<i>Pichia kudriavzevii</i>	24 strains ^c	12 E	0.1	0.5–0.8	7 / 20 / NA	50:50 ratio	NA	[69]
<i>Torulaspora delbrueckii</i>	DiSVA ⁱ 254	12.7	0.5	2.66	NA / 20 / NA	3.80 / 58.54	Fruity, citric, full-bodied	[74]
<i>Torulaspora delbrueckii</i>	DiSVA ⁱ 254	12.3	0.5	2.62	NA / 19 / 5	4.56 / 61.99	NA	[75]
<i>Torulaspora delbrueckii</i>	^f	6.6	1.5	0.50	3 / 25 / 8	0.77 / 18.1	Wort-like, honey-like,	[62]
<i>Torulaspora delbrueckii</i>	9 strains from various depositors	12 E	2	0.83–0.94	NA / 27 / 15	2.23–5.96 / 20.53–36.79	Honey, pear-like, citrusy	[73]
<i>Torulaspora delbrueckii</i>	^f	12.2	100	2.34	9 / 20 / NA	NA	Ester/fruity, high in body	[76]
<i>Zygosaccharomyces bailii</i>	^f	6.6	1.5	0.42	4 / 25 / 8	1.00 / 23.1	Wort-like, honey-like, grassy, fruity, white wine	[62]
<i>Zygosaccharomyces kombuchaensis</i>	^f	6.6	1.5	0.48	4 / 25 / 8	1.00 / 22.0	Wort-like, honey-like, diacetyl	[62]
<i>Zygosaccharomyces rouxii</i>	5 DBVPG ^g strains	12	0.05	0.93–3.32	10 / 20 / NA	2.16–71.15 / 61.80–196.77	NA	[58]
<i>Zygosaccharomyces rouxii</i>	DSM ^k 2531 ^l	6.5	0.2	2.0	10 / 7 / NA	NA	NA	[55]
				3.3	7 / 12 / NA			
<i>Zygosaccharomyces rouxii</i>	DSM ^k 2531	6	NA	0.36–0.40	4 / 12,24 / 10 ^m	NA	NC	[77]
	DSM ^k 2535							
<i>Zygosaccharomyces rouxii</i>	DSM ^k 70535, DSM ^k 70531	6	NA	0.04–0.40	2 / 4,12,24 / 10,40 anaerobic or periodic aeration	NA	Low acceptance	[56]

NA, not available; E, wort from malt extract; NC, not comparable.

^aChina Industrial Culture Collection; ^bNational Collection of Agricultural and Industrial Microorganisms, Hungary; ^cisolated from fermented masau fruit [89]; ^dNational Collection of Yeast Cultures, Norwich, UK; ^eplus addition of glucose; ^fisolated from kombucha; ^gIndustrial Yeast Collection, DBVPG (University of Perugia, Italy); ^hNational Measurement Institute, South Melbourne, Australia; ⁱYeast Collection of the Department of Life and Environmental Sciences (DiSVA), Polytechnic University of Marche, Italy; ^jisolated from wine; ^kDeutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; ^limmobilized on brewer's spent grain (BSG); ^msecondary fermentation after *S. cerevisiae* (48 h).

Bellut & Arendt (2019)

Uso de leveduras especiais: não- *Saccharomyces*

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		9	200			
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<i>Zygosaccharomyces rouxii</i>	DSM ^k 2531 ⁱ	6.5	0.2			[55]
<i>Zygosaccharomyces rouxii</i>	DSM ^k 2531	6	NA	0.3		[77]
	DSM ^k 2535					
<i>Zygosaccharomyces rouxii</i>	DSM ^k 70535, DSM ^k 70531	6	NA	0.0	otance	[56]

NA, not available; E, wort from malt extract; NC, not comparable.

^aChina Industrial Culture Collection; ^bNational Collection of Agricultural and Industrial Micro-organisms; ^cYeast Collection of the Department of Life and Environmental Sciences (DiSVA), Polytechnic University of Marche, Italy; ^disolated from wine; ^eDeutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; ^fimmobilized on brewer's spent grain (BSG); ^gsecondary fermentation after *S. cerevisiae* (48 h).

Bellut & Arendt (2019)

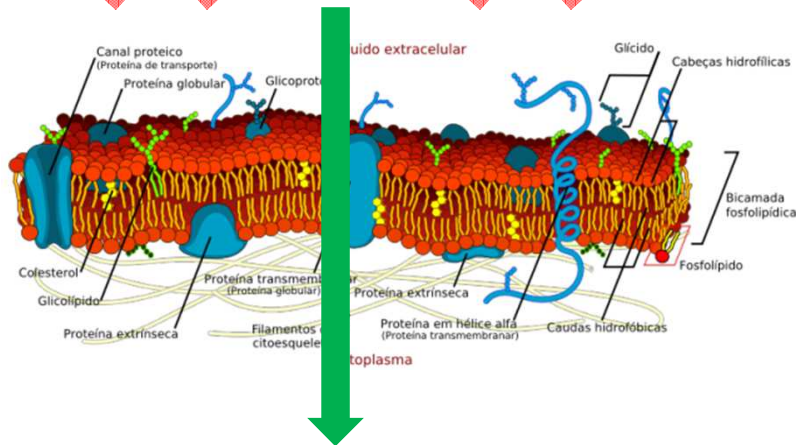
Uso de leveduras especiais: *Saccharomyces ludwigii*

MALTOSE

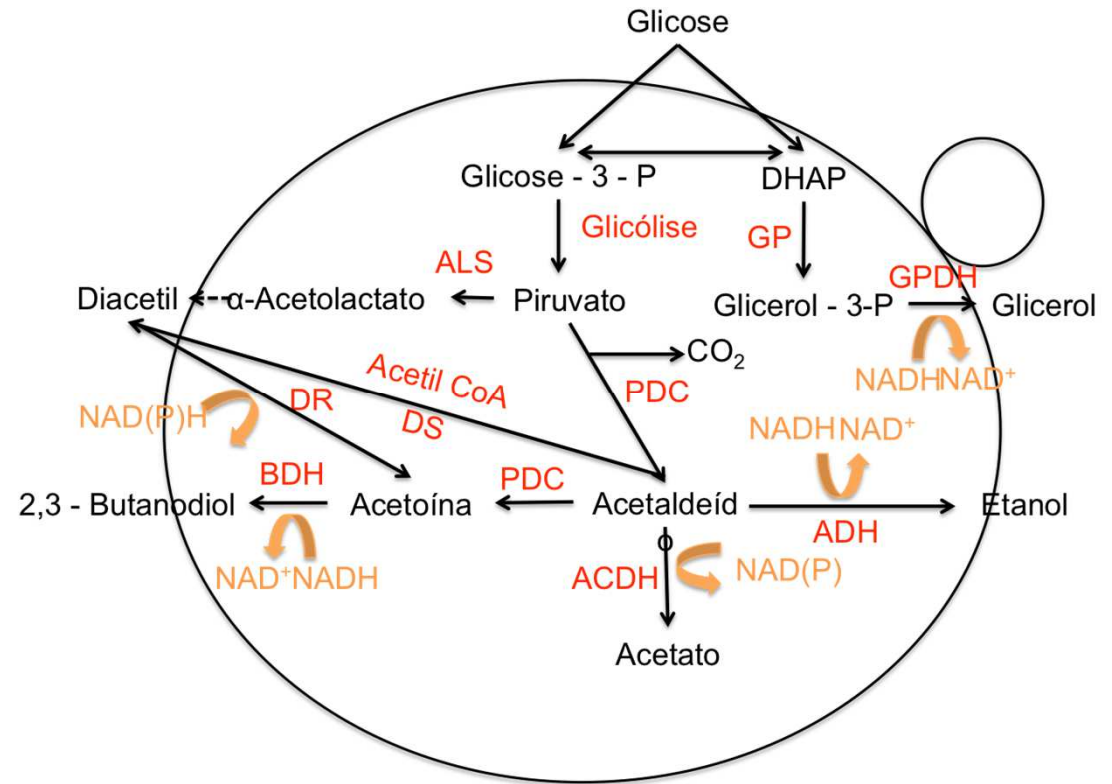
SACAROSE

MALTASE

INVERTASE



GLICOSE
FRUTOSE



Uso de leveduras especiais: *Saccharomyces ludwigii*

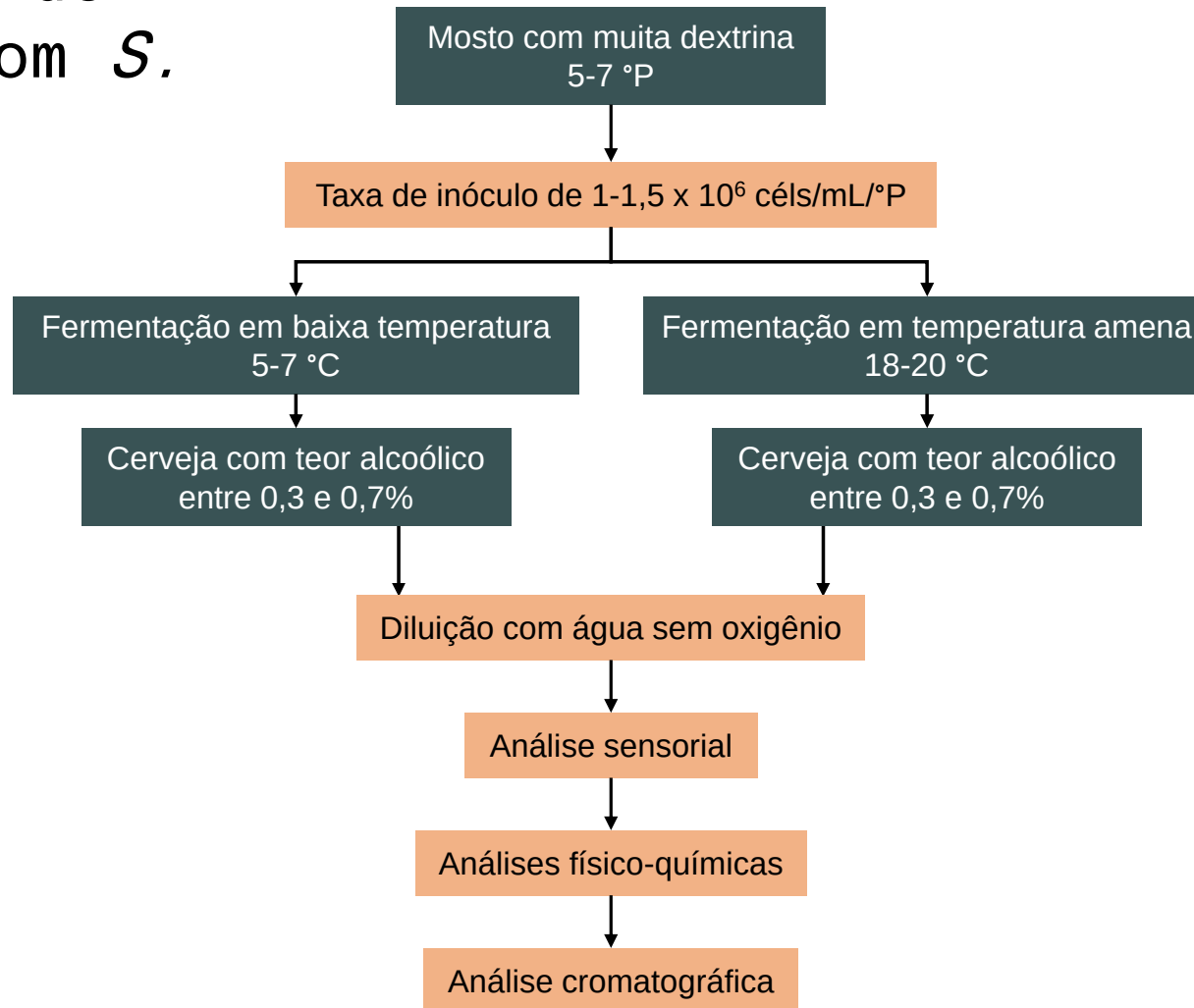
Table 1. *Saccharomyces ludwigii* strains in wort substrates.

<i>Saccharomyces ludwigii</i>	Wort gravity	Scale	Ethanol content	Fermentation conditions	Secondary metabolites	Sensory	Reference
Strain designation	°P	L	% ABV	Time (d) / Temperature (°C) / Pitching rate ($\times 10^6$ cells/mL)	Σ Esters (mg/L) / Σ Higher alcohols (mg/L)		
6 DPVPG ^a strains	12.0	0.05	0.51–1.36	10 / 20 / NA	1.21–14.92 / 43.31–76.62	NA	[58]
WSL 17 (=TUM SL 17 ^b)	12.0	25	1.23–1.32	10 / 23 / 0.4	9.3–14.9 / 42.2–43.4	Cereal, malty	[61]
NA	8.1	2	0.47	NA / 12 / NA	1.88 / 39.10	Weak aroma, sweet	[53]
#303 ^c	12.2	2000	<0.5	NA / 18 / 15	7.95 / 8.70	NA	[63]
DSM 3447 ^d	6.5	0.2	1.7	10 / 7 / NA	NA	NA	[55]
			2.7	7 / 12 / NA			
TUM SL 17 ^b	12.8	ca. 2	0.99–1.16	6 / 15&20 / 8	NA	NA	[57]
	7.0		0.50–0.62		0.75 / 22.94		
TUM SL 17 ^b	6.6	1.5	0.50	3 / 25 / 8	0.80 / 21.05	Worty, honey, bread-like, sweet	[62]
NA	11.5	NA	0.68	5 / 20 / NA	1.88 / 31.80	Worty taste, diacetyl	[52]
DSM 3447 ^e	6.0	NA	0.15–1.2	2 / 4 / 10 (periodic aeration) 2 / 24 / 40 (anaerobic)	NA	Low acceptance, lactic acid sourness; sweet and immature flavor	[56]
NA	7.0 E	0.5	0.3	5 / 20 / 1	NA	NA	[60]

NA, not available; E, wort from wort extract.

^aIndustrial Yeast Collection (DBVPG), University of Perugia, Italy; ^bResearch Center Weihenstephan for Brewing and Food Quality, Freising, Germany; ^cDoemens Academy, Germany; ^dimmobilized on brewers' spent grain (BSG); ^eDeutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany.

Estratégia de produção com *S.* *tudwigii*



Análise sensorial

Painel com 6 pessoas (teste vs controle)

Avaliação de intensidade de 16 parâmetros

Adstringência
Retrogosto
Efervescência
Turbidez
Consistência espuma
Persistência espuma
Cor espuma
Malte
Levedura
Caramelo
Banana/Frutado
Lúpulo
Amargor
Dulçor
Acidez
Corpo

Análises físico-químicas

Analytica
EBC
9.43.2

Álcool
(% m/m)

Álcool
(% v/v,
ABV)

Densida
de
(mg/mL)

Gravida
de
específ
ica (SG)

*remoção
do gás*



destilação



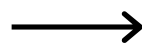
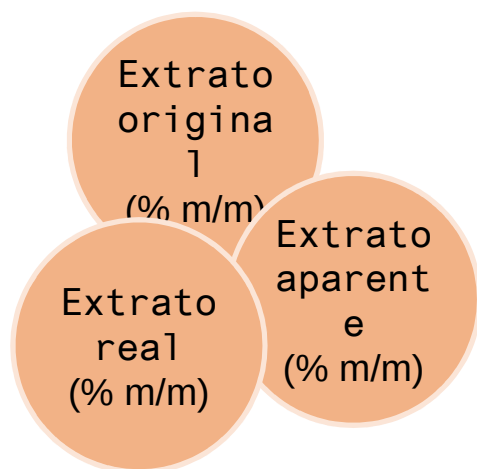
*leitura em densímetro
digital*



*leitura em densímetro
digital*

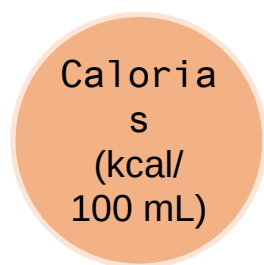
Análises físico-químicas

Analytica
EBC
9.4



*cálculo a
partir dos
dados de
álcool,
densidade e SG*

Analytica
EBC
9.45



*cálculo a
partir dos
dados de
álcool,
densidade e SG*

Análises físico-químicas

Analytica
EBC
9.6

Cor
(EBC)

remoção
do gás

diluição



leitura em
espectrofotômet
ro
a 430 nm

Analytica
EBC
9.8

Amargor
(IBU)

remoção
do gás



extração com
isooctano



leitura em
espectrofotômetro
a 275 nm

Análises físico-químicas

Analytica
EBC
9.24.1

Diceton
as
vicinai
s (VDK)



*remoção
do gás*



*destilaç
ão*



*reação com
o-
fenilenodiami
na em meio
ácido*



*leitura em
espectrofotômetro
a 335 nm*

Analytica
EBC
9.35

pH



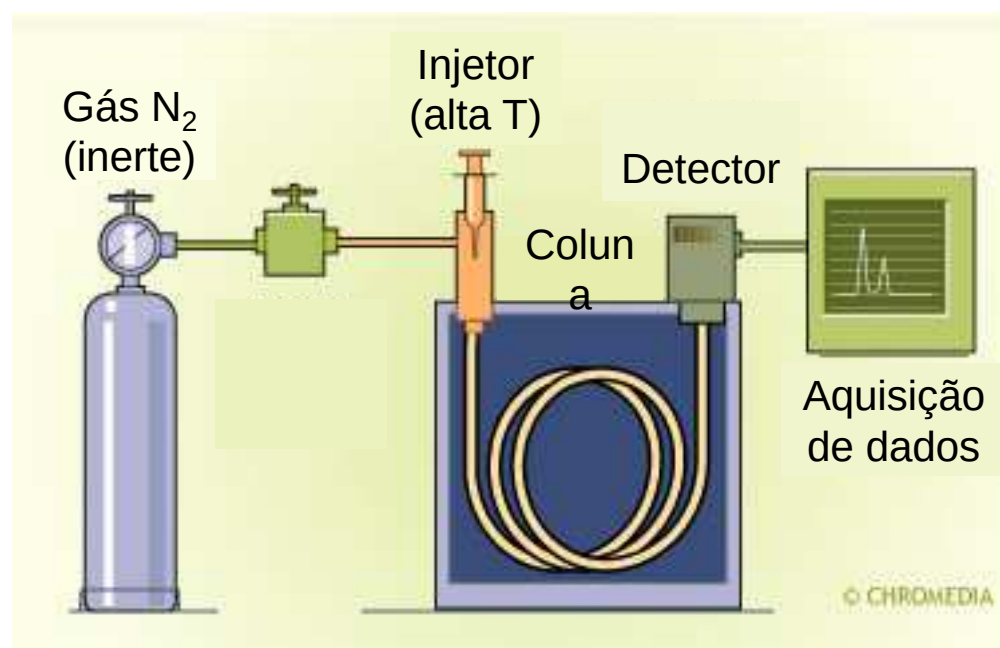
*remoção
do gás*



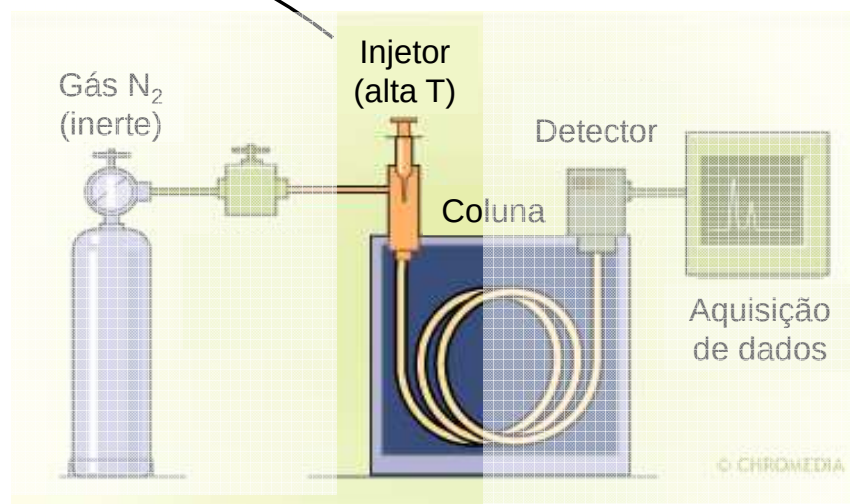
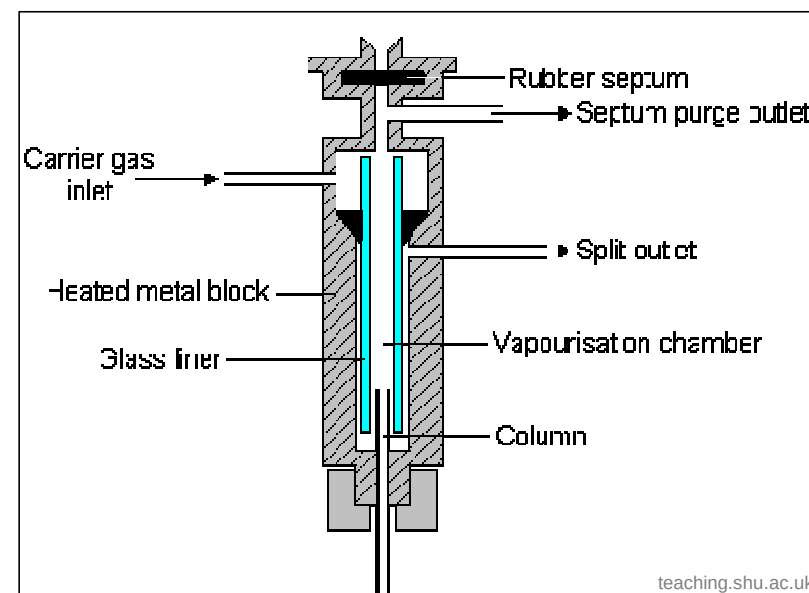
leitura em pHmetro

Cromatografia gasosa

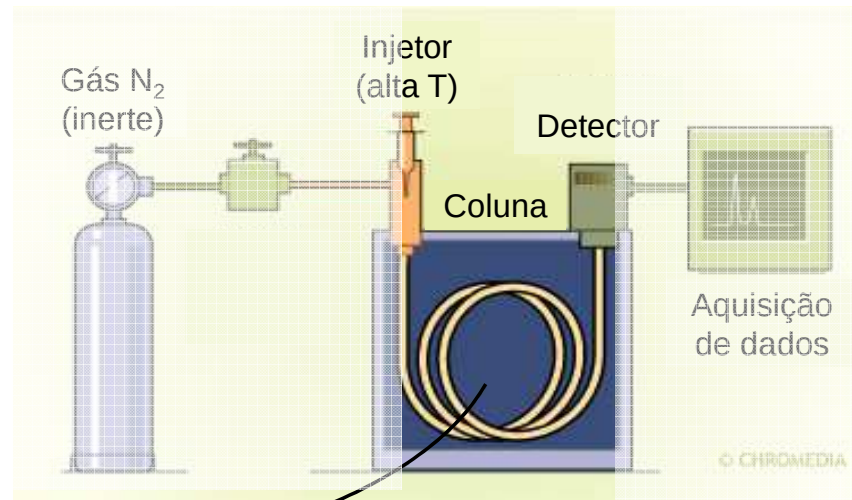
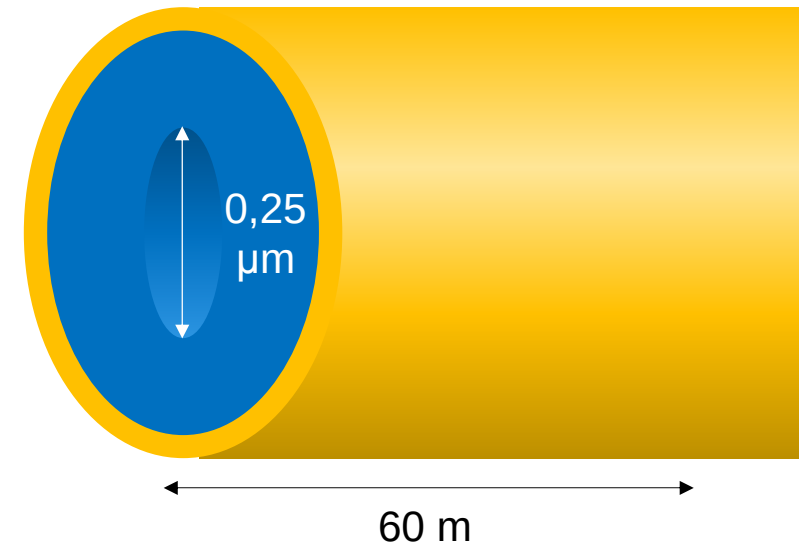
Adaptado de
Analytica EBC 9.39
e MAPA Método 14



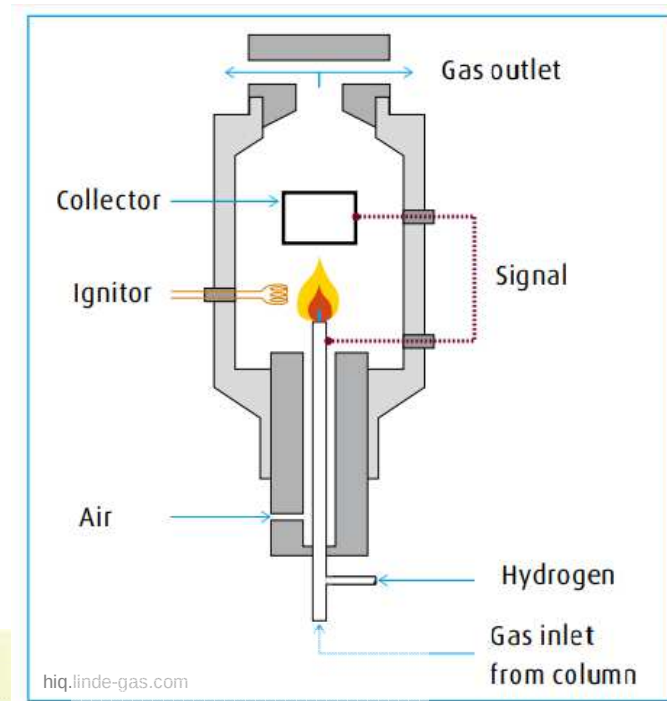
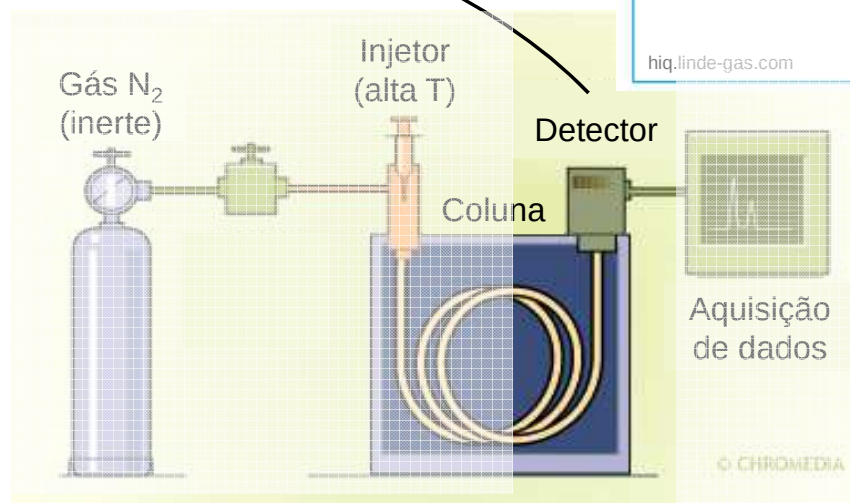
Cromatografia gasosa



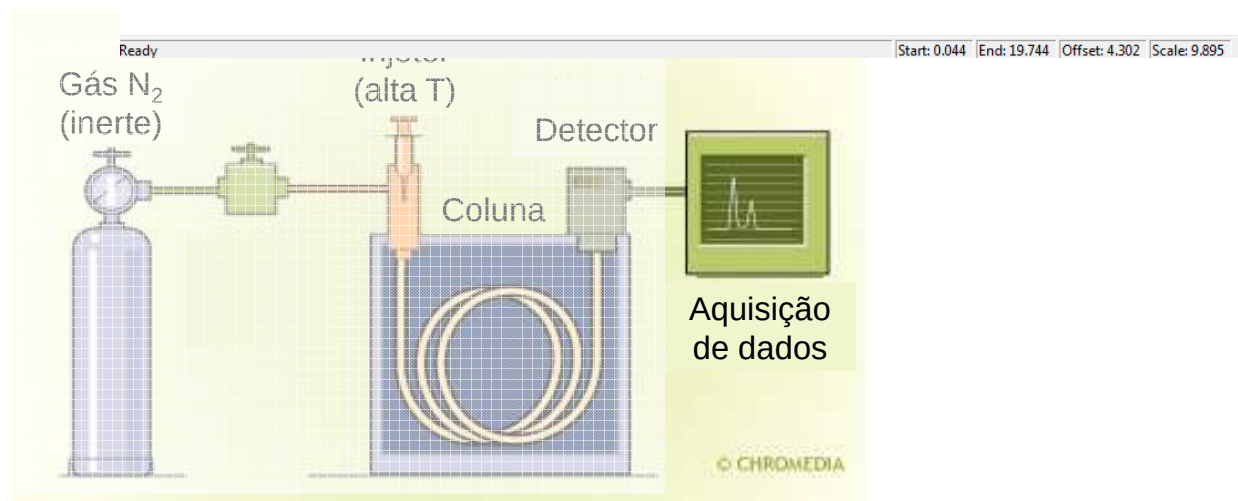
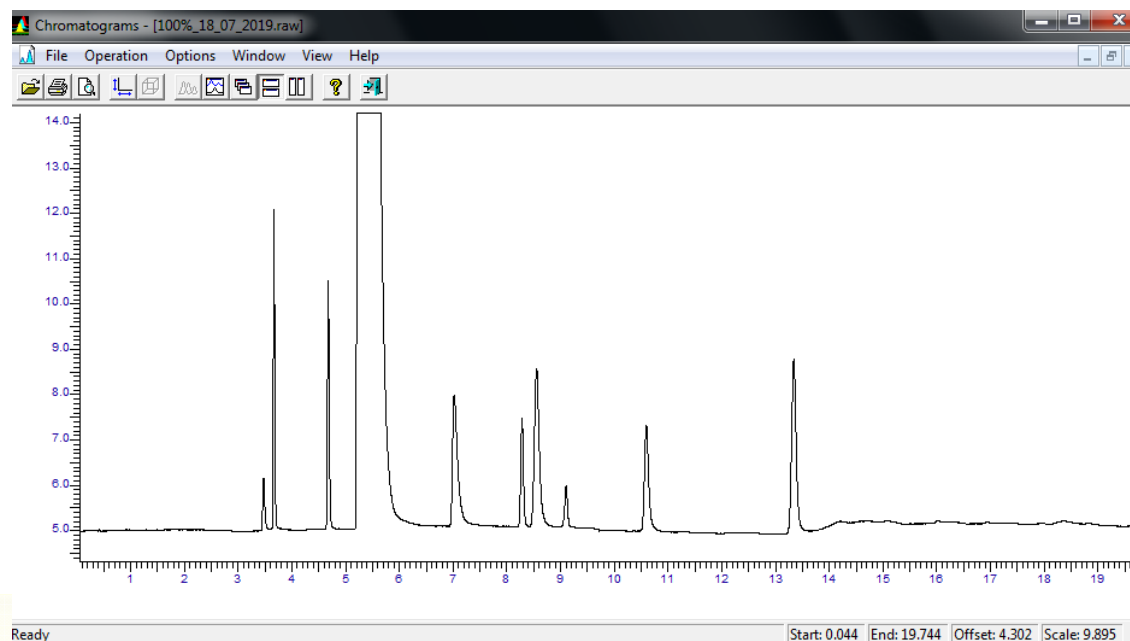
Cromatografia gasosa



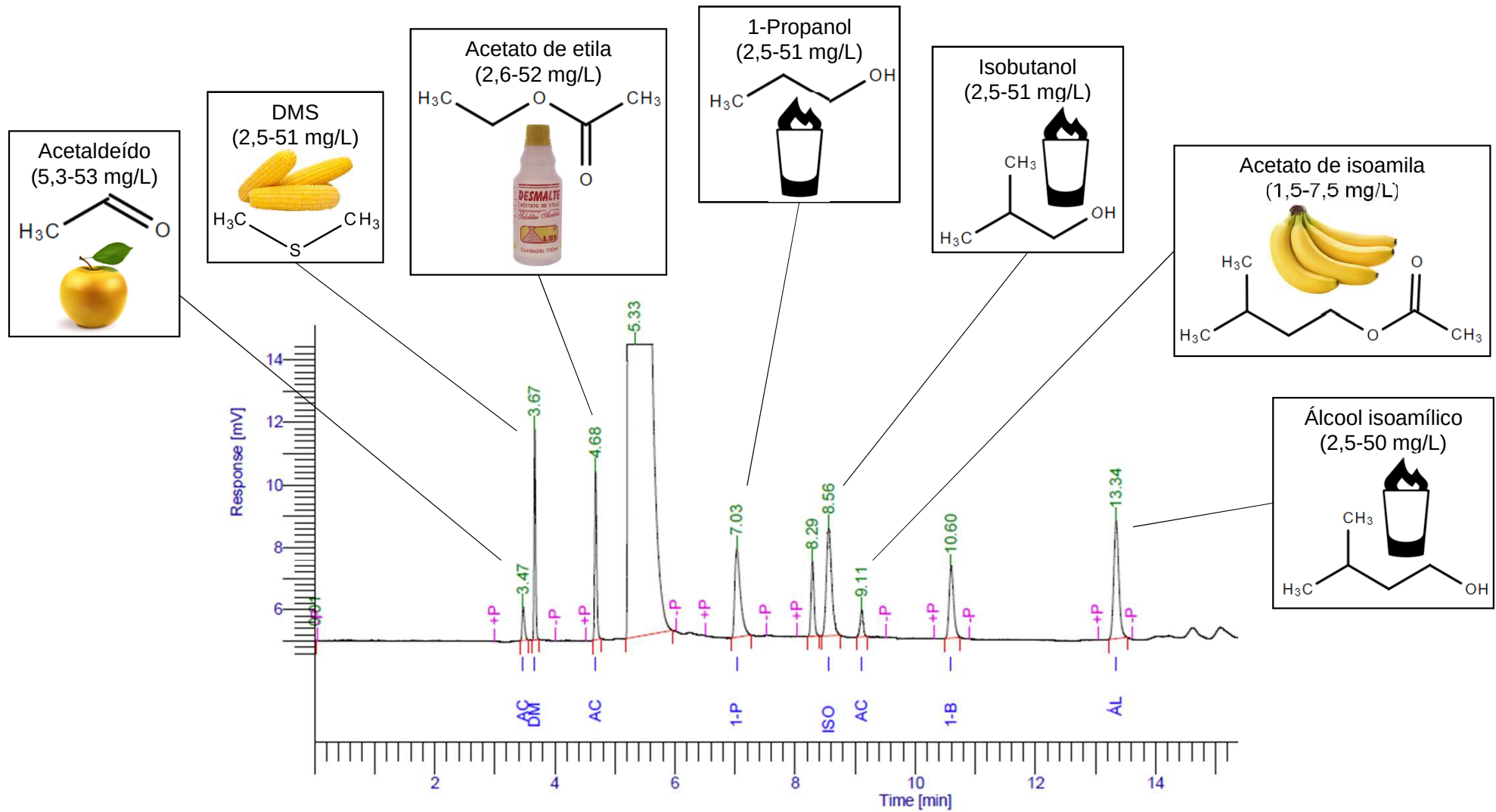
Cromatografia gasosa



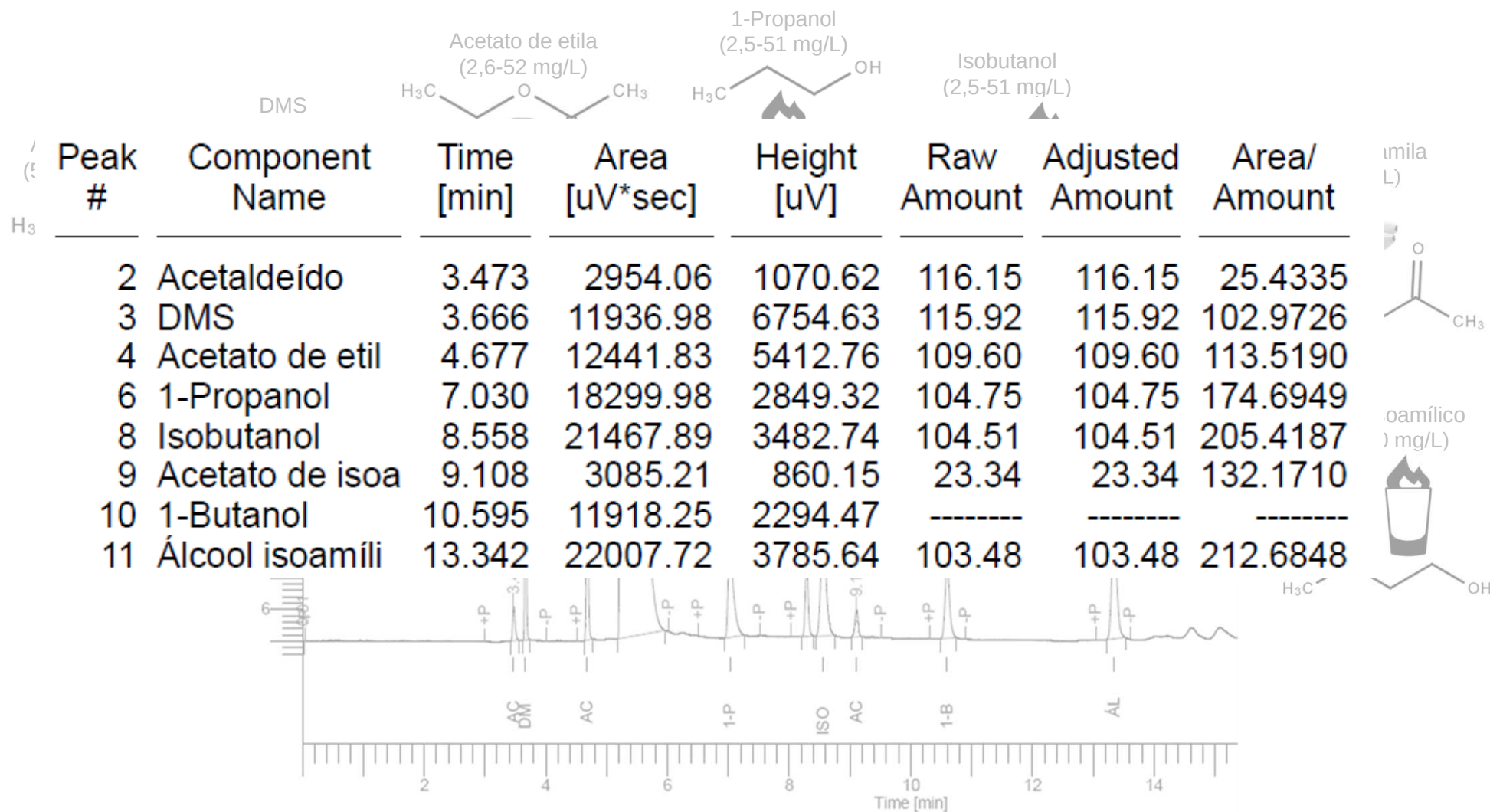
Cromatografia gasosa



Cromatografia gasosa



Cromatografia gasosa



Testes de cerveja sem álcool

IPA *NAB*
(Agrária)



Contato a frio

Pilsen *NAB*
(Way)



Saccharomyces
ludwigii

Pilsen *LAB*
(Way)



Controle

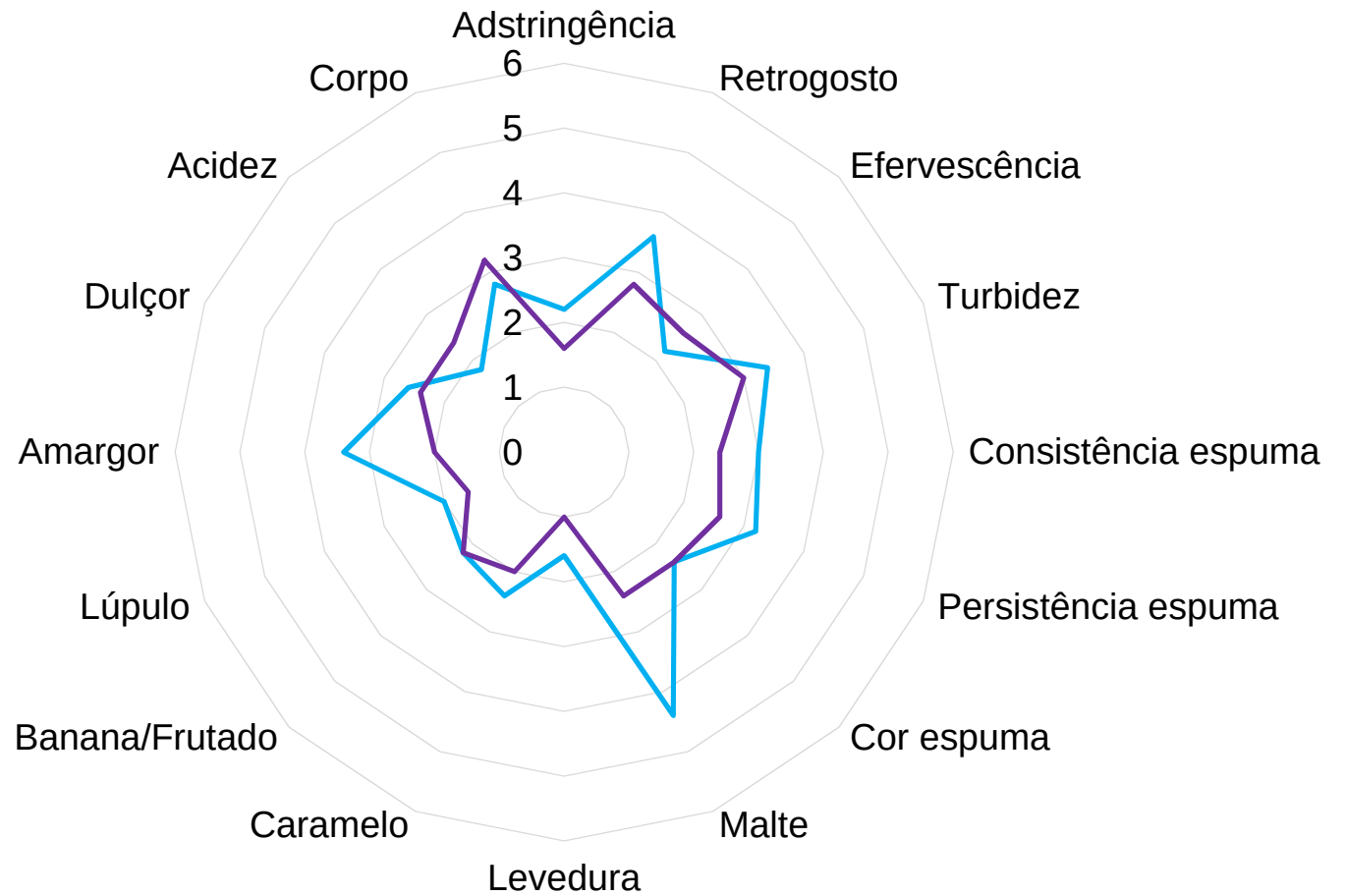
	IPA <i>NAB</i>	Pilsen <i>NAB</i>	Pilsen <i>LAB</i>
	Contato a frio	<i>Saccharomyces ludwigii</i>	Controle
Volume de teste	250 L	15 L	15 L
Malte	7,2 kg/hL Pale Ale 2,4 kg/hL Weyermann Carahell 2,0 kg/hL Crisp Dextrin 0,4 kg/hL Weyermann Caraaroma	8,8 kg/hL Weyermann Pilsner 1,1 kg/hL Château Abbey	8,8 kg/hL Weyermann Pilsner 1,1 kg/hL Château Abbey
Lúpulo (fervura)	90 g/hL Comet 62 g/hL Eldorado 57 g/hL Citra	133 g/hL Hallertauer Mittelfrueh 67 g/hL Magnum	133 g/hL Hallertauer Mittelfrueh 67 g/hL Magnum
Lúpulo (dry hopping)	100 g/hL Equinox 100 g/hL Eldorado 50 g/hL Comet	---	---
OG	7,2 °P (1,028 SG)	5,6 °P (1,022 SG)	5,6 °P (1,022 SG)
pH inicial	4,70 (ácido láctico)	4,82 (ácido láctico)	5,60
Inóculo	3,5 x 10 ⁶ céls/mL/°P Lallemand Nottingham	0,25 x 10 ⁶ céls/mL/°P Lallemand S. ludwigii BRY 437	2,5 x 10 ⁶ céls/mL/°P Bio4 SY001 Pilsner Lager
Fermentação	2 dias @ 4 °C	7 dias @ T ambiente	7 dias @ T ambiente
Maturação	3 dias @ 0 °C + CO ₂ Filtração 3 semanas @ 0 °C + CO ₂	10 dias @ 0 °C	10 dias @ 0 °C

Pilsen *NAB*

Saccharomyces ludwigii

Pilsen *LAB*

Controle



	IPA <i>NAB</i> Contato a frio	Pilsen <i>NAB</i> <i>Saccharomyces ludwigii</i>	Pilsen <i>LAB</i> Controle
Álcool (% ABV)	0,26	0,10	1,50
Densidade (mg/mL)	1040,38	1017,74	1006,15
Extrato original (% m/m)	11,02	5,18	5,04
Calorias (kcal/100 mL)	40,16	18,43	17,69
pH	4,66	4,75	4,40
Cor (EBC)	38	8	6
Amargor (IBU)	58	29	25
Dicetonas vicinais (mg/L)	0,14	0,00	0,22

	IPA <i>NAB</i> Contato a frio	Pilsen <i>NAB</i> <i>Saccharomyces ludwigii</i>	Pilsen <i>LAB</i> Controle
Acetaldeído (mg/L)	7,12	29,25	83,91
DMS (mg/L)	< LD	< LD	< LD
Acetato de etila (mg/L)	< 2,58	< 2,58	4,69
1-Propanol (mg/L)	3,67	< 2,53	9,04
Isobutanol (mg/L)	4,06	< 2,54	8,13
Acetato de isoamila (mg/L)	< LD	< LD	< LD
Álcool isoamílico (mg/L)	8,91	2,85	36,58

Próximos testes

BRASSAGEM

Alterar T para aumentar açúcares fermentescíveis

WHIRLPOOL

Adicionar PVPP e/ou polifenóis para remoção dos aldeídos de Stecker

FERMENTAÇÃO

Aumentar taxa de inóculo

Injetar CO₂

Diminuir T

Agradecimentos



Obrigado!

Ms Marcelo Calide Barga | marcelo@bio4.com.br

Dra Bianca Eli Della Bianca | bianca@bio4.com.br

+55-41-99661-5850

