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Influence of raw milk microflora and starter cultures in cheese on protein hydrolysis and peptide generation during digestion

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Context

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Do bacterial strains in cheese have an impact on protein hydrolysis during human digestion, and if so, does a higher microbial diversity lead to the generation of a higher number of different peptides after digestion? To answer this question, four different cheeses made from milk with or without microflora, plus or minus the addition of a proteolytic strain were produced and ripened for 120 days, followed by *in vitro* digestion experiments, applying a static (Infogest) and a dynamic (DIDGI) oro-gastrointestinal *in vitro* model. The digesta were characterized at a multiscale level, such as, confocal laser microscopy, gel electrophoresis, mass spectrometry, and HPLC, to investigate the influence of the pre-proteolysis occurring during cheese ripening, on the global proteolysis during the digestion.

Objectives

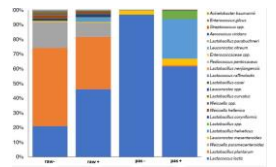
Cheese fabrication and composition

Dynamic and Static *in vitro* digestions

Swiss Raclette cheeses were produced either from raw or pasteurized milk, with or without the addition of a proteolytic bacterial strain (*Lactobacillus helveticus*) and ripened during 120 days. 2 cheeses of each condition were produced:
- raw milk without starter (raw -) n=2
- raw milk plus starter (raw +) n=2
- pasteurized milk without starter (past -) n=2
- pasteurized milk plus starter (past +) n=2

characterization after 120 days of ripening

	fat	NaCl	true protein	NPN	pH	OPA
	g/kg	g/kg	g/kg	g/kg		mmol/kg
raw -	290.3	38.9	244.4	5.7	5.6	151.3
raw +	295.8	37.0	250.3	5.6	5.6	151.5
past -	284.8	21.0	247.4	5.7	5.6	143.2
past +	288.3	38.9	238.3	5.8	5.6	145.2



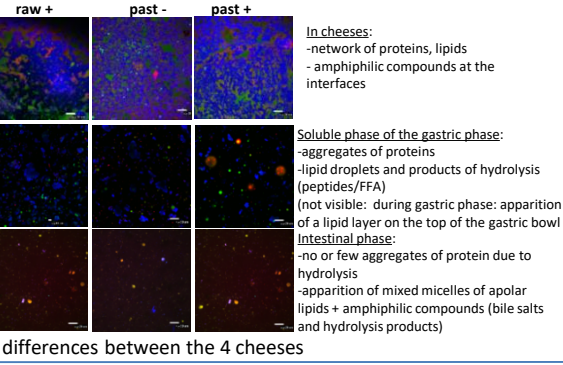
A standardised static *in vitro* digestion method suitable for food – an international consensus!
dig n=2 cheeses: 4X2
dig n=1 cheeses: 4X2

Parameters	Parameters
Gastric: -Pepsin (2000U/ml) :0.5ml/min -HCL1M to achieve:pH=1.68±3.52 ^(1,4) -Emptying t _{1/2} =85min β=1.8 -duration: 150min	Gastric: -Pepsin (2000U/ml) -pH=3 -duration: 120min
Intestinal digestion: -Bile (2%) 0.5ml/min -Pancreatin (7%)0.25ml/min -NaHCO ₃ 1M to achieve pH=6.8 -Emptying t _{1/2} =250min β=2.5 -duration: 240min	Intestinal digestion: -Bile salts 10mM -Pancreatin: to cover 100 U of trypsin/ml -pH=7 -duration: 120min

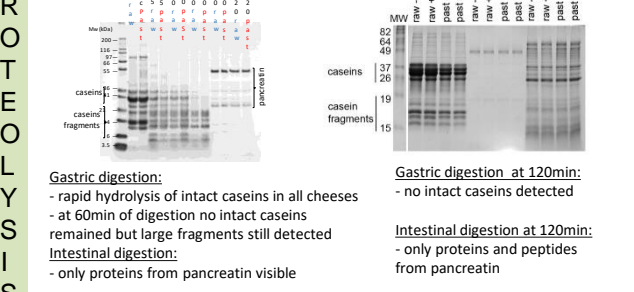
Results

Confocal microscopy (CSLM)

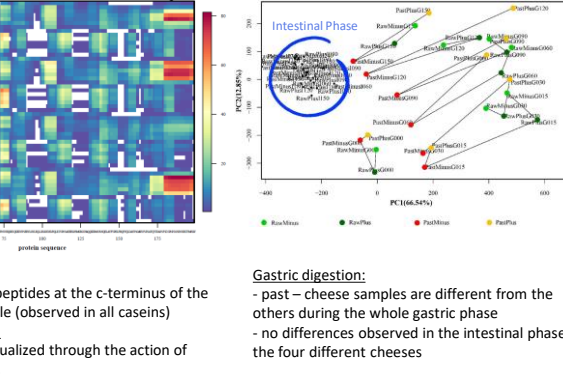
Observations of cheeses and after 85min (t_{1/2}) of dynamic gastric and intestinal digestions



PROTEOLYSIS



Mass spectrometry: Peptide patterns and PCA of dynamic digestions



Conclusion

1. A lower bacterial diversity (past - cheese) leads to a decreased peptide generation during the gastric phase which is equalized at the beginning of the intestinal phase
2. Dynamic vs static digestion:
Gastric phase: no difference between the 2 digestion models due to a low proteolysis at the molecular level
Intestinal phase: different pattern is observed with a progressive increase of FAA and Free NH₃ in the dynamic system compared to a drastic and instantaneous liberation of FAA and Free NH₃ in the static model. The results are in accordance with previous studies (Egger et al; 2018 Digestion of milk proteins: comparing static and dynamic *in vitro* digestion systems with *in vivo* data)