General information	
PPP-number	AF-17101
Title	Protein Functionality
Theme	Voeding & Gezondheid, Hoogwaardige Producten
Implementing institute	Wageningen UR / WFBR NIZO Food Research
Project leader research (name + e-mail address)	Marcel Meinders marcel.meinders@wur.nl
Coordinator (on behalf of private partners)	Thom Huppertz (FrieslandCampina) Cornelly van der Ven (Danone/Nutricia Research) Hugo Streekstra (DSM)
Project-website address	**https://topsectoragrifood.nl/project/af-17101-protein-functionality-ii-stability-of-highly-concentrated-protein-mixtures/**
Start date	1-1-2018
Final date	31-1-2019

Approval by the coordinator of the consortium The annual report must be discussed with the coordinator of the consortium. The "TKI's" appreciate additional comments concerning the annual report.	
Assessment of the report by the coordinator on behalf of the consortium:	Approved
Additional comments concerning the annual report:	Due to a delay of the project in 2019, the project has been extended with 6 months. The new end date is 31-6-2020.

Summary of the project	
Problem definition	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. There is a need to develop 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 map protein-protein interactions and aggregation in various complex mixtures high in protein content using various experimental techniques. This 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.
Project goals	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 complex dairy protein mixtures (containing casein and whey proteins, mixed with hydrolysates, and mixed with plant proteins) from ingredient properties and initial aggregation.

Results	
Planned results 2019	WP1: Protein-protein interactions and aggregation dynamics in protein mixtures • Perform experiments on chosen techniques (Light Scattering (DWS, MALS(DLS,SLS), Nanosizer), FTIR spectroscopy, Rheology) on chosen samples (Micellar Casein Isolates and Whey Protein Isolates, caseinates, varying in heat treatment, enzyme treatment and concentration (ratio's)). Perform experiments, early after preparation and follow their behaviour over storage(under different conditions). • Explore the possibilities of a new dynamic light scattering technique, the NanoFlowSizer, to measure particle size characteristics and aggregation (kinetics) in chosen samples WP2: Protein-protein interactions and aggregation dynamics in protein mixtures • Perform digestion experiments and analysis on chosen samples (model systems with varying ratios casein/whey and different enzymatic treatments)
Achieved results 2019	WP1: • Planned experiments are performed • Real time FTIR showed effects of acidification and protease action; however the effects are obscured by background drift, discarding real time FTIR as a technique. Because aggregating and dissolved protein develop different spectra in time, separate analysis of serum and pellet after centrifugation were concluded to be essential. • Clear trend in rheological behaviour, turbidity, and light-scattering with aging time could be observed, which seem to correlate well with expected aggregation mechanism • Light scattering techniques seem suitable to measure initial aggregation • NanoFlowSizer promising technique to measure fast particle size characteristics, also in turbid samples WP2:

	• Digestion experiments on chosen samples are performed • Clear effect observed in aggregation behaviour during digestion depending on casein-whey ratio • Effect of digestion of best seen with SDS-PAGE, FTIR and Gel particle size measurements • In general little or no effect on heat treatment • Considering FTIR, most useful feature to study aggregation in digested samples is Amide-I peak. It shows changes in folding/structure of peptide bonds on change in pH. For pepsin action most sensitive feature is position amide II peak in the serum. • No clear parallel observed between SDS-page and FTIR data
Planned results 2020	WP1: Protein-protein interactions and aggregation dynamics in protein mixtures • Further develop, validate, and compare the light-scattering techniques NanoFlowSizer, MALS and Nano(zeta)Sizer protocols and data analysis. Gain more insights in the predictive power and boundaries of application of these techniques to measure initial aggregation correlate the above with prediction of shelf-life on model protein samples (MCI, WPI, caseinate, enzyme) and more complex systems, by combining proteins with other components (e.g. fat droplets). • Report WP2: Protein-protein interactions and aggregation dynamics in protein mixtures • Use NanoFlowSizer in flow-mode to see to what extend early aggregation can be observed during digestion of WPI-Caseinate samples • Validate FTIR experiments • Report

Deliverables/products in 2019 (provide the titles and /or a brief description of the products/deliverables or a link to a website.
Scientific articles:
External reports: • 190128PCCSIP2_UpdateWP1.pdf • 190128PCCSIP2_UpdateWP2.pdf • 190215PCCSIP2_Minutes_Wayforward.pdf • 190215PCCSIP2_PlanFurtherWorkWP2.pdf • 1902PCCSIP2_WBS_ProgressSummary.xlsx • 190423PCCSIP2_UpdateWP1.pdf • 190423PCCSIP2_UpdateWP1.pdf • 190423PCCSIP2_UpdateWP2.pdf • 190607PCCSIP2_Planning.xlsx • 190815PCCSIP2_IntermediateUpdateWP1.pdf • 191022PCCSIP2_Update.pdf • 191031PCCSIP2_Minutes_Wayforward • 191031PCCSIP_WayForward.pdf

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