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# The fate of salivary proteins-apple polyphenols complexes during gastric digestion

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## Introduction

### Context

- Despite recognized health benefits, some polyphenols referred to as **tannins** exhibit **anti-nutritional properties**.
- Binding of tannins by **salivary proteins** (especially Proline-Rich Proteins: PRPs) prior to the stomach may therefore act as a **protective mechanism** against their deleterious effects on digestion.
- It is not entirely clear how the complexes formed in the oral cavity behave in the harsh digestive environment.

### Objectives

- To describe the interactions between salivary proteins and apple polyphenols
- To study the impact of gastric digestion on the resulting complexes

## Materials and methods

### Polyphenols (PO) extract from Dous Moën cider apples



Polyphenols: 730g /kg  
Procyanidins: 322 g/kg  
Mean DP: 3.2

Phenolic compound	Concentration (g/kg)
CAT : (+)-catechin	29.5
EC: (-)-epicatechin	63.5
PA-B1 : procyanidin dimer B1	22.4
PA-B2 : procyanidin dimer B2	44.0
PA-B5 : procyanidin dimer B5	2.9
PA-C1 : procyanidin trimer C1	21.1
DP4 : procyanidin tetramer	12.3
CGA : 5'-O-caffeoylquinic acid	237.9
PCQ : 4-O- <i>para</i> -coumaroylquinic acid	37.0
XPL : phlorizin 2'-O-xyloglucoside	24.8
PLZ : phlorizin	13.9

**Human saliva (S)**  
proteins: 0.72 g/L



Polyphenols to protein ratios: 0.16/0.32/0.48/0.80  
incubation 10 minutes  
centrifugation  
supernatants and pellets analyzed

**Saliva alone**  
**Polyphenols alone**  
**Mixtures**



Polyphenols to protein ratios: 0.16 / 0.80  
static INFOGEST gastric digestion  
centrifugation of end-point samples  
supernatants and pellets analyzed

### Protein profiles & identification

(supernatants and pellets)

SDS-PAGE + nano LC ESI MS/MS

PRP detection: Coomassie blue R staining

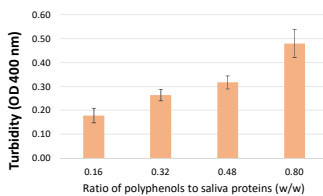
### Native polyphenols analysis

(supernatants)

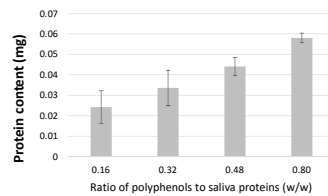
UPLC-DAD-MS

## Results

### Interactions polyphenols-saliva proteins

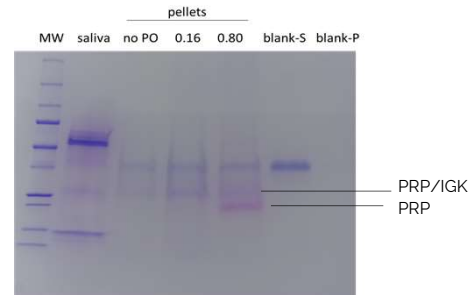


Turbidity of whole samples increases with PO/S ratio: formation of increasingly large aggregates



Amount of proteins increases in pellets with PO/S ratio: dose-dependent precipitation

### Impact of gastric digestion

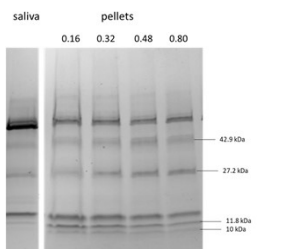


The complexes formed with CA6 and SA100-A8 before digestion are proteolyzed, while the one containing PRP/IGK persist  
Formation of a new PRP-PO complex.

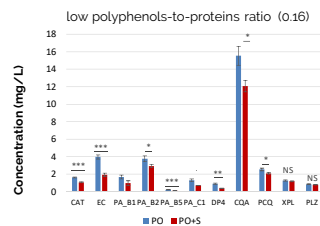
**Table 1.** Changes induced by digestion on concentrations of procyanidins in supernatants. Values are presented only when significant.

	PA-B1	PA-B2	PA-B5	PA-C1	DP4
low PO load	PO	+48 % **	+51 % ***		-17 % *
	PO+S		-100 % **	-70 % **	-63 % **
high PO load	PO	+56 % ***	+16 % ***		
	PO+S	+20 % *		-23 % *	

Digestion in presence of saliva: insolubilization of a large proportion of PA-B5, PA-C1 and DP4 (tannins highly reactive towards saliva proteins). Interaction with proteins or proteolytic fragments.



Four bands enriched in pellets: PRPs/IGK and two zinc-binding proteins (carbonic anhydrase 6 and SA100-A8)



**Low PO/S ratio:** all polyphenols classes except dihydrochalcones are decreased in supernatants in presence of saliva.

**High PO/S ratio:** only procyanidins with high DP precipitate.

## Conclusions

- PRPs can efficiently precipitate apple polyphenols. Two zinc-binding proteins can also form insoluble complexes with polyphenols.
- The classes of polyphenols involved in such complexes depend on the polyphenols-to-protein ratio.
- *In vitro* gastric digestion leads to extensive proteolysis of salivary proteins. Some resulting fragments can interact with and precipitate procyanidins.

Saliva may partly modulate bio-accessibility of procyanidins in the gastric compartment.