PPP Project Annual Report 2018
The PPP-projects that have been established under the direction of the top sectors must submit an annual report on their technical and financial progress. This format is to be used for reporting the technical progress. A separate format ('PPP final report') is available for PPP-projects that have been completed in 2018.
The annual reports will be published in full on the websites of the TKIs/top sector, excluding the blocks 'Approval coordinator/consortium' and 'Planning and progress' . Please ensure that no confidential matters are left in the remaining blocks.
The PPP Project Annual Reports must be submitted by 15 February 2019 to Hans van der Kolk

General information	
PPP number	AF-17101 (TKI toeslag project)
Title	Protein Functionality
Theme	Voeding & Gezondheid
Executive knowledge institution(s)	Wageningen UR / WFBR NIZO Food Research
Research project leader (name + e-mail address)	Marcel Meinders marcel.meinders@wur.nl
Coordinator (on behalf of private parties)	Thom Huppertz (Friesland Campina) Cornelly van der Ven (Danone/Nutricia Research) Hugo Streekstra (DSM)
Government contact person	Marjan van Creij (Min. van LNV)
Total project size (k€)	€ 1.400.000 (€ 300.000 in kind, € 400000 cash, € 700.000 subsidy)
Address project website	
Start date	1-1-2018
End date	31-1-2019

Approval coordinator/consortium The annual report should be discussed with the coordinator/the consortium. The TKIs appreciate being informed of possible feedback on the annual report.	
The coordinator has assessed the annual report on behalf of the consortium:	approved
Possible feedback on the annual report:	

Short content description/aim PPS What is going on and how is this project involved? What will be delivered by the project and what is the effect of this?
Background The long-term goal of the industrial partners is to be able to better predict functionality of high protein liquid food systems containing a complex mixture of natural ingredients. Functionality includes aggregation characteristics that are important for both shelf life stability and digestion. These are topics of great interest from a fundamental point of view and as well as from an application point of view. The aim of this PPP is to predict stability (on a time scale between hours and months) and enzymatic digestibility of highly concentrated complex dairy protein mixtures (containing casein and whey proteins, mixed with hydrolysates, and mixed with plant proteins) from ingredient properties and initial aggregation. Protein based ingredients are high in priority within the strategic innovation program (SIP) Customised Processed Food. This program has been defined in 2015 to strengthen the innovation

potential within the Netherlands by promoting collaboration between TNO and Wageningen FBR (WFBR). The previous Protein Functionality project (PPP) has been completed and disseminated. In view of the successful outcome this follow-up project has been defined by the participating knowledge Institutes (TNO, WFBR and NIZO) and three industrial partners to further increase the innovation potential of protein based ingredients. We will investigate (new) methods to quantify and characterize protein-protein interactions in complex dairy protein mixtures (as such, mixed with hydrolysates, and mixed with plant proteins). These methods will allow investigation of the role of aggregation and its dynamics in relation to storage stability and digestibility.
The project consists of two subprojects:
1. Protein-protein interactions and aggregation dynamics in protein mixtures;
2. Role of protein-protein interactions and aggregation on protein digestion kinetics.

Important part of the project is the development of a methodology to measure (initial) aggregation and predict shelf life stability of these protein mixtures. Therefore we will map protein-protein interactions and aggregation in various complex mixtures high in protein content by various experimental techniques. This will give important insights in the relation between protein properties and aggregation propensity and dynamics as well as on the role of other non-protein components. Furthermore, aggregation will be studied during in-vitro digestion. Various enzymes will be studied to control aggregation during shelf-life and/or during digestion. These learnings will allow industry to better predict the functionality of proteins in terms of generic measurable protein properties and thus better predict and control the stability and digestibility of their end products. This will result in higher product quality, less waste, and more flexibility in ingredient use. Increased ingredient flexibility also allows industry to choose for more sustainable produced ingredients while maintaining the high quality of their end products.

Planning and progress (if there are changes to the project plan, please explain)	
Is the PPP going according to plan?	yes
Have there been changes in the consortium/project partners?	no
Is there a delay and/or deferred delivery date?	no
Are there any substantive bottlenecks?	no
Are there any deviations from the projected budget?	no

Results in 2018 so far

WP0 overall project
- Project well on schedule
- During kick-off meeting begin January 2018 scoping of WP1 and WP2 as well as the total Work Breakdown Structure including tasks defined and finalized.
- Aim of overall project defined in more detail: To develop methods to measure the process of protein-protein interaction and aggregation in non-diluted concentrated systems during production, shelf-life, and (in-vitro) digestion, as well as the influence of enzymes on these processes and to derive insights from the measurements including the resulting impact on (in-vitro) digestibility of proteins.

WP1: Protein-protein interactions and aggregation dynamics in protein mixtures
- Long list compiled of experimental techniques that seem to have a good potential measure protein aggregation at a very early stage and have the potential to serve as predictor for protein aggregation at a later stage, on basis of literature study and discussion with experts.

- Selection made from long list of techniques that will be tested within the project based on discussion between science and industrial partners. These are Light Scattering (DWS, DLS, MALS), Raman IR spectroscopy, FTIR spectroscopy, Low field NMR, Rheology
- Model systems defined to test short list of experimental techniques (Micellar Casein Isolates (MCI) at concentrations of 7% and higher. Gelling induced using GDL or rennet)
- Aggregation experiments performed. From the results the most promising techniques chosen that will be continued with in the project: Light Scattering (NanoSizer and MALS when needed), FTIR spectroscopy, Rheology.
- New set of model systems defined for shelf-life tests and potential to measure initial aggregation
- WPI at different concentrations and different pH, with and without the enzyme protein glutaminase, stored at RT for 7 days (measured in between).
- MCI and MCI-caseinate mixtures at 8% stored at different temperatures (4, 25, 60 ^{o}C) for 1 day (measured in between)
- Shelf life tests performed
- Nanosizer autocorrelation function at forward and backward scattering and MALS seems good methodologies to measure initial aggregation. Also rheology.
- Data analysis running to obtain better insights on statistics and predictive power of the different techniques

WP2: Protein-protein interactions and aggregation dynamics in protein mixtures

- Materials and methods chosen that will be (and have been) used during digestion experiments
  - Infant formulation (about 1.5 wt% protein and 3.5 wt% fat, with and without enzyme treatments) combined with an infant in vitro gastric digestion protocol
  - Adult formulation (about 6 wt% protein and 6 wt% fat, with and without enzyme treatments) combined with an adult in vitro gastric digestion protocol
- Detailed work plan compiled for digestion experiments (including sieving and centrifugation methods to detect aggregates, soluble molecules and fat; FTIR to detect possible protein conformational change and/or differences in composition in the various phases; imaging and image analysis to measure aggregate amounts and sizes in various phases; mass balances, SDS-PAGE (reduced and non-reduced) to obtain insights in the protein composition in the different phases
- Digestion experimental methodology now in place and digestion experiments and analysis running
- Several model systems with varying ratios casein/whey and with different enzymatic treatments are already measured showing interesting effects of protein composition, processing and enzyme modifications on aggregate formation and rate of digestion in the in vitro digestion model under adult conditions.

Number of delivered products in 2018 / so far *(in an appendix, please provide the titles and/or description of the products or a link to the products on public websites)*			
Academic articles	Reports	Articles in journals	Introductions/workshops
0	8	0	1

Appendix: Names of the products or a link to the products on a public website

- 1802WBS_PCC_SIP_II_ProteinFunctionality.xlsx (Work Breakdown Structure)
- 180220PCCSIP_Phase1.pdf (Summary of phase 1 including long list of experimental techniques and suggestion for short list)
- 180220MinutesPCCSIP2phase1.pdf (Conclusions phase 1 including short list of experimental techniques)
- 1805PCCSIP_All.pdf (Summary results)
- 1809PCCSIP_All.pdf (Summary results, including detailed work plan to measure role of aggregation in gastric digestion)
- 181022PCCSIP.pdf (Summary results)
- 181022MinutesPCCSIP2update.pdf (Minutes and conclusions update meeting 22-10-2018)

- 181109MinutesPCCSIP2PC.pdf (Minutes and conclusions first Project Council meeting)