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Influence of protein source on the functionality and the digestibility of infant formulas

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HAL Authorization



ICEF13
INTERNATIONAL CONGRESS
ON ENGINEERING AND FOOD

MELBOURNE, AUSTRALIA
23-26 SEPTEMBER 2019

*Engineering Innovations
for Food Supply Chains*



INRA
SCIENCE & IMPACT

INFLUENCE OF PROTEIN SOURCE ON THE FUNCTIONALITY AND THE DIGESTIBILITY OF INFANT FORMULAS

Linda Le Roux¹, Olivia Ménard², Raphaël Chacon¹, Didier Dupont²,
Amélie Deglaire², Romain Jeantet², Françoise Nau²

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²STLO, INRA, Agrocampus Ouest

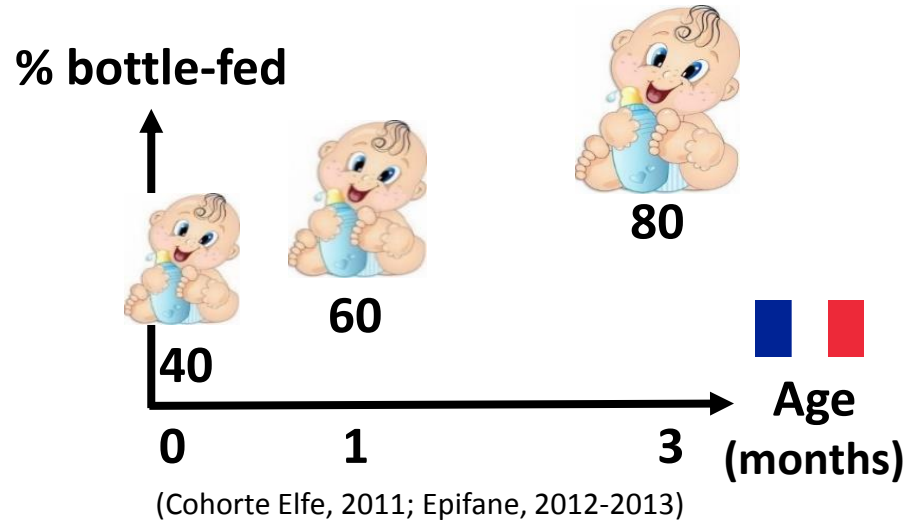


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CONTEXT



- Infant formula is the main source of nutrition for infants who cannot be breastfed



- Growing interest in plant proteins

- Ethical, health, sustainability

(Baroni et al., 2018; Ferrara et al., 2017)



- Importance of protein nutritional quality:

- Infant optimal growth (Michaelsen & Greer, 2014)



- Essential amino acid profile and protein digestibility (Friedman, 1996)

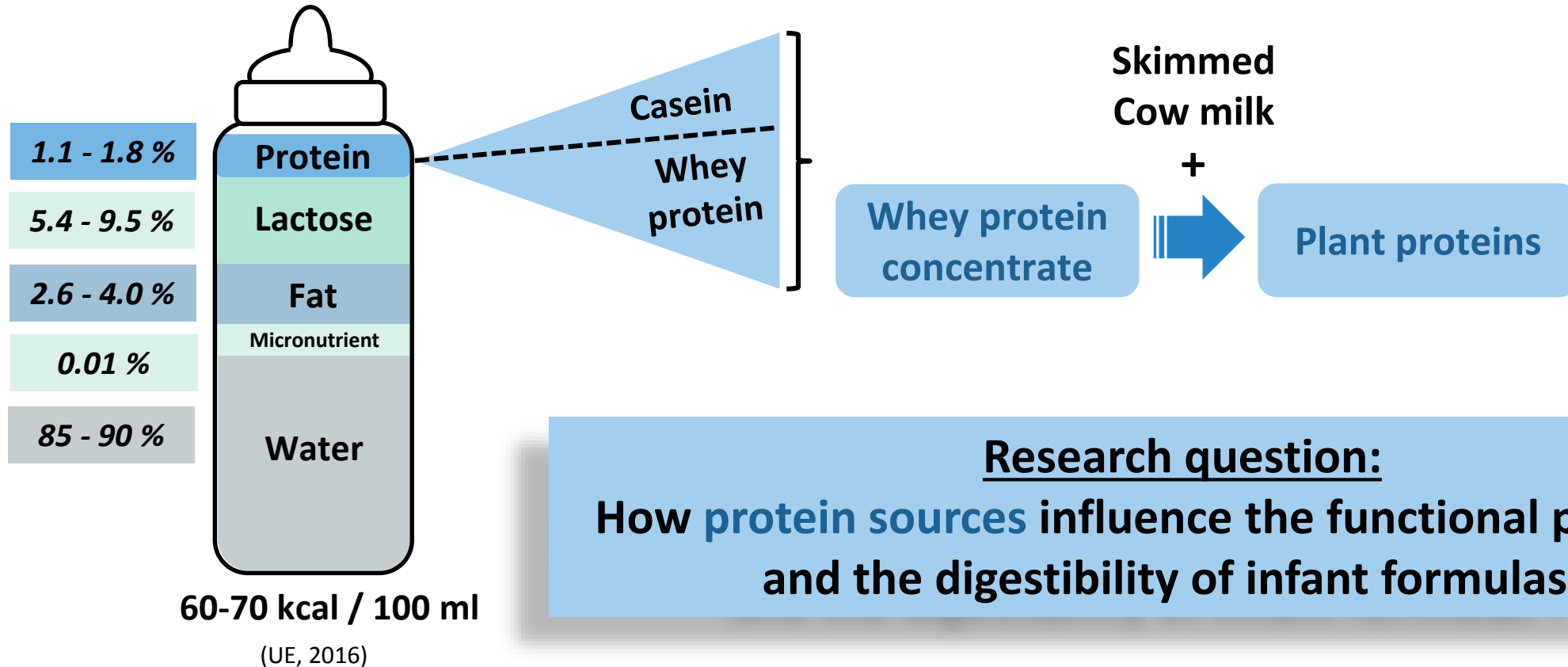
(FAO, 2013)

Age Group	His	Ile	Leu	Lys	SAA	AAA	Thr	Trp	Val
	<i>scoring pattern mg/g protein requirement</i>								
Infant (birth to 6 months) ¹	21	55	96	69	33	94	44	17	55
Child (6 months to 3 year) ²	20	32	66	57	27	52	31	8.5	43
Older child, adolescent, adult ³	16	30	61	48	23	41	25	6.6	40

OBJECTIVE

Standard 1st age infant formula

New 1st age infant formulas



STRATEGY

1. Selection of new protein sources

Whey <i>Whey protein</i>	Pea <i>Pisum sativum</i>	Faba bean <i>Vicia faba</i>	Rice <i>Oriza sativa L.</i>	Potato <i>Solanum tuberosum</i>
				



2. Development of new infant formulas



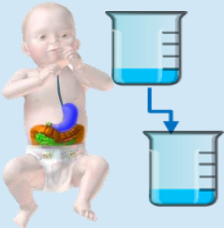
Pilot scale



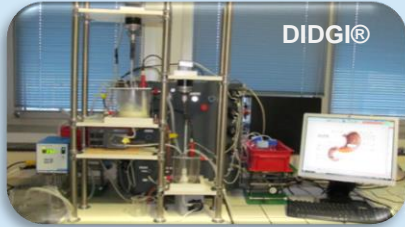
Semi-industrial scale



3. Digestion of new infant formulas

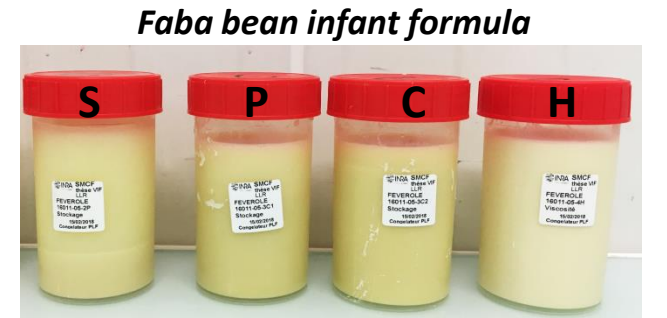
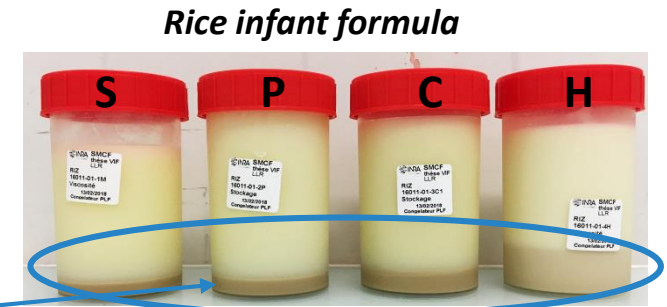
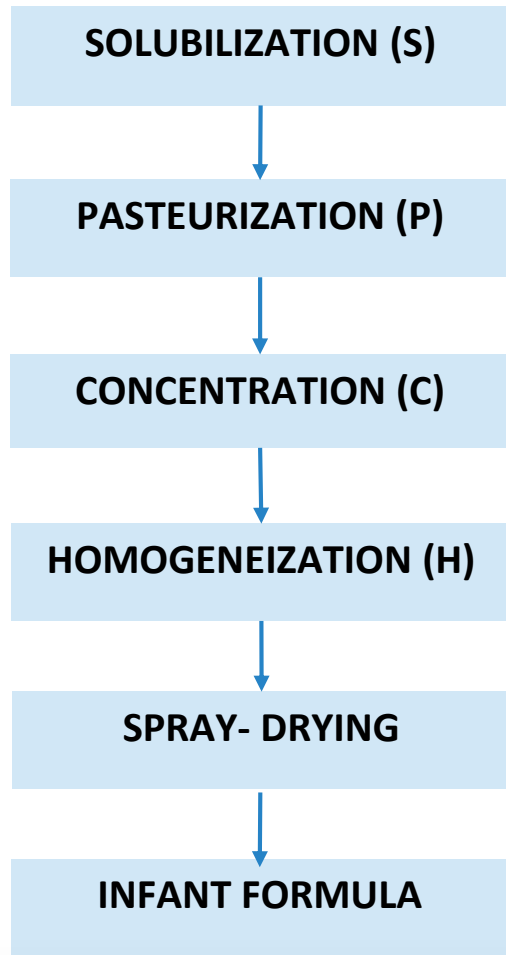


In vitro static



In vitro dynamic

PILOT SCALE MANUFACTURING



Solubility limits

Viscosity limits



Potato infant formula



RICE

POTATO

SCALE-UP



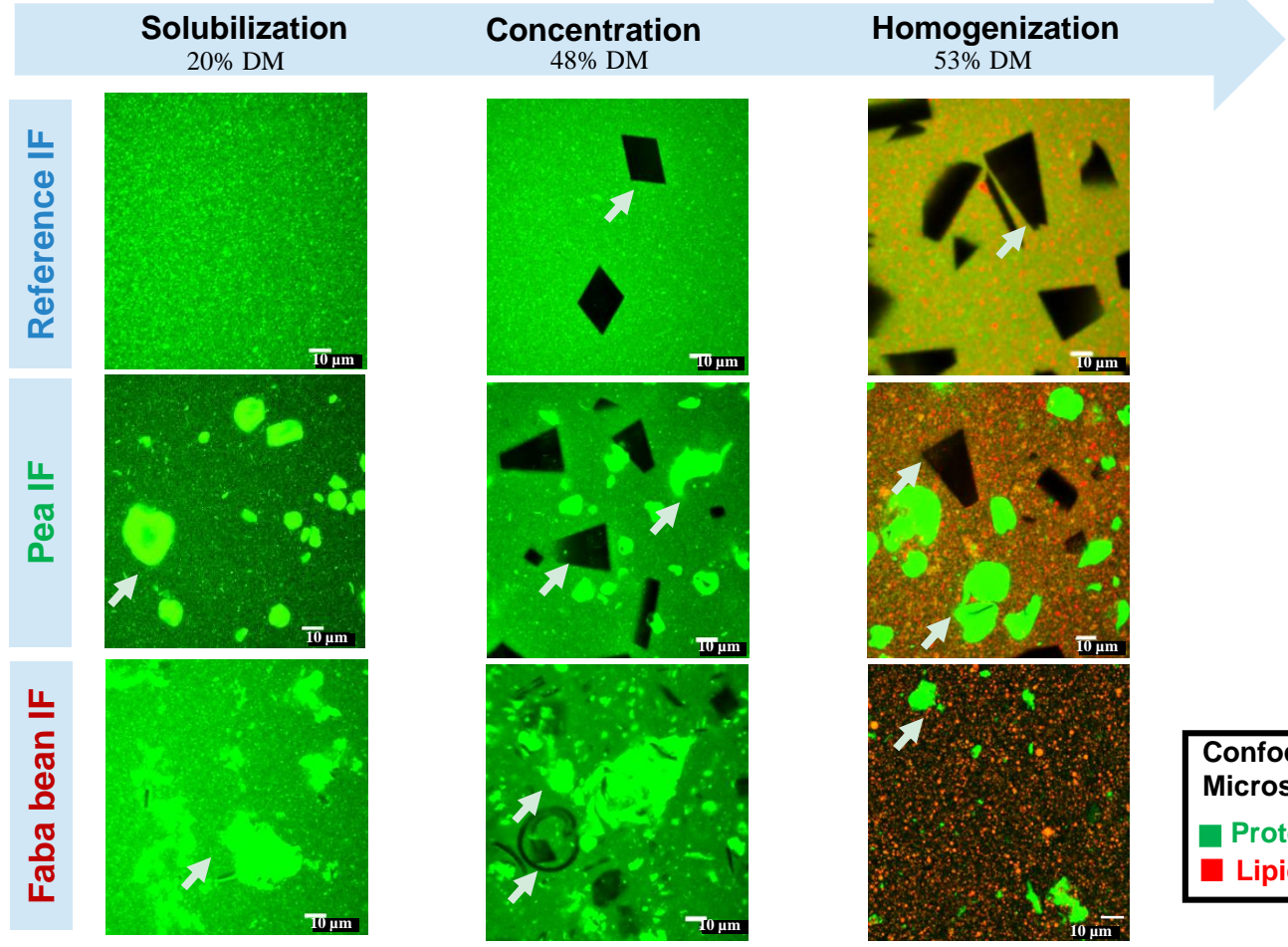
Evaporation capacity 5 kg/h



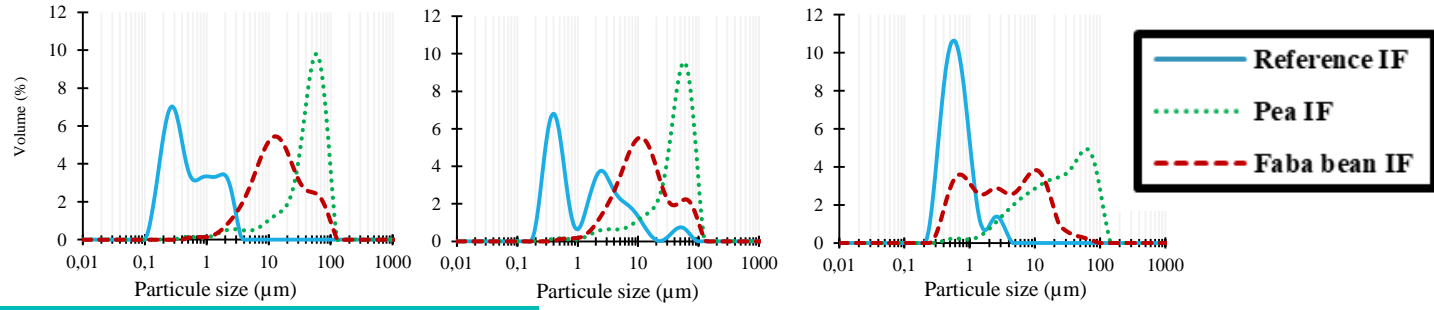
Evaporation capacity 90 kg/h



SEMI-INDUSTRIAL SCALE MANUFACTURING



Confocal Microscopy
■ Proteins
■ Lipids



- Bigger particles in plant based IF
- Lactose crystals are observed at high dry matter
- Particle size ↓ thanks to process

DM: dry matter

CONFORMITY OF INFANT FORMULA

- Functional properties

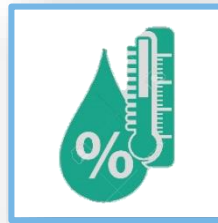
Identical composition of infant formulas



Soluble powders



a_w around 0.2



Low Free fat



Preventing from phenomena as caking, browning, lipid oxidation...

- Nutritional properties

EUROPEAN REGULATION

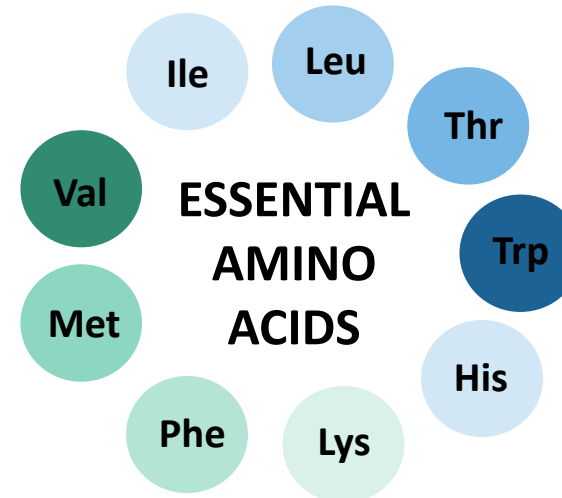


RÈGLEMENT DÉLÉGUÉ (UE) 2016/127 DE LA COMMISSION

du 25 septembre 2015

complétant le règlement (UE) n° 609/2013 du Parlement européen et du Conseil en ce qui concerne les exigences spécifiques en matière de composition et d'information applicables aux préparations pour nourrissons et aux préparations de suite et les exigences portant sur les informations relatives à l'alimentation des nourrissons et des enfants en bas âge

(UE, 2016)



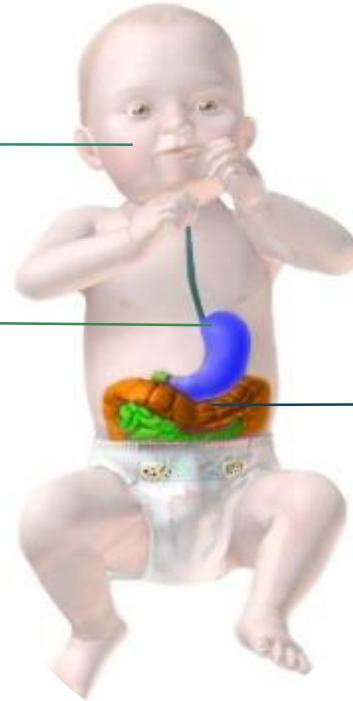
IMMATURITY OF NEWBORN DIGESTIVE SYSTEM

Mouth

- Limited oral capacity

Stomach

- Relatively high pH (3.2 – 6.5)
- Mature secretion of human gastric lipase (HGL)
- **Immature secretion of pepsin and limited proteolysis**



Intestine

- **Immature secretion of human pancreatic lipase (HPL)**
- Mature secretion of trypsin but immature secretion of chymotrypsin and carboxypeptidase B

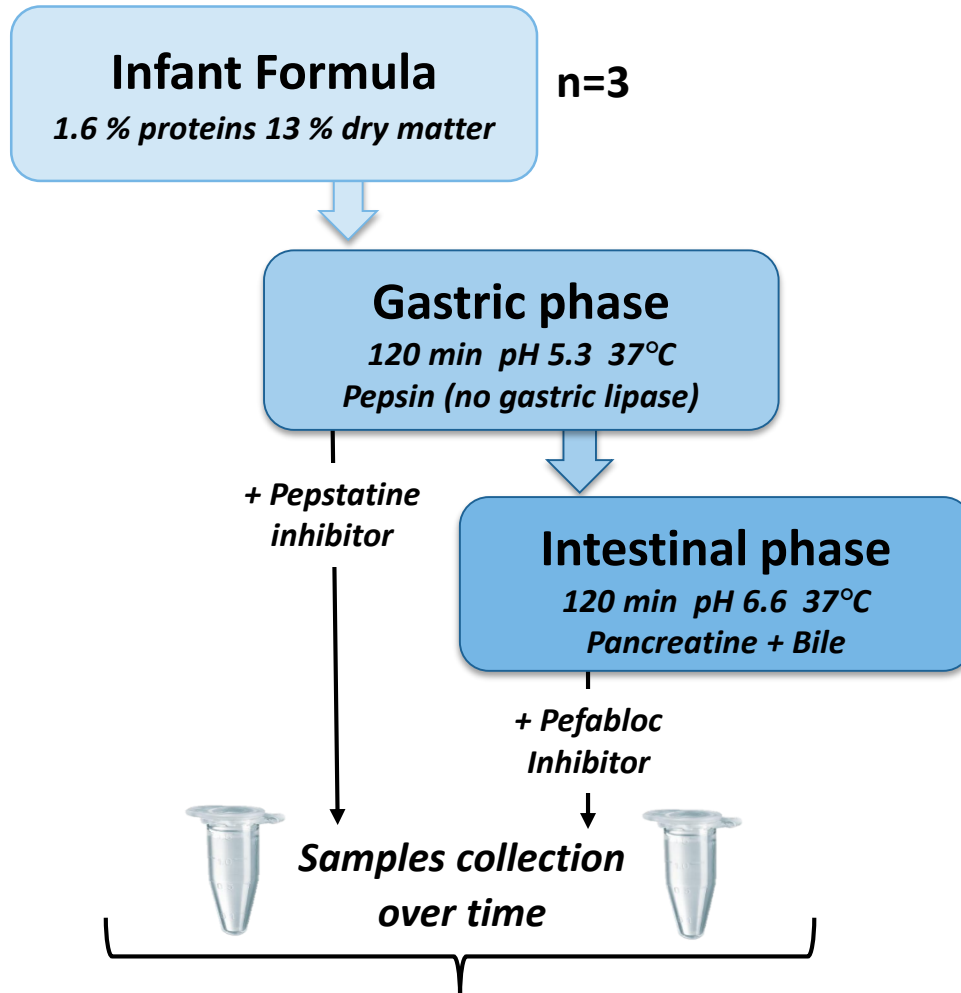
This digestive immaturity has to be taken into account to apply relevant *in vitro* digestion models

(Shani-Levi et al. 2016; Bourlieu et al., 2014)

IN VITRO STATIC DIGESTION



- Reference IF
- Pea IF
- Faba bean IF
- Rice IF
- Potato IF



In vitro static model

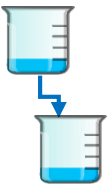
(Ménard et al. 2018)

PROTEOLYSIS :

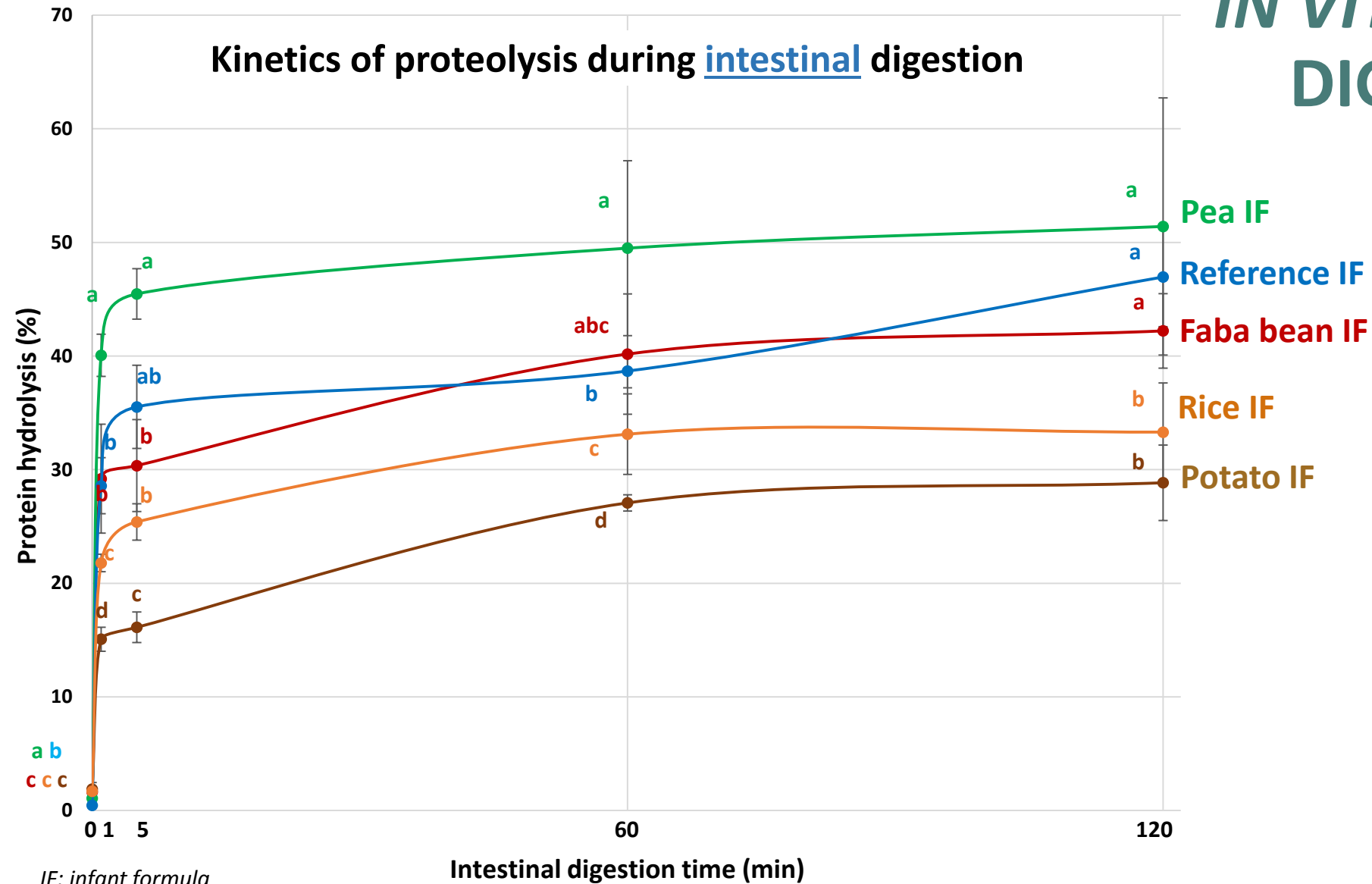
Free amino groups and Free amino acids

IF: infant formula

IN VITRO STATIC DIGESTION



Kinetics of proteolysis during intestinal digestion

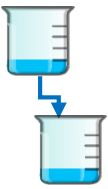


IF: infant formula

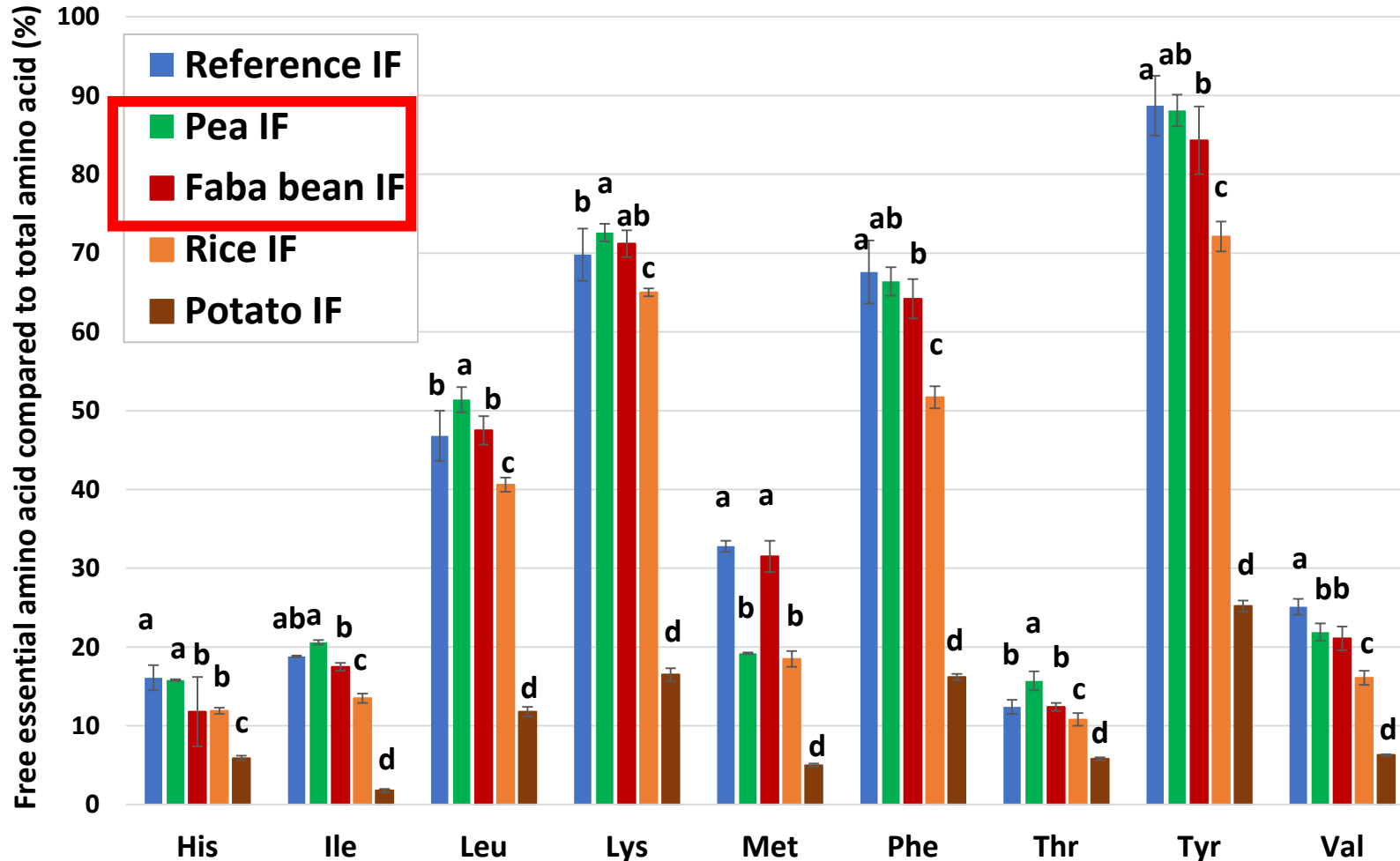
- Proteolysis is very limited at the beginning (<2%)
- A plateau is reached at 60 min of intestinal digestion except for the **Reference IF**
- **Pea IF**, **Reference IF** and **Faba bean IF** have higher proteolysis than **Rice IF** and **Potato IF** ($p < 0.05$)

(Darrouzet-Nardi, Ladd, & Weintraub, 2013)

IN VITRO STATIC DIGESTION



Bioaccessibility of amino acids at the end of the digestion



- Bioaccessibility is higher for **Pea IF**, **Reference IF** and **Faba bean IF** compared to **Rice IF** and **Potato IF** ($p < 0.05$)
- **Pea IF** and **Faba bean IF** similar to the **Reference IF** → **Good candidates**
- **Rice IF** and in particular **Potato IF** are less digestible → **Antinutritional factor (trypsin inhibitor)**

IF: infant formula

(Moore, Spackman, & Stein, 1958)

Reference IF

Pea IF

Faba bean IF

IN VITRO DYNAMIC DIGESTION



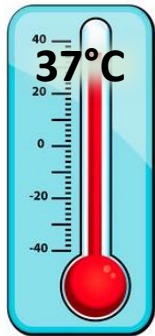
Infant formula

13 % dry matter and 1.6 % proteins

n=3

Stomach

$t_{1/2} = 78 \text{ min}$; $\beta = 1.2$
 $\text{pH} = -0.0155 * \text{time} + \text{pH meal}$
+ Pepsin (no gastric lipase)



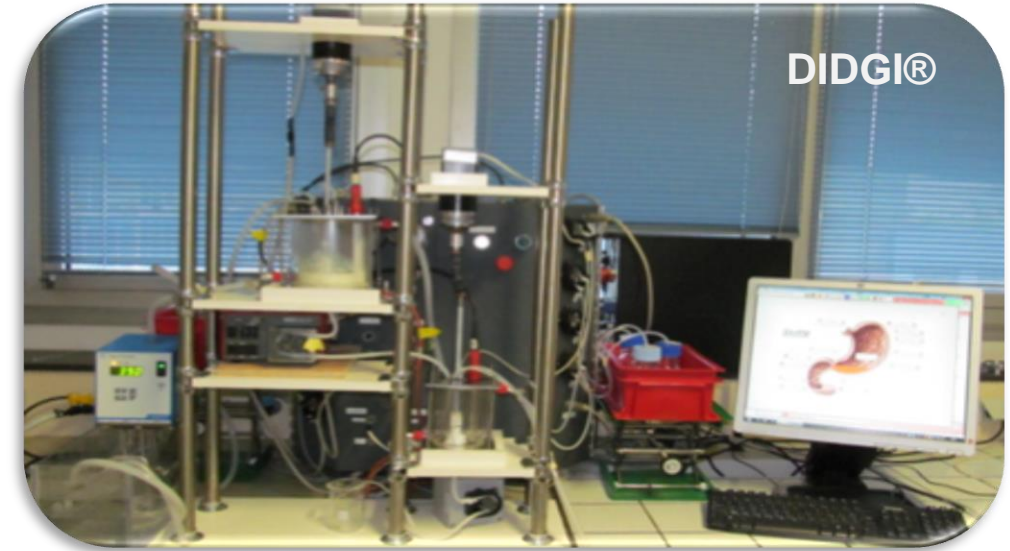
+ Pepstatine inhibitor

Intestine

$t_{1/2} = 200 \text{ min}$; $\beta = 2.2$
 $\text{pH} = 6.2$
+ Bile + Pancreatin

+ Pefaloc inhibitor

Samples collection
over time



In vitro dynamic model
(Ménard et al. 2014)

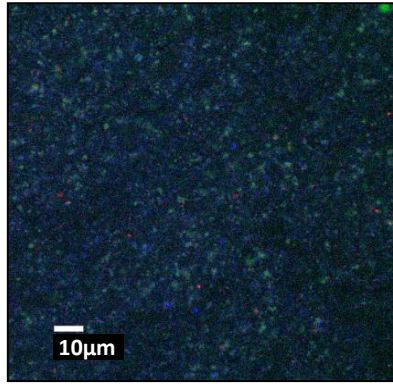
Validated from in vivo studies

- **PROTEOLYSIS:** Free amino groups and Free amino acids (%)
 - Microstructure analysis

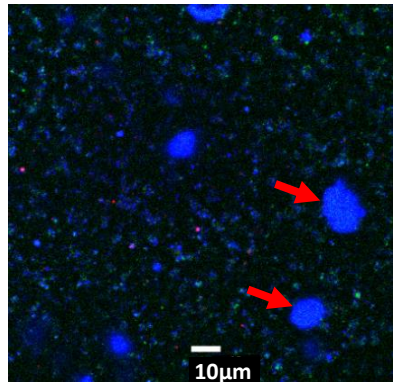
0 min
pH=6.8

GASTRIC

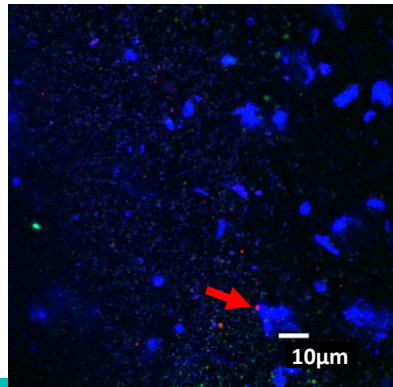
REFERENCE IF



PEA IF

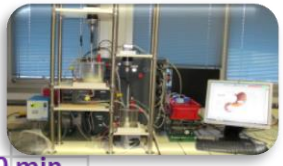
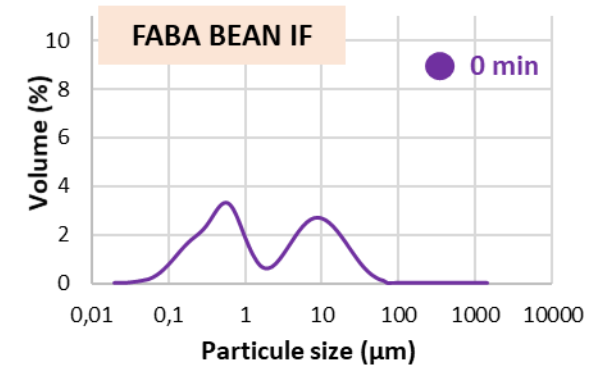
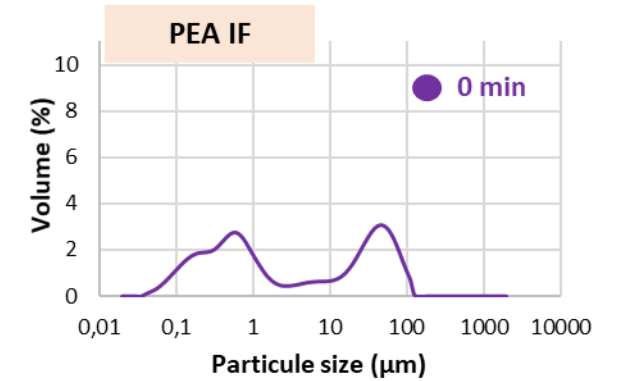
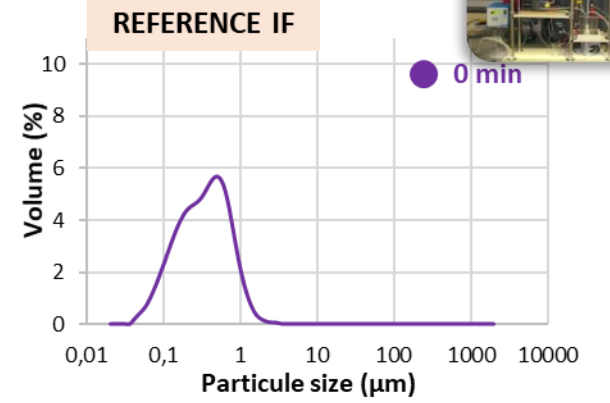


FABA BEAN IF



Confocal microscopy
X100 zoom1
■ Proteins
■ Lipids
■ Amphiphilic

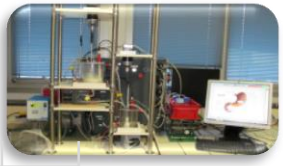
IF: infant formula



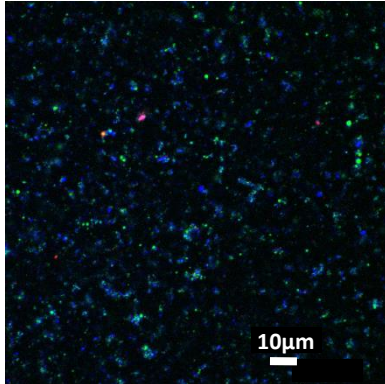
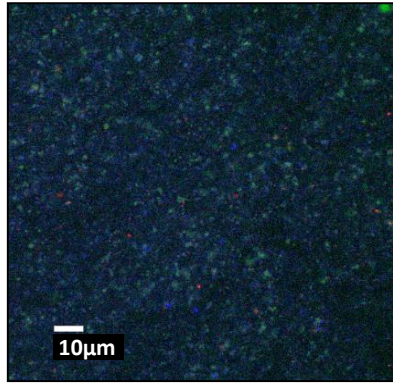
0 min
pH=6.8

60 min
pH=5.9

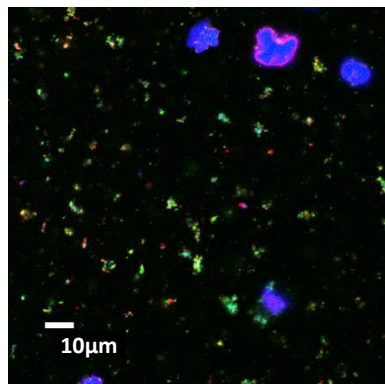
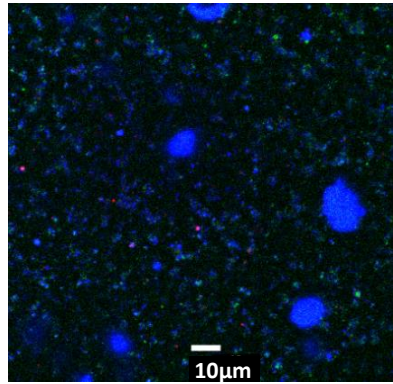
GASTRIC



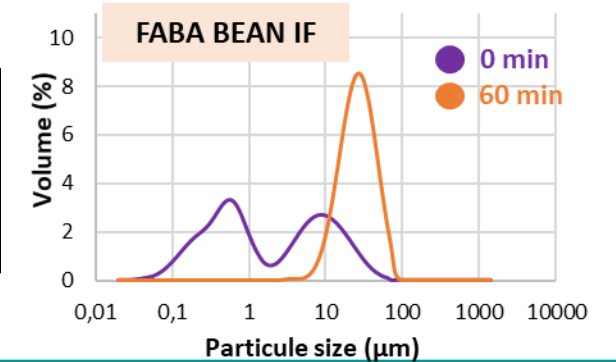
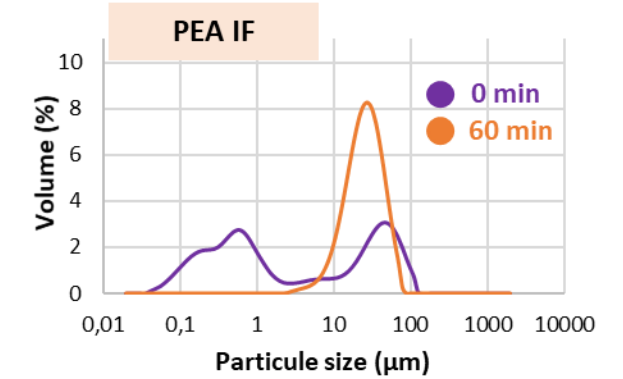
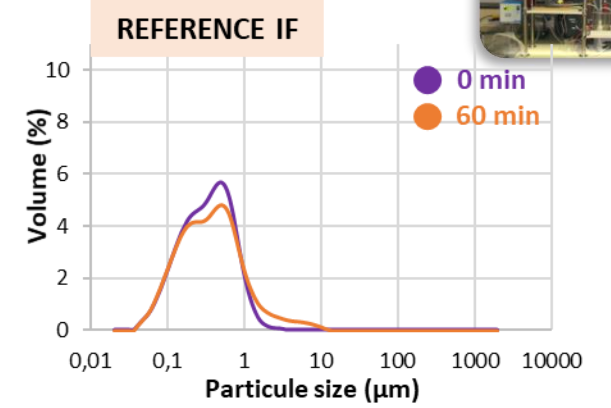
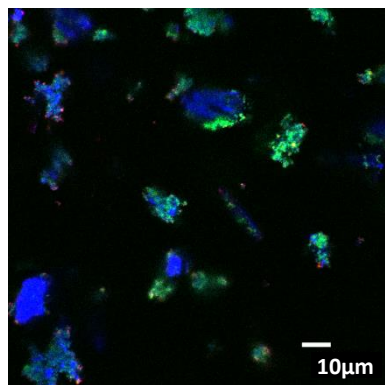
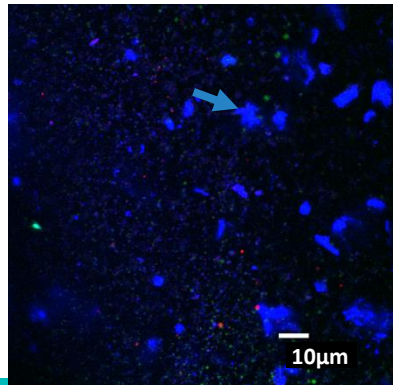
REFERENCE IF



PEA IF



FABA BEAN IF



Confocal microscopy
X100 zoom1
■ Proteins
■ Lipids
■ Amphiphilic

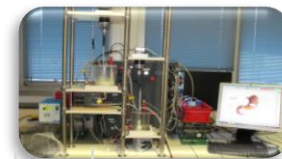
IF: infant formula

0 min
pH=6.8

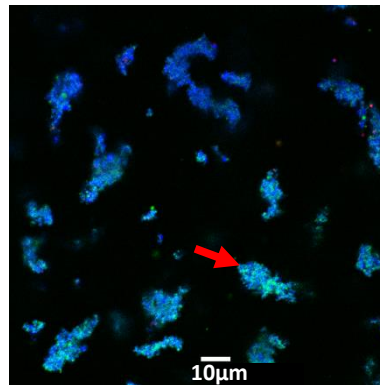
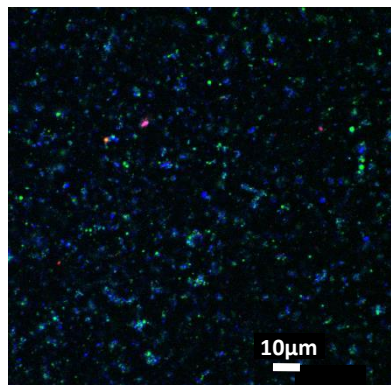
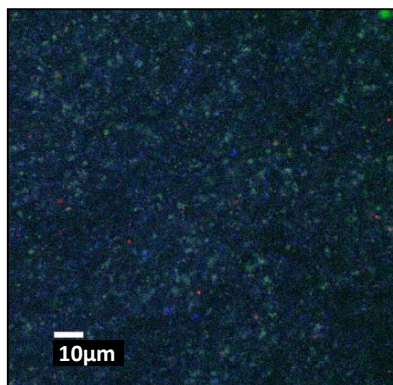
60 min
pH=5.9

120 min
pH=4.9

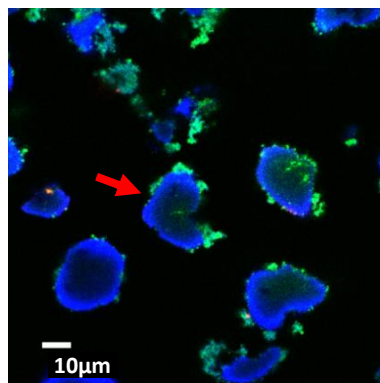
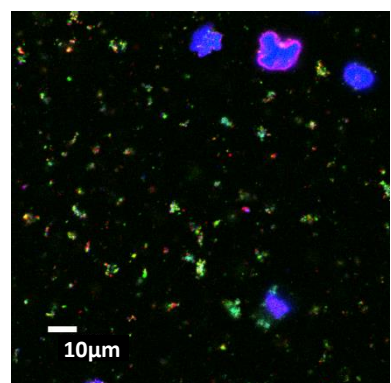
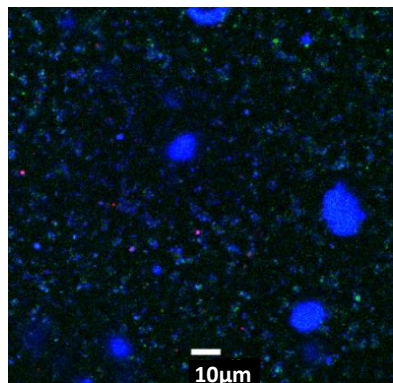
GASTRIC



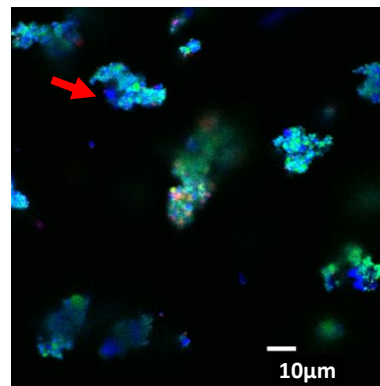
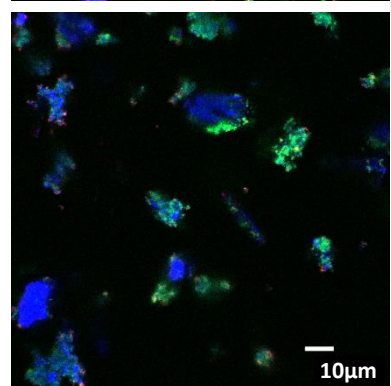
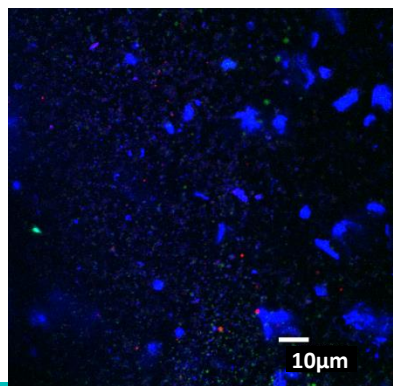
REFERENCE IF



PEA IF

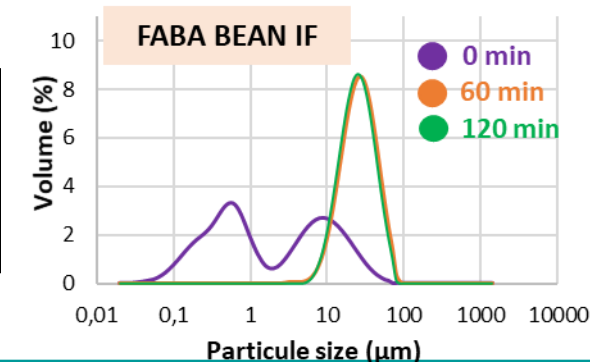
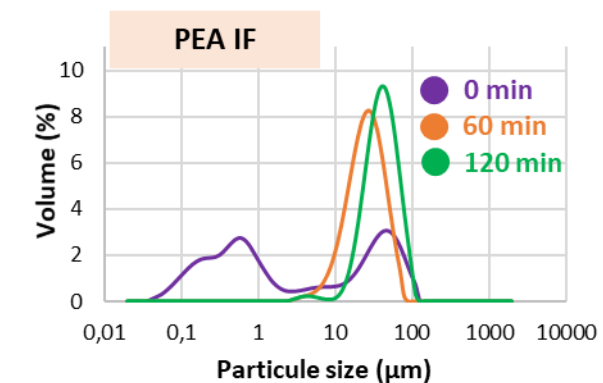
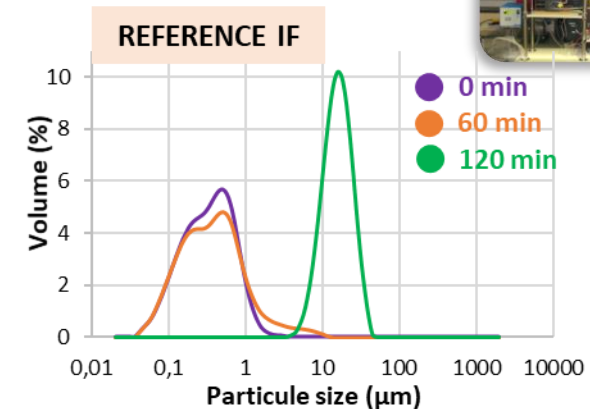


FABA BEAN IF



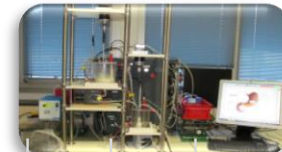
Confocal microscopy
X100 zoom1
■ Proteins
■ Lipids
■ Amphiphilic

IF: infant formula

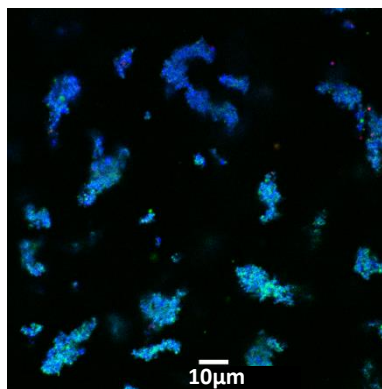


120 min (gastric)
pH=4.9

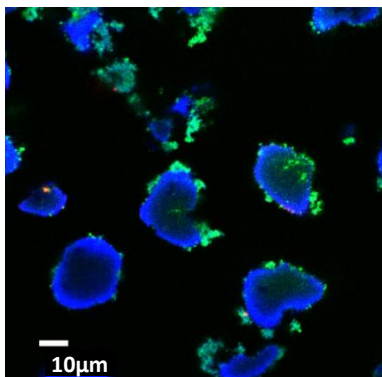
INTESTINAL



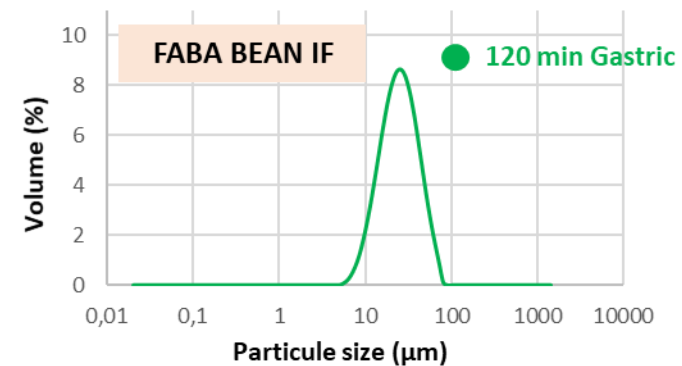
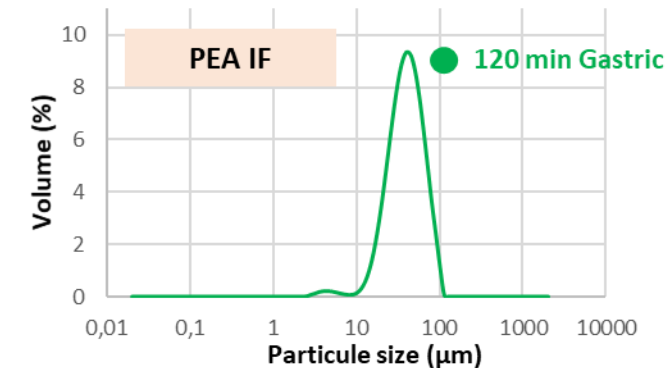
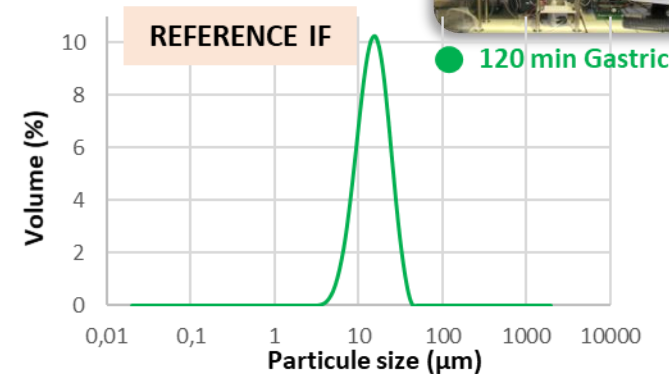
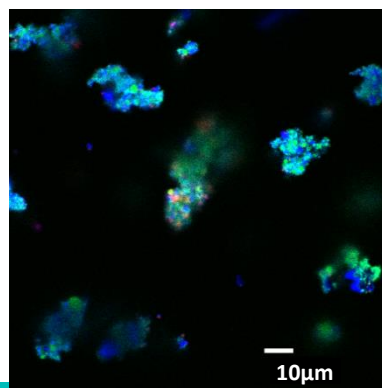
REFERENCE IF



PEA IF



FABA BEAN IF



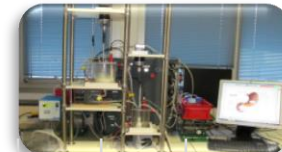
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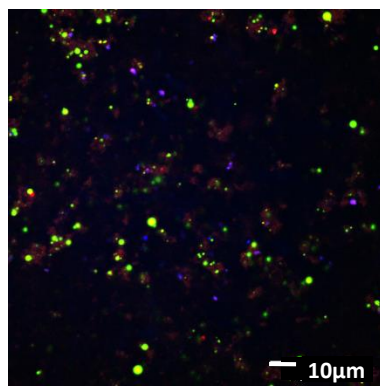
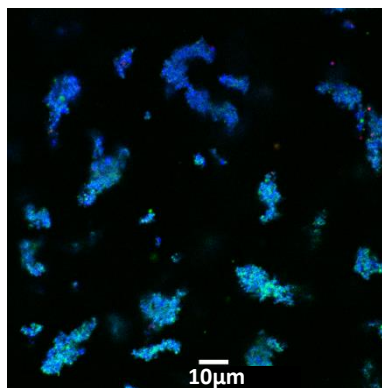
120 min (gastric)
pH=4.9

60 min
pH=6.2

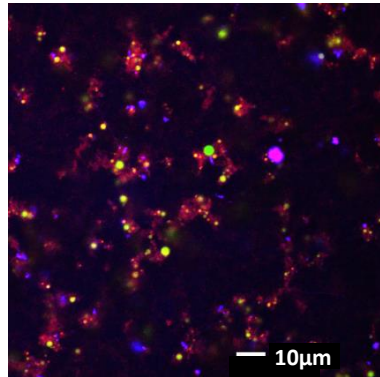
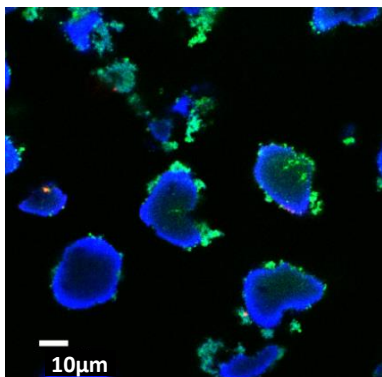
INTESTINAL



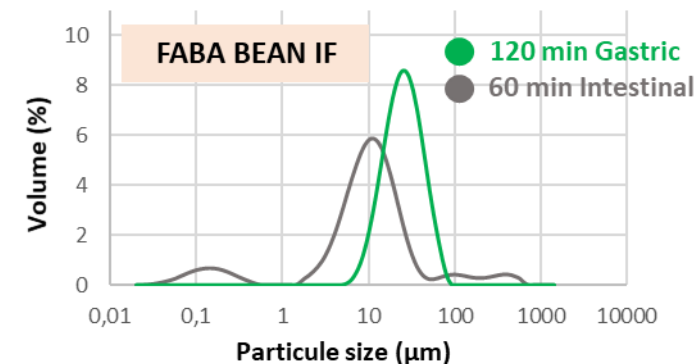
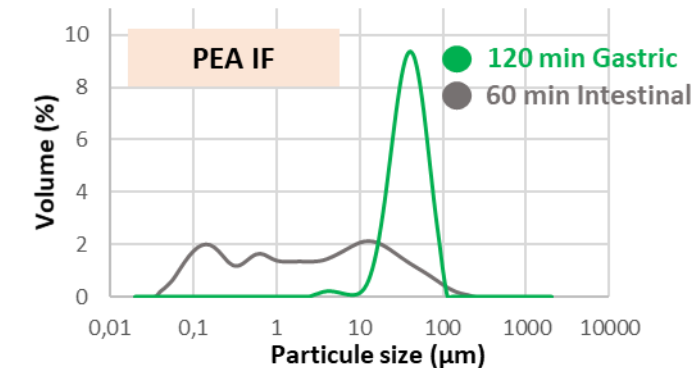
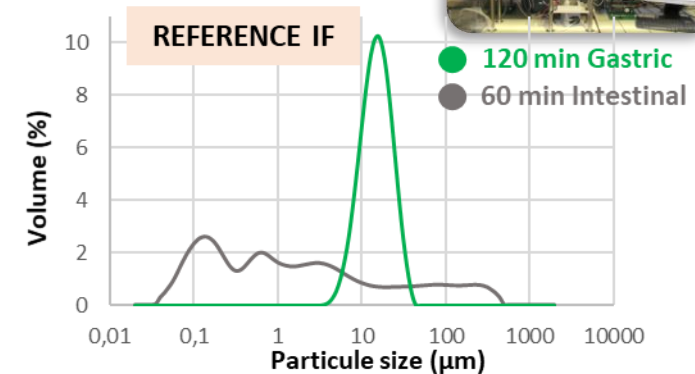
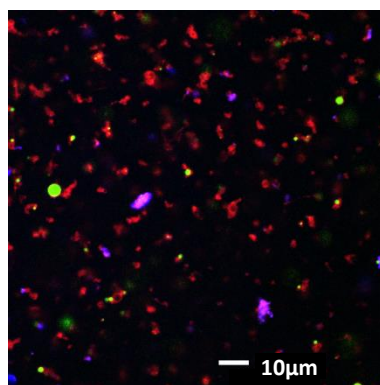
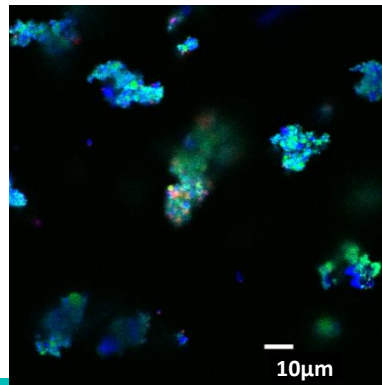
REFERENCE IF



PEA IF



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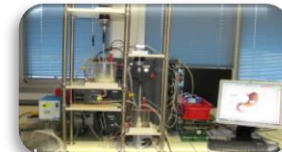
IF: infant formula

120 min (gastric)
pH=4.9

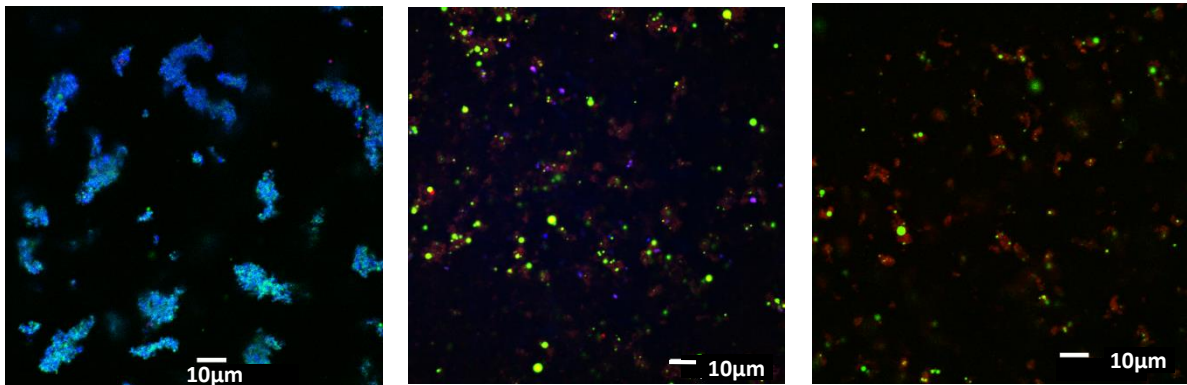
60 min
pH=6.2

120 min
pH=6.2

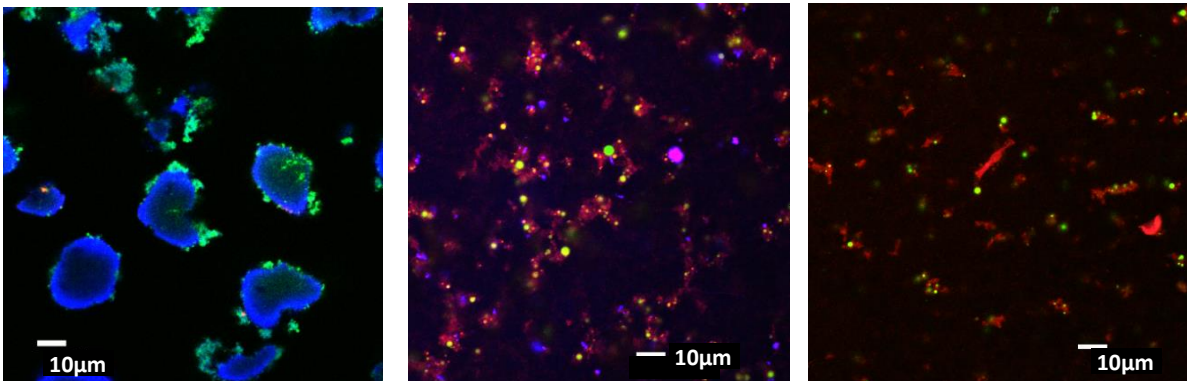
INTESTINAL



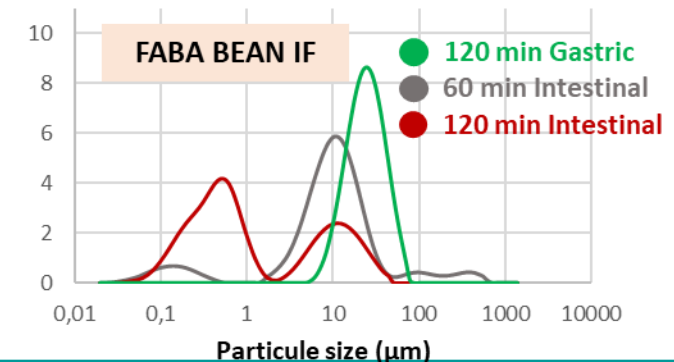
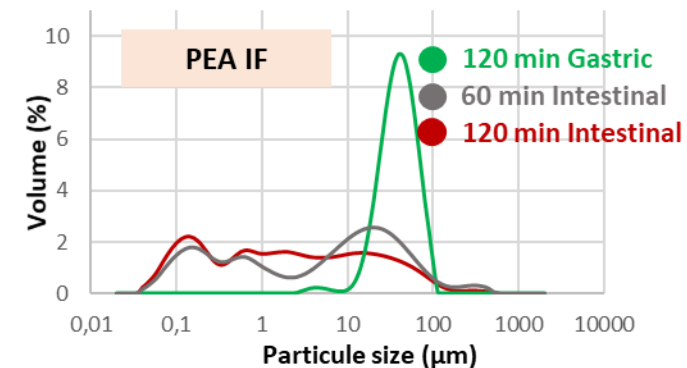
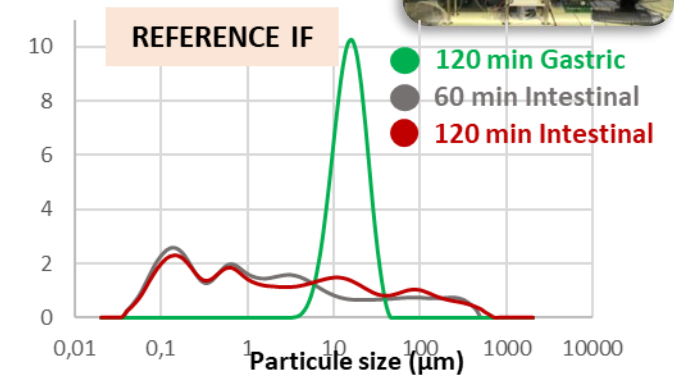
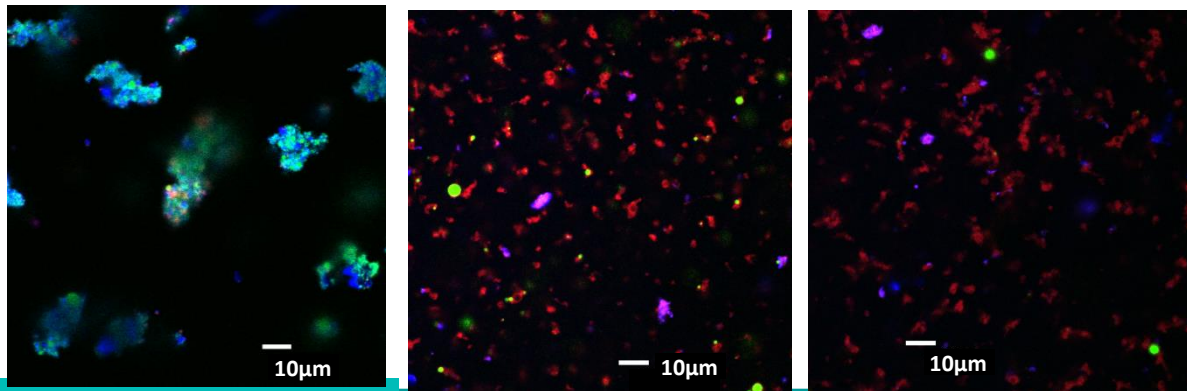
REFERENCE IF



PEA IF



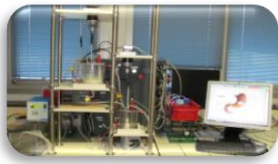
FABA BEAN IF



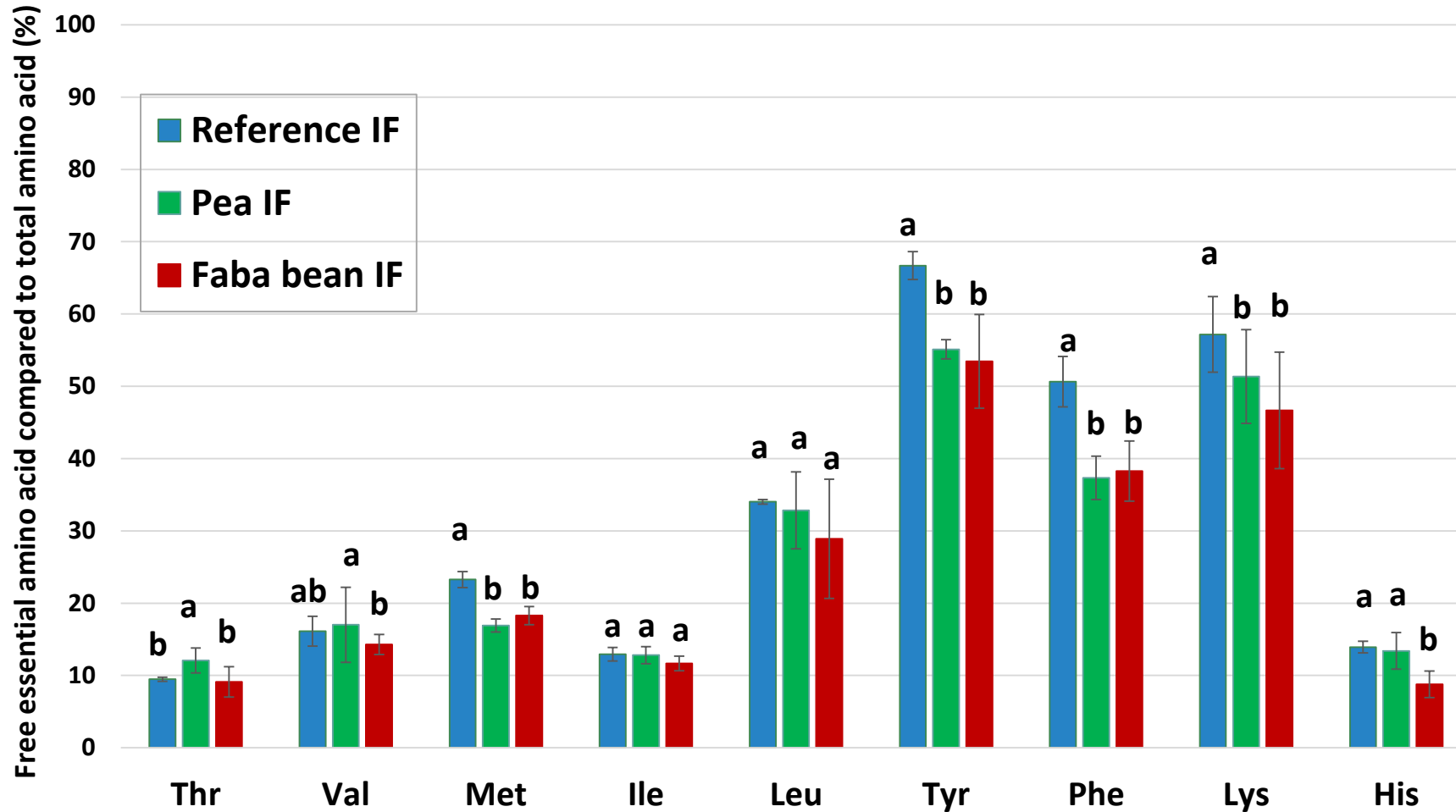
Confocal microscopy
X100 zoom1
■ Proteins
■ Lipids
■ Amphiphilic

IF: infant formula

IN VITRO DYNAMIC DIGESTION



Bioaccessibility of amino acids at the end of the digestion



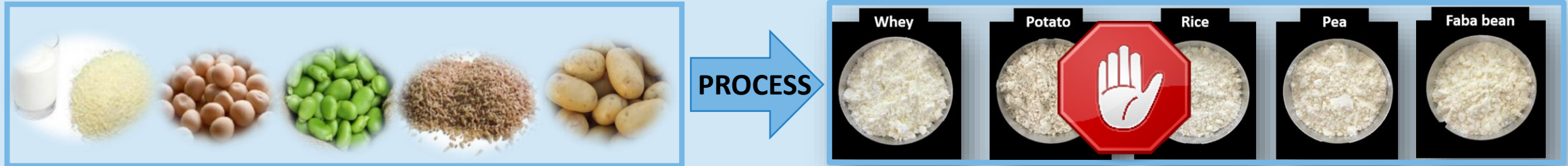
Bioaccessibility is higher for the **Reference IF** and **Pea IF** compared to **Faba bean IF** for some amino acids

IF: infant formula

(Moore, Spackman, & Stein, 1958)

CONCLUSION

- Producing plant protein based infant formulas 



- Proteolysis using *in vitro* STATIC digestion model

→ Pea IF and Faba bean IF = Reference IF 

→ Rice IF and Potato IF < Reference IF 

- Microstructure using *in vitro* DYNAMIC digestion model

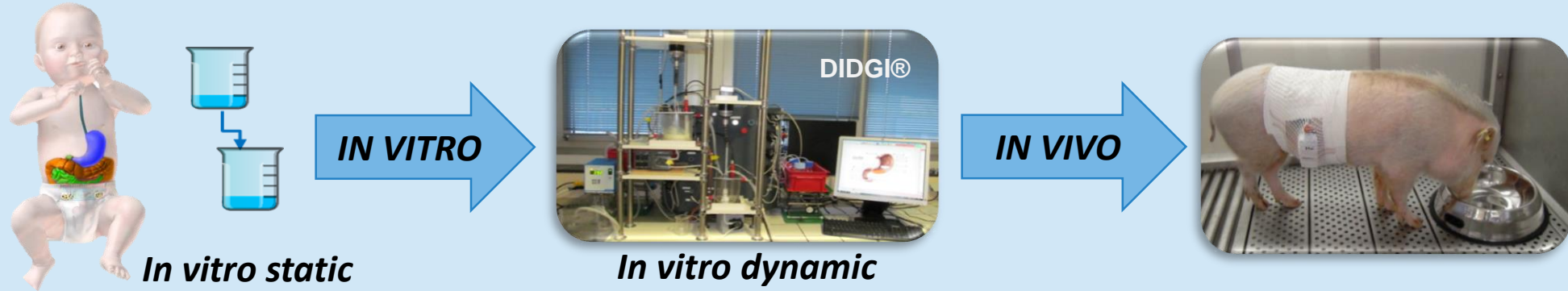


- Bioaccessibility of EAA using *in vitro* DYNAMIC digestion model

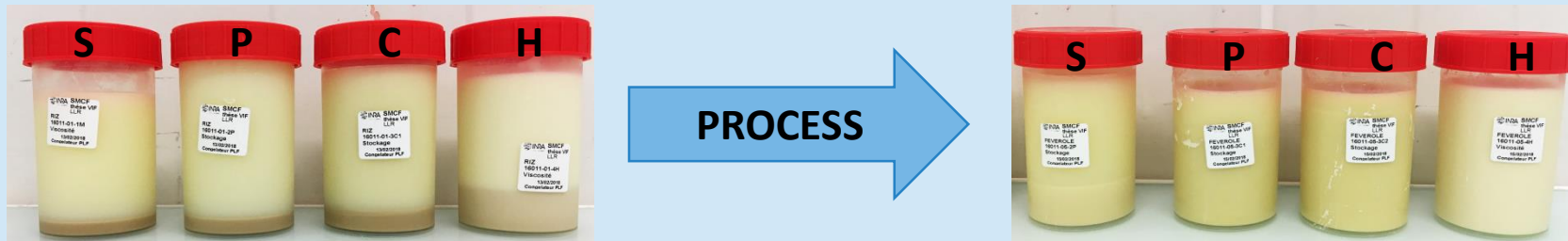
→ Reference IF \approx Pea IF \approx Faba bean IF → Comparable digestibility

PERSPECTIVES

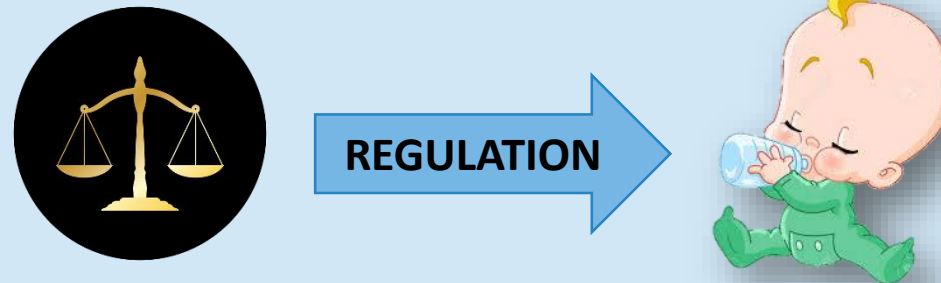
- *In vivo* studies → Allergenicity, microbiota, proteins absorption



- Functional improvement → Dispersion of plant proteins



- Evidence of conformity regarding the European regulation 2016/127



Team project & Supervisors



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