



Modelling cocoa bean fermentation processes

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Overview

1 Introduction

2 Mathematical modelling

3 Results

4 Conclusions

Introduction

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Introduction

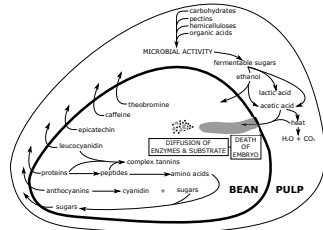
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- Its fermentation is considered a key step in cocoa processing.
- In contrast to other fermentations processes, cocoa bean fermentation is highly non-controlled and non-standardized.
- Its main role is to facilitate **a series of biochemical reactions** → precursors of aroma and flavor of chocolate.
- A mathematical model can provide a deeper mechanistic understanding of the process.

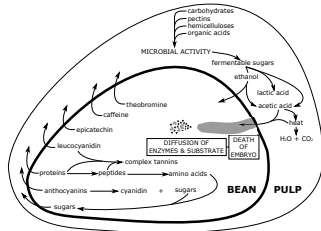
Fermentation of Cocoa Beans

- It occurs mainly in the pulp by intervention of:
 - Micro-organisms: Yeast (Y), lactic acid bacteria (LAB) and acetic acid bacteria (AAB).

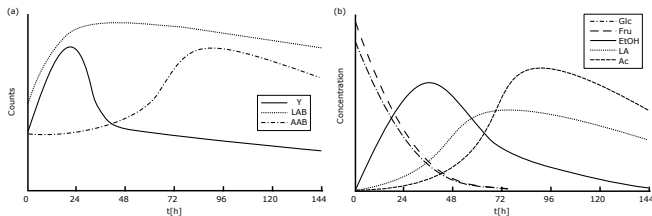


Fermentation of Cocoa Beans

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 - Micro-organisms: Yeast (Y), lactic acid bacteria (LAB) and acetic acid bacteria (AAB).
 - Metabolites: Glucose (Glc), fructose (Fru), ethanol (EtOH), lactic acid (LA) and acetic acid (Ac).

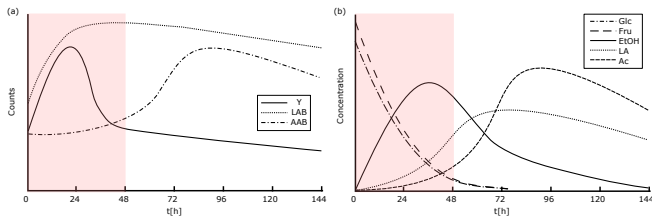


Microbial Succession and Biochemistry during the fermentation



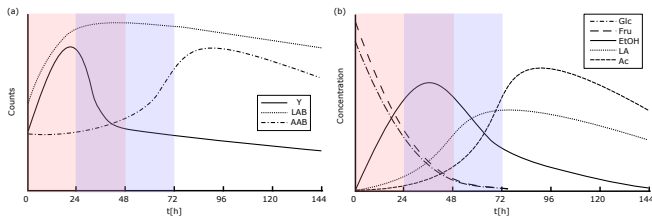
Phase	MO	Time [h]	Carbon sources	Product(s)	Negative feedback by	Temp. [°C]	pH
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Microbial Succession and Biochemistry during the fermentation



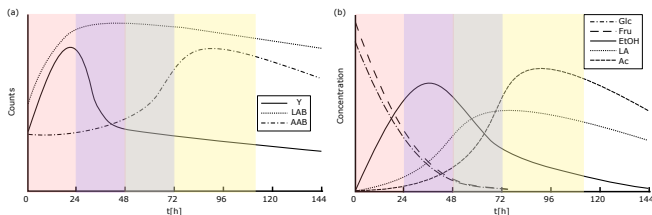
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I	Y	0 - 48	Glc, Fru	EtOH	EtOH, LA, Ac, IT	25-30 to 35-48	< 4.0

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II	LAB	24 - 72	Glc, Fru, EtOH	LA, Ac	EtOH, IT	40	3.5-4.0 to 4.2-5.0
III	AAB	48 - 112	EtOH, LA	Ac*	IT	45-50	4.2-5.0

* As consequence of over-oxidation of acetic acid, CO₂ and H₂O can be produced too.

De Vuyst & Weckx (2016) *J App Microbiol.* 121:1, 5-17

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Setting the baseline

- From the dynamics of the process we can expect to account with coupled ODE's.
- Microbial growth and death are governing the process → consumption and production of metabolites.
- Classical equations for microbial growth: Monod, Contois, Haldane, ...
- Death rates simply can be modelled by linear or non-linear relationships with products.

Setting the baseline

Monod

$\mu = \mu_{max} \frac{[S]}{K_s + [S]}$ \rightarrow relates microbial growth with substrate consumption.

Contois

$\mu = \mu_{max} \frac{S}{K_s [X] + [S]}$ \rightarrow $[X]$ is microbial concentration. Hence, as microbes reproduce, μ decreases.

Linear death rate

$$d = k [X]$$

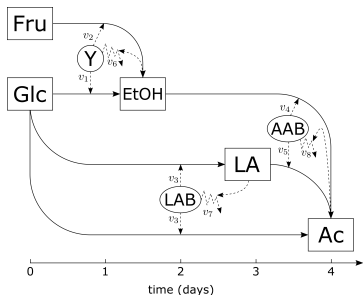
Non-linear death rate

$d = k [X] [S]^n$ \rightarrow death rate d of X is subject to non-linear interactions with a substrate (product) S .

Summary of model's iterations

Model	Multiple substrate	Product toxicity	Population size effect for LA consumption
M1	X	X	X
M2	✓	X	X
M3	✓	✓	X
M4	✓	✓	✓

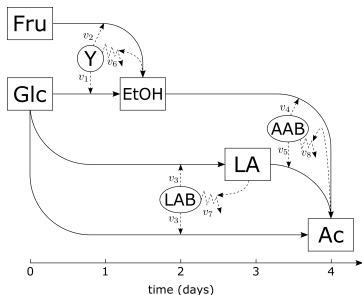
Mathematical formulation, M4



Model's Network diagram

MO	Growth rate equation	Mortality rate equation
Y	$v_1 = \frac{\mu_{\max}^Y [\text{Glc}]}{[\text{Glc}] + K_{\text{Glc}}^Y} [\text{Y}]$ $v_2 = \frac{\mu_{\max}^Y [\text{Fru}]}{[\text{Fru}] + K_{\text{Fru}}^Y} [\text{Y}]$	$v_6 = k_Y [\text{Y}] [\text{EtOH}]$
LAB	$v_3 = \frac{\mu_{\max}^{\text{LAB}} [\text{Glc}]}{[\text{Glc}] + K_{\text{Glc}}^{\text{LAB}}} [\text{LAB}]$	$v_7 = k_{\text{LAB}} [\text{LAB}] [\text{LA}]$
AAB	$v_4 = \frac{\mu_{\max}^{\text{AABEtOH}} [\text{EtOH}]}{K_{\text{EtOH}}^{\text{AAB}}} [\text{AAB}]$ $v_5 = \frac{\mu_{\max}^{\text{AABLA}} [\text{LA}]}{K_{\text{LA}}^{\text{AAB}} [\text{AAB}] + [\text{LA}]} [\text{AAB}]$	$v_8 = k_{\text{AAB}} [\text{AAB}] [\text{Ac}]^2$

Mathematical formulation, M4



Model's Network diagram

$$\frac{d[\text{Glc}]}{dt} = -Y_{\text{Glc}|\text{Y}} v_1 - Y_{\text{Glc}|\text{LAB}} v_3$$

$$\frac{d[\text{Fru}]}{dt} = -Y_{\text{Fru}|\text{Y}} v_2$$

$$\frac{d[\text{EtOH}]}{dt} = Y_{\text{EtOH}|\text{Y}}^{\text{Glc}} v_1 + Y_{\text{EtOH}|\text{Y}}^{\text{Fru}} v_2 - Y_{\text{EtOH}|\text{AAB}} v_4$$

$$\frac{d[\text{LA}]}{dt} = Y_{\text{LA}|\text{LAB}} v_3 - Y_{\text{LA}|\text{AAB}} v_5$$

$$\frac{d[\text{Ac}]}{dt} = Y_{\text{Ac}|\text{LAB}} v_3 + Y_{\text{Ac}|\text{AAB}}^{\text{EtOH}} v_4 + Y_{\text{Ac}|\text{AAB}}^{\text{LA}} v_5$$

$$\frac{d[\text{Y}]}{dt} = v_1 + v_2 - v_6$$

$$\frac{d[\text{LAB}]}{dt} = v_3 - v_7$$

$$\frac{d[\text{AAB}]}{dt} = v_4 + v_5 - v_8$$

Experimental data

- Experimental data from published works by Papalexandratou *et al.* (2011) and Camu *et al.* (2007) about spontaneous fermentations conducted in Brazil (box 1 and box 2) and Ghana respectively.

Camu *et al.* (2007) *App Environ Microbiol*, 73:6, 1809-1824
Papalexandratou *et al.* (2011) *Food Microbiol*, 28, 1326-1338

Experimental data

- Experimental data from published works by Papalexandratou *et al.* (2011) and Camu *et al.* (2007) about spontaneous fermentations conducted in Brazil (box 1 and box 2) and Ghana respectively.
- In Papalexandratou *et al.* (2011) study, there exist little differences between the conditions under which both trials were performed (e.g., location, temperature and protection of fermenting masses towards environmental conditions).

Camu *et al.* (2007) *App Environ Microbiol*, 73:6, 1809-1824
Papalexandratou *et al.* (2011) *Food Microbiol*, 28, 1326-1338

Stan model

```

functions {
  real[] cbf(real t,
             real[] x,
             real[] theta,
             real[] y_r,
             int[] y_i) {

    real dxdt[8];

    dxdt[1] = - theta[1]*theta[12]*x[1]*x[6]/(theta[17]+x[1]) -
              theta[2]*theta[14]*x[1]*x[7]/(theta[19]+x[1]);
    .
    .
    .
    dxdt[8] = theta[15]*x[3]*x[8]/(theta[20]+x[3]) + theta[16]*x
              [4]*x[8]/(theta[21]*x[8]+x[4]) - theta[24]*x[8]*x[5]^2;

    return dxdt;
  }
}

```

Stan model

```

data {
  int<lower=1> T;
  real<lower=0> x[T,8];
  real t0;
  real ts[T];
  real x0[9];
}

transformed data {

  real y_r[0];
  int y_i[0];
  real<lower=0> x0_1[8];
  real<lower=0> xn[T,8];
  for (t in 1:T)
    for (n in 1:8)
      xn[t,n]=x[t,n]/scl[n];

  for (n in 1:8)
    x0_1[n] = x0[n]/scl[n];
}

```

Stan model

```

parameters {
  real <lower=0> ycl;
  .
  .
  .
  real <lower=0> sigma;
}

transformed parameters {
  real x_hat[T,8];
  {
    real theta[24];
    theta[1] = ycl;
    .
    .
    .
    theta[24] = k3;

    x_hat = integrate_ode_rk45(cbf, x0_1, t0, ts, theta,
                             y_r, y_i, 1.0E-6, 1.0E-6, 1.0E6);
  }
}

```


Stan model

```

model{
  yc1 ~ normal(0.5,0.3);
  .
  .
  .
  k3 ~ normal(0.5,0.3);
  sigma ~ cauchy(0,1);

  for (t in 1:T)
    xn[t] ~ normal(x_hat[t],sigma);
}

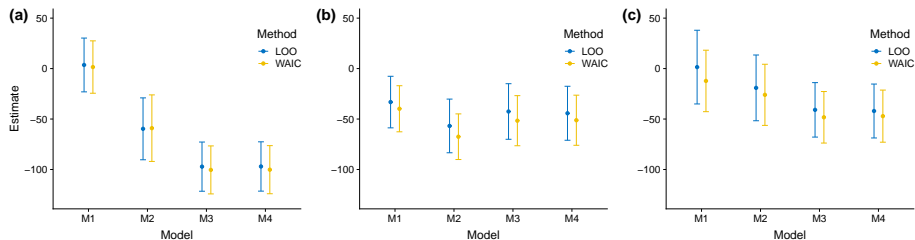
generated quantities{
  real log_lik[T,8];
  for (t in 1:T)
    for (n in 1:8)
      log_lik[t,n]=normal_lpdf(xn[t,n]|x_hat[t,n],
                                sigma);
}

```

Overview

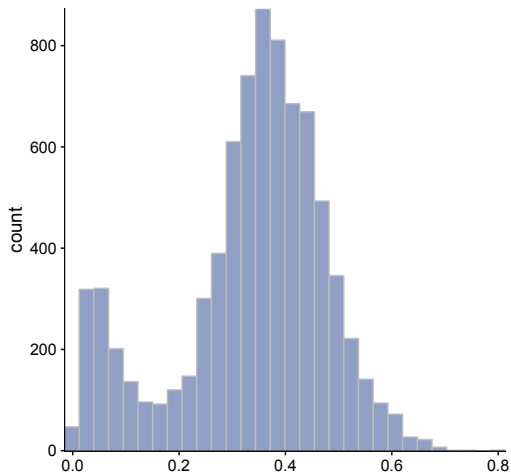
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Model selection



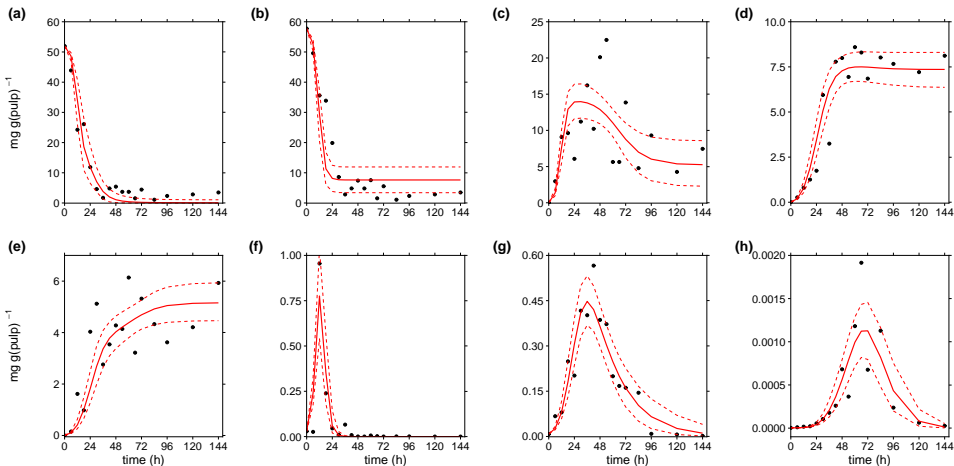
Leave-one-out cross-validation (LOO) and widely applicable information criterion (WAIC) for the models. (a) Camu *et al.* data, (b) Papalexandratou *et al.* box 1 data and (c) Papalexandratou *et al.* box 2 data.

Model 3 or 4?



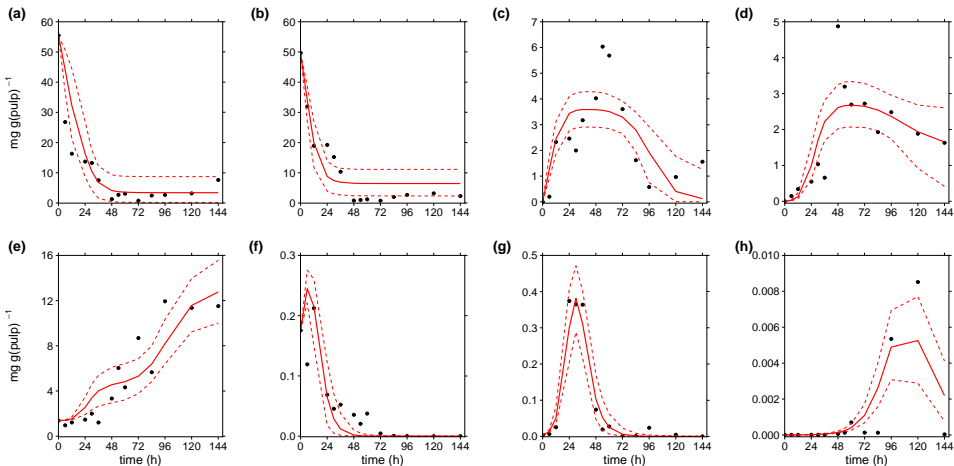
Posterior distribution of the maximum specific growth rate of AAB on EtOH ($\mu_{\max}^{\text{AABEtOH}}$) of model M3 with the data from Camu *et al.* (2007).

Model's simulations M4



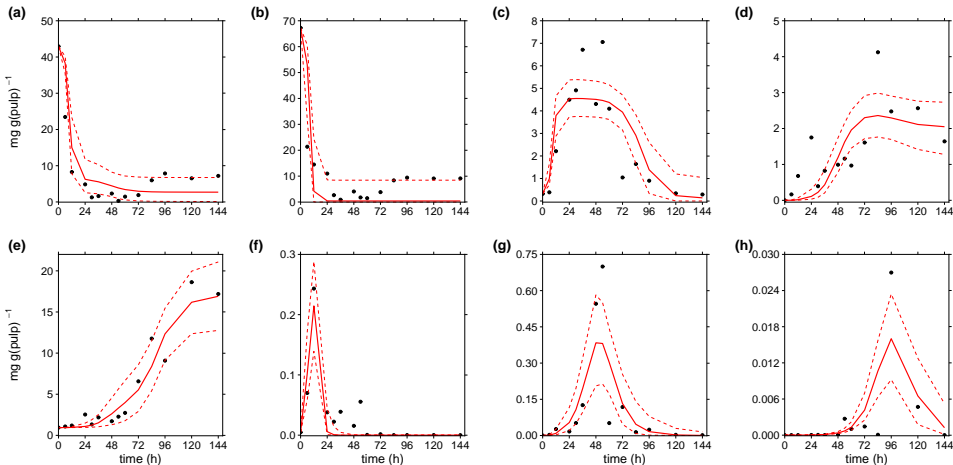
Results of the model for data reported by Camu *et al.* (2007). (a) Glc, (b) Fru, (c) EtOH, (d) LA, (e) Ac, (f) Y, (g) LAB and (h) AAB.

Model's simulations M4



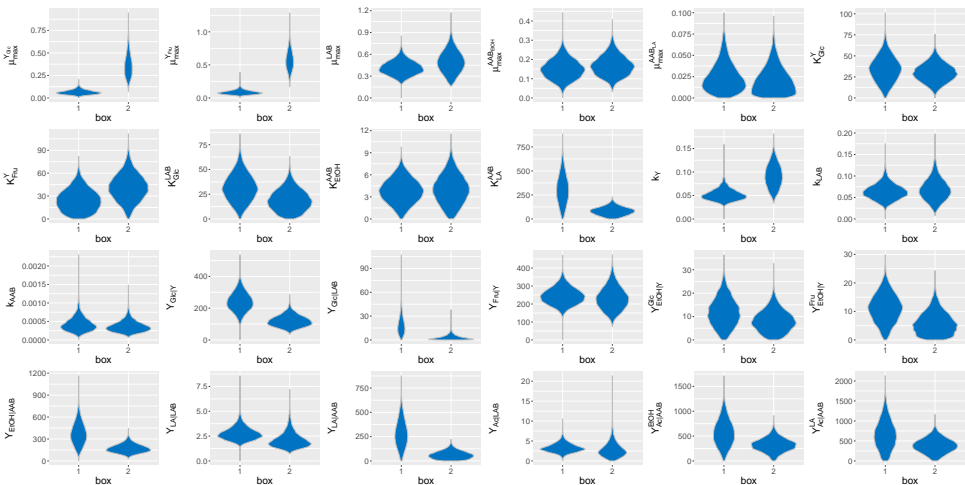
Results of the model for data reported for Box 1 by Papalexandratou *et al.* (2011). (a) Glc, (b) Fru, (c) EtOH, (d) LA, (e) Ac, (f) Y, (g) LAB and (h) AAB.

Model's simulations M4



Results of the model for data reported for Box 2 by Papalexandratou *et al.* (2011). (a) Glc, (b) Fru, (c) EtOH, (d) LA, (e) Ac, (f) Y, (g) LAB and (h) AAB.

Differences between parameters from boxes 1 and 2



Re-scaled posterior distributions of the 24 parameters of boxes 1 and 2.

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- Provides further mechanistic understanding on the interactions that were purely qualitatively described before.
- Currently we are working in extensions of the model (e.g., including temperature dependencies and ways to determine differences depending on the fermentation method used).

Acknowledgements

Barry Callebaut
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Stan Community

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Paper: <https://doi.org/10.1098/rsos.180964>
Code: <https://dx.doi.org/10.5061/dryad.321d33v>
and StanCon notebook