Properties and applications of enzymatically modified cocoa butter

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UGent Cacaolab

Modification of CB

Cocoa Butter

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Cocoa butter DAG

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Functional ingredient in chocolate ?

Modification of CB

Various routes for the production of DAG

- Glycerolysis between TAG and glycerol, chemical or enzymatically (with or without organic solvent)
- Esterification of fatty acids to glycerol (with or without organic solvents)
- Selective hydrolysis of TAG
- Combination of the above described methods

Modification of CB

Reaction toolbox

- Glycerolysis
  - ~~Chemical~~
  - Enzymatic
- Esterification
  - ~~Chemical~~
  - ~~Enzymatic~~
- ~~Hydrolysis~~

LIMITATIONS

- Simple reaction
- Substrate cocoa butter
- Food applications
- Easy to control

→ Enzymatic glycerolysis

Enzymatic modification CB

Cocoa Butter

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Enzymatic glycerolysis --------> Optimization

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Separation --------> Optimization

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Cocoa butter DAG

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Functional ingredient in chocolate ? -------> Evaluation

Experimental set up

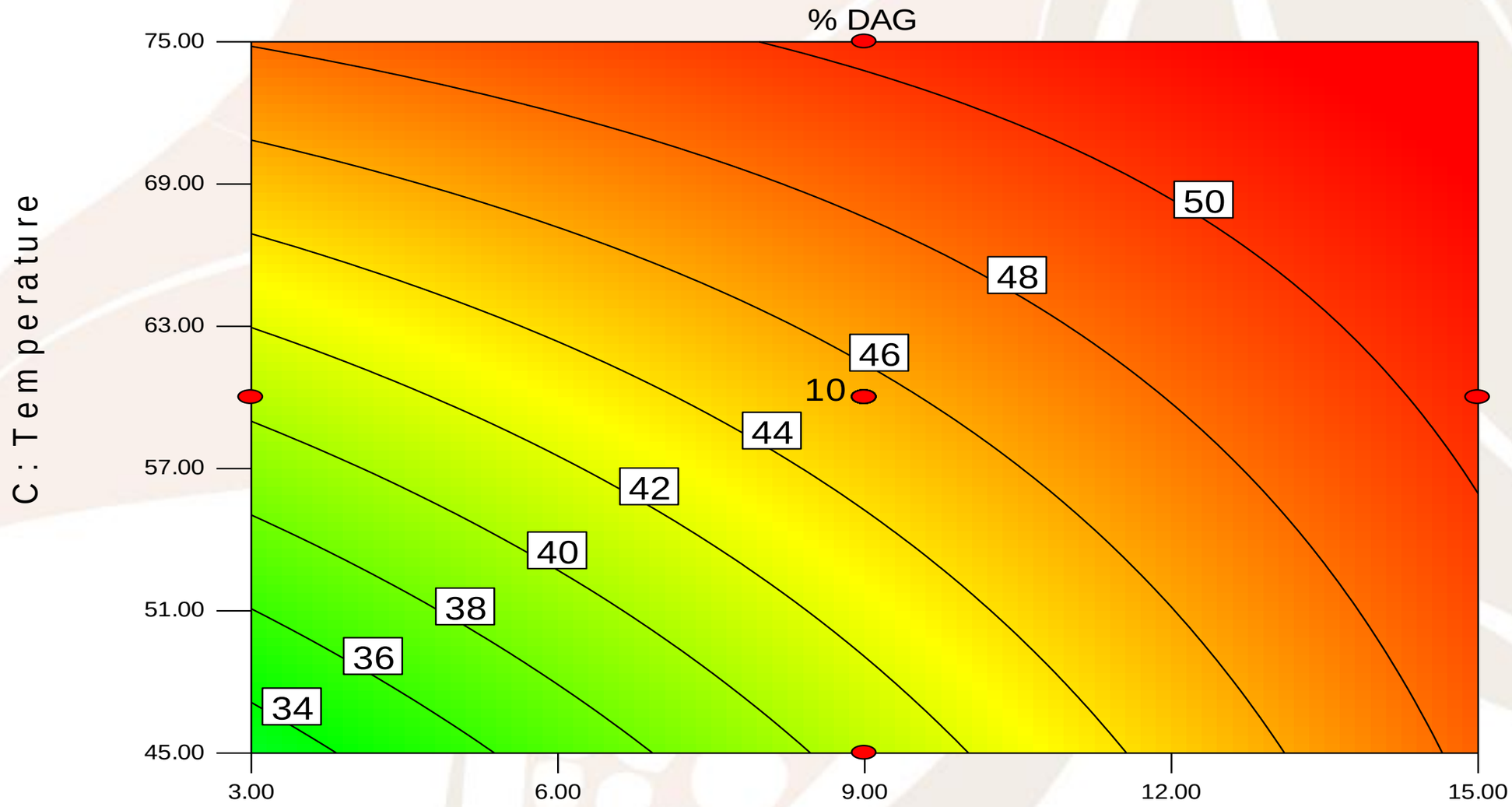
- Enzyme
  - *Candida antarctica*= Novozym 435
- Response surface methodology
  - Variables
    - ✓Reaction time: 3 to 15 hours
    - ✓Reaction temperature: 40°C to 75°C
    - ✓Enzyme load: 3 to 15 wt% of oil mass
    - ✓Substrate molar ratio, oil/glycerol: 0.25-2.00
    - ✓Water content: 0 to 6 wt% of glycerol mass
  - Response: % DAG
  - Fractional Face-Centered Composite Design: 36 experiments

Response surface methodology

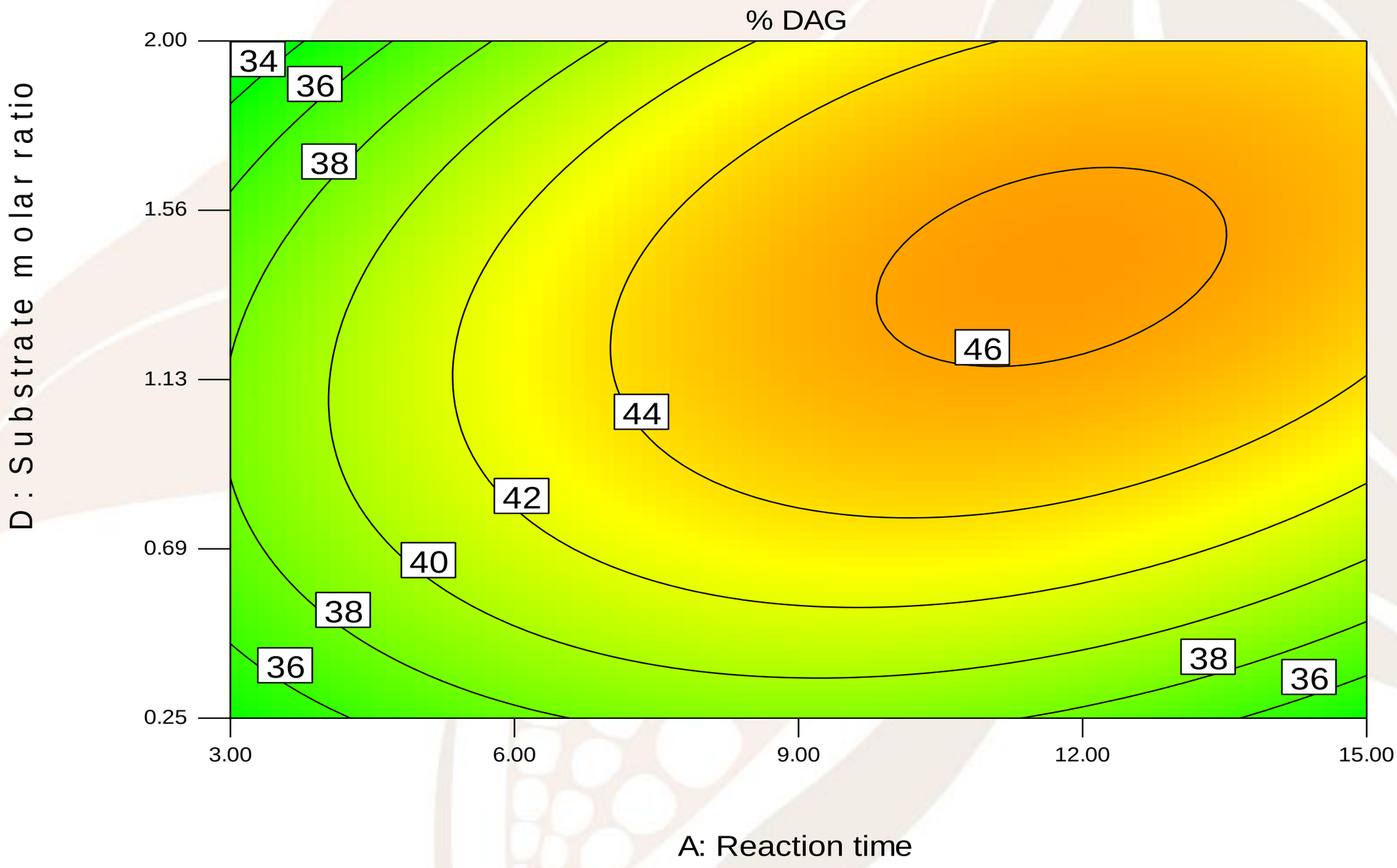
- Regression calculations: Quadratic model
- Regression coeffiencts
  - Factor water removed → no influence

Variable	Regression coefficients	p-values
Intercept	45.54	0.001
A: Reaction time	2.88	0.0033
B: Enzyme load	5.03	<0.0001
C: Temperature	4.86	<0.0001
D: Substrate molar ratio	1.89	0.0436
AC	-1.79	0.0694
AD	2.97	0.0041
BC	-2.73	0.0076
A^2	-4.39	0.0404
D^2	-4.83	0.0255

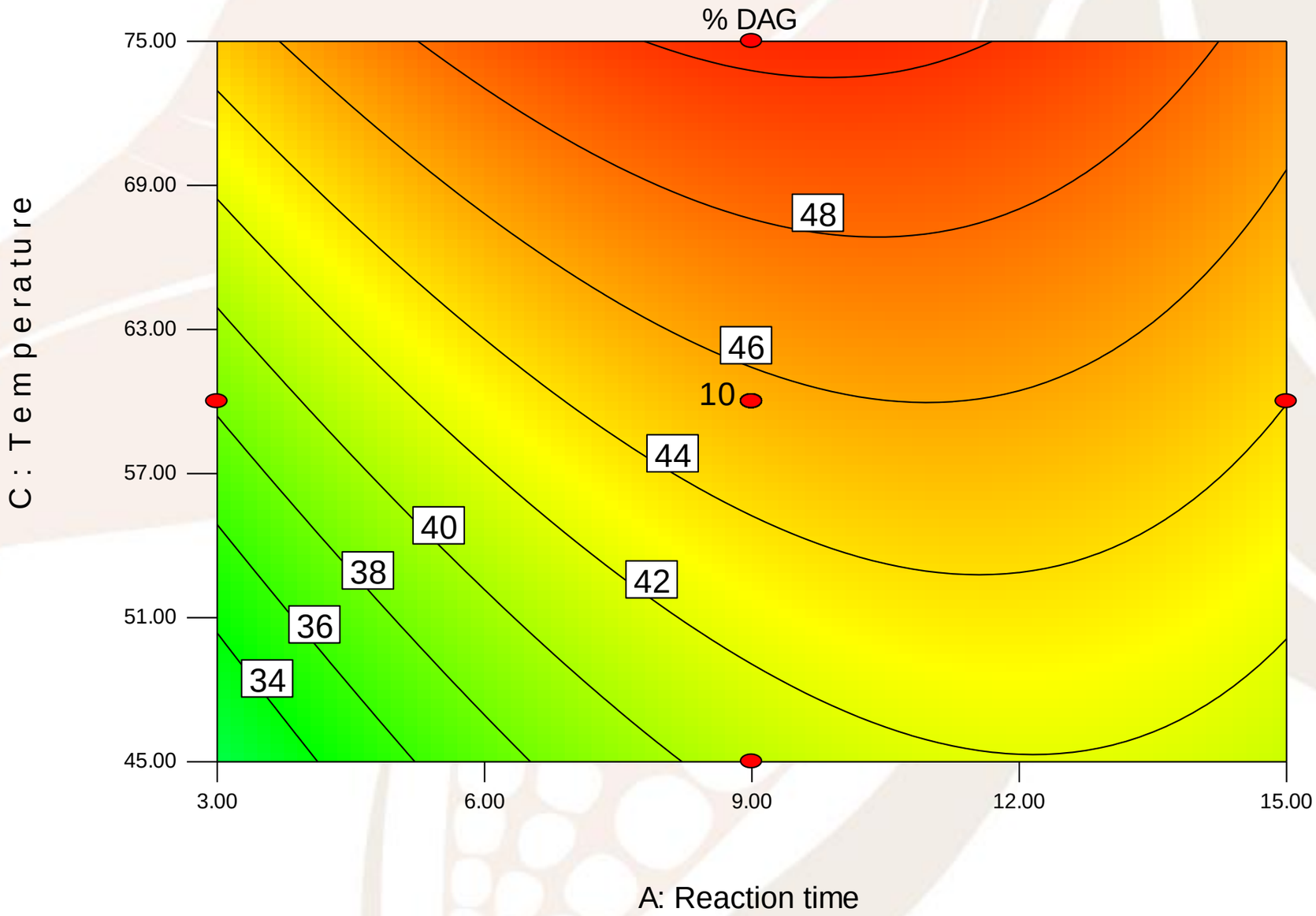
Response surface methodology



Response surface methodology



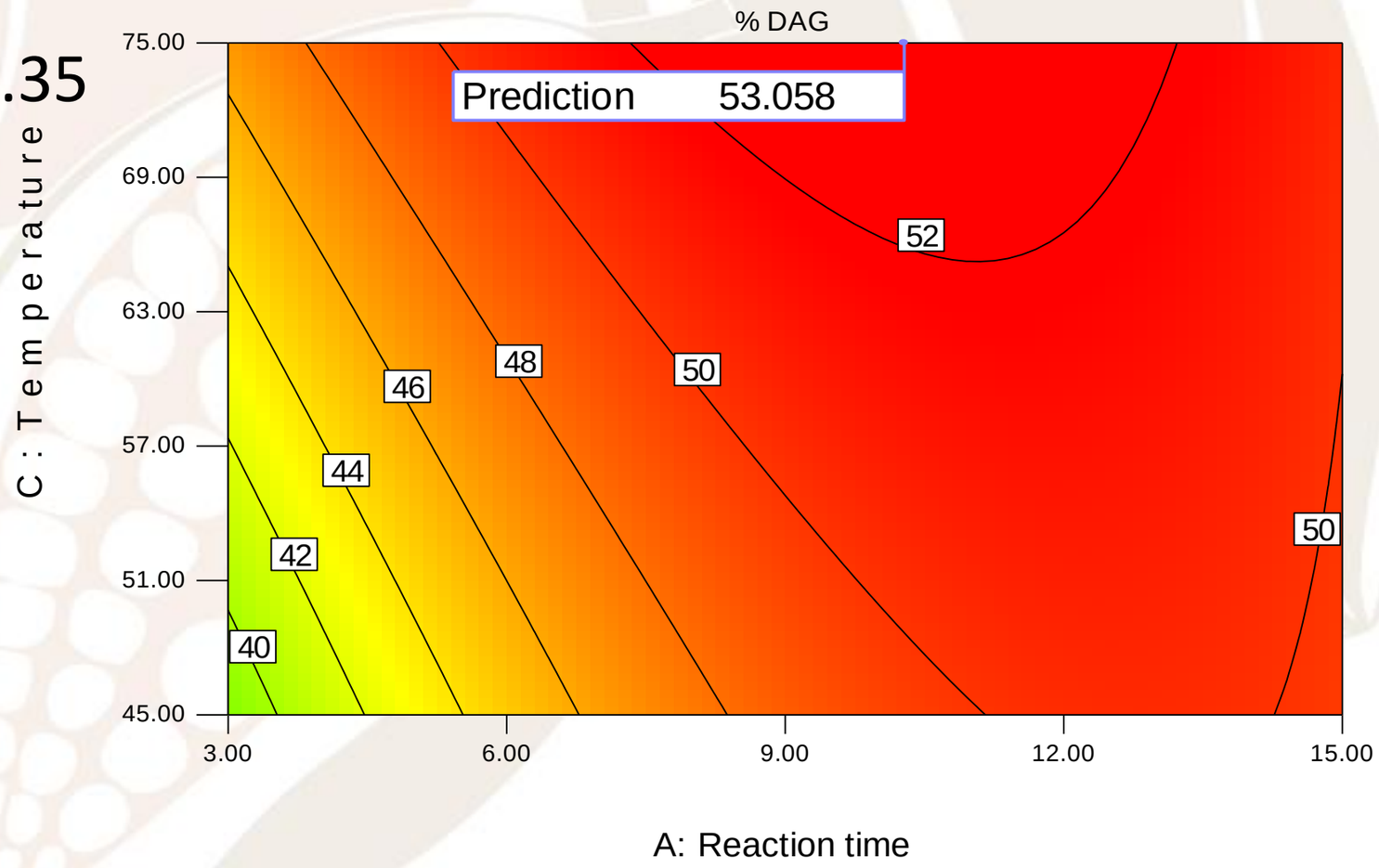
Response surface methodology



Response surface methodology

Design expert: optimal conditions: 53% DAG

- Reaction time: 10.26 hours
- Enzyme load: 15%
- Temperature: 75°C
- Substrate molar ratio: 1.35



Reaction optimization

Further optimization and model verification

Reaction Follow up: 4 h – 5 h – 6 h – 7 h – 8 h – 9 h

Temperature

55°C

75°C

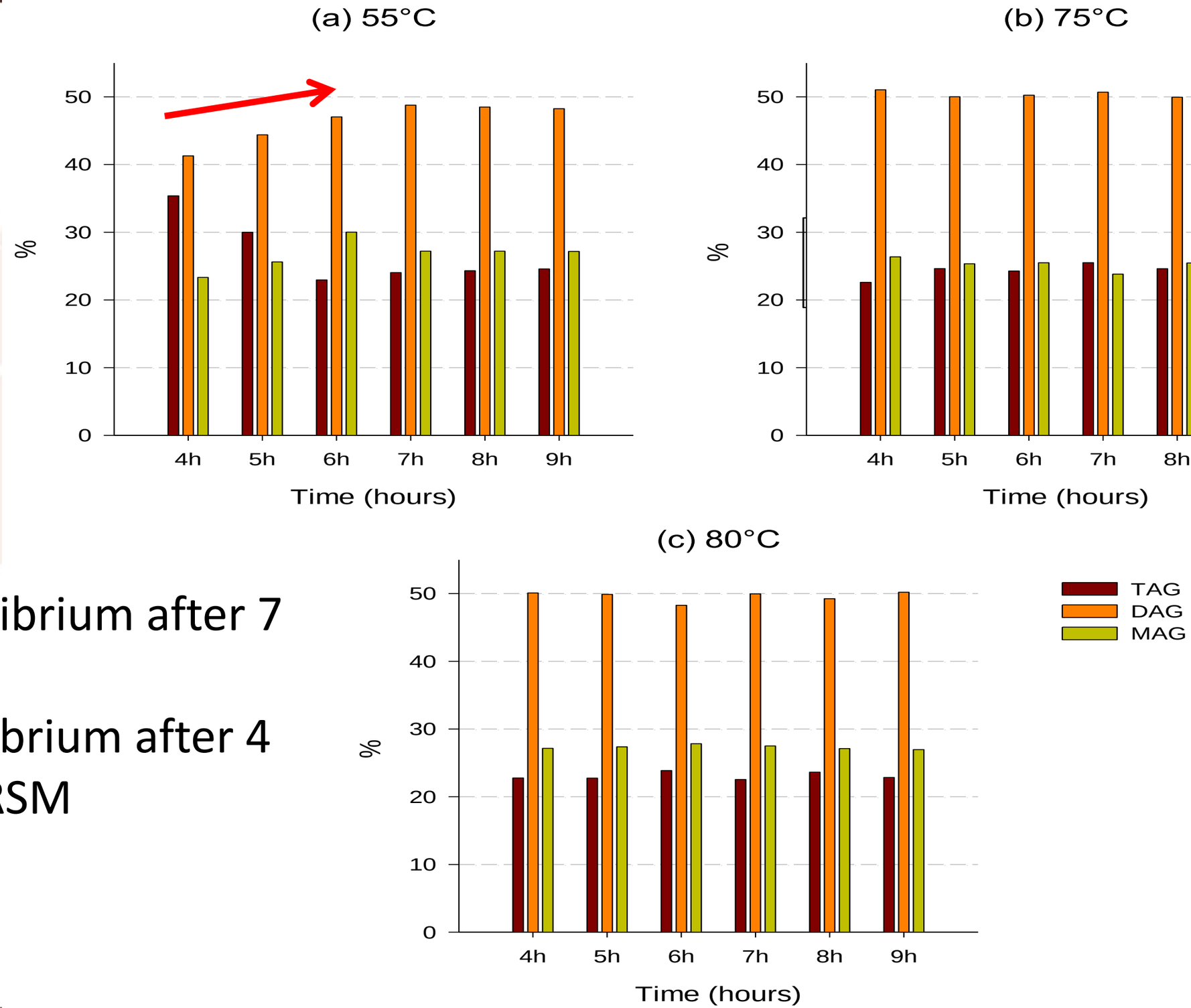
80°C

→ Substrate molar ratio

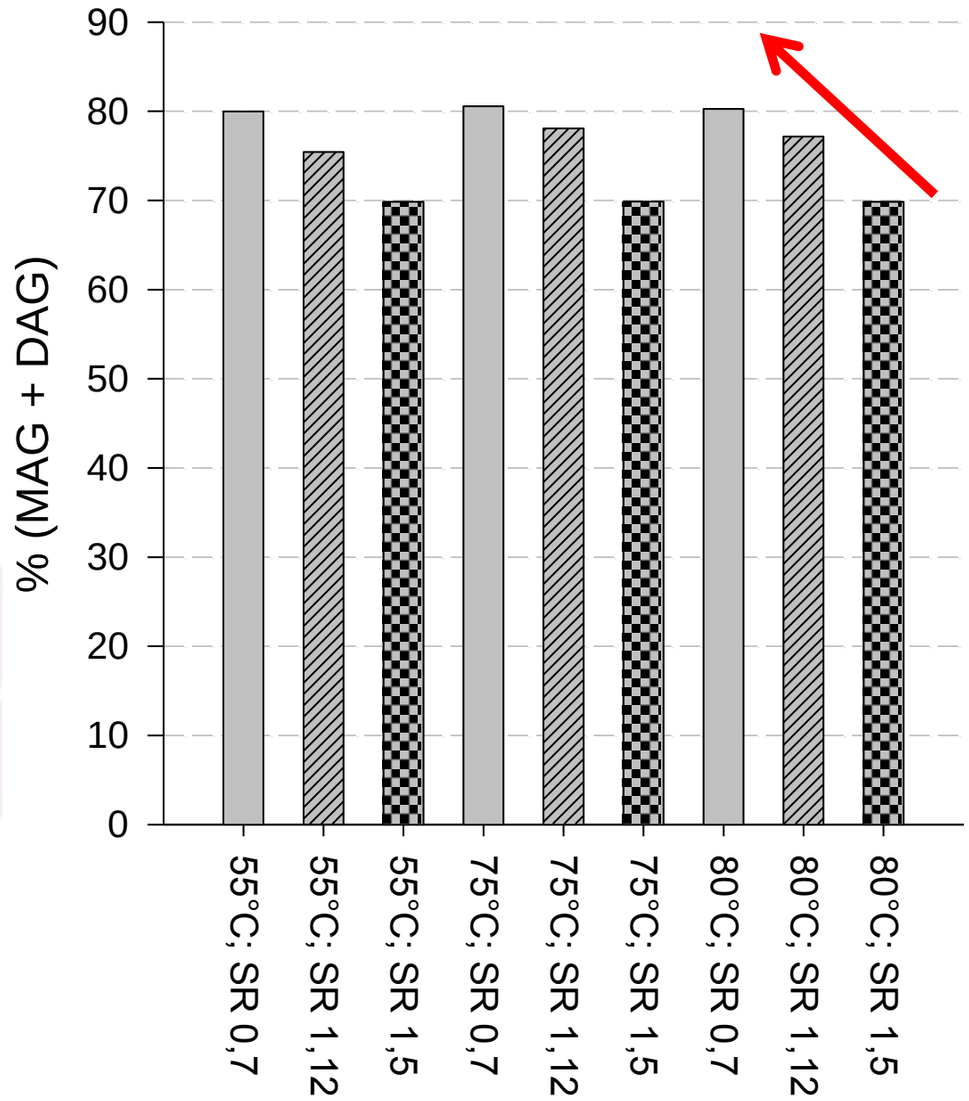
0,7 – 1,12 - 2

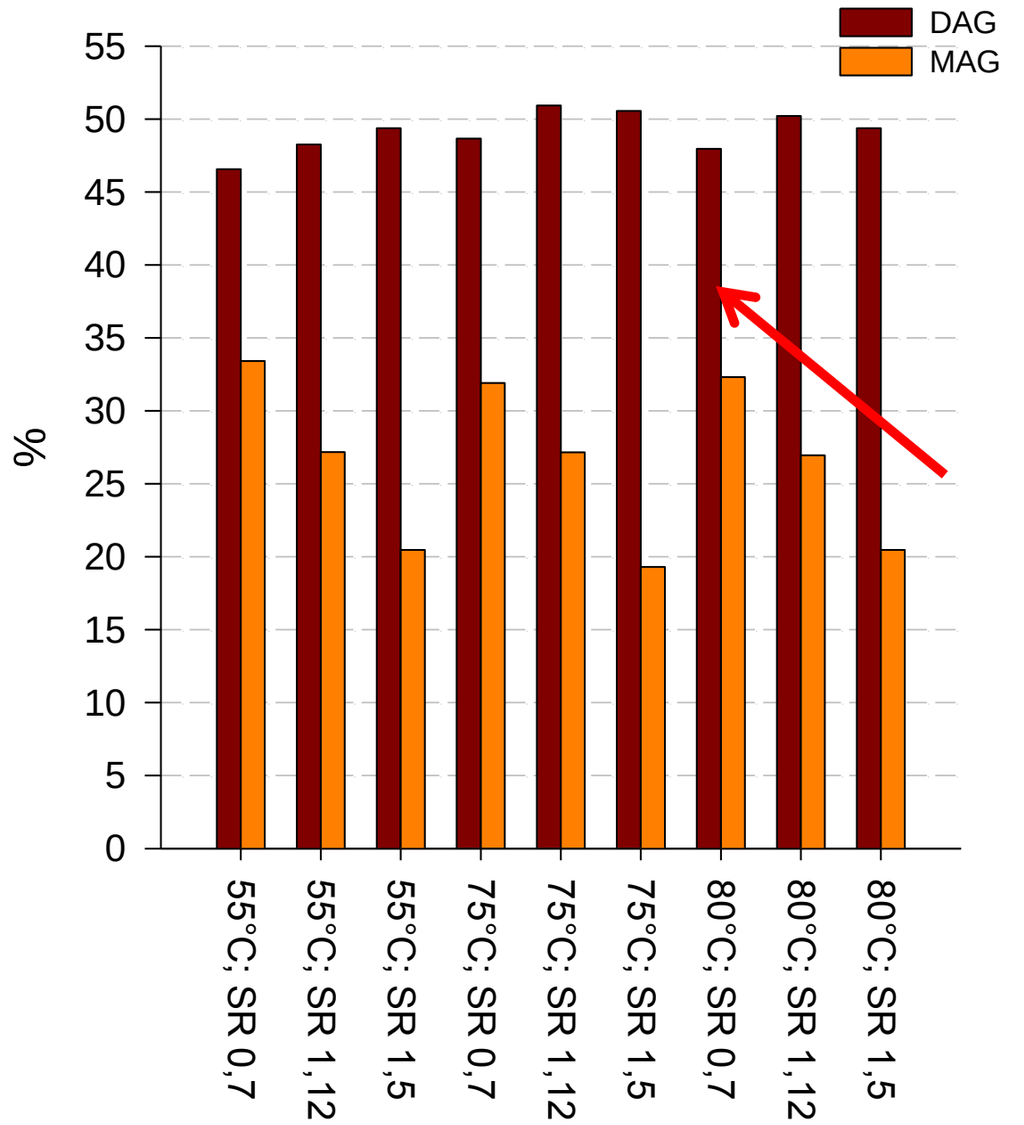
Effect of reaction time

- 55°C: equilibrium after 7 hours
- 75°C: equilbrium after 4 hours ⟺ RSM



Effect of substrate ratio





More glycerol (lower substrate ratio): higher conversion degree, higher amount of MAG but lower amount of DAG

Reaction optimization

- No water necessary
- Reaction time: 6 hours: a reasonable time to obtain equilibrium
- Reaction temperature :70°C being still in the range of maximum enzyme activity
- Enzyme load: 15 wt% of the oil mass: high amount of enzyme didn’t had negative effects
- Substrate molar ratio: 1.12 combination of a reasonable conversion with a reasonable amount of glycerol

Cocoa butter DAG

Cocoa Butte

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Enzymatic glycer

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Separation: short path

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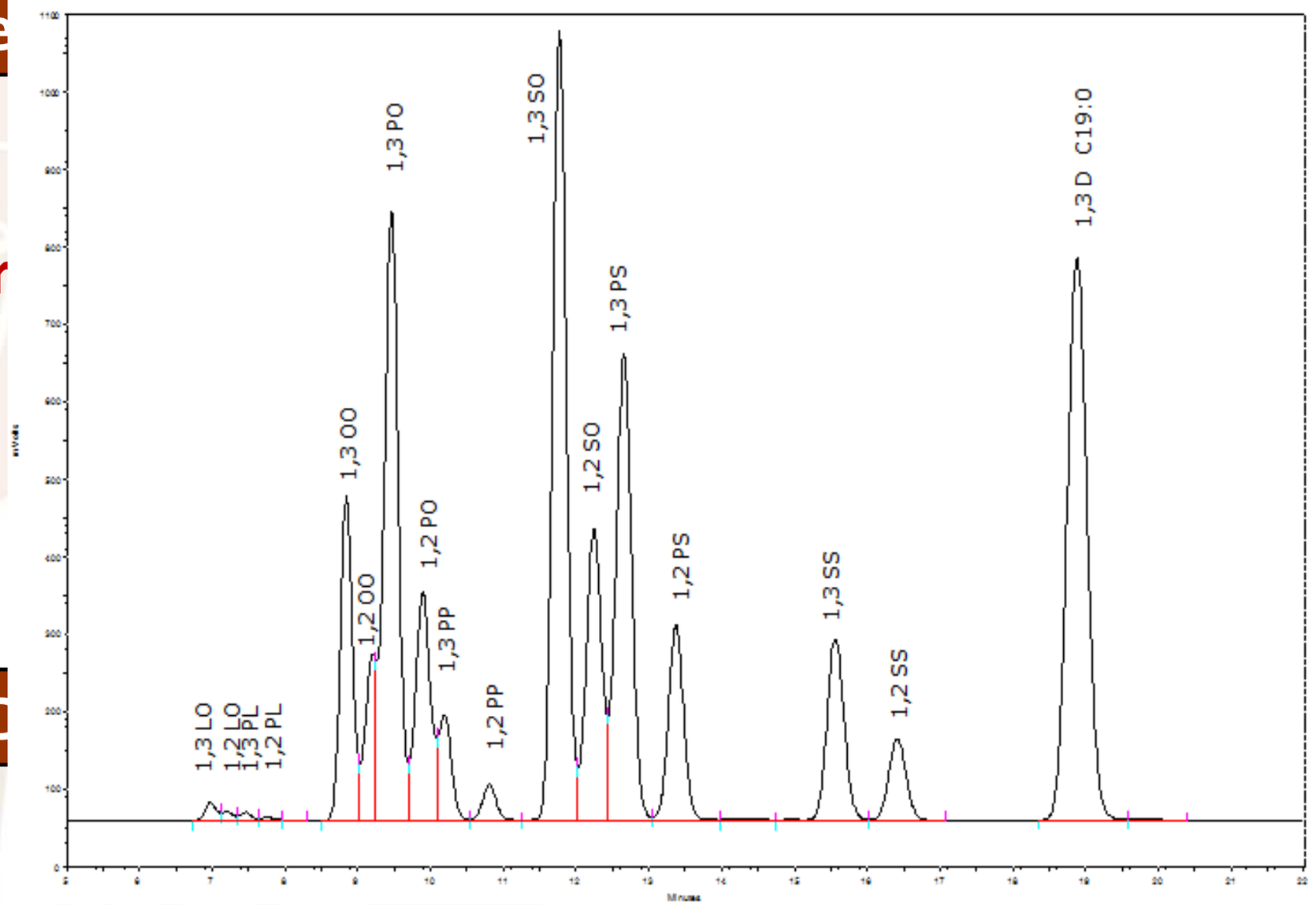
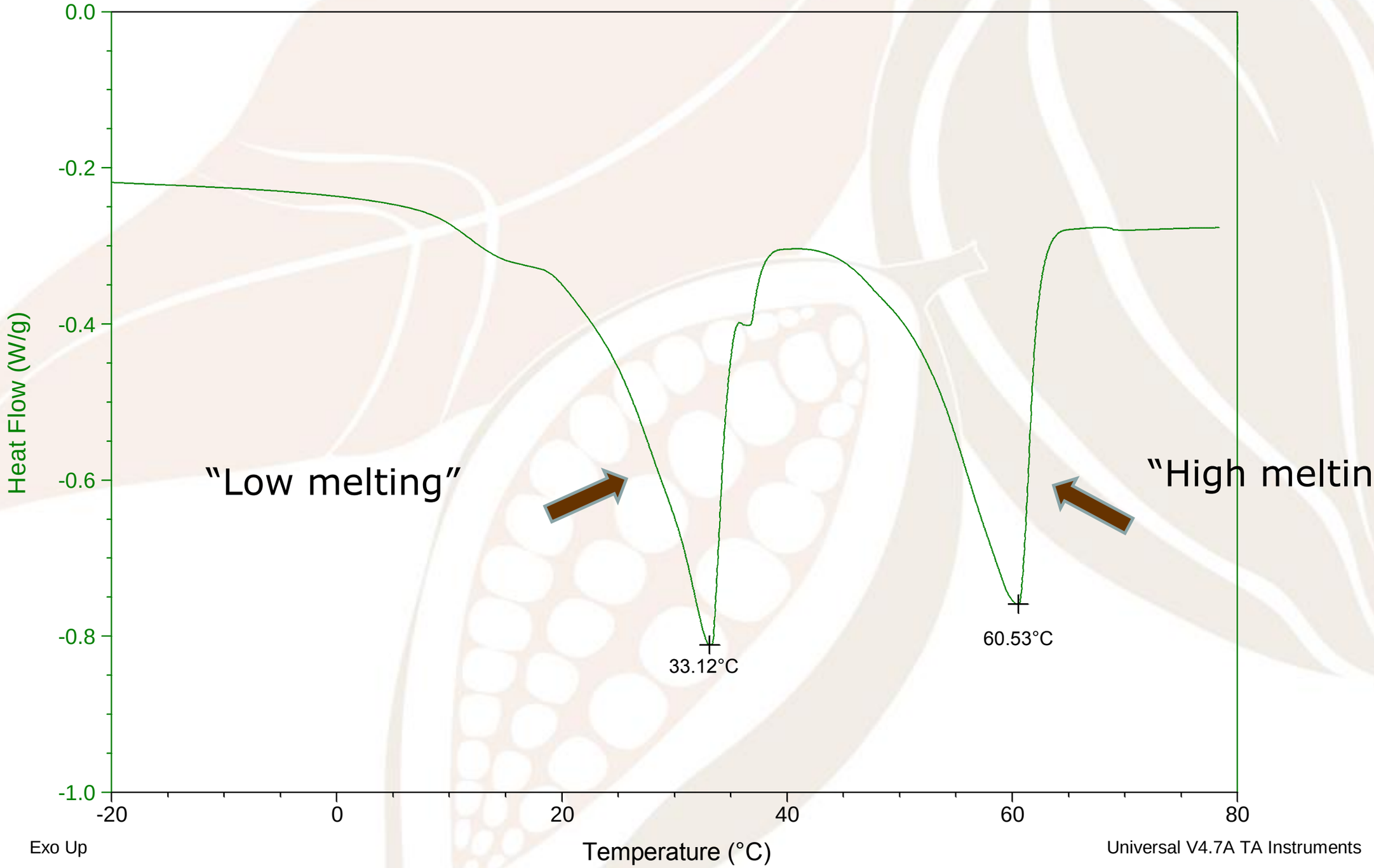
Cocoa butter D



Cocoa butter DAG

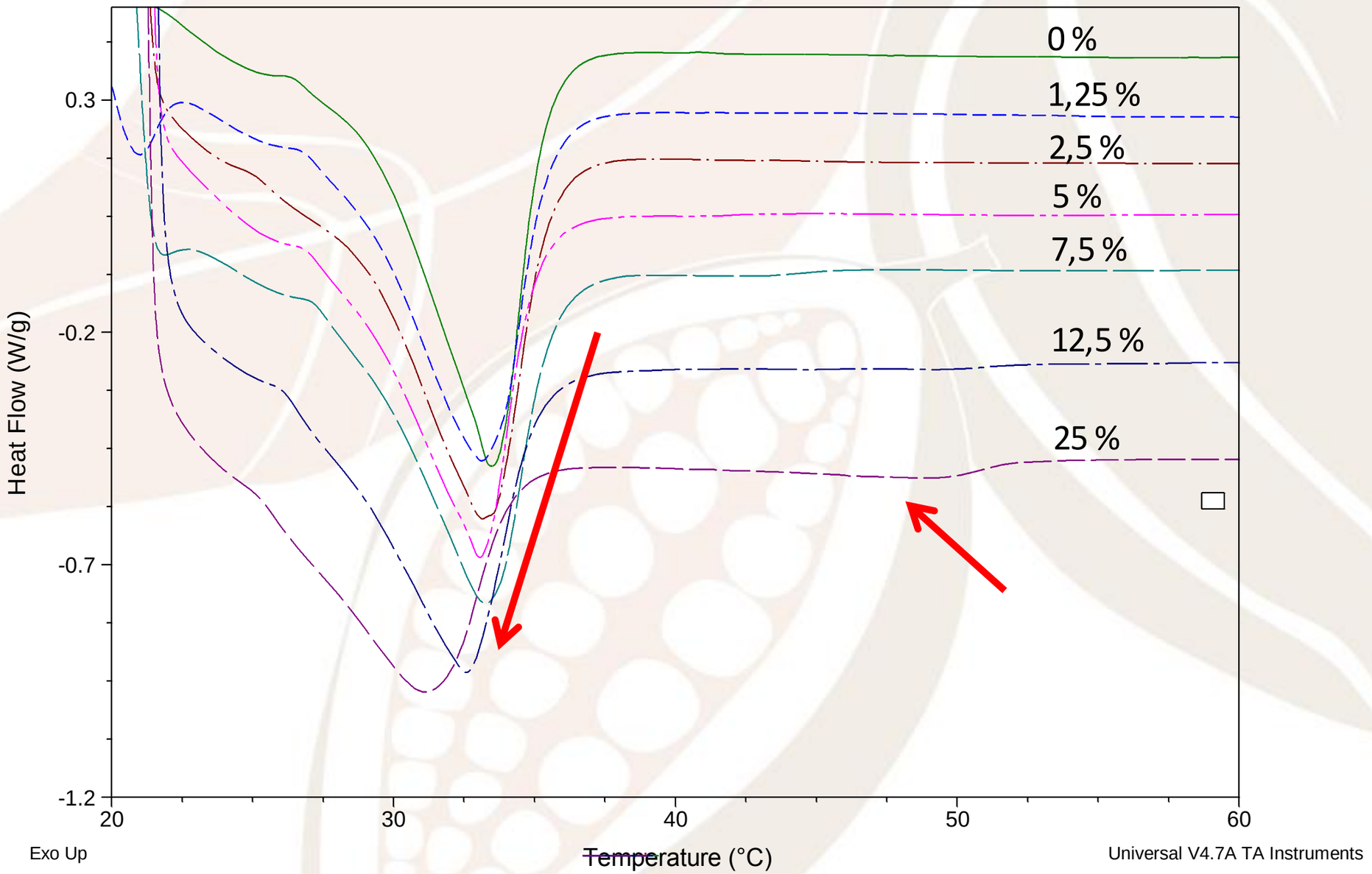


Chocolates containing DAG

- Chocolates
  - 0, 1.25, 2.5, 5, 10, 12.5 and 25 % DAG on fat base
  - 0.4% to 8.75% DAG on product base

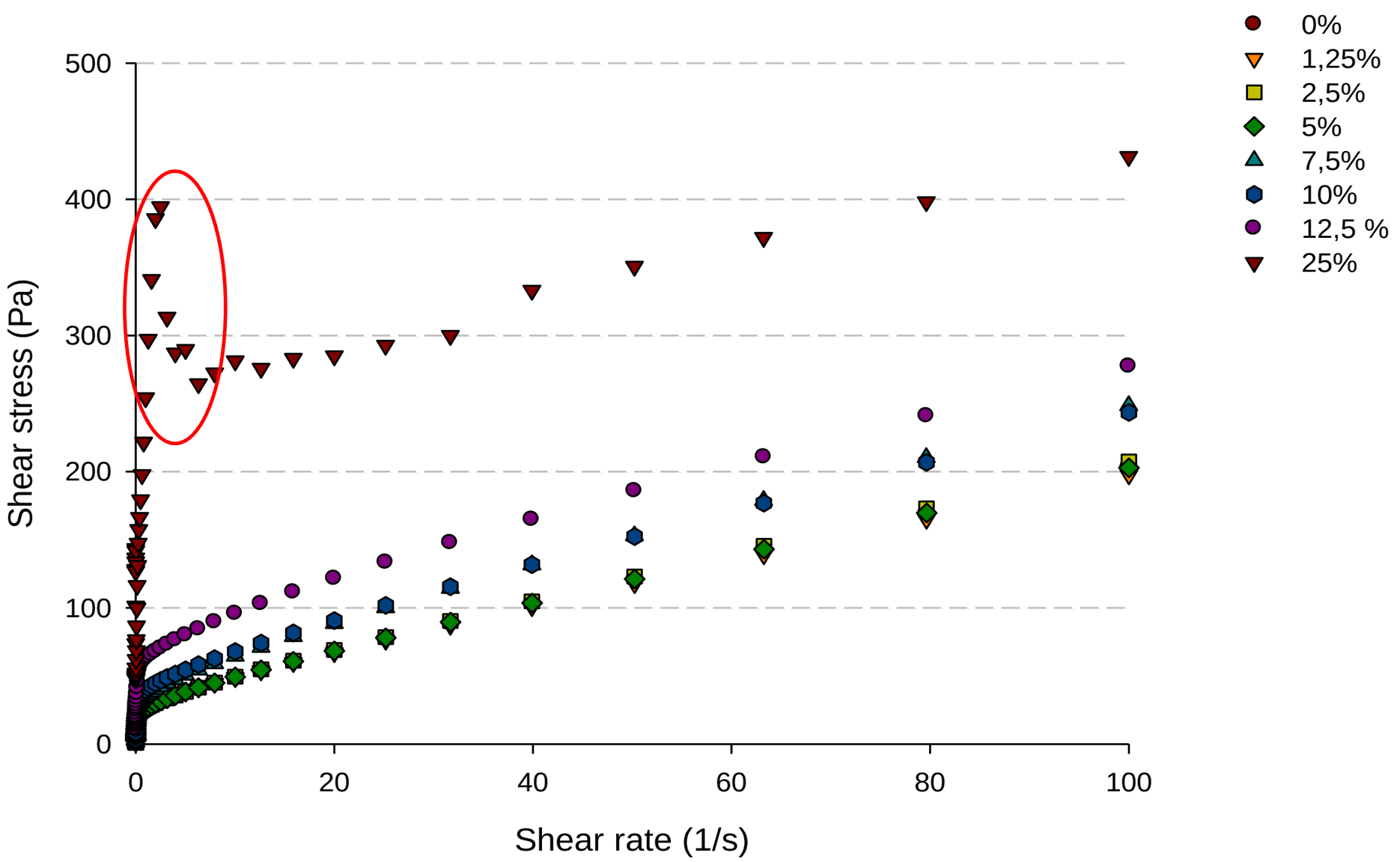
- Melting behaviour
- Rheological behaviour
- Texture analysis

Chocolates containing DAG



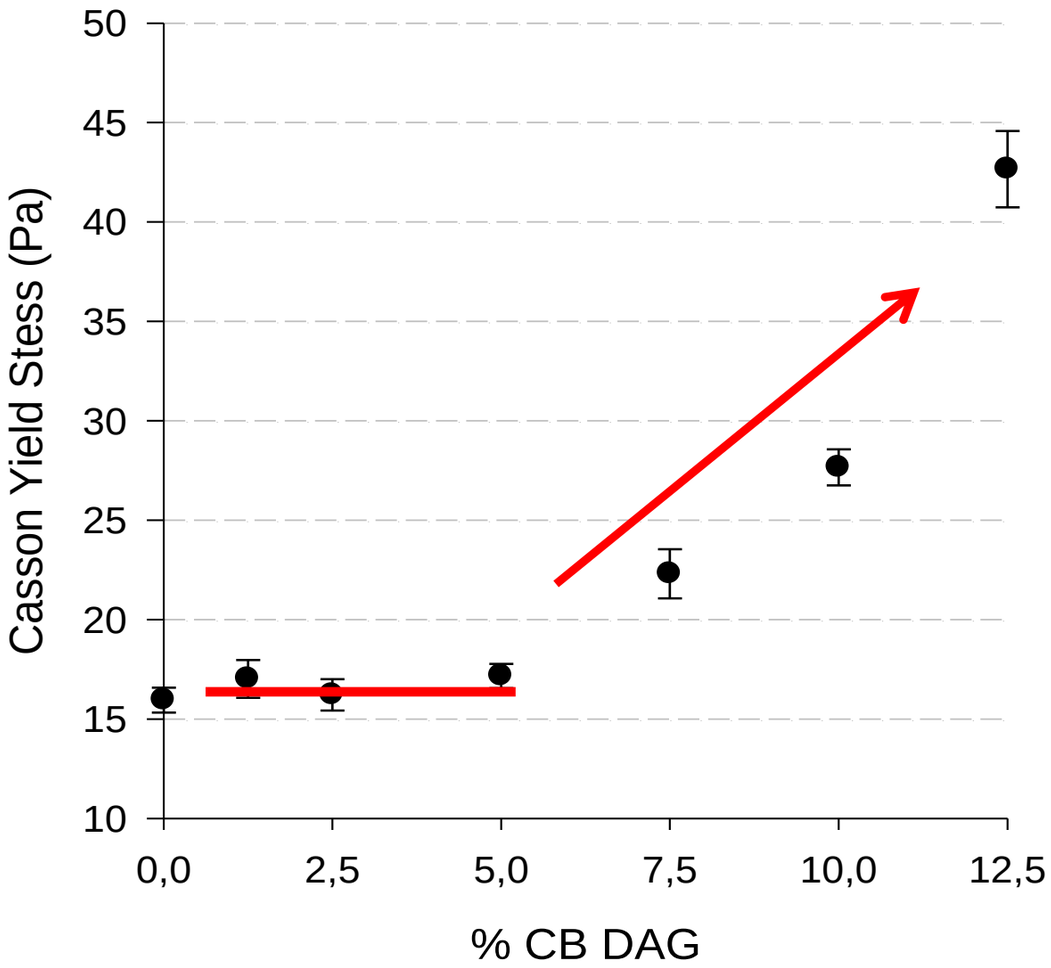
Chocolate flow behaviour

Flow behaviour: plate-plate geometry at 40°C

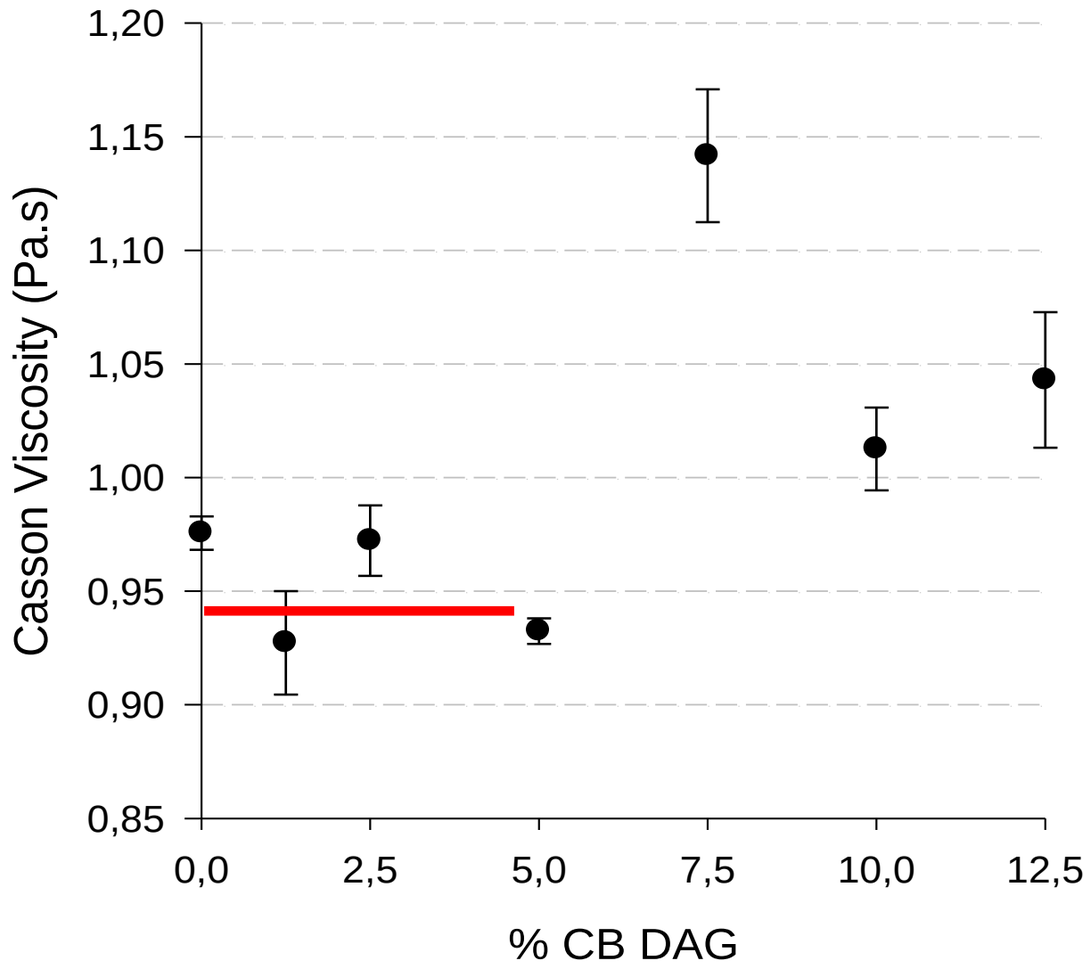


Chocolate flow behaviour

Casson Yield stress



Casson Viscosity



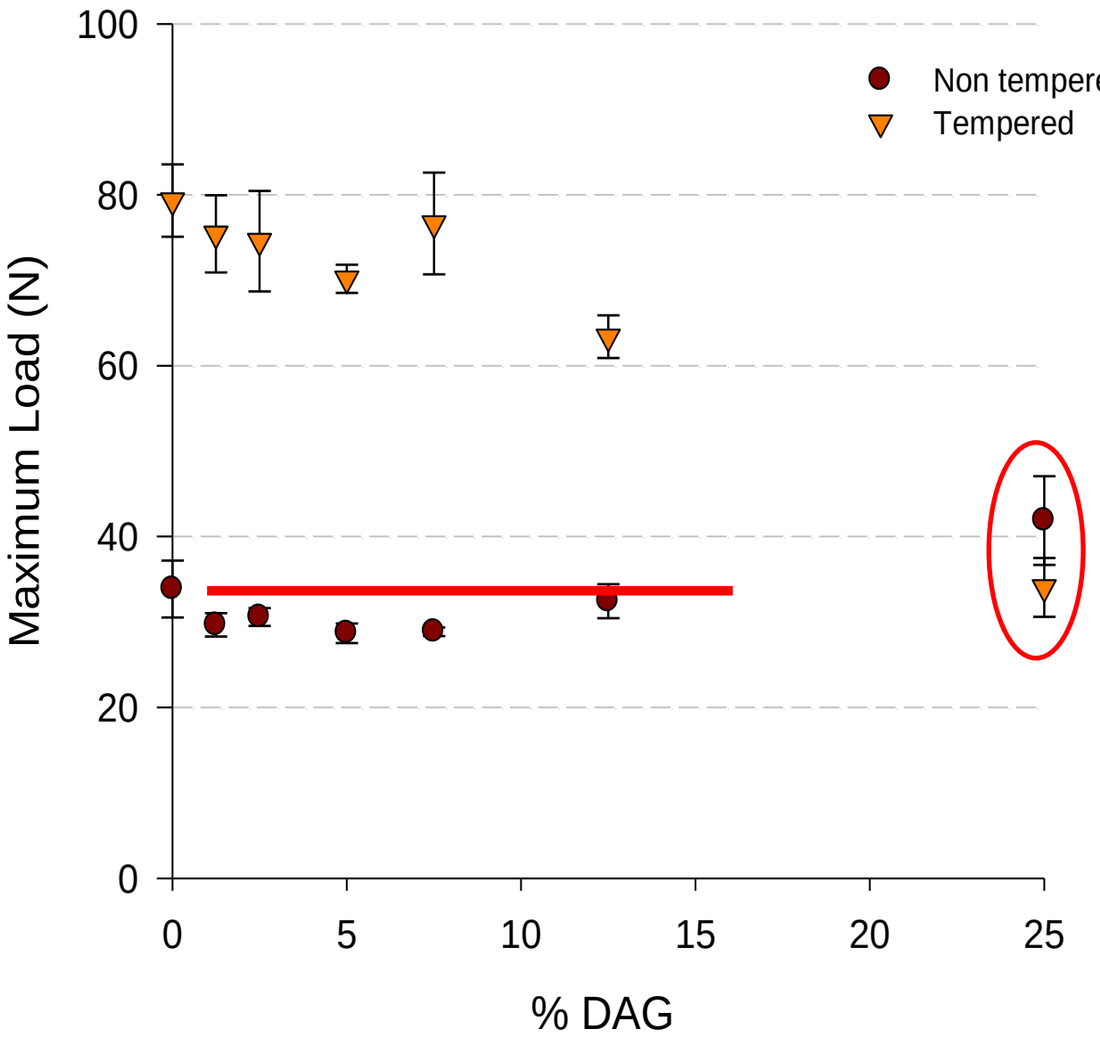
- Up to 5% no influence on rheological parameters
- > 7.5%: DAG interact at the interphase → micelle formation and/or multilayers → increased yield stress
- > 7.5%: higher viscosity: remaining crystals at 40°C

Texture analysis

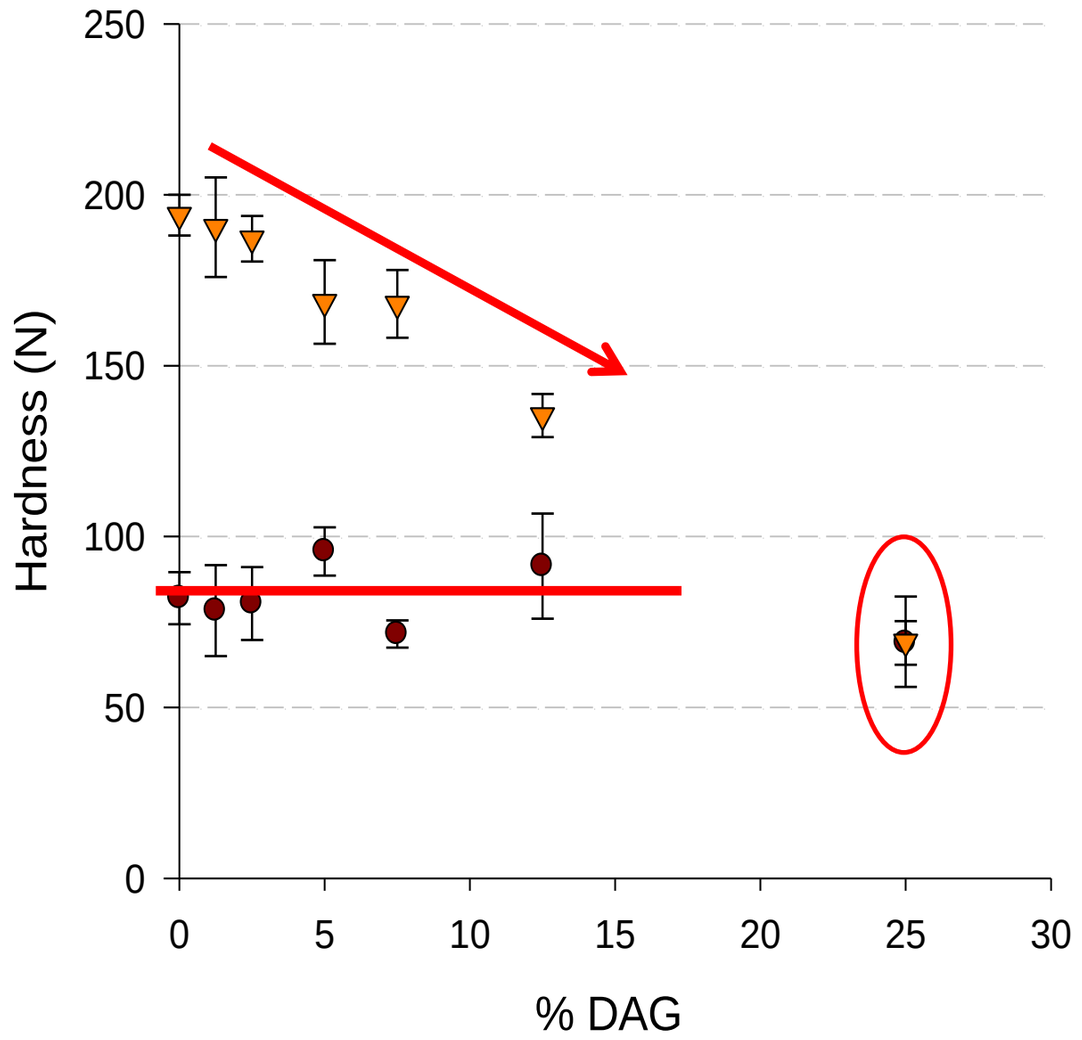
- Chocolate were hand tempered and compared with non tempered
- Texture analysis after **24** hours

Texture analysis

Three point bend test



Penetration test



Conclusions

- Enzymatic glycerolysis is a good technique to produce CB based DAG

  CB + enzyme + glycerol → 50% yield DAG

- CB DAG in chocolate
  - Up to 5%: limited influence on chocolate properties
  - Possibility to adjust yield stress with a CB based emulsifier

Acknowledgements:

Barry-Callebaut

FWO Flanders

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