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pOP-1

Harnessing polysaccharide-lectin interactions to functionalize emulsions: Insights from emulsions stabilized by waxy starch and Concanavalin-A

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In recent years there is a growing interest in legumes, legume-based proteins and lectins as an innovative toolbox to shape functionality and health benefits or risks of orally consumed products. Lectins are robust proteins known to uphold specific interactions with various carbohydrates, to inhibit digestive amylases and to possess various biological activities. Specifically, this study sought to harness Concanavalin A (Con-A) from jack beans and its interactions with processed waxy starch to generate and stabilize canola oil in water emulsions. The working hypothesis was that Con-A tertiary and quaternary structures along with Con-A interactions with waxy starch offer the potential to fabricate responsive emulsions with modulated susceptibility to digestive lipolysis.

First, seventeen different emulsion formulations were produced using canola oil, Con-A (0.25-2% w/w), waxy starch (4% w/w) and various levels of divalent ions (Mn, Ca and Zn) known to affect Con-A-starch interactions. The stability and pH responsiveness (2<pH<9) of the emulsions was analyzed by analytical centrifugation (2000 RPM, 35^{o}C, 28 hr) and emulsion viscosity was monitored using a rotational plate rheometer. These experiments showed that highly viscous emulsions with shear thinning behavior were obtained. Moreover, emulsion stability to varying pH conditions was tightly linked to the type of counter ions present. This indicates that Con-A-starch interactions were detrimental to emulsion stability.

Second, selected formulations were subjected to an in vitro pH stat intestinal lipolysis model. These experiments revealed that Con-A-starch interactions can inhibit or enhance the release of free fatty acids from the lipid droplets during simulated intestinal lipolysis. An effect that was again linked to the metal ion present in the formulation and governing Con-A-starch interactions. Overall, this research demonstrates a novel strategy to functionalize emulsions through the utilization of lectin-carbohydrate specific interactions, offering a potential means to shape emulsion functionality and digestibility.

pOP-2

Interfacial engineering of droplet interfaces towards controlled digestive fates in infants, adults and the elderly

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Emulsions are cost effective delivery systems for lipophilic nutraceuticals. However, there is a constant need to ensure their effectiveness to deliver desirable bioaccessibility, bioavailability and bioefficacy tailored consumer needs. This lecture will overview findings seeking to understand the impact of colloid emulsifiers and consumer physiology on emulsion behaviour and breakdown in the gastro-intestinal system of different human age groups using various in vitro digestion models.

The first part will show how different nano-colloids (hydrophobically modified inulin, lactoferrin nano-particles and lactoferrin-polysacchride nano-particles) affect emulsion susceptibility to adult gastric and intestinal breakdown [1-3]. These studies exemplify how gastric proteolysis may lead to droplet coalescence while gastric pH profiles may induce flocculation due to diminished electrostatic repulsion near the protein isoelectric point. New findings into the importance of the droplet lipid type in affecting emulsion differential lipolysis and the free fatty acids released from emulsified olive, hemp or pomegranate seeds bioactive oils. The second part will illustrate the importance of understanding the consumer physiological capabilities to digest emulsions based on findings from semi-dynamic and dynamic in vitro digestion experiments recreating digestion in infants, adults and the elderly [4, 5]. These will show that accounting for dynamic events, such as gastric pH profiles and emptying as well as bile and pancreatic secretions are essential when interrogating protein-stabilized emulsion breakdown and the relevant destabilization mechanisms. Particularly, the use of mammalian gastric lipase (100 u/mL in adults and 18u/mL in the elderly) and the implementation of a novel elderly digestion model will show that extrapolating identification of fast and slow digesting formulations based on adult conditions do not necessarily maintain these traits under elderly conditions.

Overall, the findings depicted will indicate that it is imperative to understand implications of digestion dynamics on emulsion digestive fate in different humans. Current knowledge limits our ability to design formulations for "non-healthy-adult" groups based solely on information obtained in adults. Standardized systematic research and in vivo validations are key milestones on the path to develop and tweak the functionality of age-tailored emulsion formulations and perhaps even personalized formulations.

References:

1. Meshulam, D. and U. Lesmes, *Responsiveness of emulsions stabilized by lactoferrin nano-particles to simulated intestinal conditions.* Food & Function, 2014. **5**(1): p. 65-73.

2. Shimoni, G., et al., *Emulsions stabilization by lactoferrin nano-particles under in♀ vitro digestion conditions.* Food Hydrocolloids, 2013. **33**(2): p. 264-272.

3. Meshulam, D., J. Slavuter, and U. Lesmes, *Behavior of Emulsions Stabilized by a Hydrophobically Modified Inulin Under Bio-Relevant Conditions of the Human Gastro-Intestine.* Food Biophysics, 2014. **9**(4): p. 416-423.

4. Levi, C.S. and U. Lesmes, *Bi-compartmental elderly or adult dynamic digestion models applied to interrogate protein digestibility.* Food & Function, 2014. **5**(10): p. 2402-2409.

5. Shani-Levi, C., S. Levi-Tal, and U. Lesmes, *Comparative performance of milk proteins and their emulsions under dynamic in♀ vitro adult and infant gastric digestion.* Food Hydrocolloids, 2013. **32**(2): p. 349-357.

pOP-3

Can we mimic *in vivo* digestion of the preterm newborn by an *in vitro* dynamic model?

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Understanding human milk digestion is a key step for developing biomimetic infant formulae. Ethical and technical reasons limit the possibility of *in vivo* trials. Relevant *in vitro* models are thus necessary. Our aim was to compare a dynamic *in vitro* digestion system developed by the French National Institute for Agricultural Research[1] (DIDGI ®) against *in vivo* data collected in preterm newborns.

Supported by an exhaustive literature review[2], the dynamic digester parameters were set in terms of types and amounts of enzymes, secretions, pH decrease and emptying rate to mimic as closely as possible the gastric digestive conditions of preterm newborns (1.9 kg). Raw or pasteurized (62.5°C, 30 min) preterm human milks (n=5 donors) were digested *in vitro* in triplicate. An *in vivo* study was conducted on hospitalized preterm newborns at Rennes Hospital (NCT02112331; n=12; mean ± SD: 28 ± 17 days of life, 1.5 ± 0.2 kg of bodyweight). *In vitro* and *in vivo* gastric digesta were sampled regularly (30 or 35 min, 60 min, 90 min). Structural changes were evaluated by confocal microscopy and laser light scattering. Lipolysis and proteolysis were determined by SDS-Page, thin-layer and gas chromatography methods. Gastric volume, pH and emptying rates were monitored.

During *in vitro* and *in vivo* digestions, gastric digestive kinetics were in overall similar and were not affected by pasteurization ($p > 0.05$), although this impacted the structural evolution of digesta. At 90 min of digestion, there was a similar degree of lipolysis ($p>0.05$) *in vitro* (11.3 ± 3.8%) and *in vivo (*11.5 ± 5.9%) and a similar proportion of residual proteins for β-casein and serum albumin (25.8 and 34%, in average). The latter was somewhat lower *in vitro* for lactoferrin (-11%) and for serum albumin (-20%). Curves of pH decrease and emptying rates could be improved to adjust closer *in vivo* gastric conditions.

Overall, our model appears as a relevant tool to study digestive kinetics of human milk and could also be applied for infant formula digestion. This will be useful to the scientist community and food manufacturers who focus on neonatal gastric digestion and infant formula optimization.

References:

[1]Ménard O., et al. (2014). *Food Chem*, 145, 1039-45.

[2]Bourlieu C., et al. (2015). *Crit Rev Food Sci Nutr*, 54(11), 1427-57.

pOP-4

How the interfacial and aggregation behaviour of bile salts influences in vitro lipid digestion

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Bile salts are naturally occurring biosurfactants synthesised in the liver from cholesterol. They adsorb to the surface of dietary fat during duodenal digestion. They facilitate the adsorption and activity of pancreatic lipase, and the removal and transport of hydrolysis products. This is critical for effective lipid digestion and absorption in humans. However the mechanisms underlying their effectiveness are not clear. From a colloidal viewpoint, it is difficult to understand how a surfactant on the one hand can promote the adsorption of a protein (lipase), yet on the other hand, can displace the products of lipolysis (free fatty acids and monoglycerides etc). Previous work showed how different bile salts possessed different adsorption – desorption kinetics[1] and it was thought that this could help explain this paradox.

Following on from this work, we have attempted to investigate how the interfacial properties of bile salts affects the production of lipolysis products by lipase at the interface. Using the pendant drop method under simulated duodenal conditions, together with pH autotitration, we have tried to separate the process of interfacial accumulation of lipolysis products from partitioning of these products from the oil phase into the micellar phase.

We studied two common bile salts Sodium Taurocholate (NaTC) and Sodium Glycodeoxycholate (NaGDC)as we showed previously that they have different interfacial properties in terms of their adsorption and desorption kinetics. NaGDC adsorbs more rapidly, has a slightly lower cmc, and a lower equilibrium interfacial tension. NaGDC was found to promote the release of free fatty acids from the oil phase during lipolysis more quickly than NaTC. In addition, NaGDC could achieve this at higher interfacial tensions than NaTC. The rates of accumulation of product at the interface appeared to be similar between the bile salts, suggesting that desorption kinetics may not be important for lipase activity as first postulated. This suggests that it is the aggregation behaviour of the bile salts, and their ability to displace and solubilise lipolysis products which is a limiting factor for fat digestion. These results could give insights into the critical stages of lipid digestion that could be targeted in order to help slow the rates of digestion and prolong energy release, both of which are linked with reducing incidence of obesity and cardiovascular disease.

1. Parker R, Rigby NM, Ridout MJ, Gunning AP and Wilde PJ. The adsorption–desorption behaviour and structure function relationships of bile salts. *Soft Matter*, 2014, **10**, 6457-6466.

pOP-5

Bioaccessibility of quercetin encapsulated in solid lipid microparticles: influence of the oil core composition and of the interactions among bioactive and surfactants

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This study investigated the bioaccessibility of quercetin in solid lipid microparticles produced with different percentages of babacu oil (50% medium chain triglycerides, MCTs) in the lipid core, as well as to evaluate the influence of the amount of surfactant in the digestion of the particles. Solid lipid microparticles (4% total oil, 4 or 2% total surfactants, in a mixture of 70% span 80 and 30% tween 60 in the interface) were produced by high shear method, with different mass percentages of babacu oil (1.2, 2.0 and 2.8% w/w, respectively, 30, 50 and 70% of the lipid core). The particles presented an average diameter in the range 1.0-1.5 µm. The gastric and intestinal simulated digestion was carried out using the *in vitro* pH-stat protocols published by Minekus et al. (2014). The digestion process was evaluated by average particle size, zeta potential and release of free fatty acids. Bioaccessibility of quercetin was assessed by quantifying the bioactive present in the micelles after intestinal digestion. The results showed the amount of surfactant and MCTs in the lipid core did not influence in the total release of fatty acids (30-35% in all cases), but in the fatty acid release rate. The interfacial characteristics together with the amount of MCTs were essential to determine the bioaccessibility of quercetin. A higher bioacessibility value (69%) was observed for formulations produced with a lower surfactant amount (2.0%) and higher percentages of babacu oil (70% in the lipid core), in comparison to particles produced with 4.0% surfactant and less babacu oil (30% in the lipid core), which showed a bioacessibility value of 53%. However, it was noticed that one more factor could have influenced in the results of bioacessibility; experiments of interfacial tension (pendant drop method) showed quercetin formed complexes with the mixture of surfactants tween 60/span80, and, therefore, could be located in the interface of the microparticles, contributing significantly to the increase in the bioaccessibility, especially in the cases of dispersions produced with 2.0% surfactant.

References:

Minekus, M. et al. A standardised static in vitro digestion method suitable for food-an international consensus. Food & Function, 5, 1113-1124, 2014.

pOP-6

How the body reacts to food: an innovative *In Silico* tool to model and predict digestion, absorption and physiological responses

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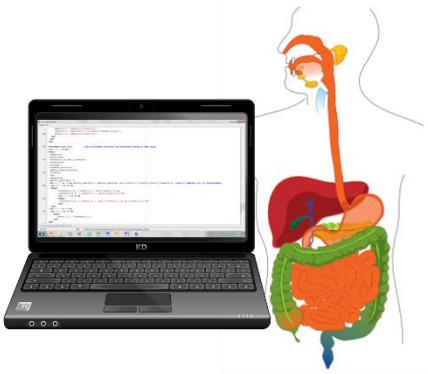
In vitro digestion models give information on the way food composition and structure affect the amount and kinetics of release of absorbable nutrients. However, the *in vivo* digestive processes are constantly adapted in response to the body's physiological feedback on the receptors that monitor the state of digestion and the release of absorbable nutrients in the gastrointestinal tract. This systemic feedback is absent in *in vitro* systems.



In order to introduce the complex and dynamic nature of digestion, a computer simulation model has been developed that describes the physiological feedback mechanisms by which the body optimizes the digestive processes in response to various stimuli from the food along the gastrointestinal tract [a]. This innovative model describes and reproduces these processes on an extensive basis of physiological literature.

The model allows a translation of the results of relatively static *in vitro* digestion studies to realistic dynamic *in vivo* pre- and post-absorptive physiological responses to food. Food-specific data from in vitro digestion experiments may be fed into the model to predict food-specific physiological responses. The model can also support an optimization of *in vitro* experimental instrumentation and digestive parameters to deliver more realistic results.

The model has demonstrated ability to independently reproduce *in vivo* data on the kinetics of amino acid release to the bloodstream for different protein supplements ("fast" versus "slow") and the difference in time-dependent fullness and hunger ratings after meals varying in protein content, and can be used to model the release of blood and breath tracers used for measuring gastric emptying and glycaemic effects of foods (blood glucose and insulin levels). In principle, the model can also be used to evaluate digestion and bioavailability in specific subpopulations (elderly, infants, diabetics, diseased states and animals) by adapting the relevant physiological parameters.

The oral presentation will introduce and discuss the model, provide some modelling examples and give an outlook to further development and application.

Reference:

a. information on the principles of the model can be found on www.insightfoodinside.com

pOP-7

Study on pepsin diffusion in protein gel digestion

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Protein is one of the essential macronutrient and its digestion starts in the stomach. Numerous in vitro studies on protein digestion are based on experiments with protein solutions, however the majority of proteins exists in solid food. Therefore, our research is aimed at understanding how the structures of food affects the digestion of protein.

In our study, we used whey protein gel as a model for protein based food matrix. We found that the structure of hydrogel was hindering the hydrolysis of protein. However, the hindrance is not simply slowing down the hydrolysis, but also altering the enzyme kinetics to some extent(1). We inferred that the diffusion limitation in the gel matrices had led to the difference in the hydrolysis kinetics.

Our current study is aiming at measuring the diffusivity of pepsin in gel matrices. Pepsin was labelled with an Alexa Fluor® dye, its diffusion was measured by Fluorescent Correlation Spectroscopy (FCS). A degree of hydrolysis assay and Size Exclusion Chromatography (HPSEC) were used to characterize the hydrolysis in the gel digestion. Scanning Electron Microscopy (SEM) was used to observe the surface of the protein gels.

We measured the diffusion coefficients of pepsin in different solutions and gel matrices. Degree of hydrolysis and HPSEC of digested gels indicated the effect of pepsin diffusion on hydrolysis extent. SEM images showed the microstructural change of gel during digestion.

By quantifying the diffusion of pepsin, we gained more insight on the action of pepsin and effect of gel structure in protein digestion. Moreover, this approach makes it possible to bridge the digestion process with established physical-chemistry theories and models, which may lead to better knowledge on the underlying mechanisms of gastric digestion.

Reference:

(1) Luo, Q., Boom, R.M., & Janssen, A.E.M. (2015). LWT - Food Science and Technology, 63, 161–168

pOP-8

Oral processing of mixed biopolymer gel with different degrees of inhomogeneity

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Bolus swallowing is a complex process that has been studied mainly from human physiology and coordination point of view by clinicians. It is well known that food consistency affects the risk of aspiration, and increasing the time at mouth has been largely addressed with viscosity optimization. Beside rheology, the degree of structure is also an essential variable in oral processing. The aim of this study was to address this research gap by examining the influence of gel structuring on oral residence time by designing mixed biopolymer gels with different degrees of inhomogeneity. Ten model gels with varying mechanical and microstructural properties were prepared using *K*-carrageenan and Na-alginate at concentrations ranging from 0-4 wt%. In few of the mixed gel systems, structural inhomogeneity was introduced by incorporation of calcium alginate beads of different particle sizes, later made by syringe extrusion or spraying techniques. The gels were characterized by dynamic oscillation, fracture behaviour and the microstructural details were revealed by cryo-scanning electron microscopy (cryo-SEM) and transmission electron microscopy. In parallel, gels were characterized by quantitative descriptive analysis (QDA™). Oral processing behaviour was assessed in terms of oral residence time, number of chews and difficulty perceived by eleven young participants. The food structural properties, oral processing behaviour and difficulty perception of the gels were compared using Principal Component Analysis. A decrease in the gel fracture point with the addition of alginate beads was attributed to the interruption of the continuous gel network, as revealed in the Cryo-SEM and TEM images and with narrower linear viscoelastic region. When the mixed gel network included *κ*-Carrageenan with Na-alginate, the linear viscoelastic range was extended, but the gel strength was lower than *K*-carrageenan alone highlighting the incompatibility between the biopolymers. Oral residence time was highly dependent on the number of chews and to a certain extent on the difficulty perceived. Oral residence time and number of chews were positively correlated with gel strength, degree of network inhomogeneity in terms of patricle size of the beads (Figure 1). This novel insight of incorporating structuring defects in gel can be an effective design strategy for future food formulations.

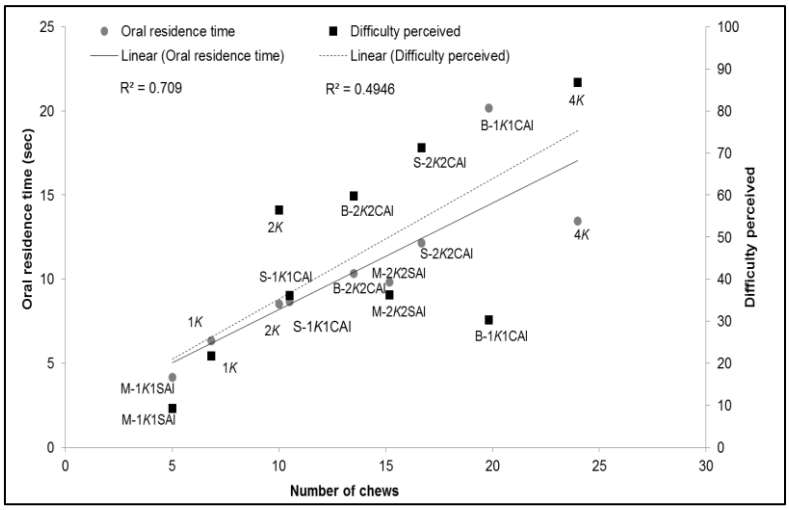

Figure 1: Number of chews (participant's average) in relation with A) the time at swallow of the different gels created and difficulty perceived, and B) the time at swallow and the level of inhomogeneity.