Unraveling the molecular mechanism of *in vitro* gastric lipolysis through the advanced multi-response modeling technique

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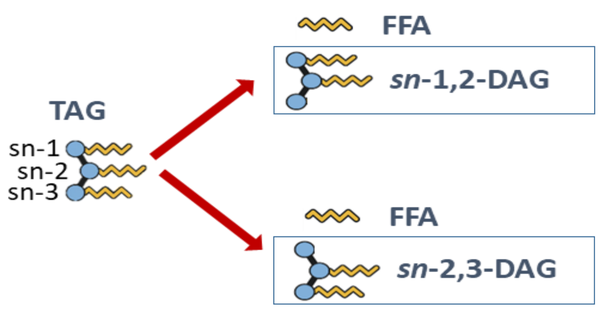
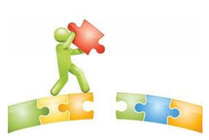
Background and research objective

Human gastric lipase (HGL) stereospecificity



DAGS identified and quantified through expensive and laborious techniques

Research gap → Gastric lipid digestion mechanism?



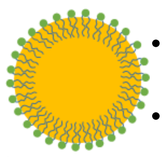
- Lack of relevant and convenient substitute of HGL
- End-point quantification of digestion product(s)
- FFA release by titration
- Simple and reliable analytical platform is missing.

Research objective

To elucidate the molecular mechanism of *in vitro* gastric lipid digestion by integrating multiple lipolysis responses quantified as a function of digestion time.

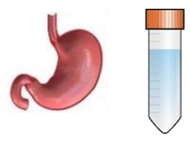
Experimental design

1. Static *in vitro* digestion

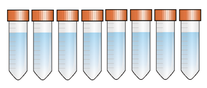


- 5% triolein (chiral substrate)
- 1% emulsifier

HGL substitute: Rabbit gastric lipase (RGL)



Chyme samples taken after 5, 10, 15, 30, 45, 60, 90, and 120 minutes of digestion



Details on emulsions composition and digestion conditions in Table 1

Table 1: Overview of data sets considered for the multi-response modeling

Emulsifier type	Emulsion initial droplet size d(4,3) (μm)	Emulsion name	*In vitro* gastric conditions
Data set 1:Effect of emulsion droplet size [1]			pH 5.5, RGLA 20 U/mL (Minekus *et al.*, 2014 and Sams *et al.*, 2016)
Sodium taurodeoxycholate	0.58 ± 0.03	FE	
Sodium taurodeoxycholate	1.82 ± 0.02	ME	
Data set 2: Effect of emulsion interfacial composition [2]			pH 3, RGLA 60 U/mL (Brodkorb *et al.*, 2019)
Soy lecithin	1.38 ± 0.09	LEC	
Soy protein isolate	1.36 ± 0.02	SPI	
Citrus pectin	1.17 ± 0.01	CP	

RGLA: rabbit gastric lipase activity

2. Analytical platform

Normal phase chromatography → HPLC-Charged aerosol detector

- Triolein (TAG)
- *sn*-1,2/2,3-diolein (sn-1,2/2,3-DAG)
- *sn*-1,3-diolein (sn-1,3-DAG)
- *sn*-2-monoolein (sn-2-MAG)
- *sn*-1/3-monoolein (sn-1/3-MAG)
- Oleic acid (FFA)

3. Multi-response kinetic modeling

Literature + own data → Reaction scheme → Differential equations

Reaction scheme 7

$TAG + H_2O \xrightarrow{k_1} sn\text{-}1,2/2,3\text{-}DAG + FFA$

$TAG + H_2O \xrightarrow{k_2} sn\text{-}1,3\text{-}DAG + FFA$

$TAG + 2H_2O \xrightarrow{k_3} sn\text{-}2\text{-}MAG + 2FFA$

$sn\text{-}1,2/2,3\text{-}DAG \xrightarrow{k_4} sn\text{-}1,3\text{-}DAG$

$sn\text{-}1,3\text{-}DAG + H_2O \xrightarrow{k_5} sn\text{-}1/3\text{-}MAG + FFA$

$sn\text{-}2\text{-}MAG \xrightarrow{k_6} sn\text{-}1/3\ MAG$

$sn\text{-}1/3\text{-}MAG + H_2O \xrightarrow{k_7} FFA + GLY$

Reaction scheme 1

Start from the simplest scheme

Numerical method 'proc model' SAS

Iterative process

Model convergence?

$$\frac{d(TAG)}{dt} = -(k_1+k_2+k_3)(TAG - TAG_f)$$

$$\frac{d(sn\text{-}1,2/2,3\ DAG)}{dt} = k_1(TAG - TAG_f) - k_4(sn\text{-}1,2/2,3\ DAG)$$

$$\frac{d(sn\text{-}1,3\ DAG)}{dt} = k_2(TAG - TAG_f) + k_4(sn\text{-}1,2/2,3\ DAG) - k_5(sn\text{-}1,3\ DAG)$$

$$\frac{d(sn\text{-}2\ MAG)}{dt} = k_3(TAG - TAG_f) - k_6(sn\text{-}2\ MAG)$$

$$\frac{d(sn\text{-}1/3\ MAG)}{dt} = k_5(sn\text{-}1,3\ DAG) + k_6(sn\text{-}2\ MAG) - k_7(sn\text{-}1/3\ MAG)$$

$$\frac{d(FFA)}{dt} = k_1(TAG - TAG_f) + k_2(TAG - TAG_f) + 2k_3(TAG - TAG_f) + k_5(sn\text{-}1,3\ DAG) + k_7(sn\text{-}1/3\ MAG)$$

$$\frac{d(GLY)}{dt} = k_7(sn\text{-}1/3\ MAG)$$

- Parity plots
- Residual plots
- Estimates error

Results and discussion

✓ Model corresponding to reaction scheme 7 converged

Table 2: Estimated parameters after subjecting data sets to multi-response modeling

	Data set 1		Data set 2		
	FE	**ME**	**LEC**	**SPI**	**CP**
k_1	0.081 ± 0.004	0.098 ± 0.005	0.035 ± 0.003	0.039 ± 0.003	0.080 ± 0.005
k_2	0.034 ± 0.003	0.051 ± 0.007	0.019 ± 0.004	0.010 ± 0.002	0.040 ± 0.005
k_3	0.058 ± 0.003	0.071 ± 0.004	0.023 ± 0.002	0.009 ± 0.001	0.017 ± 0.001
k_4	0.004 ± 0.000	0.002 ± 0.001	0.003 ± 0.001	0.003 ± 0.001	0.001 ± 0.001
k_5	0.541 ± 0.285	0.097 ± 0.027	0.033 ± 0.013	0.086 ± 0.036	0.024 ± 0.005
k_6	0.004 ± 0.000	0.002 ± 0.001	0.003 ± 0.001	0.003 ± 0.001	0.001 ± 0.001
k_7	0.037 ± 0.003	0.024 ± 0.003	0.018 ± 0.003	0.059 ± 0.004	0.043 ± 0.005
TAG_f	49.68 ± 0.06	51.44 ± 0.05	48.36 ± 0.19	41.18 ± 0.29	42.47 ± 0.27

$k_{1,3,5,7}$: *sn*-1/3 positions cleavage

k_2: *sn*-2 position cleavage

$k_{4,6}$: isomerization reactions

$k_1 > k_2 > k_{4,6}$

Conclusions

- The reaction mechanism was the same for all conditions. This demonstrates the validity of the mechanistic multi-response model under different static conditions (e.g. different pH and lipase activity values).
- These findings may be the starting point to develop *in silico* models to predict the gastric lipolysis based on emulsion design properties.

This poster is based on:

[1] Infantes-Garcia *et al.* (2020), *Food Chemistry* 326 (126895)

SCAN ME

[2] Infantes-Garcia et al. (2021), *Food Hydrocolloids* 110 (106163)

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