



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

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► To cite this version:

Olivia Ménard, Reto Portmann, Didier Dupont, Helena Stoffers, Hélène Berthoud, et al.. Influence of raw milk microflora and starter cultures in cheese on protein hydrolysis and peptide generation during digestion. 17. Food Colloids Conference: Application of Soft Matter Concepts, May 2018, Leeds, United Kingdom. , 2018. hal-01783984

HAL Id: hal-01783984

<https://hal.science/hal-01783984>

Submitted on 2 May 2018

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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 (DIDIG) 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

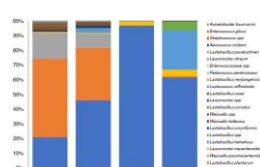
Cheese fabrication and composition

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	18.9	244.4	5.7	5.6	151.3
raw +	295.8	17.9	250.3	5.6	5.6	151.5
past -	284.8	21.0	247.4	5.7	5.6	143.2
past +	288.3	18.9	238.3	5.8	5.6	145.2

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

Bacterial RNA at 120 days



Dynamic and Static *in vitro* digestions

A standardised static *in vitro* digestion method suitable for food – an international consensus¹

INFOGEST dig n=1 cheeses: 4X2

DIDIG dig n=2 cheeses: 4X2

Parameters

Gastric:
 -Pepsin (2000U/ml) :0.5ml/min
 -HCL1M to achieve:pH=1.68±3.52^(1,40)
 -Emptying: t_{1/2}=85min β=1.8
 -duration: 150min

Intestinal digestion:
 -Bile (2%) 0.5ml/min
 -Pancreatin (7%)0.25ml/min
 -NaHCO₃ 1M to achieve pH=6.8
 -Emptying: t_{1/2}=250min β=2.5
 -duration: 240min

Parameters

Gastric:
 -Pepsin (2000U/ml)
 -pH=3
 -duration: 120min

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

raw - raw + past - past +

In cheeses:
 -network of proteins, lipids
 - amphiphilic compounds at the interfaces

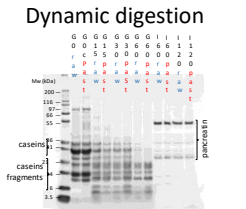
Soluble phase of the gastric phase:
 -aggregates of proteins
 -lipid droplets and products of hydrolysis (peptides/FFA)
 (not visible: during gastric phase: apparition of a lipid layer on the top of the gastric bowl)

Intestinal phase:
 -no or few aggregates of protein due to hydrolysis
 -apparition of mixed micelles of apolar lipids + amphiphilic compounds (bile salts and hydrolysis products)

no structural differences between the 4 cheeses

PROTEOLYSIS

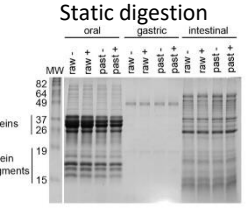
Dynamic digestion



Gastric digestion:
 - rapid hydrolysis of intact caseins in all cheeses
 - at 60min of digestion no intact caseins remained but large fragments still detected

Intestinal digestion:
 - only proteins from pancreatin visible

Static digestion

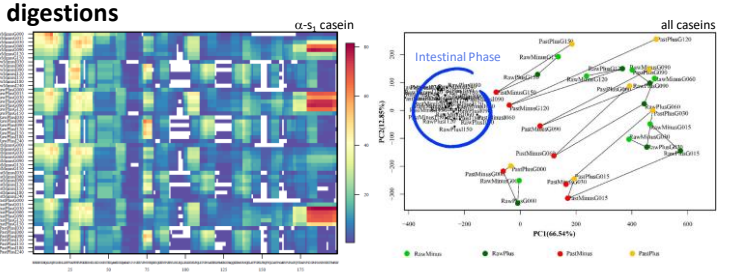


Gastric digestion at 120min:
 - no intact caseins detected

Intestinal digestion at 120min:
 - only proteins and peptides from pancreatin

PROTEOLYSIS

Mass spectrometry: Peptide patterns and PCA of dynamic digestions



Gastric digestion:
 - lower number of peptides at the c-terminus of the past – cheese sample (observed in all caseins)

Intestinal digestion:
 - differences are equalized through the action of intestinal proteases

Gastric digestion:
 - past – cheese samples are different from the others during the whole gastric phase
 - no differences observed in the intestinal phase for the four different cheeses

PROTEOLYSIS

Gastric digestion:
 - very low liberation of amino-acids (FAA) during the gastric phase
 - liberation of Free NH₃ after 15min but decrease from 60min due to increase in gastric emptying

Intestinal digestion:
 - high proteolysis with a maximal value after 180 min
 - at t240min decrease due to intestinal emptying

In both digestion systems :no difference between the four cheeses

Conclusion

- 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
- 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)