

Project Information Reporting Format for Agriculture, Water, and Food

Public-Private Partnerships

Version date: 7 December 2020

From the project plan (fill in as much as possible)

1. Project Information

1.1 Funding	PPS toeslag TKI-A&F
1.2 Project number	AF 18017
1.3 Project title	Protein Compass
1.4 Project leader	Floor Boon (floor.boon@wur.nl)
1.5 Start date	01-01-2019
1.5 End date	30-06-2021
1.7 Primary MMIP	A4 Eiwitvoorziening
1.8 Secondary MMIP	x

2. Project Description

2.1 Summary

The protein intake of humans is primarily animal based. From sustainability point of view, the resources required for animal protein production are too high, particularly in view of the current population growth and societal trends, such as increased standard of living. This calls for a transition from animal to plant-based protein and therefore, for the development of new sustainable and healthy food products. A major challenge, however, is the lack of overview and predictability of the intrinsic functional, nutritional and sensorial properties of the protein raw materials, which hampers fast and successful identification of the best plant protein for a specific application.

Protein Compass targets to collect and generate a systematic overview of the characteristics of protein rich raw materials and protein isolates. This information is brought together in a database containing all relevant characteristics, including the future potential of the production chain and the economic viability, of the most promising plant protein sources. The data base will contain publicly available information, retrieved by smart digital technologies, and newly generated data from standardized, preferably high throughput, methods to ensure reliability in comparing data from different sources. This collection of data on the raw protein rich materials will be a start for a comprehensive tool for the food industry in which also other data like full nutritional content information, CO₂ foot prints, sustainability and examples for applications can be added to reach an efficient toolbox to support the industry in more effective and faster development of more sustainable food products.

2.2 Project target

Protein Compass targets to collect and generate a systematic overview of the characteristics of protein rich raw materials and protein isolates. This information is brought together in a database containing all relevant characteristics of the most promising plant-protein sources. The database will contain publicly available information, retrieved by smart digital technologies, and newly

generated data from standardized, preferably high throughput, methods to ensure reliability in comparing data from different sources.

2.3 Motivation

Protein Compass addresses the Agri & Food Sector's themes Consumer & Society, Healthy & Safe and Smart Technologies. It is also anticipated that from the database and the newly developed high throughput methods, ideas for new technologies for protein processing and total use of raw materials can be developed to link to the themes Climate neutral and Circularity. For these reasons, the project is of importance to the food industry and science community to embark on big data and data analysis technologies for speeding up innovation in healthy and sustainable food production, with high chance of consumer acceptance.

2.4 Result

Deliverable 1: reliable database on nutritional, functional and sensorial characteristics

- Selection of plant protein sources to be used for method development;
- Setting up structure of the database;
- Selection of properties of interest for nutritional, functional and sensorial characteristics;
- Fill the first version of the database with literature data.

Deliverable 2: Standardized methods for further systematic generation of information that is compatible with database comparison strategies

- Obtain protein ingredients for experimental work;
- Selection of measurement methods for the properties of interest and dividing them into straightforward, standard methods and methods for higher throughput.

Annual Report

3. Project Status

3.1 Project status	Project is on schedule
3.2 Explanation	No major changes have been made. Due to corona measures the capacity on the lab was reduced. This has resulted in an overall 3 months delay.

4. Achieved results

4. Brief description of the results

Deliverable 1: reliable database on nutritional, functional and sensorial characteristics

- Import Excel files to enter data in the database;
- Tool for importing excel data into the SQL database
- SQL database containing over 50 tables with dedicated views (3 versions have been released);
- Export PowerPivots as an example of what data can be extracted from the database;
- Database with data from literature and experimental data from WFBR, Unilever, Cargill and DSM;
- Data consist of 78 references with 1824 samples. Resulting in 1557, 401 and 2582 records on nutritional, sensorial and functional properties respectively;
- In total there are over 40.000 data points entered in the database.

Deliverable 2: Standardized methods for further systematic generation of information that is compatible with database comparison strategies

- Nutritional. Assays were evaluated for phytate, lipase, lipoxygenase, trypsin inhibition and α -amylase inhibition. Evaluation was based on suitability for various protein sources, reproducibility and time involved. All methods are suitable for high throughput setups using a pipette robot.
 - Phytate analysis is straightforward using a commercial assay.
 - Lipase analyses uses a commercial assay with a fluorescent label which showed a good reproducibility with a relative pure lipase sample but showed a relative large standard deviation when commercial samples were analyzed.
 - A lipoxygenase assay was set up based upon literature. The method was easy in indicating that most samples had no lipoxygenase activity, however the method requires a large set of dilutions to calculate the lipoxygenase activity for samples that test positive.
 - Trypsin inhibition was measured using a method based upon literature. This method, in most cases, could generate values for the amount of inhibitor. However some commercial protein samples showed inhibition curves that could not be used directly for calculation. In these cases a much more time consuming protocol has to be used in which the concentration of the protein samples and the fluorescent substrate are varied.
 - α -Amylase inhibition was measured using a commercial kit. The kit can be used to indicate presence of α -amylase inhibitor (yes or no). Quantification of the concentration at which half of the maximum inhibition is reached (IC_{50}) is not straightforward.
- Functional. In this part the techno-functional properties of different proteins are characterized. Results for each property were compared between benchmark and high throughput techniques. Evaluation was based on suitability for various protein sources, reproducibility and time involved.
 - Denaturation temperature: well established Differential Scanning Calorimetry (DSC) was used. The DSC equipped with a robotic arm for loading samples on the instrument was used to generate results in a high throughput way.
 - Viscosity and flow behavior: benchmark were compared to methods performed with a pipette robot (96 tips). We applied rotational rheology as benchmark technique obtaining results on the viscosity at different shear rates. The same samples were tested using a pipette robot (96 tips), measuring the pressure during pipetting as a function of the pipetting time. The maximum pressure drop was used as a quantitative parameter for viscosity. The results obtained from various protein sources measured at different conditions (variations in pH and salt) demonstrate a good correlation between viscosity as obtained from the benchmark technique with the maximum pressure drop as obtained from the high throughput method.
 - Particle size distribution: the benchmark technique Dynamic Light Scattering (DLS) was used (commercial available instrument (Zetasizer - Malvern)). From this we obtained results on the particle characteristics (Z-average and particle size distribution (PSD)). For the benchmark technique sample conditions (e.g. pH, salt

content etc.) have to be adjusted prior to the measurement. We developed a new set-up which makes use of the same first principles (DLS) for in-line measurements (e.g. adjusting pH or salt concentration) including a new commercially available instrumentation (NanoflowSizer – Inprocess LSP). From these method the particle characteristics (Z-average and PSD) were also acquired. Results obtained between the two set ups were in good agreement.

- Color: benchmark color measurements were performed on suspensions using a Hunter color lab instrument obtaining data $L^*a^*b^*$ values. High throughput measurements on dispersions performed with a pipette robot (96 tips) equipped with a photo camera. Same parameters ($L^*a^*b^*$) were determined through image analysis using a custom made software.
- Emulsifying capabilities: as an indicator we used the size characteristics of the emulsion droplets and the macroscopic stability.
 - For the size characteristics of the emulsion droplets we applied as benchmark technique diffraction scattering using a well-established commercial instrument (Mastersizer - Malvern). From this we obtained results on the particle characteristics (D[4,3] and PSD). For the benchmark technique, sample conditions (e.g. pH salt content etc.) have to be adjusted prior to the measurement. We used the developed set-up, described earlier for particle size distribution, to probe the size characteristics of the emulsion droplets in a high-throughput way. Results obtained between the two methods were in good agreement.
 - For the macroscopic stability we used transmission and backscattering data for the benchmark. This data was collected simultaneously using instrumentation namely 'Turbiscan'. For the high throughput method, photos collected on samples (with and without using a pipette robot (96 tips)) were analyzed using a developed Matlab script. Despite of the image analysis performed on a limited data set, results showed that the principle for a high throughput method is in place.
- Solubility: the same protocol was followed for the benchmark and the pipette robot (96 tips). The protocol included centrifugation of protein sample (30 min at 3200 g) and analysis of the supernatant using Bradford method (BSA used as reference). Results from both methods were in good agreement.
- Gelation: storage and loss modulus are important parameters to evaluate gelling properties of proteins upon heating. For accessing them we applied oscillatory rheology. No high throughput techniques were developed or used to determine the same parameters in a high throughput way. Measurements were performed using three instruments simultaneously for the generation of data in a fast way. A fast qualitative screening test was developed for evaluating the gelling properties. The qualitative results from the fast screening method corresponded well to the quantitative results of the oscillatory rheology.
- Sensorial. The aim of correlating sensory attributes and the presence of volatile and non-volatile compounds, was to evaluate which compounds would be most suitable to use as these marker compounds for 'quality'. A sensory test was done. A GC-MS method using SPME was developed for the measurement of volatile compounds (related to aroma), and

<p>a LC-MS method was developed for the measurement of non-volatile compounds (related to taste and mouthfeel).</p> <p>The limitations for GC-MS are the run time of a chromatographic measurement, and the data analysis if many compounds need to be analyzed. The limitation for LC-MS lays in the sample preparation, the run length of a chromatographic measurement, and in data analysis. Still, compared to traditional methods, these protocols are very fast. For both techniques the run lengths might be shortened even further when a more limited set of marker compounds would be chosen. Unfortunately, the set of samples for sensory analysis was not sufficiently representative to determine marker compounds (e.g. flavour quality is largely determined by oxidation, increasing attributes like oxidation, beany and green in aroma and bitterness/astringency in taste) and thus reach the aim.</p> <p>Therefore, it is recommended to use a large set of ingredients for sensory evaluation (e.g. >10 samples per crop, with as different sensory properties as possible), and check which compounds correlate with the most relevant sensory parameters using the whole data set. The expectation would be that general marker compounds for proteins from diverse crops can be found for lipid oxidation products, namely specific aldehydes on the volatile side, and trihydroxy fatty acids on the non-volatile side. Other important marker compounds are expected to vary between crop types. Therefore, the data set also needs to be analyzed per crop type. A selection of ~5 markers per crop (aroma / taste) might be appropriate.</p>
4.2 Deliverables
- Final version of the database, import Excel files and export PowerPivots
4.3 Communication
4.3.1 Scientific articles and their DOI
x
4.3.2 Reports/articles in journals
<ul style="list-style-type: none"> - Pyett S. 2019. Towards sustainable & equitable global protein systems. World Food Ingredients, October/November, pp 128-131. - Nieuwland M. 2021. Snel meten en vergelijken – Meer eiwit-keuzevrijheid in productontwikkeling. Voedingsmiddelenindustrie, April, 32-33. - Vogels J. 2021. Protein Compass – Database technical documentation. Report WFBR. - Vogels J. et al. 2021 Protein Compass – Database structure and explanation of data fields. Report WFBR. - Nieuwland M et al. 2021. Protein Compass – Standardized methods for systematic generation of information. Report WFBR.
4.3.3 Other communications
<ul style="list-style-type: none"> - Protein Compass. SFI Newsletter March 2021. https://www.sfifood.nl/blog/protein-compass - Presentations at regular SFI meetings
4.4 Other results
x
4.5 Project website
x

Final report

5. TRL upon project completion

5.1 Main category	Experimental development
5.2 Detail category at the start of the project	TRL3
5.3 Detail category at the end of the project	TRL4

6 Project status upon completion

Project status	The project has been completed in accordance with the original scope and all milestones were achieved.
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7 Output across the whole project

		Number
7.1	Number of scientific publications achieved	0
7.1 List		
7.2	Number of anticipated scientific publications	0
7.2 List		
7.3	Number of non-scientific publications achieved	5
7.3 List	<ul style="list-style-type: none"> - Pyett S. 2019. Towards sustainable & equitable global protein systems. World Food Ingredients, October/November, pp 128-131. - Nieuwland M. 2021. Snel meten en vergelijken – Meer eiwitkeuzevrijheid in productontwikkeling. Voedingsmiddelenindustrie, April, 32-33. - Vogels J. 2021. Protein Compass – Database technical documentation. Report WFBR. - Vogels J. et al. 2021 Protein Compass – Database structure and explanation of data fields. Report WFBR. - Nieuwland M et al. 2021. Protein Compass – Standardized methods for systematic generation of information. Report WFBR. 	
7.4	Number of requested patents	0
7.4 List		
7.5	Number of licences granted	0
7.5 List		
7.6	Number of prototypes	0
7.6 List		
7.7	Number of demonstrators	0
7.7 List		
7.8	Number of spin-offs/spin-outs	0
7.8 List		
7.9	Number of new or improved products/processes/services introduced	3

7.9 List	<ul style="list-style-type: none"> - Protein Compass database - Import Excel files - Export PowerPivots 	
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8 Impact

Describe the impact of the project

The project partners have quick and easy access to a database containing information for pre-selecting protein source in the development of healthy and sustainable plant-based protein food products, regarding nutritional, sensorial and functional aspects. Methods are available to fill the database with data obtained by high throughput methods. This will accelerate the food industry's innovation to efficiently replace current protein sources with more sustainable sources that have similar functionality and maintained taste preferences for consumers. Furthermore, the database is a good starting point to identify opportunities for development of new processing technologies to further enhance the properties of plant-based protein fractions and to make new sources suitable for specific food applications by minimal/dedicated processing methods. This will be addressed in the new TKI-proposal *LWV21.44 Design rules for novel protein sources in dairy alternatives* submitted in September 2021. Demonstrating that the database supports the industry in more effective and faster development of sustainable food products.