A new in vitro gastrointestinal system to evaluate the effect of exogenous molecules

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OUR AIM (PRESENT AND FUTURE)

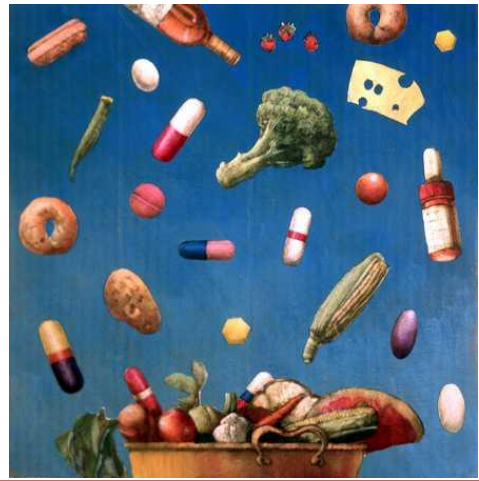
- Compound analysis → new gastrointestinal model
- Dynamic system → Reduction?
- Validation → Replacement?

BIOLOGICAL AND TOXICOLOGICAL STUDIES

- **Food** components/products:
  - ✓ nutrient (proteins, fatty acids, vitamins, minerals)
  - ✓ active (polyphenols, carotenoids…)
  - ✓ toxin

- **Drugs** or **Natural products**:
  - ✓ activity/therapeutic effect
  - ✓ toxicity

- Industrial/agro-chemicals (**Contaminants**):
  - ✓ toxicity



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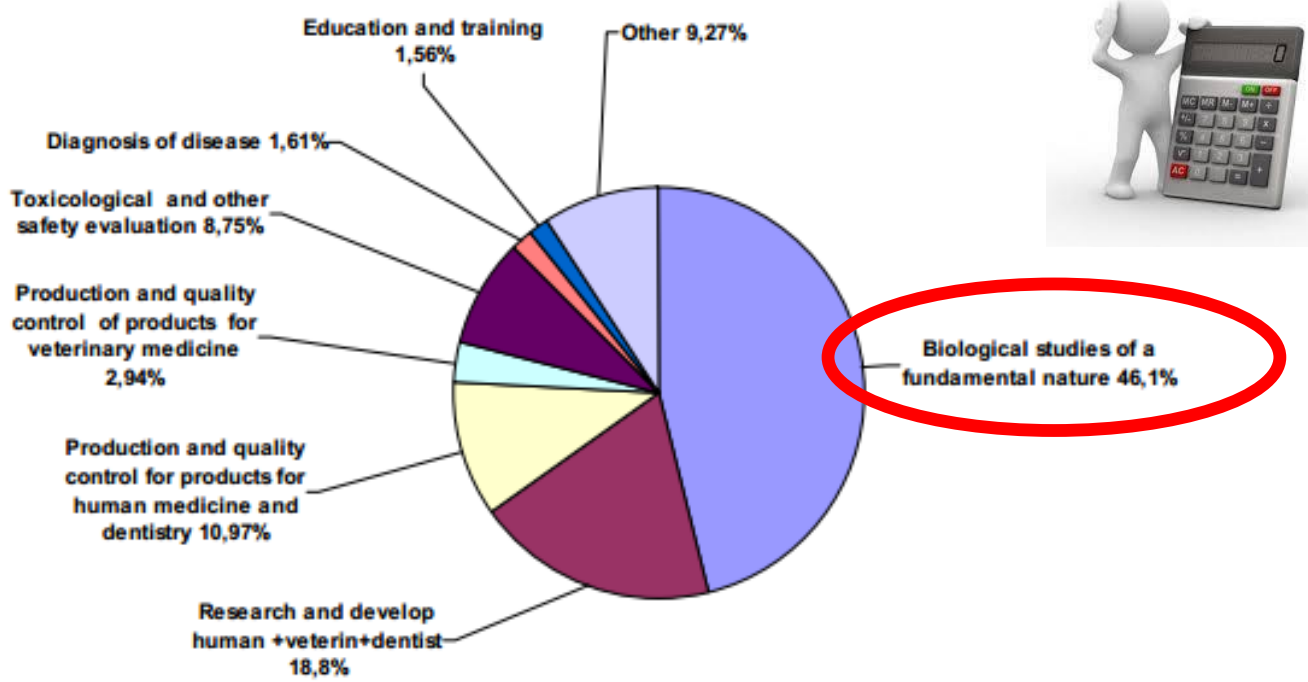
HOW MANY ANIMAL MODELS Vs AIM

12 million

*UE Commission 2013_*



3RS PRINCIPLES/MODEL: REDUCTION

In vitro MODELS

Compound screening/
Compound testing

BIOCHEMICAL

NO PHYSICAL PROCESS

SPEED
VERY LOW COST

IN SILICO-METHODS
COMPUTATIONAL MODELLING

PHYSICOCHEMICAL PROPERTIES/
MOLECULAR MECHANISMS

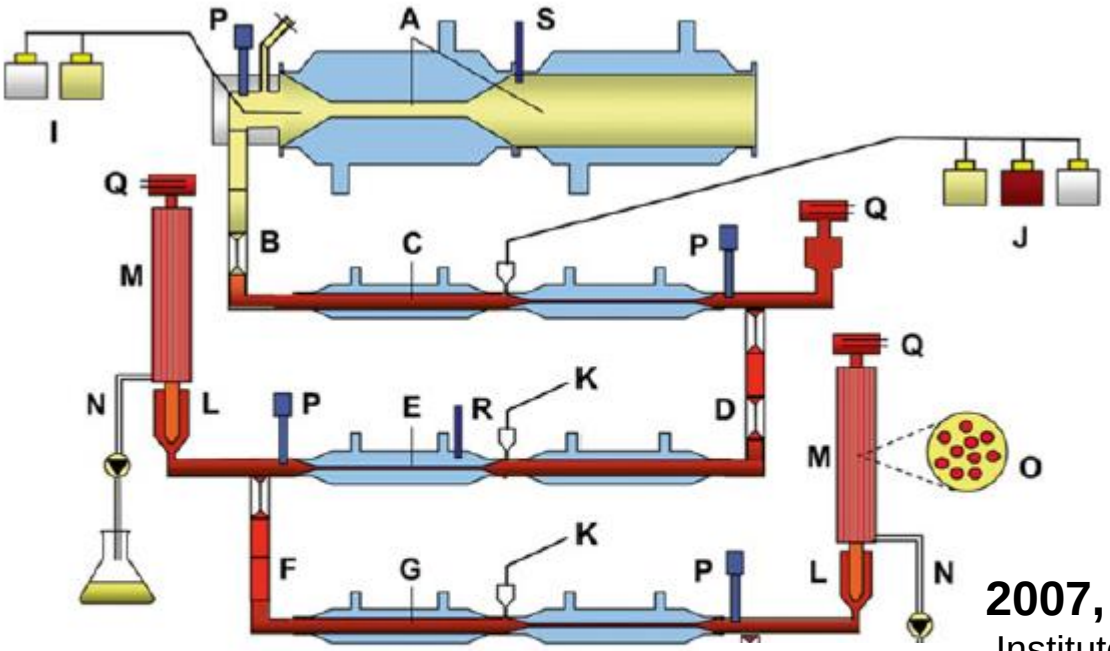
SPEED
THE LEAST EXPENSIVE

CELL-BASED

FIRST REPRESENTATION OF
LIVING SYSTEMS

SPEED
LOW-HIGH COST

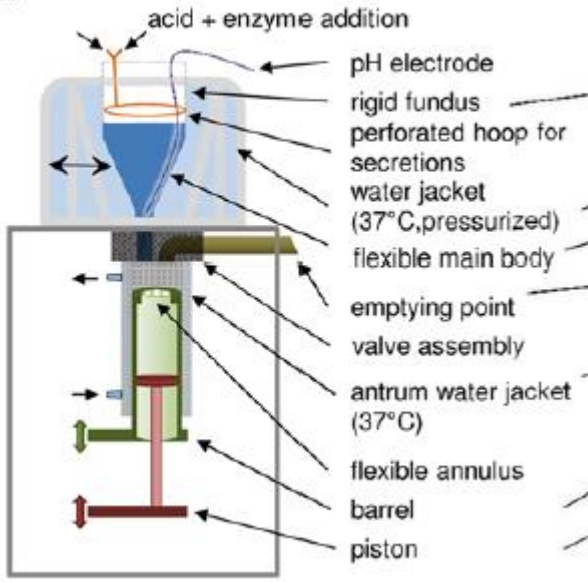
1990, TNO gastro-Intestinal Model (TIM)



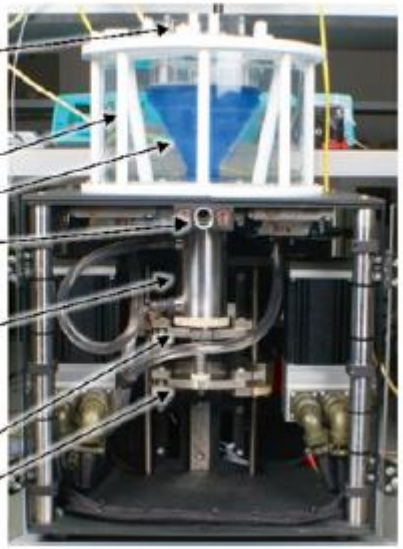
2007, Dynamic Gastric Model (DGM)

Institute of Food Research (Norwich, UK)

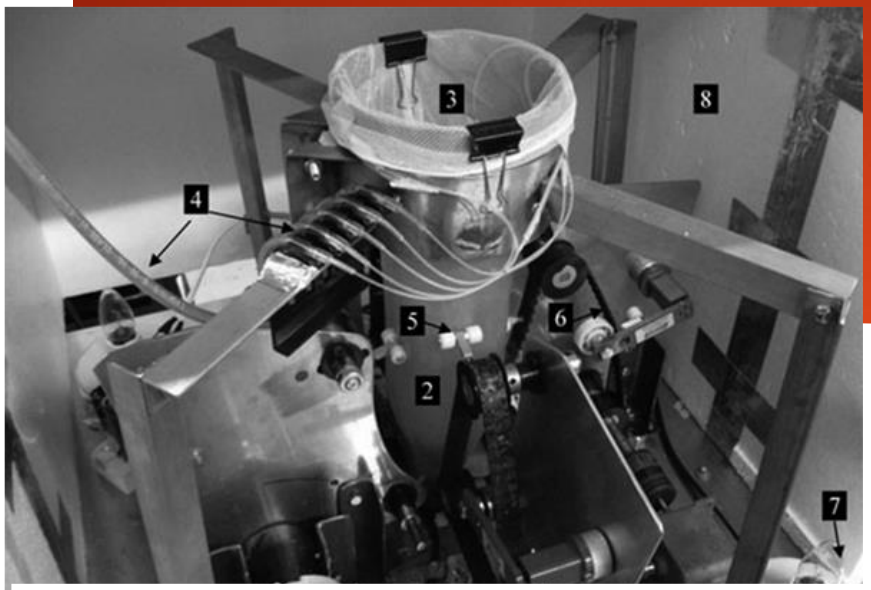
a



b

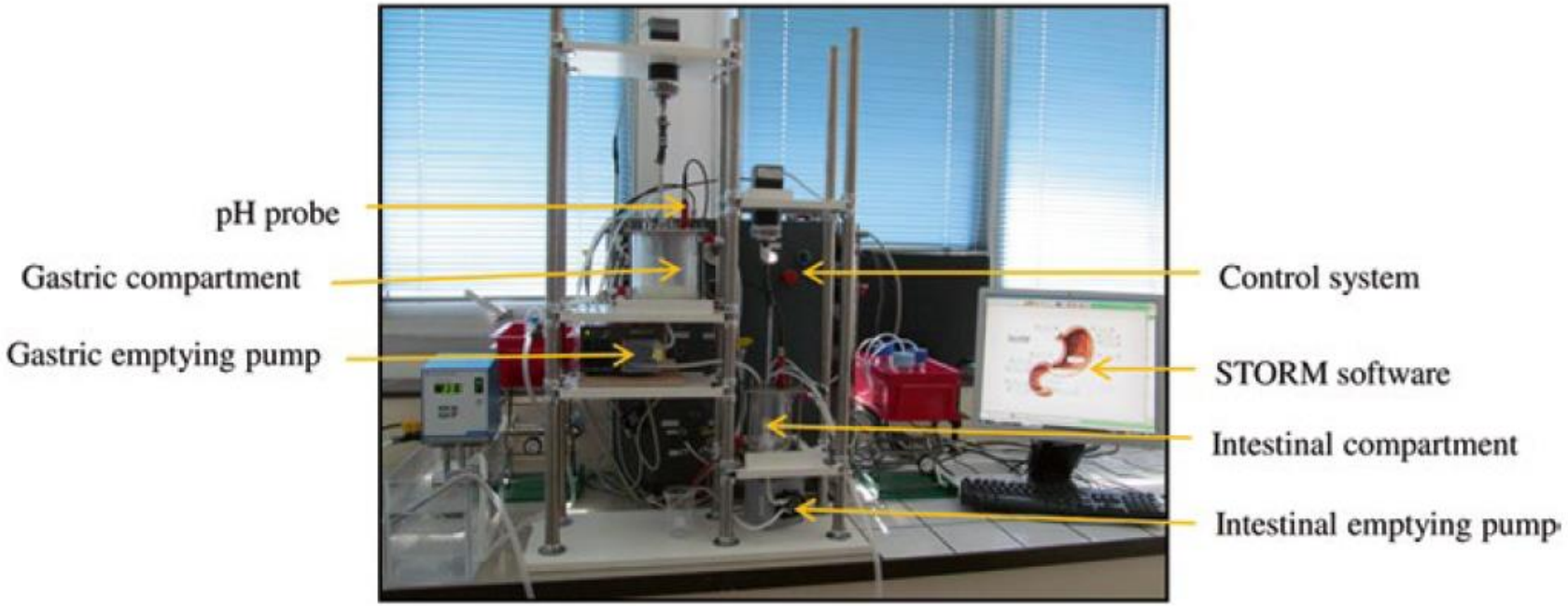


2010, Human Gastric Simulator (HGS) Riddet Model



2014, DIDGI® SYSTEM

Institut national de la recherche agronomique (INRA)



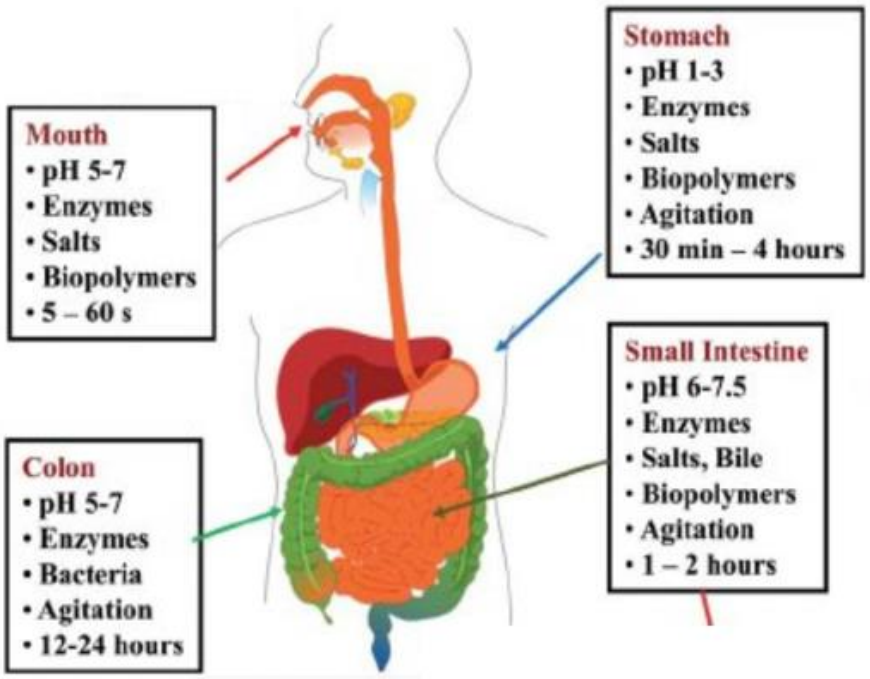
MILLIFLUIDIC SYSTEM

Two pumps
Two interconnected circuits
Independent experiments
Flow rate range (100-450 µl/min)
Flow direction
Compatible with incubators and hoods

Interconnected cell co-culture

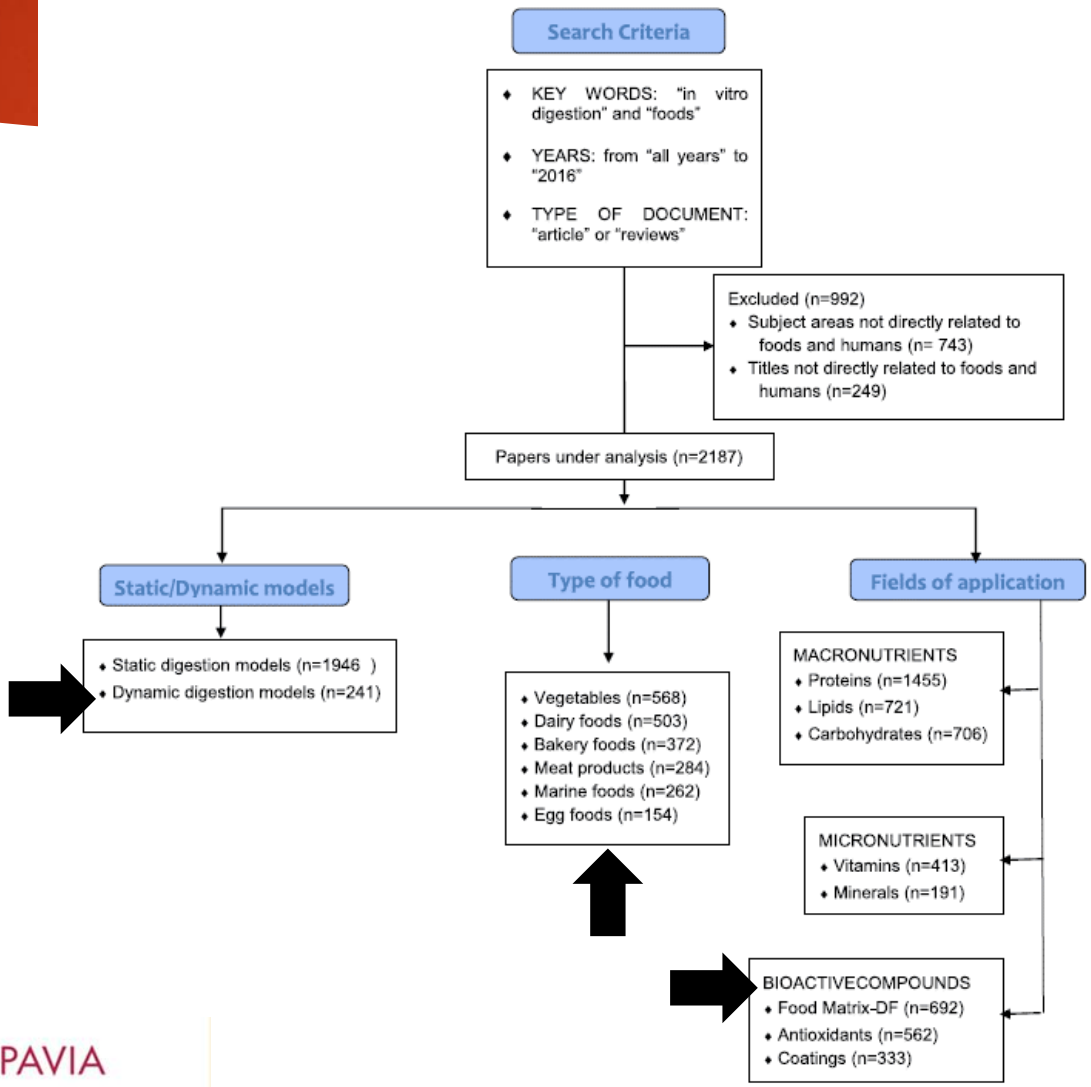
LiveBoxes

INGESTED FOOD (OR DRUG) BIOAVAILABILITY



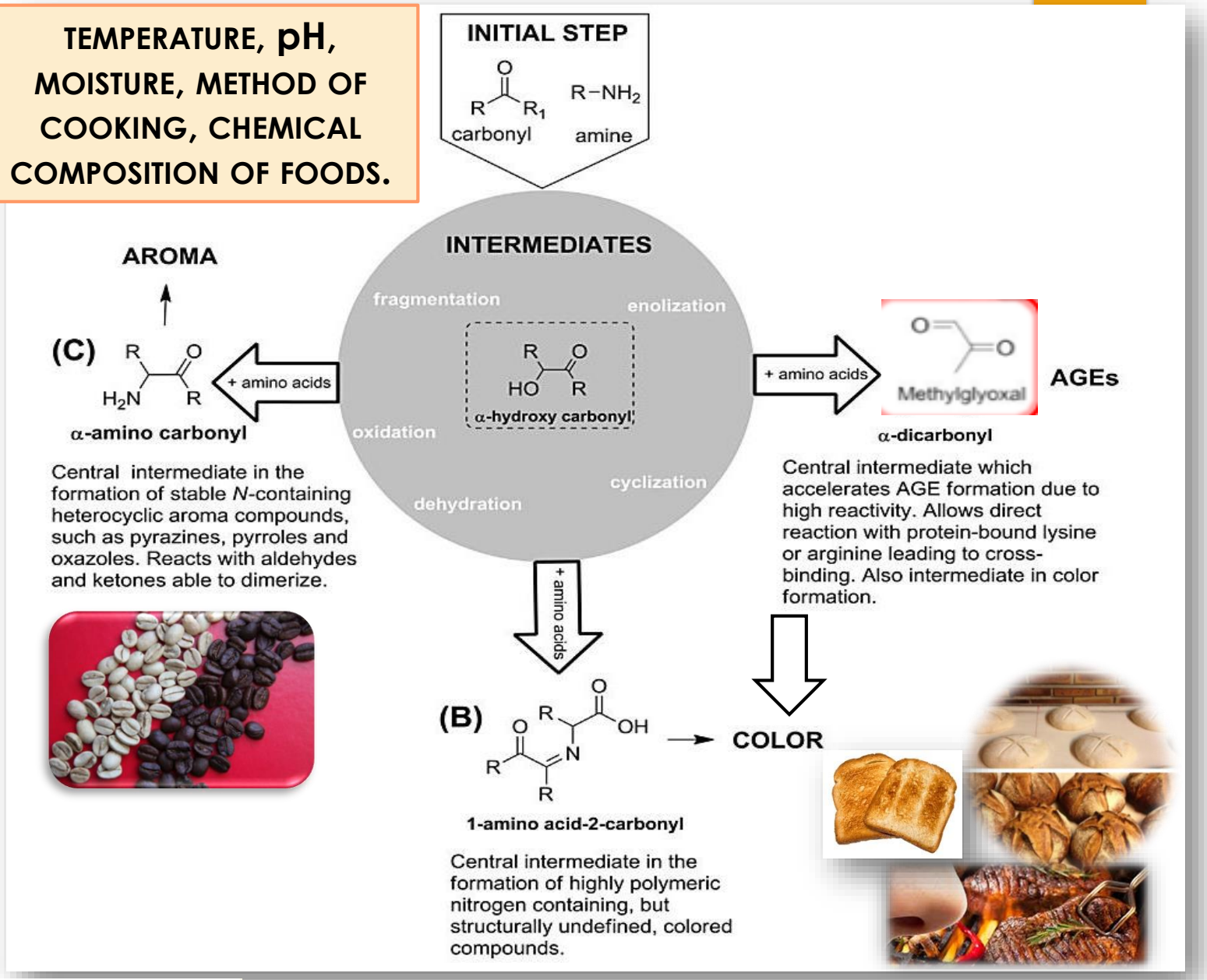
1. **Digestibility and solubility**
2. **Absorption/metabolization** and transport
3. From the circulation to target

BACKGROUND

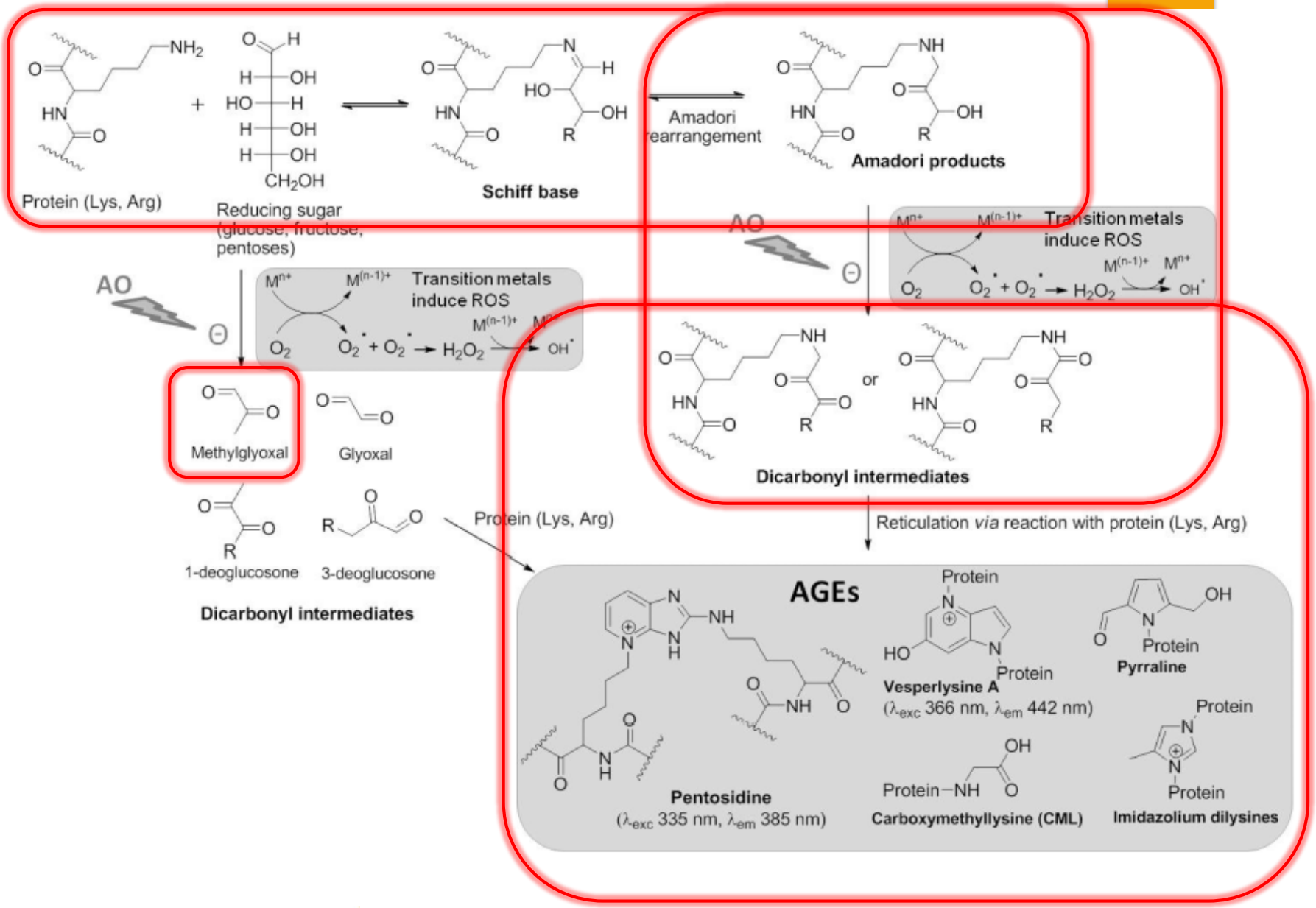


Lucas-González et al *Food Res Int*, **2018**, *107*, 423-436

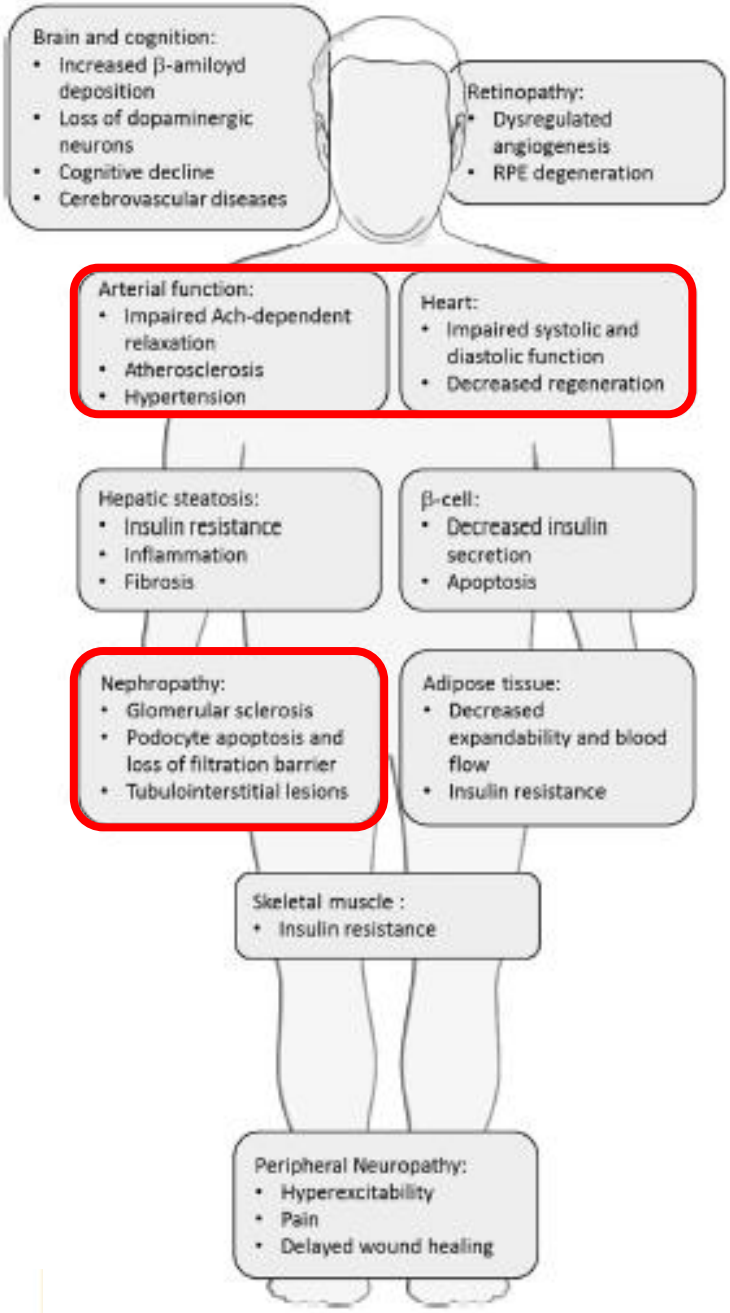
TEMPERATURE, pH, MOISTURE, METHOD OF COOKING, CHEMICAL COMPOSITION OF FOODS.



Poulsen et al *Food Chem. Toxicol.*, **2013**, *60*, 10-37



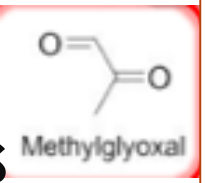
Boisard et al *J Agric Food Chem*, **2014**, 62, 1344—1351



Matafome et al *Med Res Rev*, **2017**, *37*, 368-403

AIM

TO INVESTIGATE THE FATE OF EXOGENOUS METHYLGLYOXAL (MGO) DURING THE DIGESTIVE PROCESS



TWO different APPROACHES

WITH THREE DIFFERENT MGO INTAKES

STATIC process
(biochemical approach)

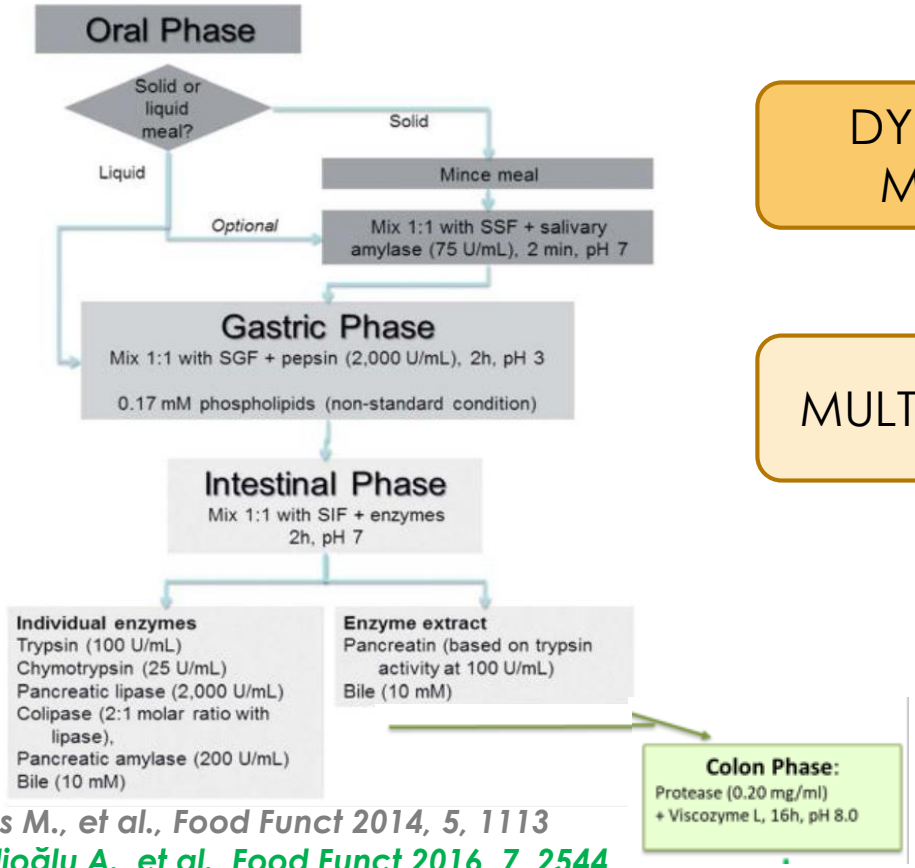
DYNAMIC process
(cell-based assay)

Colombo R., Paolillo M., Papetti, A. accepted *Food and Function*

MULTI-APPROACH
Gastrointestinal system

BIOCHEMICAL

CELL-BASED



DYNAMIC MODEL

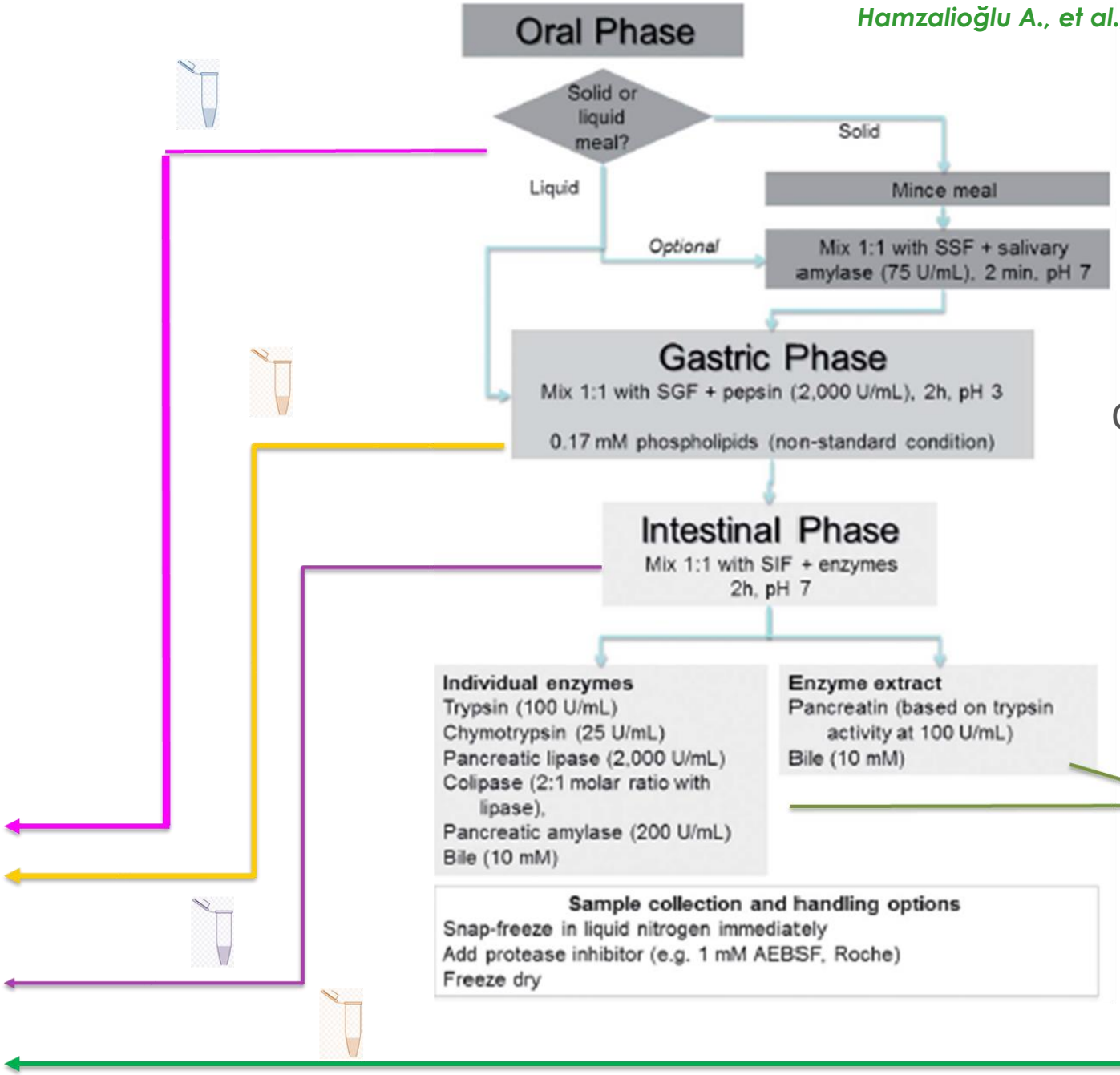
MULTI-ORGAN

LiveFlow®

IVTech In-vitro technologies

Minekus M., et al., Food Funct 2014, 5, 1113
Hamzalioğlu A., et al., Food Funct 2016, 7, 2544

Minekus M., et al., Food Funct 201, 5, 1113
Hamzalioğlu A., et al., Food Funct 2016, 7, 2544



COST **INFOGEST** network

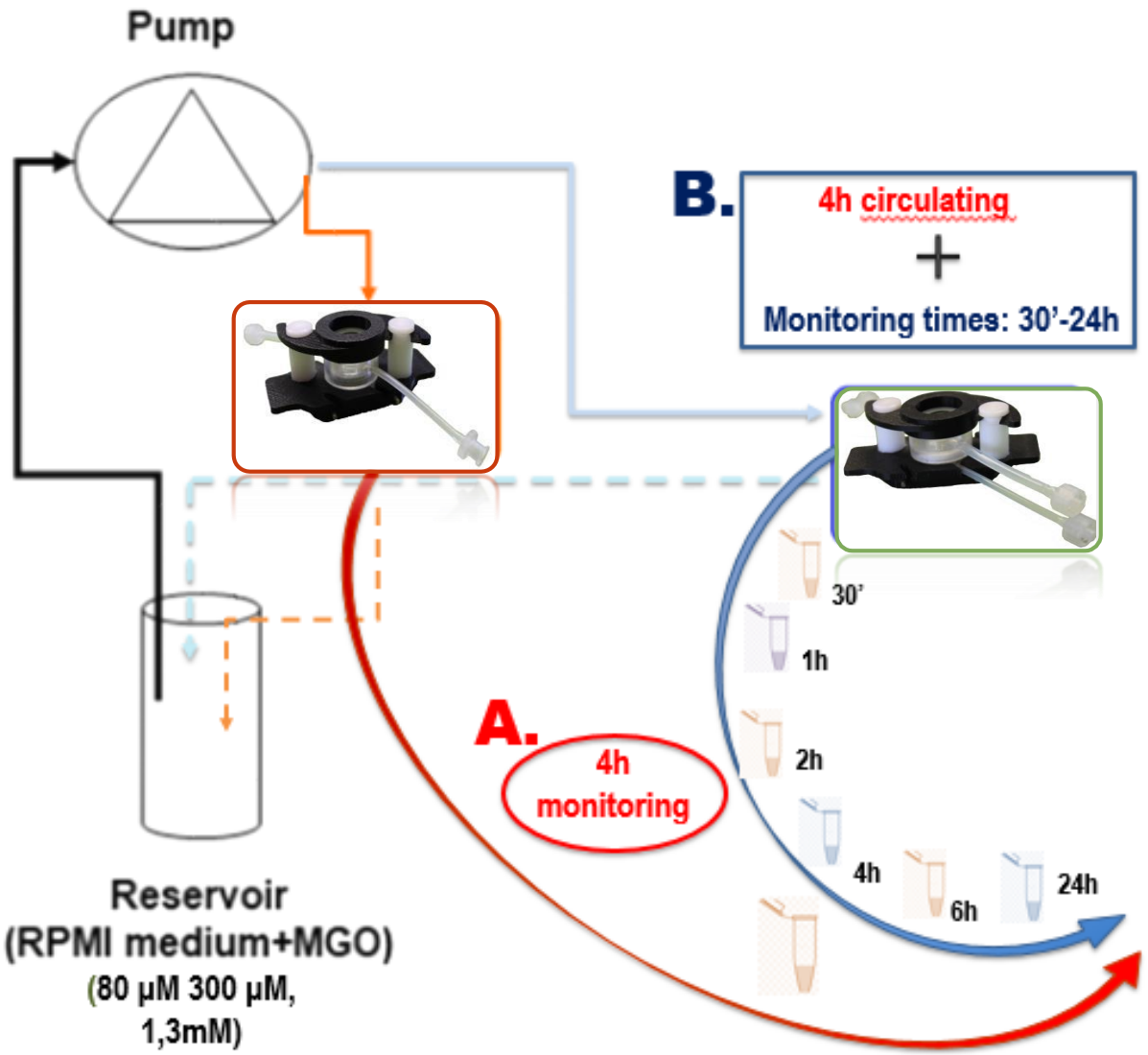
LiveFlow®

In-vitro technologies IVTECH

HUMAN CELLS

INTESTINAL phase
CaCo-2 cells

GASTRIC phase
GIST882 cells



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SAMPLE PREPARATION

MGO digested samples

↓ 5000 rpm, 10 min, 25 °C

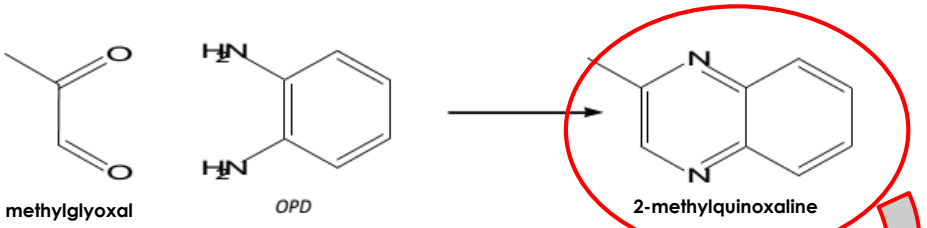
Deproteinization
with PCA (0.5 M), 1:9 v/v
4 °C, 10 min

↓ 5000 rpm, 5 min, 4 °C

Derivatization
with OPD 0.25% w/v,
37 °C, 1 h

↓

Filtration



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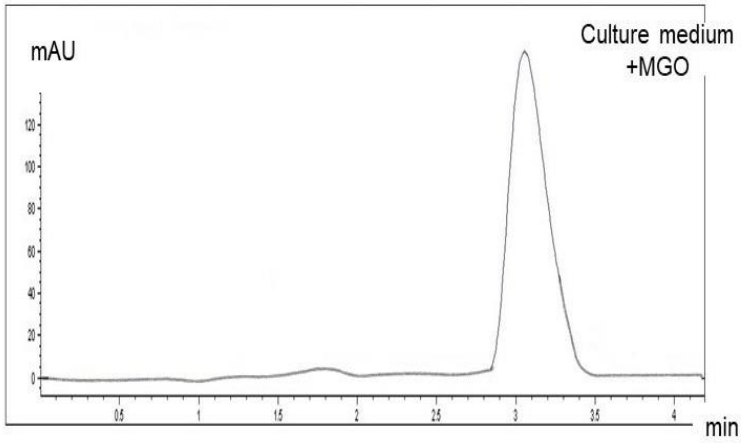
METHOD SET-UP AND VALIDATION

HPLC-DAD

- Column: Gemini 5µm C18 110Å, 150 x 2.00 mm, (Phenomenex® Torrance, CA, USA)
- Loop: 20 µl
- Mobile phase: 0,5 % CH_3COOH- MeOH, 50:50 (v/v)
- Isocratic elution
- Flow: 0.3 ml/min
- T=25°C
- λ 315 nm

[Modified method of Nemet *et al.* (2004), *Clin Biochem, 37*. 875 – 881]



INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

VALIDATION OF ANALYTICAL PROCEDURES: TEXT AND METHODOLOGY
Q2(R1)

- Specificity
- Selectivity
- Linearity — 5.0-405.9 µM; 500-1500 µM; R^2>0.9900
- Accuracy — 94.02 to 102.60%
- Precision (intra and inter-day) — Intra-day < 2%, Inter-day < 2%
- Limit of detection (LOD) — LOD 1.1 µM
- Limit of quantification (LOQ) — LOQ 3.5 µM

HPLC-DAD MONITORING

MGO concentration (μM)			
Phase	80 μM *acute*	300 μM *daily*	1300 μM *weekly*
oral	48.39 ± 3.44	207.87 ± 4.57	959.66 ± 38.96
gastric	82.22 ± 9.22	300.24 ± 12.46	1316.90 ± 21.60
duodenal	15.64 ± 0.55	63.84 ± 5.48	265.85 ± 5.96
colon	4.30 ± 0.01	10.29 ± 0.09	148.98 ± 2.34

Hypotized that the reduction of MGO level registered after oral phase could be due to a potential interaction between salivary a-amylase and MGO

Following static process, MGO metabolization rate reached the highest peak after **duodenal phase**

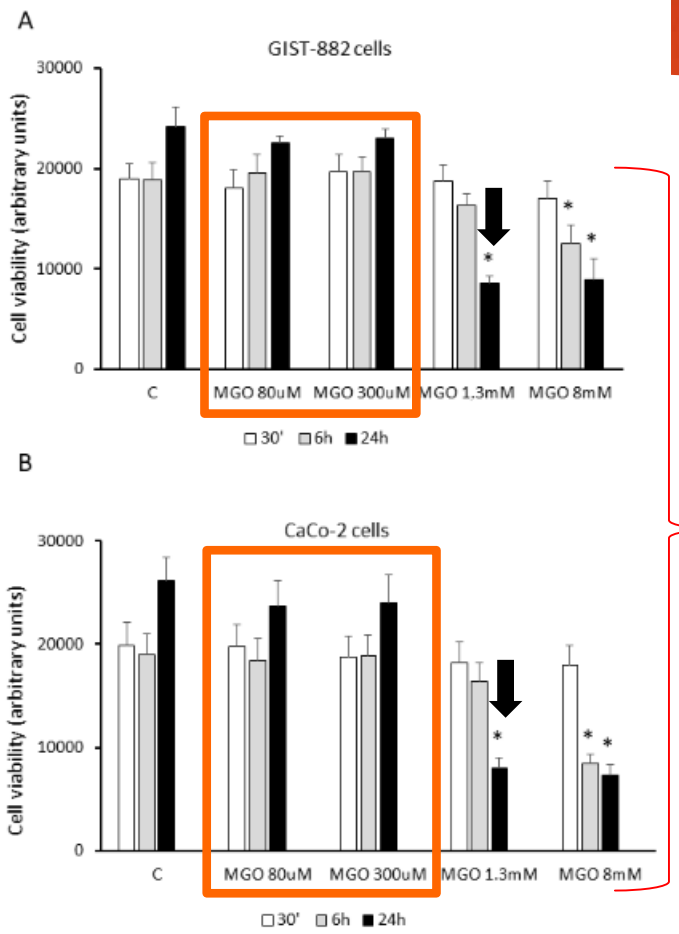
a) passage through gastric chamber; b) passage through intestinal chamber

	Time (h)	MGO concentration (μM)		
		80 μM ***acute***	**300 μM** ***daily***	**1300 μM** ***weekly***
A.	**4[a]**	21.33 ± 3.24	85.75 ± 2.40	556.79 ± 45.14
B.	0.5[b]	16.42 ± 1.47	72.33 ± 0.91	496.47 ± 60.86
	1[b]	14.66 ± 1.05	66.87 ± 1.11	473.59 ± 56.43
	2[b]	13.48 ± 1.20	54.06 ± 3.24	411.45 ± 36.68
	4[b]	10.03 ± 1.01	43.14 ± 3.46	304.72 ± 34.15
	6[b]	7.56 ± 1.26	28.26 ± 1.97	231.14 ± 21.70
	24[b]	-	-	39.26 ± 5.28

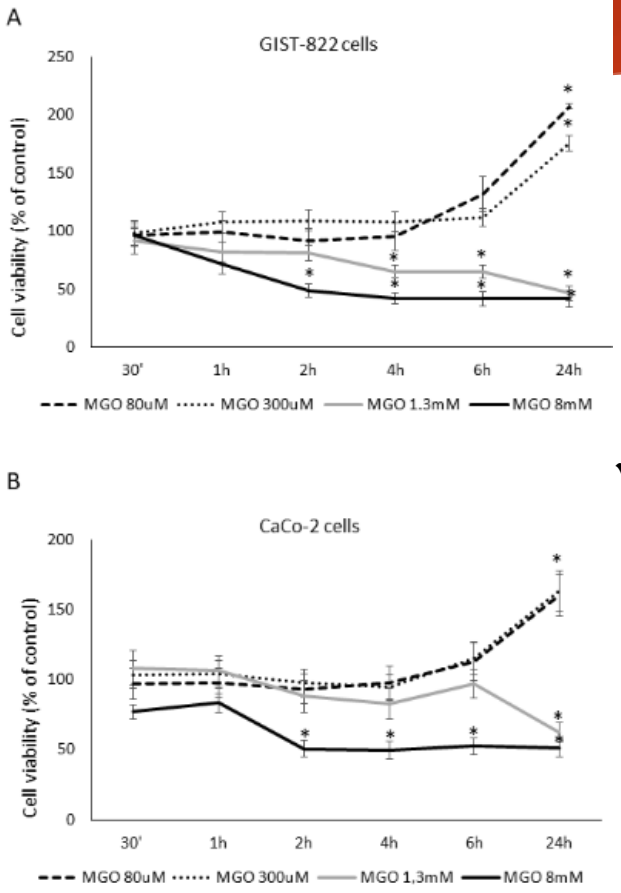
MGO is always submitted to a strong metabolization rate at gastric level

MGO at 80 and 300 concentrations are totally metabolized after 24h

CELL TOXICITY ASSAYS



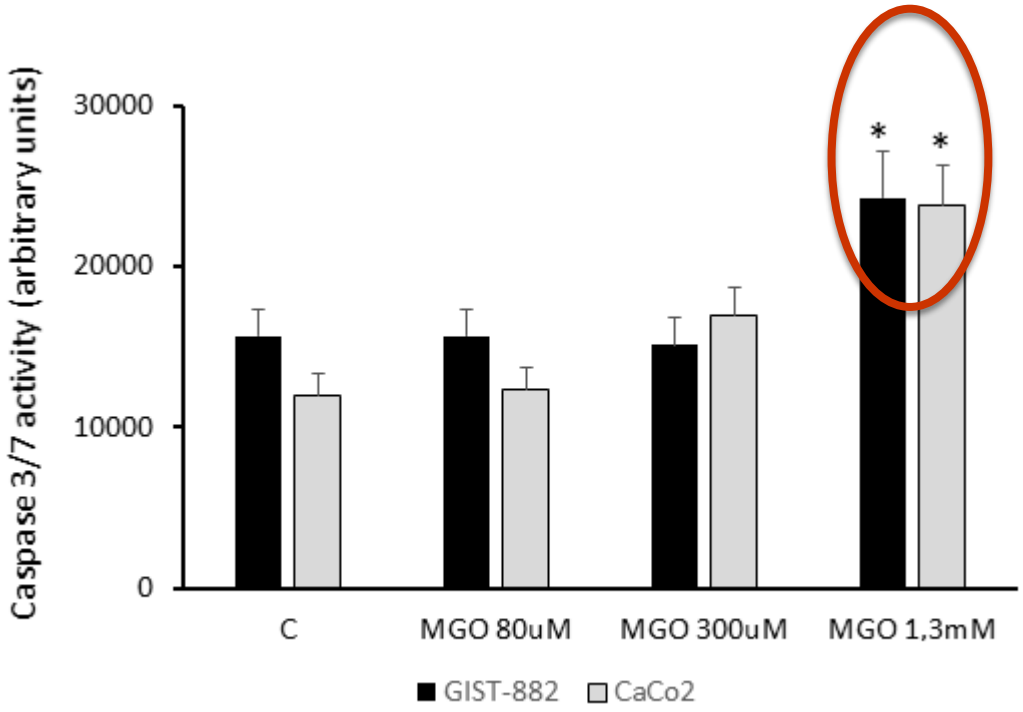
ALAMAR blue test



MTS assay

- ✓ Exogenous MGO in acute and daily dose has not a toxic effect
- ✓ MGO exerted a strong toxic effect only at 1.3 mM and at 24hours
- ✓ MGO at the concentration of 8 mM was used as positive toxicity control

CASPASE ACTIVITY



A significant increase of caspase 3/7 activity at **1.3 mM** concentration was observed in both cell lines, so the observed **decrease of cell viability** in presence of MGO **is due to apoptosis**

Protective action of intestinal cells!

CONCLUSIONS

MGO static digestion approach

Complete metabolization of MGO at intestinal level

(literature data confirmed)

MGO dynamic digestion approach

Gastric compartment's role (NOVELTY)

BOTH APPROACHES ARE NEEDED

Future…

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- Compound analysis → new gastrointestinal model
- Dynamic system → Reduction?
- Validation → Replacement?

THANKS TO…

UNIPV-DDS

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I-Care network

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