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

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Engineering Innovations for Food Supply Chains

INRA
SCIENCE & IMPACT

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

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Sill DAIRY INTERNATIONAL

AGRO CAMPUS OUEST

STLO

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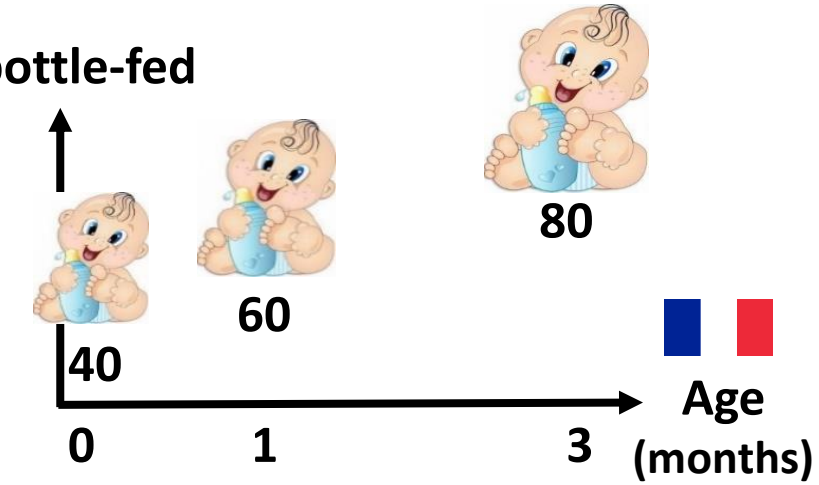
CONTEXT

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



(Cohorte Elfe, 2011; Epifane, 2012-2013)

- **Importance of protein nutritional quality:**
  - Infant optimal growth (Michaelsen & Greer, 2014)
  - Essential amino acid profile and protein digestibility (Friedman, 1996)

Age Group	His	Ile	Leu	Lys	SAA	AAA	Thr	Trp	Val
	scoring pattern mg/g protein requirement								
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

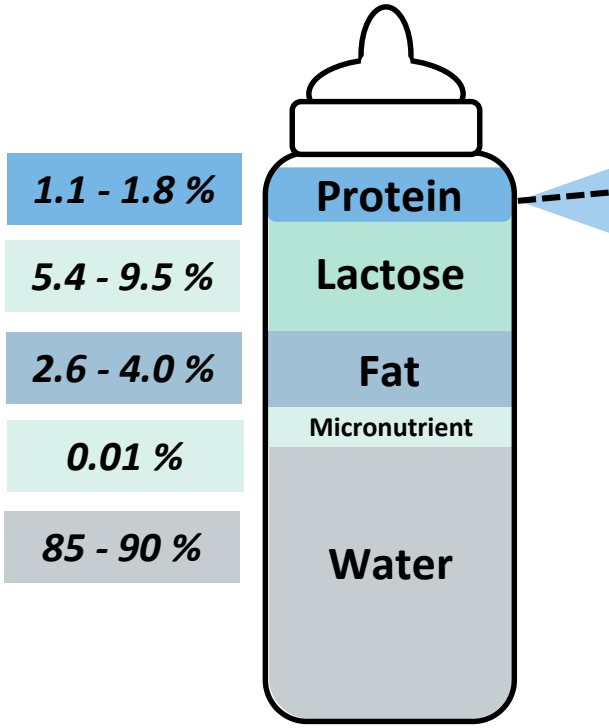
(FAO, 2013)

- **Growing interest in plant proteins**
  - Ethical, health, sustainability

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

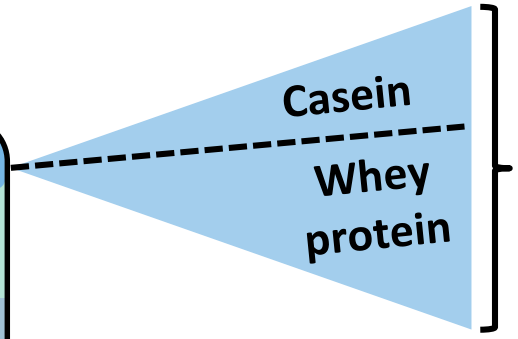
OBJECTIVE

Standard 1st age infant formula → **New 1st age infant formulas**



60-70 kcal / 100 ml

(UE, 2016)



Skimmed Cow milk

+

Whey protein concentrate → **Plant proteins**

<u>Research question:</u>

How protein sources influence the functional properties and the digestibility of infant formulas ?

STRATEGY

1. Selection of new protein sources

Whey	Pea	Faba bean	Rice	Potato
Whey protein	*Pisum sativum*	*Vicia faba*	*Oriza sativa L.*	*Solanum tuberosum*



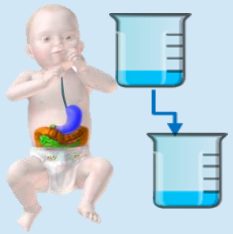


2. Development of new infant formulas

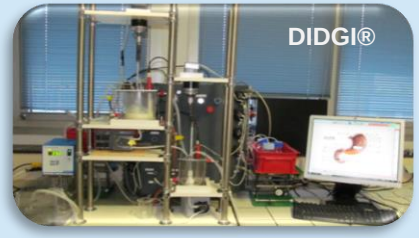
Pilot scale

Semi-industrial scale

3. Digestion of new infant formulas



In vitro static



In vitro dynamic

PILOT SCALE MANUFACTURING

SOLUBILIZATION (S)

PASTEURIZATION (P)

CONCENTRATION (C)

HOMOGENEIZATION (H)

SPRAY- DRYING

INFANT FORMULA

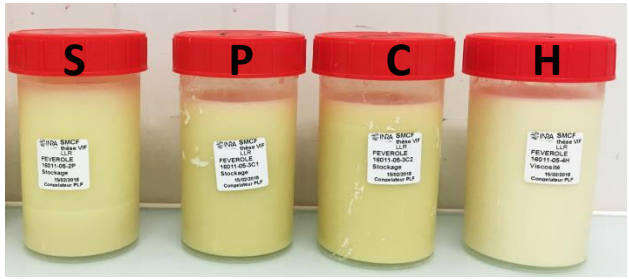
Rice infant formula

Solubility limits

Viscosity limits

Potato infant formula

Faba bean infant formula



Evaporation capacity 5 kg/h

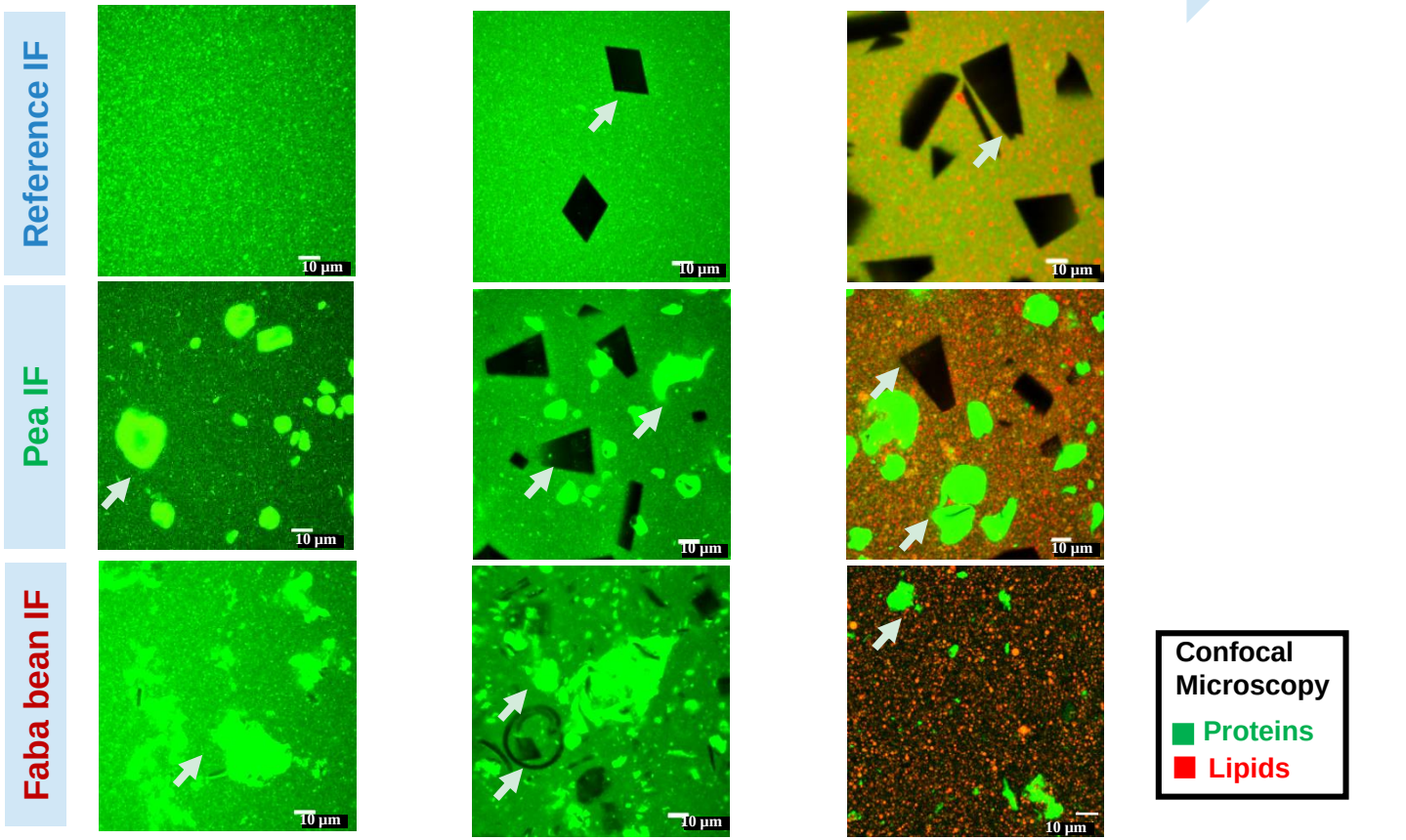
RICE

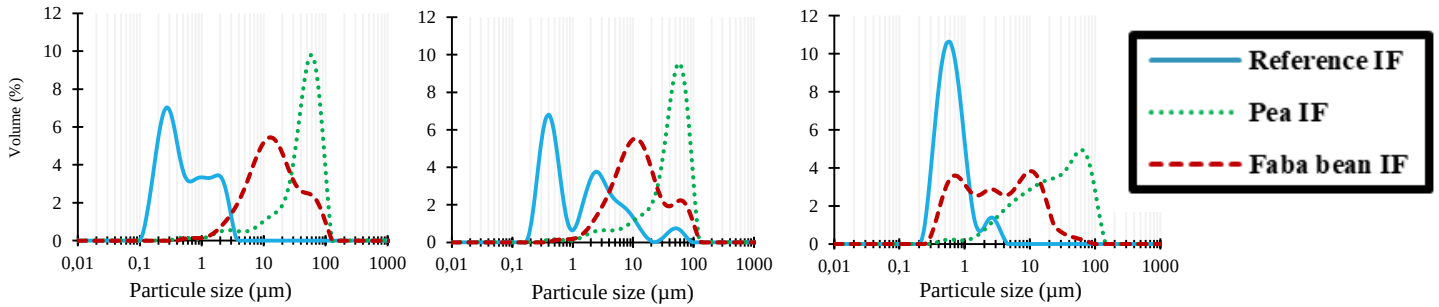
POTATO

SCALE-UP

Evaporation capacity 90 kg/h

SEMI-INDUSTRIAL SCALE MANUFACTURING





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

DM: dry matter

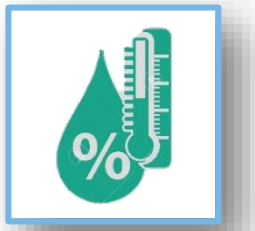
CONFORMITY OF INFANT FORMULA

- **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**

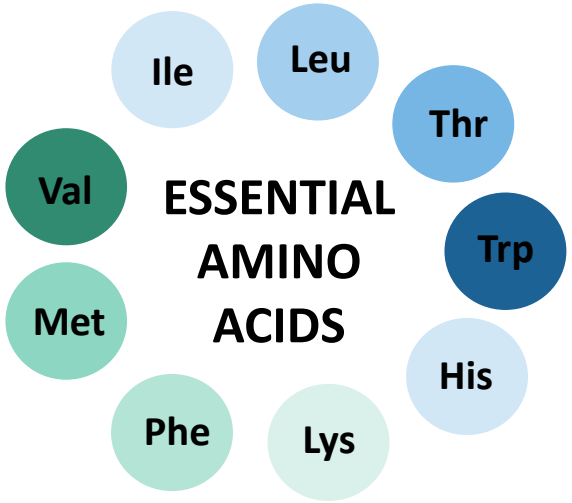
EUROPEAN REGULATION

RÈGLEMENT DÉLÉGUÉ (UE) 2016/127 DE LA COMMISSION

du 25 septembre 2015

complétant le règlement (UE) nº 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)



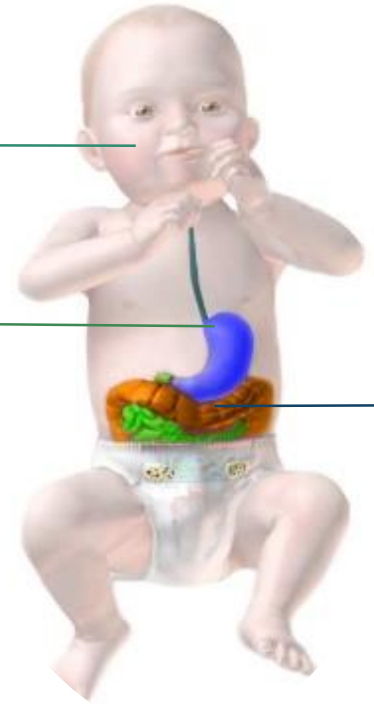
IMMATURITY OF NEWBORN DIGESTIVE SYSTEM

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

Infant Formula
1.6 % proteins 13 % dry matter

n=3

Gastric phase
120 min pH 5.3 37°C
Pepsin (no gastric lipase)

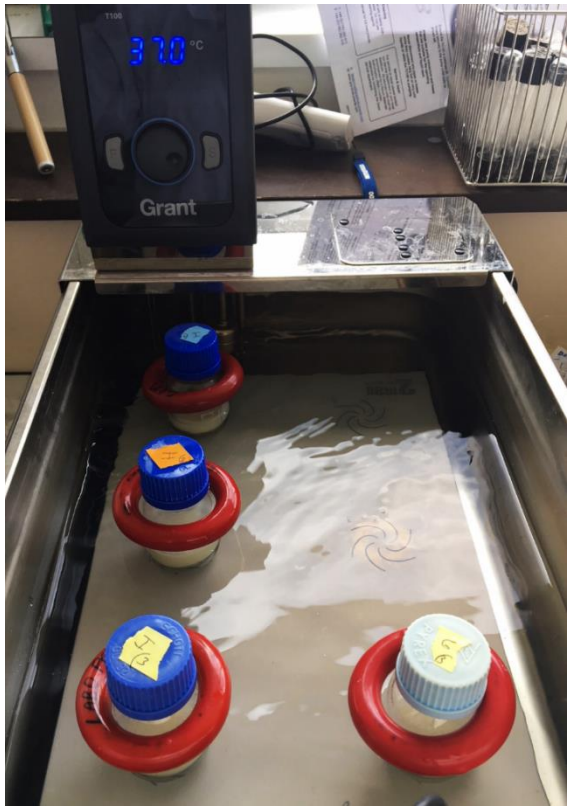
+ Pepstatine inhibitor

Intestinal phase
120 min pH 6.6 37°C
Pancreatine + Bile

+ Pefabloc Inhibitor

Samples collection over time

PROTEOLYSIS :
Free amino groups and Free amino acids

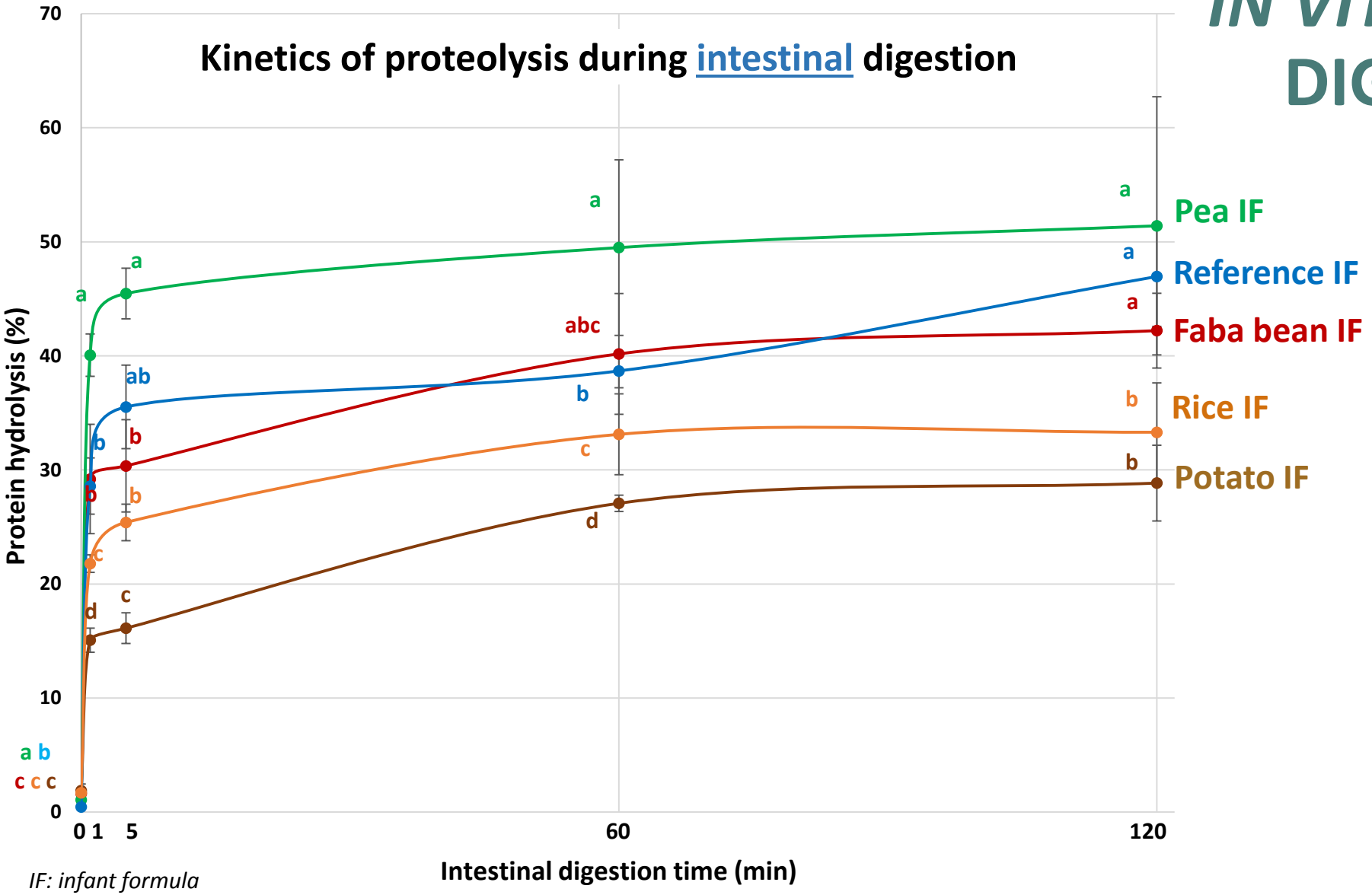


In vitro static model

(Ménard et al. 2018)

IF: infant formula

IN VITRO STATIC DIGESTION



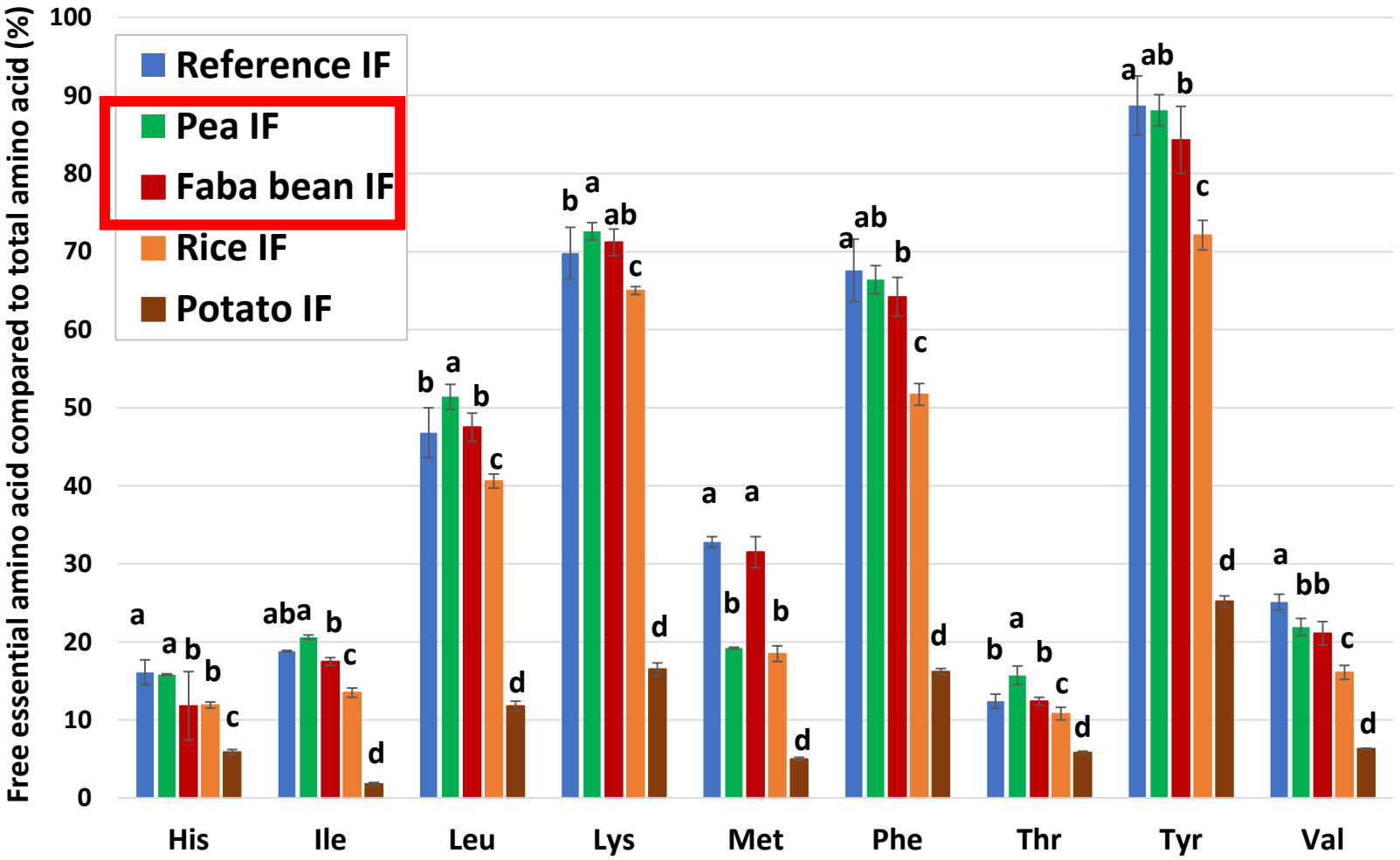
IF: infant formula

- 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)

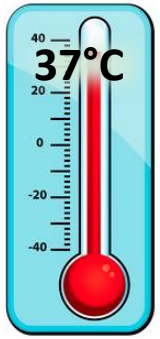
IN VITRO DYNAMIC DIGESTION

Reference IF | Pea IF | Faba bean IF

Infant formula
13 % dry matter and 1.6 % proteins

n=3

Stomach
t1/2 = 78 min ; β=1.2
*pH = -0.0155 * time + pH meal*
+ Pepsin (no gastric lipase)

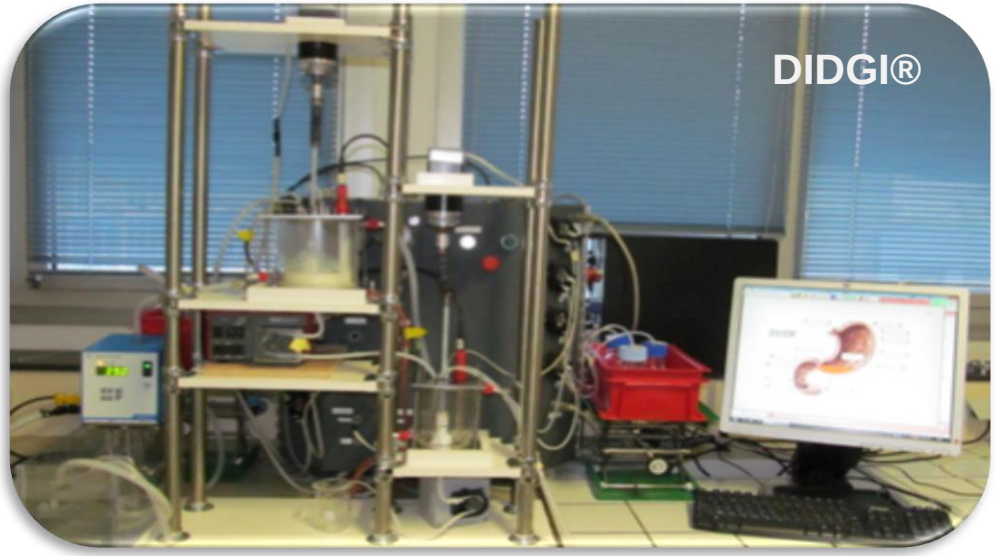


+Pepstatine inhibitor

Intestine
t1/2 = 200 min ; β = 2.2
pH = 6.2
+ Bile + Pancreatin

+ Pefaloc inhibitor

Samples collection over time



In vitro dynamic model
(Ménard et al. 2014)

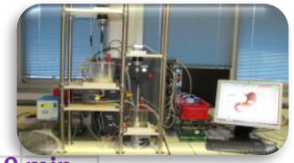
Validated from in vivo studies

- **<u>PROTEOLYSIS</u>: Free amino groups and Free amino acids (%)**
- **Microstructure analysis**

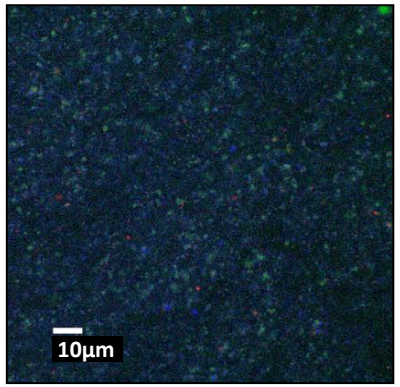
F: infant formula

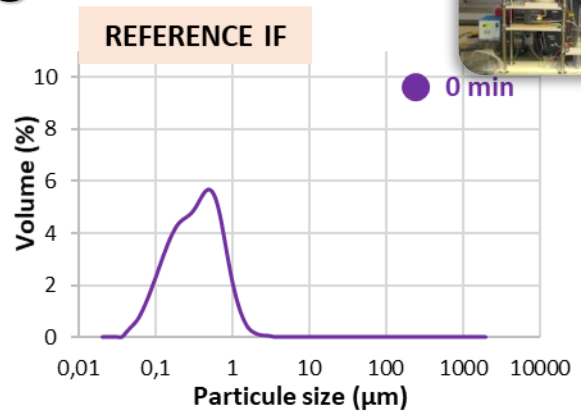
0 min
pH=6.8

GASTRIC

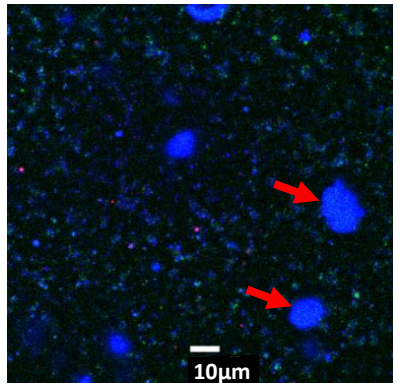


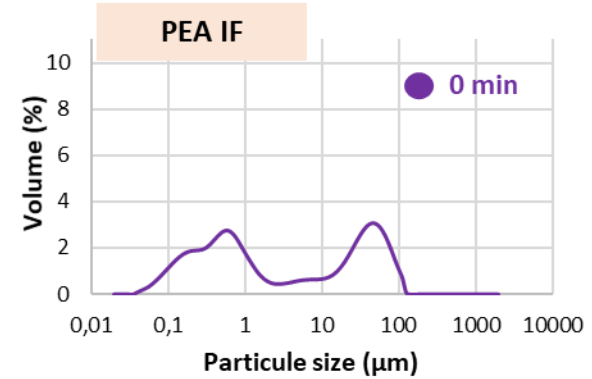
REFERENCE IF



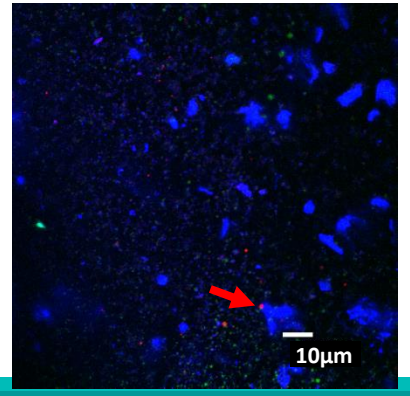


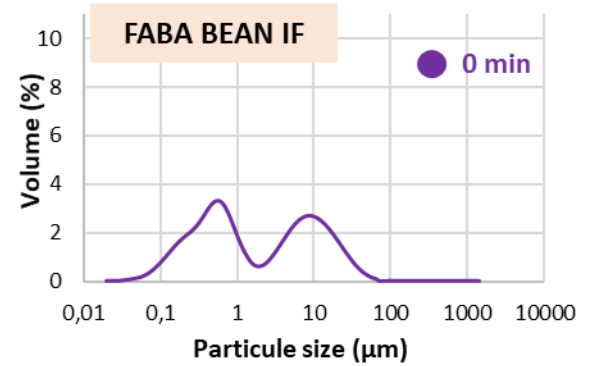
PEA IF





FABA BEAN IF

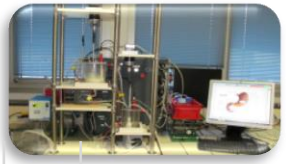


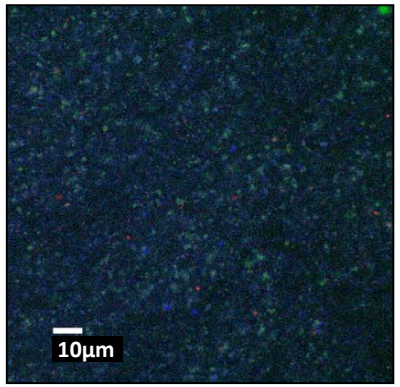
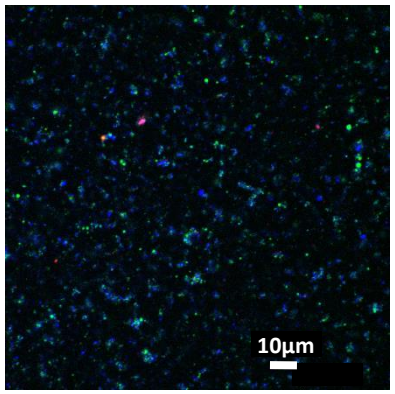
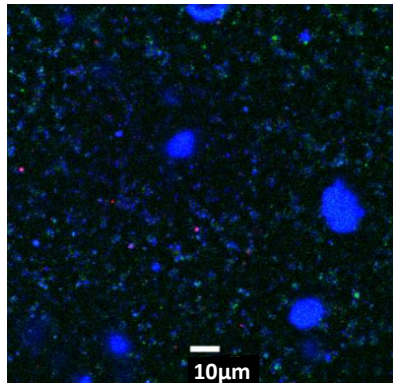
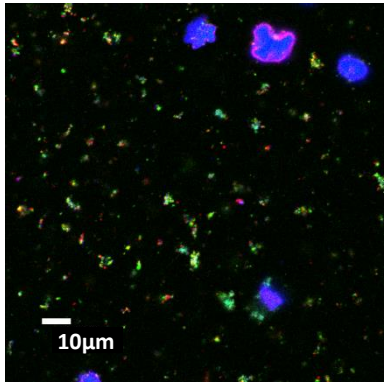
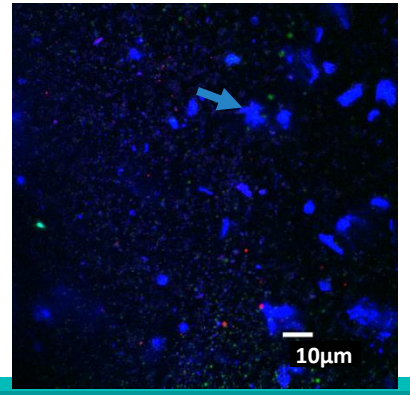
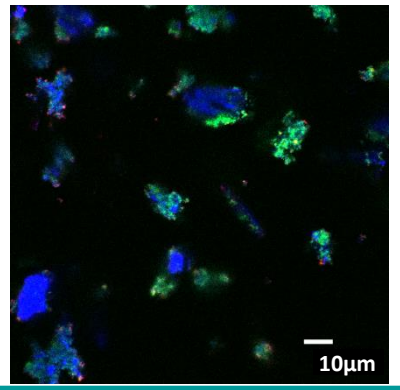


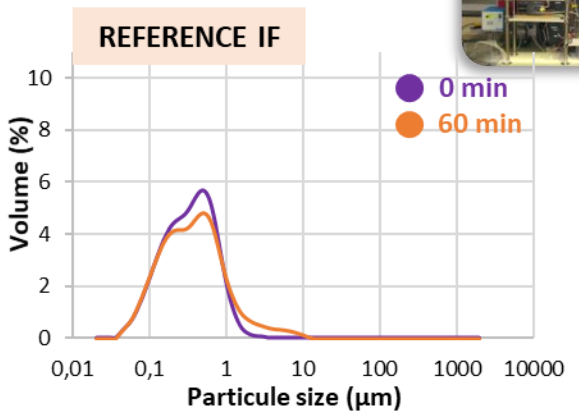
Confocal microscopy
X100 zoom1
Proteins
Lipids
Amphiphilic

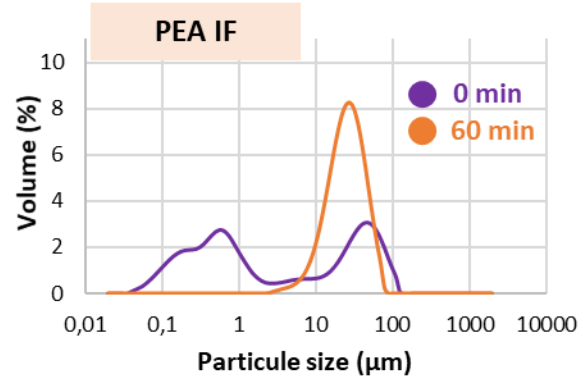
IF: infant formula

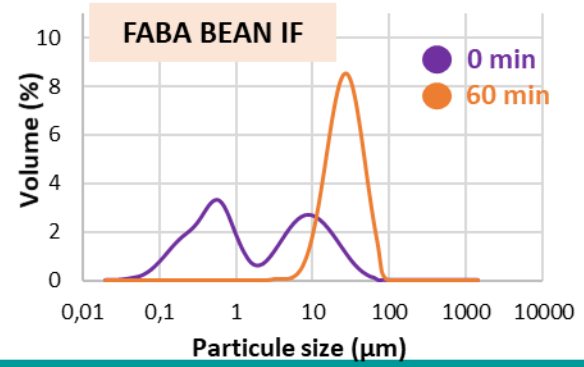
GASTRIC



	0 min pH=6.8	60 min pH=5.9
REFERENCE IF		
PEA IF		
FABA BEAN IF		



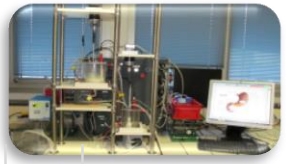


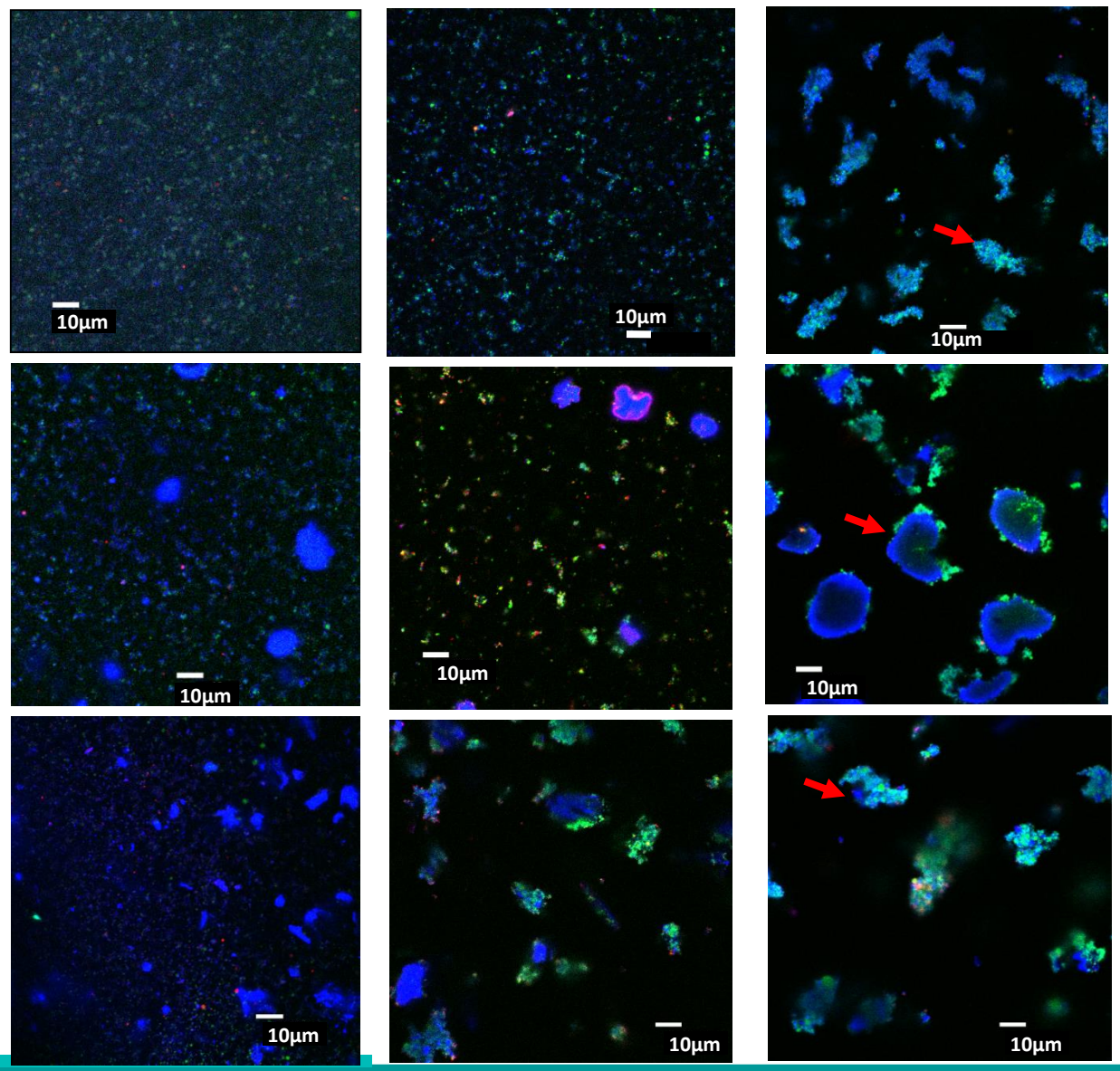


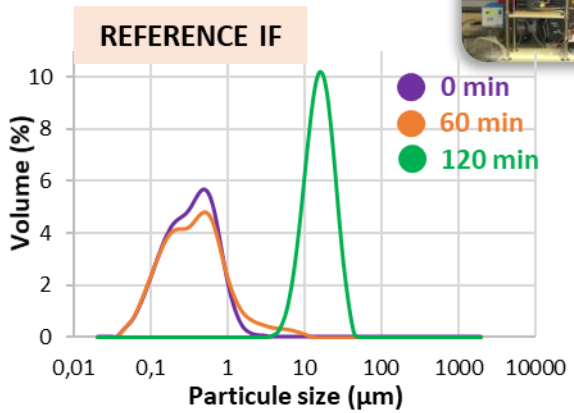
Confocal microscopy
X100 zoom1
Proteins
Lipids
Amphiphilic

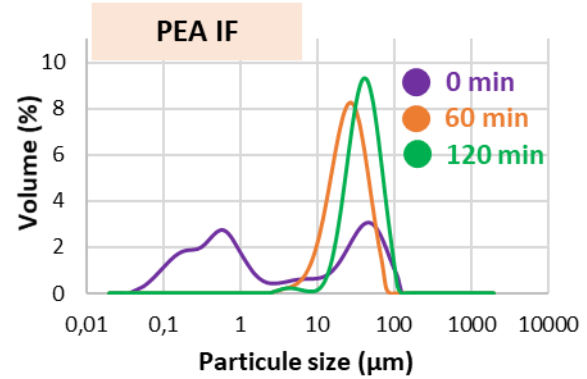
IF: infant formula

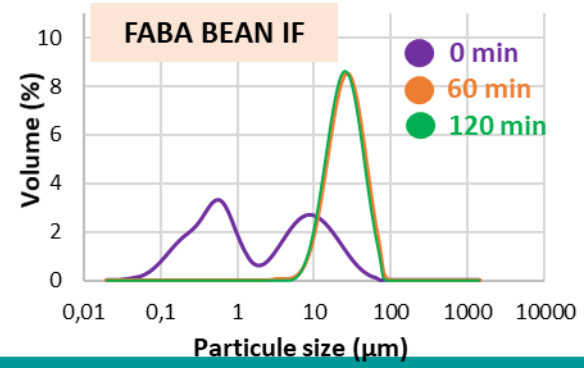
GASTRIC









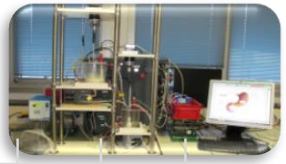


Confocal microscopy
X100 zoom1
Proteins
Lipids
Amphiphilic

IF: infant formula

120 min (gastric)
pH=4.9

INTESTINAL



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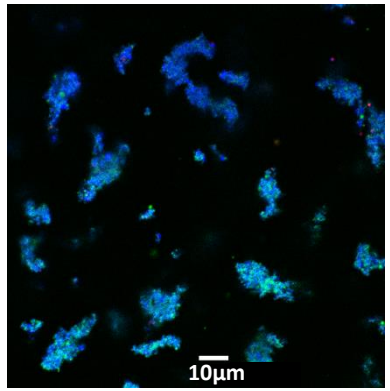
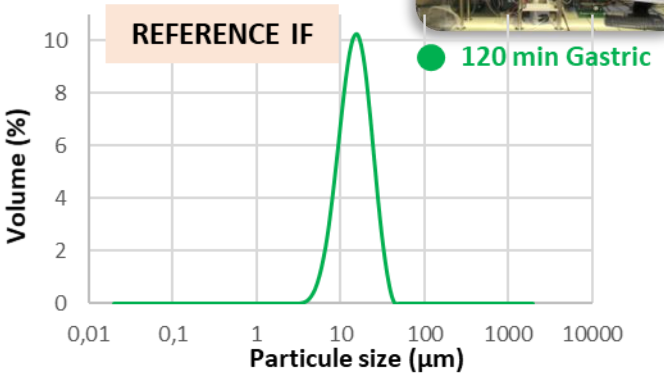
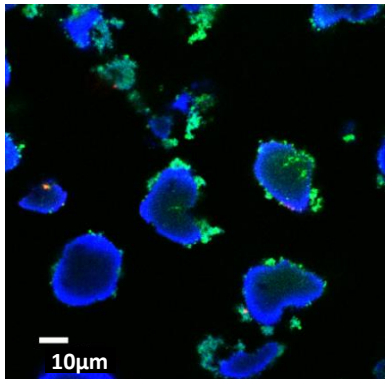
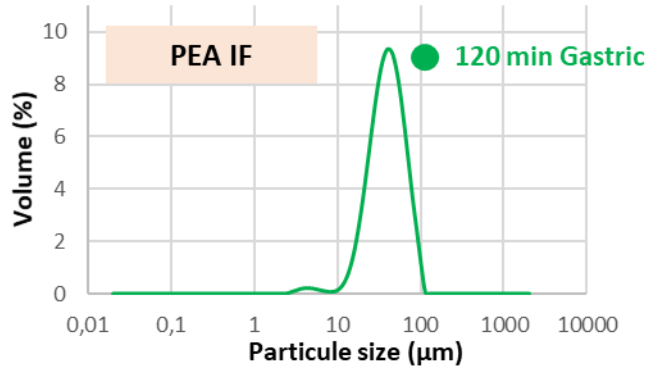


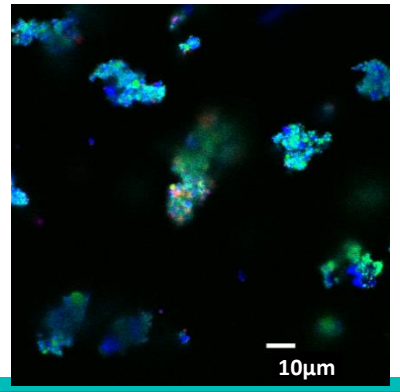


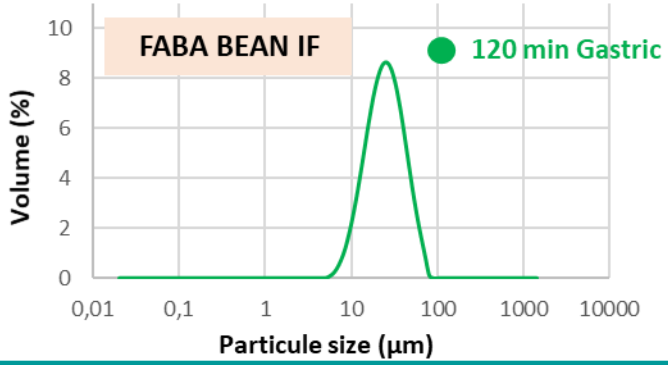
PEA IF





FABA BEAN IF

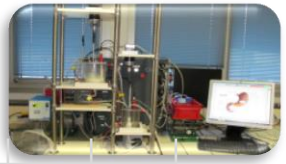


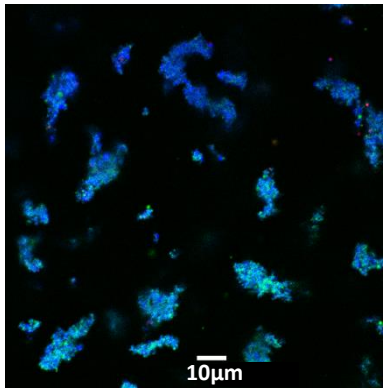
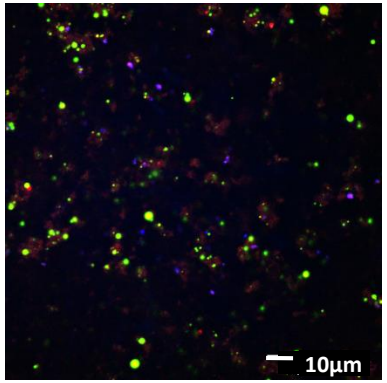
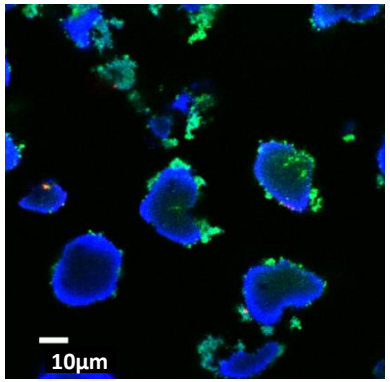
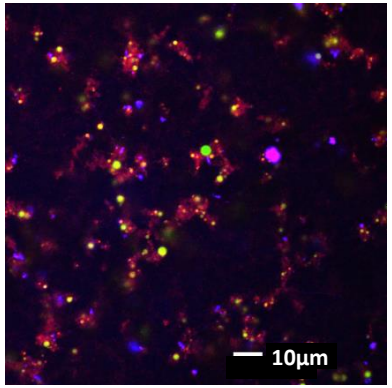
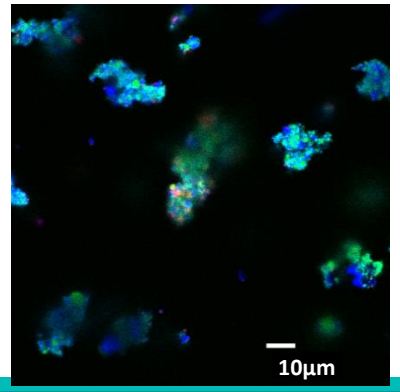
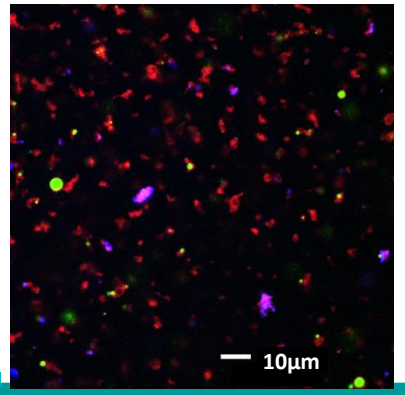


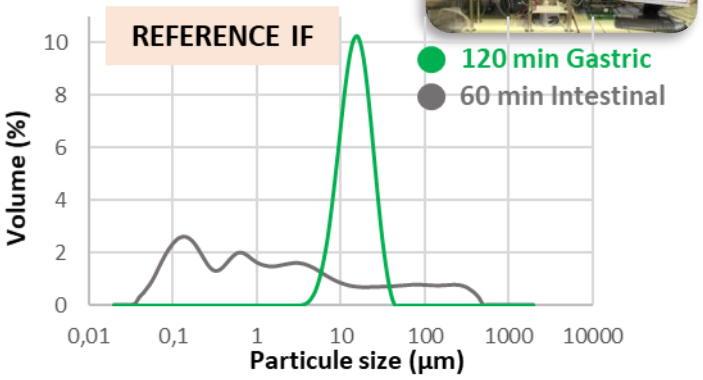
Confocal microscopy
X100 zoom1
Proteins
Lipids
Amphiphilic

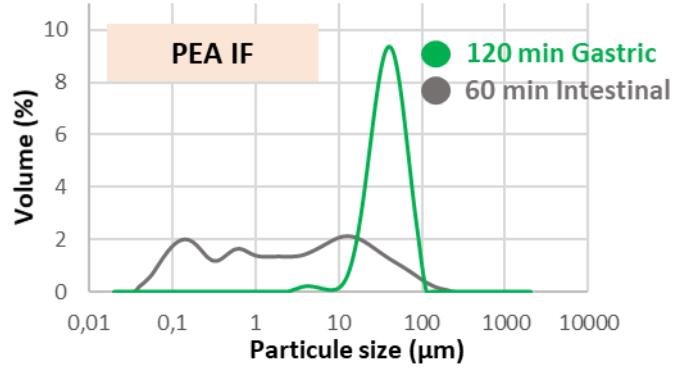
IF: infant formula

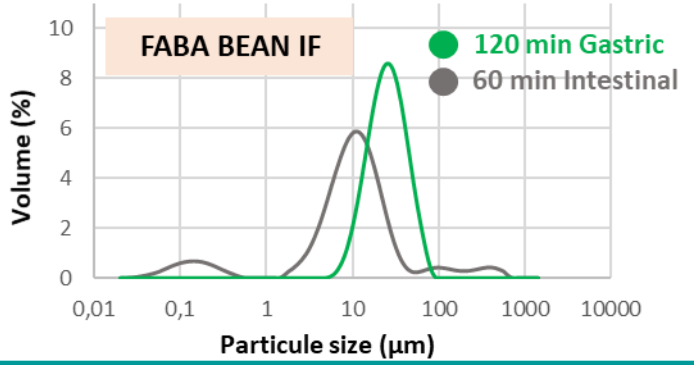
INTESTINAL



	120 min (gastric) pH=4.9	60 min pH=6.2
REFERENCE IF		
PEA IF		
FABA BEAN IF		



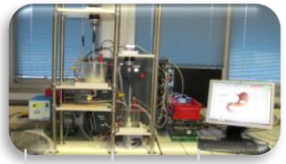


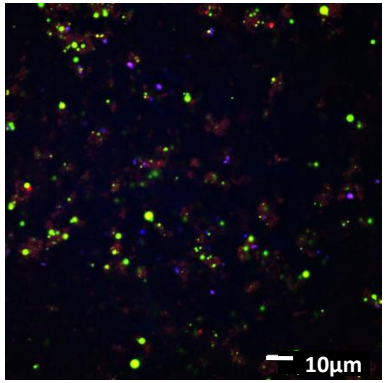
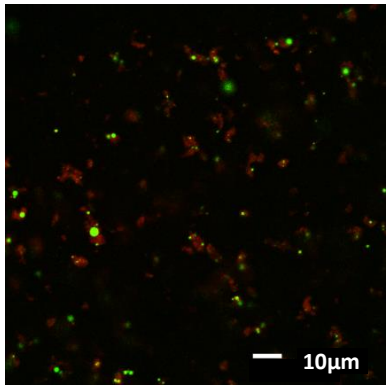
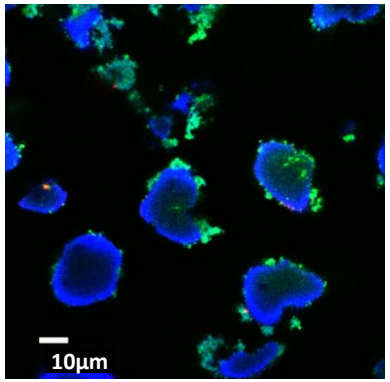
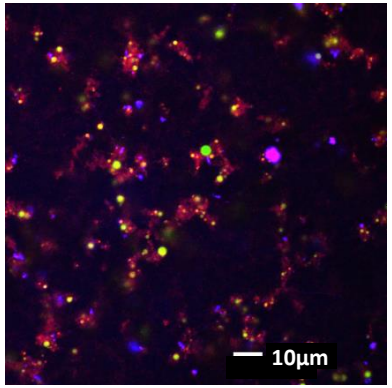
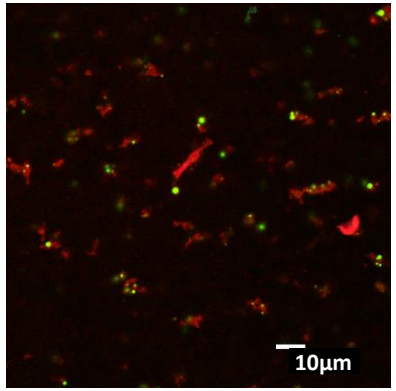
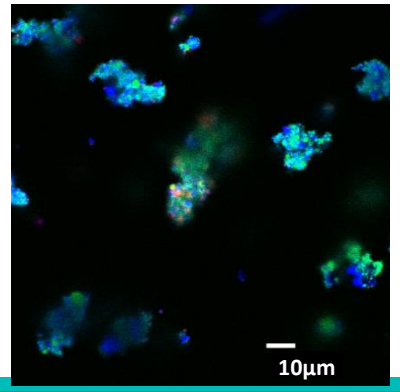
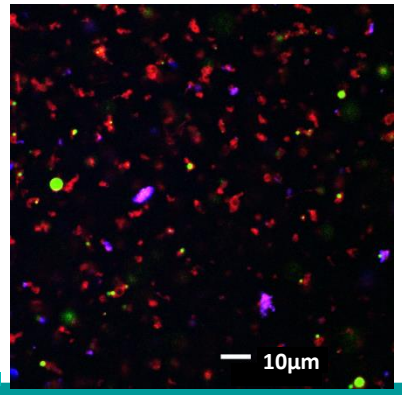
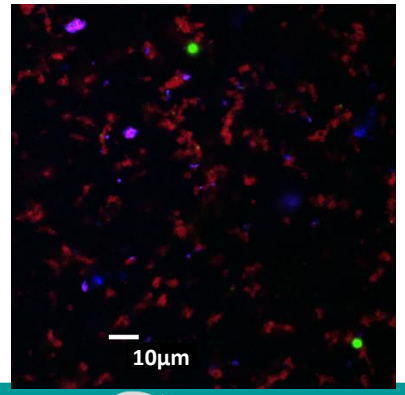


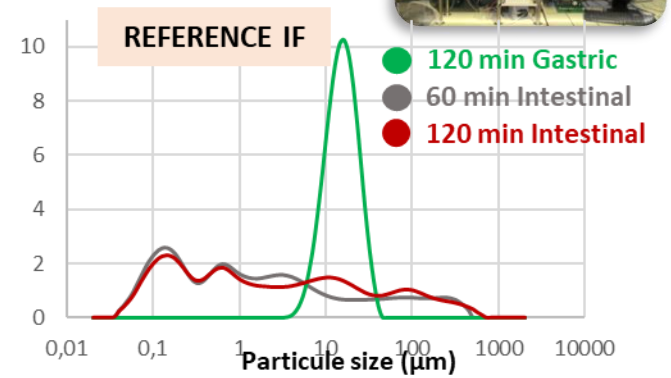
Confocal microscopy
X100 zoom1
- **Proteins**
- **Lipids**
- **Amphiphilic**

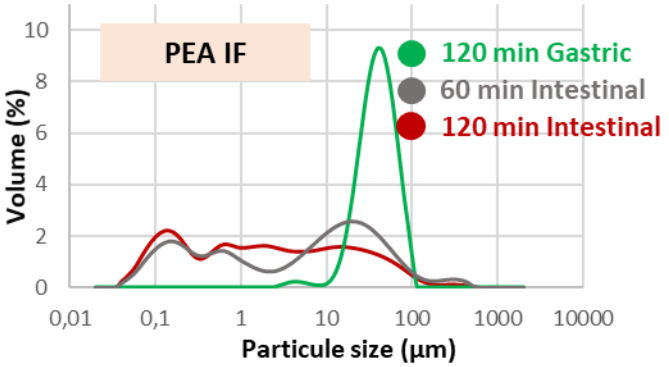
IF: infant formula

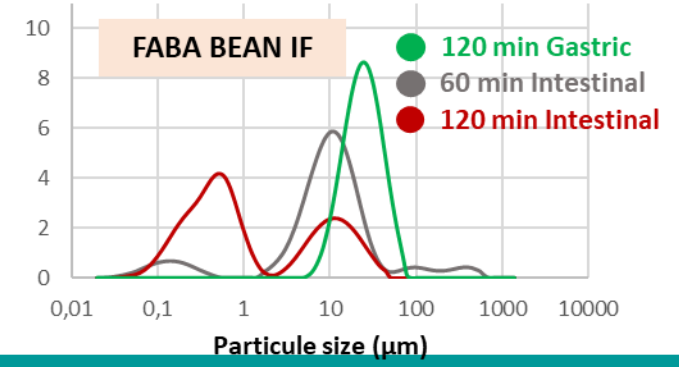
INTESTINAL



120 min (gastric) pH=4.9	60 min pH=6.2	120 min pH=6.2
REFERENCE IF			
PEA IF			
FABA BEAN IF			



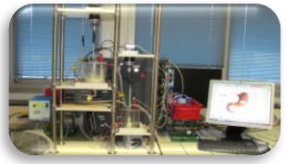




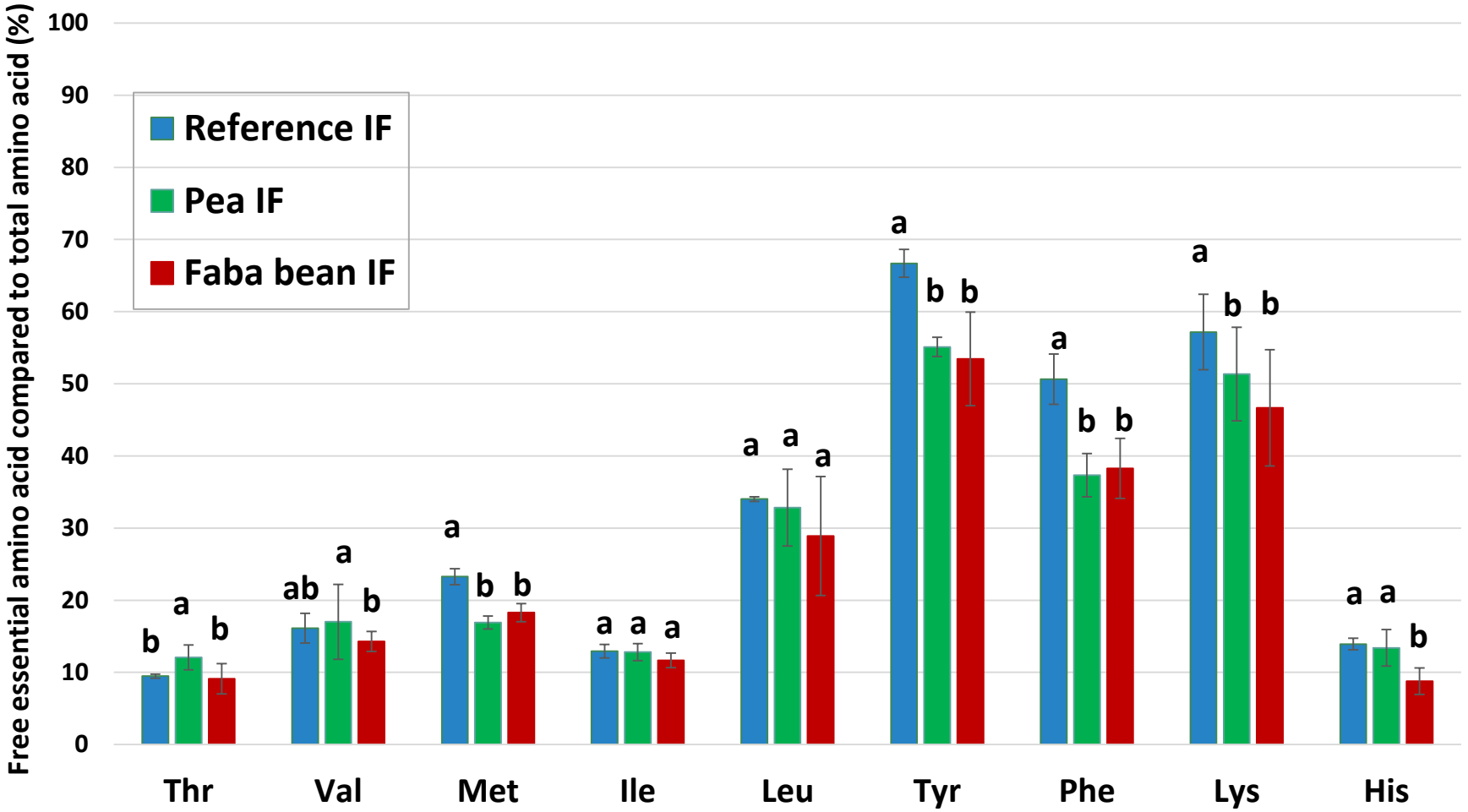
Confocal microscopy
X100 zoom1
Proteins
Lipids
Amphiphilic

IF: infant formula

IN VITRO DYNAMIC DIGESTION



Bioaccessibility of amino acids at the end of the digestion



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

IF: infant formula

(Moore, Spackman, & Stein, 1958)

CONCLUSION

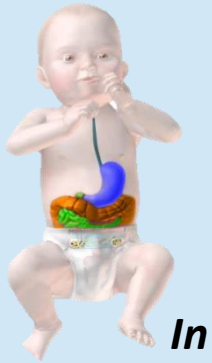
- **Producing plant protein based infant formulas**

- **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 ≈ Pea IF ≈ Faba bean IF → Comparable digestibility

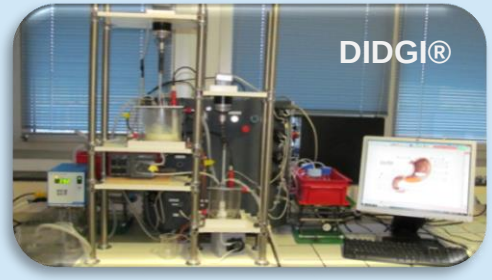
PERSPECTIVES

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



In vitro static

IN VITRO

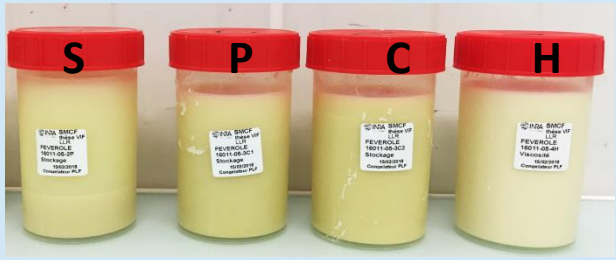


In vitro dynamic

IN VIVO

- Functional improvement → Dispersion of plant proteins

PROCESS



- Evidence of conformity regarding the European regulation 2016/127

REGULATION

THANK YOU FOR YOUR ATTENTION

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