Project acronym: PROTEIN2FOOD

Project No.: 635727

H2020-SFS-2014-2015/H2020-SFS-2014-2

Start date of project: March 2015

Duration: 5 years

Deliverable reference number and title

D2.5: Ingredient selection

Data basis for ingredient selection for food development and for sustainability assessment

Date: 28.02.2019

Organisation name of lead for this deliverable:

UCPH-FOOD

Project co-funded by the European Commission within the Horizon 2020 Programme		
Dissemination Level		
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0. Summary

In Work Package 2 (WP2), a number of dry and wet (aqueous) fractionation processes have been developed within the scope of PROTEIN2FOOD (P2F) and as a result, new high quality plant protein ingredients, not available yet on the market, have been provided as competitive alternatives to animal derived protein ingredients and soy based protein ingredients. In order to characterise these new protein ingredients, a wide range of analyses comprising compositional, functional, nutritional and sensory properties have been conducted. This report summarises the results presented in the three former reports, D2.2, D2.3 and D2.4, in order to combine the highlighted results relevant as a data basis for ingredient selection. This was mainly done by compiling most important results in a table, which aims to give a clear basis for comparison of the 10 new protein ingredients developed in WP2. Furthermore, their application potential is discussed on the basis of their compositional, functional, nutritional and sensory properties. This report serves as a valuable overview of the newly developed protein ingredients and their properties with respect to food application, yields and main processing challenges. These can be used not only within P2F, but rather by involved SME's and stakeholders, in order to highlight the potential possibilities for new market opportunities. In this respect, this report supports the goal of P2F to increase the market share of European grown protein crops by 10%, by showing that processing technologies can be developed to create excellent new protein ingredients for food applications from raw materials that support an increase in Europe's agro-biodiversity.

1. Introduction and objectives

Throughout the P2F project, there has been a close collaboration between the partners within WP2, as well as between partners from WP2 and other Work Packages, mainly WP1, WP3 and WP5, with respect to data exchanges. The close collaboration with WP1 partners has secured an optimal selection of the species and varieties used for the protein ingredient processing (i.e. low content of antinutrients, and high content of protein). In order for WP5 to perform Life Cycle Assessment analyses (LCA), it has been crucial to receive input from WP2 partners regarding processing parameters, e.g. yield, energy consumption, by-product utilisation and waste. And particularly with WP3, there has been a need for a continuous close collaboration on the characterisation of the new protein ingredients with respect to chemical, nutritional and functional characterisation, in order to secure the optimal basis for food development in WP3. The detailed results from these characterisations have been reported in D2.4 "Report on the analytical characterisation of new protein ingredients".

This present report on "Data basis for ingredient selection for food development and for sustainability assessment" aims at giving a more accessible overview of the results that were presented in D2.4. Therefore, this deliverable highlights the chemical, nutritional and functional characteristics of each of the protein ingredients, including notes about the processing yield and challenges, as reported in D2.2 "Report on dry milling methods" and D2.3 "Report on bioprocessing methods". The objectives of this deliverable is to present a table that gives a clear basis for a comparison of the 10 new protein products developed in WP2 and to provide a targeted selection of ingredients for food prototype development.

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2. Activities for solving the task(s)

As mentioned in the introduction, there has been a close collaboration between WP2 and WPs 1, 3 and 5, in order to secure an optimal exchange of data relevant for the different partners. Partners from WP1 have been closely involved in the supply of knowledge of the most optimal species and cultivars, with respect to quality and sustainability, for the ingredient processing partners in WP2. Focus has been on high protein content and low contents of antinutrients, which is of high importance for WP2, but also on best yield performance in the field, which is important with respect to the overall sustainability of the project. Furthermore, selected samples from WP1 have been analysed by WP2 partners with regard to the protein and antinutrients levels. With WP5 there has been an exchange of processing data in order for WP5 to gain information for the Life Cycle Assessment (LCA) calculations. This has included both data on product and protein yield, water and energy consumption, waste and side stream utilisation and valuation. Conference calls between partners, discussions at annual meetings, as well as a dedicated face-to-face two-day meeting between the involved WP2 and WP5 partners to discuss the different parameters in detail have been conducted.

There has been a continuous and close collaboration between WP2 and WP3 partners in order to facilitate flow of relevant information between partners, in order to secure optimal basis for development of protein rich products in WP2, as well as for food development in WP3. This has been done both on common meetings between WP2 and WP3 partners during our annual meetings, but also on a common face-to-face two-day meeting between WP2 and WP3 held midway between the two annual meetings. To facilitate a good infrastructure for sharing results, a complete table with all protein products (including side-streams, where the protein content is lower) and the associated analytical results have been generated and circulated between partners. Since analytical characterisation is still ongoing, this table is used as a working document for all WP2 and WP3 partners, thereby also giving a good data basis for the selection of protein ingredients for the food prototype development in WP3. The collaboration between partners of WP2 and WP3 has also resulted in common published scientific papers/manuscripts under preparation, as has also the collaboration with partners of WPs 1 and 5.

3. Results

3.1 Properties of the newly developed protein ingredients

The results serving as a data basis for the ingredient selection are presented in Table 1. The results are presented as a summary of the main findings from D2.2, D2.3 and D2.4, in order to give a more straightforward basis for a comparison and overview of the characteristics of the new protein ingredients developed in WP2. The detailed results can be found in the respective reports from D2.2, D2.3 and D2.4. The protein ingredients have been produced either by dry processing or wet (aqueous) processing; indicated in Table 1with either a D or W. The raw materials that have been in focus are the seeds of the pseudocereals amaranth, buckwheat and quinoa (high quality protein crops) and the legumes of faba bean, lentil and lupin (high protein containing crops) (Table 1). **Chemical composition** indicates the content of the macronutrients protein, starch and fat in the produced ingredients.

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<u>The functional properties</u> in Table 1 gives an indication of how the different ingredients will behave in a food formulation, and for which product type they are suitable. **Protein solubility** (PrSol) results are helpful when choosing ingredients for either liquid products, where protein needs to be in solution, or solid products where solubility is of less importance. The **emulsifying capacity** (EC) gives an indication of how good the ingredient is to bind fat in an emulsion. In order to know how the product behaves when dissolved in water or another appropriate food solvent, **viscosity** determinations can be used. The viscosity of an ingredient often determines its application potential, e.g. in a nutritional beverage where protein fortification is required, a low viscosity ingredient will be most suitable. Therefore, the high viscosity determined for the lentil protein ingredients may limit their use in beverage applications. The **water binding capacity** (WBC) of the ingredients has been determined both in cold and hot water. In most food products, proteins are responsible for the formation and stabilisation of foams, i.e. egg white is a commercially important foaming agent in foods. Therefore, measurement of **foaming ability** is relevant for utilisation of protein ingredients in aerated foods, such as meringues, mousses and baked goods. Main findings regarding the functional properties:

- Foaming capacities were good for protein isolates of white lupin, lentil and faba bean and faba bean concentrates.
- Protein solubility was considered good for lupin and lentil protein isolates and faba bean concentrate.
- Emulsifying capacity was good for lentil and faba bean protein isolates.
- Gelling properties were rather weak for all ingredients (results not included in Table 1).

<u>The nutritional properties</u> listed in Table 1 can be important to consider with regards to both positive and negative effects, especially when the focus is on design of foods for consumer groups with special dietary needs, i.e. infants or elderly people. **Phytic acid** is considered an antinutrient compound in food due to the negative effect on mineral and trace element bioavailability. On the other hand, however, beneficial activities of dietary phytates is also related to effects on calcification and kidney stone formation, on lowering blood glucose and lipids, as well as its antioxidative activity and its potential anticancerogenic activities. **Saponins** within protein products are considered a negative quality marker, due to its bitter taste. Saponins have not been detected in any of the new protein ingredients. **Tannins** have been considered as health-promoting components in plant-derived foods and beverages, primarily owing to anticarcinogenic- and antimutagenic potential, as well as antimicrobial properties. On the other hand, consumption of tannin-rich food and beverages is associated with the sensation described as astringency. **Phenolics**, as well as tannins, are natural sources of antioxidants in plant foods. Application of protein ingredients with high content of phenolics as additive to food can protect fat against oxidation and extend shelf life of the product. A side-effect from a high content of tannins and phenolic compounds is often seen as a dark/brown colouring of the products, negatively impacting its application potential as a food ingredient.

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Oligosaccharides from the raffinose family cannot be digested in the human small intestine, and instead pass through to the large intestine, where they promote the growth of Bifidobacteria, which are beneficial to gut health. From this point of view, its presence in protein preparations is very positive. However, application of preparations containing oligosaccharides can be limited to consumers with gastrointestinal diseases. Oligosaccharides are only relevant in the leguminous seeds, and have only been detected in the protein ingredients derived from faba beans. Faba beans are also known to contain the antinutritional compounds **vicine and convicine**. Intake of vicine and convicine are known to induce favism (an acute haemolytic disease) in certain susceptible human individuals who have an inherent deficiency in glucose-6-phosphate dehydrogenase. Vicine and convicine has been measured in all faba bean ingredients, and the results indicate that dry processing does not allow for the removal of vicine and convicine, whereas it is possible to significantly reduce the levels in the produced protein ingredients using wet processing (W). To our knowledge, there are no regulations regarding the content of vicine and convicine in food products.

Trypsin inhibitors constitute a negative quality marker as they limit the activity of the digestive enzymes trypsin and chymotrypsin, thus reducing the utilization of ingested proteins. Trypsin inhibitor levels have been measured in all protein ingredients, and the results show that trypsin inhibitor activity (TIA) is co-concentrated with proteins in the protein-rich fractions produced by dry fractionation (D), whereas TIA can be significantly reduced in selected protein isolates using wet fractionation (W). There are no official limits to the content of trypsin inhibitor levels in food. All protein ingredients with protein content above 50% have been evaluated for their **digestibility** by a fast screening method simulating the gastro-pancreatic protein digestion. With this *in vitro* protein digestibility (IVPD) method, the performance of the ingredients on a short term basis (1 hour pepsin and 1 hour pancreatin) has been compared to BSA (highly digestible milk where protein used as reference). A high TIA is negatively affecting the IVPD, as the digestive enzymes are being inhibited. This is seen e.g. for the faba bean protein concentrate, as compared to the faba bean protein isolate. Main findings can be summarised as follows:

- TIA was co-concentrated in dry fractionated protein rich flours, but TIA can be significantly reduced using wet fractionation.
- Dry fractionation and dehulling procedures does not allow for the removal of vicine and convicine in faba bean products, but removal of vicine and convicine in faba bean protein ingredients is possible when using wet (aqueous) bioprocessing.
- The highest tannin content was determined in the protein rich buckwheat flour.
- Oligosaccharides were only detected in faba beans, where they were found to be co-concentrated along with protein when dry fractionation is utilized, whereas wet processing can remove oligosaccharides.
- Protein isolates showed good short term digestibility compared to that of BSA, and the results indicate that processing significantly improves the digestibility of the protein products.

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- Phytates were detected in all examined samples. Both dry and wet fractionation is shown to increase the levels of phytates.
- Ingredients with high phenolic content also show high antioxidant potential.

The sensory properties have been determined with respect to taste, orthonasal and retronasal properties (Table 1). The term **taste** describes the five basic tastes: sweet, sour, salty, bitter and umami. **Orthonasal** is the flavour perception by sniffing through the nose, i.e. odour and smell, whereas **retronasal** is the flavour perception you get through the mouth, by chewing and tasting the sample (excluding the five basic tastes). The faba bean protein rich flour was perceived in particular as intensely sweaty/cheesy and the hay- and pea-like note dominated the ingredient prepared from lentil. The protein rich flours of amaranth, quinoa, and buckwheat were in part similar. Pea-like and earthy notes were the predominant attributes of all three ingredients. In addition, a mouldy note was detectable in the amaranth fraction. In particular the determined pea-like, earthy and mouldy notes of lentil, amaranth, quinoa, and buckwheat were assessed negatively by consumers, which is why further processing of these ingredients have to be investigated carefully with regard to the overall aroma. The sensory properties for the two buckwheat protein rich flours were evaluated similarly, but all parameters were evaluated more positive for the "buckwheat protein rich flour II" (Table 1). The findings regarding the sensory properties are summarized here:

- Faba bean protein rich flour was evaluated quite negatively with respect to orthonasal properties, whereas lupin protein isolate was evaluated less negatively.
- Protein rich flours from amaranth, quinoa, and buckwheat were in part similar, and pea-like and earthy notes were the predominant orthonasal attributes of all three ingredients.
- The determined pea-like, earthy and mouldy notes of lentil, amaranth, quinoa, and buckwheat are assessed negatively by consumers.
- Aqueous processing of lentil provided isolates with reduced flavour intensity, especially the bitterness and legume taste, compared to the lentil flour.

Processing challenges and yields for the laboratory or pilot scale processes are described in the last column of Table 1. Since each plant source has different properties, i.e. morphology, content of macronutrients as well as antinutrients, different challenges are faced when adapting the **processing** steps to the plant source. These challenges have been described in short. The **protein yield** defines how much protein (on a dry mass basis) from the starting material has been recovered in the protein ingredient. A higher protein yield is desirable from the point of utilising as much of the protein as possible. The **product yield** defines on a dry matter basis how much of the starting material is recovered in the final protein ingredient. Product yields will expectedly differ according to the composition of the starting material (what is the amount of protein and other macronutrients) as well as the composition of the final protein ingredient (i.e. protein content).

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Table 1: Chemical, functional, nutritional and sensory characterisation of new protein ingredients developed within the scope of WP2, including processing challenges and yields. Data based on reports D2.2, D2.3 and D2.4.

Plant source	**Chemical composition of new protein ingredients**	**Functional properties of new protein ingredients**	**Nutritional properties (positive and negative) of new protein ingredients**	**Sensory properties of new protein ingredients**	**Processing challenges and yields**
Amaranth protein rich flour (D) *Amaranthus caudatus* Commercial quality	Protein: 37.5 % Starch: 9.4 % Fat: 16.6 %	PrSol ~65 % EC ~350 ml/g Viscosity: very low WBC: low FA: poor	No tannins Phytic acid ~20 mg/g Phenolics ~1 mg/g TIA ~0.2 IU/g	Taste: bitter, (sweet) Orthonasal: Beetroot, steamed potatoes, pea-like and earthy notes, mouldy note Retronasal: beetroot, bitter aftertaste	Separation of protein from starch difficult due to small particle size of starch granules. Only impact milling (fragments) combined with sieve classification provided good results Protein yield: ~28% Product yield (DM): ~12%
Buckwheat protein rich flour I (D) *Fagopyrum esculentum* Commercial quality	Protein: 24.2 % Starch: 53.6 % Fat: 3.4 %	PrSol ~65 % EC ~350 ml/g Viscosity: low WBC: high (hot) FA: poor	Tannins ~15 mg/g Phytic acid ~15 mg/g Phenolics ~7 mg/g TIA ~3 IU/g	Taste: bitter Orthonasal: plant, buckwheat, flour, pea-like and earthy notes Retronasal: plant, buckwheat, bitter aftertaste	Dehulling of the seed necessary but not possible with standard equipment. Best fractionation by impact milling combined with sieve classification (see above, amaranth) Protein yield: ~55% Product yield (DM): ~41%
Buckwheat protein rich flour II (D) *Fagopyrum esculentum* Commercial quality	Protein: 20.3 % Starch: 62.5 % Fat: 2.9 %	PrSol ~70 % EC ~300 ml/g Viscosity: very low WBC: high (hot)	Tannins ~10 mg/g Phytic acid ~10 mg/g Phenolics ~5 mg/g TIA ~1.5 IU/g	Taste: bitter Orthonasal: plant, buckwheat, bitter aftertaste Retronasal: plant, buckwheat, flour	See above for buckwheat protein rich flour I Protein yield: ~31% Product vield (DM): ~28%
Quinoa protein rich flour (D) *Chenopodium quinoa* ‘Titicaca’	Protein: 35.7 % Starch: 20.5 % Fat: 14.7 %	PrSol ~75 % EC ~350 ml/g Viscosity: low WBC: low FA: poor	No tannins No saponins Phytic acid ~15 mg/g Phenolics ~5 mg/g TIA <0.1 IU/g	Taste: bitter, (sweet) Orthonasal: plant, pea-like and earthy notes Retronasal:plant, astringent, bitter aftertaste	Peeling of the seed is necessary to remove the saponins (bitter tasting components). Needs specific processing equipment. Best fractionation by impact milling combined with sieve classification (see above, amaranth) Protein yield: ~66% Product yield (DM): ~30%
Faba bean protein rich flour (D) *Vicia faba* ‘Imposa’	Protein: 67.3 % Starch: 5.0 % Fat: 4.6 %	PrSol ~75 % EC ~550 ml/g Viscosity: very low WBC: low FA: good	No tannins Vicin/convicin ~40 µmol/g Oligosaccharides ~20 mg/g Phytic acid ~15 mg/g Phenolics ~5 mg/g TIA ~2 IU/g IVPD ~75 %	Taste: bitter, sweet Orthonasal: legume, perceived as intensely sweaty/cheesy Retronasal: legume, bitter aftertaste	Fractionation not possible with standard milling equipment. Jet milling and air classification provides good fractionation of starch and protein particles. Protein yield: ~76% Product yield (DM): ~39%

Faba bean protein isolate (W) *Vicia faba* 'Imposa'	Protein: 88.7 % Starch: 1.8 % Fat: 5.2 %	PrSol ~30 % EC ~550 ml/g Viscosity: low WBC: intermediate FA: good	No tannins Vicin/convicin <0.5 µmol/g No oligosaccharides. Phytic acid ~25 mg/g Phenolics <1 mg/g TIA ~0.3 IU/g IVPD ~85 %	n.a.	Protein yield: ~71% (lab) Product yield (DM): 20% (lab)
Faba bean protein concentrate (W) *Vicia faba* 'Imposa'	Protein: 52.9 % Starch: 23.4 % Fat: 1.6 %	PrSol ~65 % EC ~400 ml/g Viscosity: intermediate WBC: low FA: high	No tannins Vicin/convicin <0.5 µmol/g No oligosaccharides. Phytic acid ~15 mg/g Phenolics ~2 mg/g TIA ~9 IU/g IVPD ~50 %	n.a.	This concentrate is developed from the side stream generated when producing the faba bean protein isolate (W), and therefore, protein yield is seemingly low. Overall though, the faba bean process is able to recover ~87% of the protein Protein yield: ~16% (lab) Product yield (DM): 23% (lab)
Lentil protein isolate IEP (W) *Lens culinaris* 'Itaca'	Protein: 85.9% Starch: 0.4 % Fat: 4.6 %	PrSol ~40 % EC ~600 ml/g Viscosity: high WBC: intermediate FA: good	No tannins Phytic acid ~20 mg/g Phenolics <1 mg/g TIA ~1 IU/g IVPD ~80 %	Taste: bitter, salt Orthonasal: legume, alkaline, hay- and pea-like note Retronasal: legume, alkaline, astringent, bitter aftertaste	High viscosity of the precipitate during pasteurization and spray drying made careful adaption of processing parameters necessary Protein yield:~ 59-61% Product yield (DM): ~ 26% Yields comparable in lab and pilot plant scale
Lentil protein isolate UF (W) *Lens culinaris* 'Itaca'	Protein: 93.4 % Starch: <0.2 % Fat: 4.3 %	PrSol ~45 % EC ~500 ml/g Viscosity: very high WBC: intermediate FA: good	No tannins Phytic acid ~15 mg/g Phenolics <1 mg/g TIA ~0.7 IU/g IVPD ~80 %	Taste: bitter Orthonasal: legume, alkaline Retronasal: legume, alkaline, astringent, bitter aftertaste	High viscosity of the retentate at higher dry matter → Adaption of pasteurization and spray drying Protein yield: ~61% Product yield (DM): ~24% Yields comparable in lab and pilot plant scale
White lupin protein isolate (W) *Lupinus albus* 'Butan'	Protein: 89.7 % Starch: n.a. Fat: 2.3 %	PrSol ~65 % EC ~250 ml/g Viscosity: very low WBC: low FA: good	No tannins Phytic acid ~15 mg/g Phenolics ~2 mg/g TIA ~0.1 IU/g IVPD ~86%	Taste: bitter, salt Orthonasal: legume, soap-like Retronasal: astringent, legume, soap-like, bitter aftertaste	In the technical process at Prolupin difficulties appeared in the protein separation which made an adaption of the process necessary. Protein yield: ~44-48% (lab) Product yield (DM): ~21-26% (lab)

D: dry processing, W: wet (aqueous) processing, n.a.: not analysed, PrSol: Protein solubility, EC: Emulsifying capacity, WBC: Water Binding Capacity, FA: foaming ability, TIA: Trypsin inhibitor activity, IVPD: *In vitro* protein digestibility, short term, compared to BSA (100%)

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3.2 Application potential of newly developed protein ingredients

As indicated above and in Table 1, the newly developed protein ingredients have different compositional, functional, nutritional and sensory properties, and therefore, the **application potential** for the different ingredients will also be governed by these properties. Depending on the sensory profile of the ingredient, consideration might need to be taken in order to mask or dilute certain “off-flavours” that otherwise will be negatively assessed by consumers. This could be relevant for most of the newly developed ingredients, except the lupin based ingredients. Ingredient applications in beverages are most sensible to taste and off-flavours, as there is limited possibilities for masking these.

There are to our knowledge no official limits to the contents of the antinutritional factors present in the protein ingredients. However, levels of these should still be taken into consideration, especially if the ingredients are to be incorporated in a high concentration or into foodstuff intended for consumer groups with special nutritional needs, e.g. infants or elderly. This is relevant e.g. when comparing the trypsin inhibitor activity (TIA) between the different faba bean protein ingredients. Also regarding the faba bean protein ingredients, attentiveness should be given to the levels of vicine and convicine in the faba bean protein rich flour, which can be a problem for certain susceptible human individuals, where they are known to induce favism (see above). Protein ingredients with high content of phenolics can be positively considered for food formulations high in fat, as the phenolics can help to protect the fat against oxidation and extend shelf life of the product. Medium to high content of phenolics is mainly seen in the protein rich flours produced by dry processing.

Protein ingredients with high protein solubility (PrSol) are suitable for liquid products, whereas products with low protein solubility have a better application potential in solid or semi-solid product types. Most of the newly developed protein ingredients have fairly high protein solubility, except the protein isolates produced from faba bean and lentil that have lower protein solubility. The protein solubility is an important criteria for the development of protein beverages. It can also have a great impact on extrusion properties and is furthermore related to the emulsifying ability of proteins. For liquid products, it is often desired to have low viscosity and ingredients, such as lentil protein isolates and faba bean protein concentrate. These however may have limited use in beverage applications, due to the intermediate to very high viscosity determined for these ingredients. High foaming ability is important in aerated foods, i.e. as a substitute for egg white protein. Here, all the ingredients with protein content above 50% show good performance, especially pronounced for the white lupin protein isolate and faba bean protein concentrate. High protein contents are usually correlated with good emulsifying properties. But not only the protein content is crucial for a good emulsifying capacity (EC), also the protein quality such as protein types, degree of denaturation and pH of the emulsifying system are influencing criteria. The determined EC-values of more than 500ml/g for most protein isolates/concentrates predict good suitability of the raw materials in food emulsions, such as spread-like meat alternatives that usually have high fat contents between 10-40%.

For some applications, a high protein content may be desired (as in the protein isolates and concentrates), but in other applications, it can also be an advantage to have a natural content of other macronutrients, such as starch or fibres, as they can add to the desired functional properties in a food formulation. Therefore, the protein rich flours can have equal good value as the protein isolates and concentrates, but for different food applications. As an example, protein ingredients with a higher content of starch, e.g. buckwheat protein rich flour, may be the better choice for more solid products like spreads, since the starch content may make it unnecessary to add binding agents, gums or thickeners, or starches from other plant sources.

A number of the newly developed protein ingredients have already been considered and tested in food applications with high protein content (at least 20% of the energy value of the food product is provided by protein) by different project partners within the scope of WP3. The majority of vegetable products that are on the market with high protein content are gluten or soy-based. Therefore, the availability of new raw materials, which present high levels of proteins and with good organoleptic characteristics, is of great importance to meet the needs of current consumers, as there is a pull towards products that are gluten-free, soy-free and with beneficial nutritional properties. COPOSA, one of the involved SMEs, has tested both functional and organoleptic characteristics of ingredients from lentil, buckwheat and lupin. They developed different formulations of veggie burgers made with oat flakes and other gluten free raw materials, always taking into account the requirement that the final product has to have a high content of protein. They concluded that the tested P2F ingredients present good handling characteristics and good organoleptic properties. The formulations used, in addition to theoretically fulfilling the requirement of high protein content, contain quality proteins, since the combination of proteins derived from legume and quinoa contains all the essential amino acids. They also found that these new formulations are correct in flavour; however, their texture has to be improved. This need for improvement is largely due to the high amount of protein isolate required to achieve a product with high protein content. Their conclusions can therefore be considered as recommendations for optimal use of the ingredients.

In addition, the newly developed ingredients[1] have been tested successfully in high protein applications, by the WP3 research partners, as listed below (see D3.2, D3.3 and D3.6):

- Spread-like meat products: Mixtures of amaranth, quinoa and buckwheat flours with legume protein isolates/concentrates (lentil, lupin, faba bean) – best results were achieved by spreads composed of a mixture of protein rich flour of quinoa and-faba bean or by a mixture of buckwheat flour and lupin protein isolate.
- Protein-rich extrudates (breakfast cereals): It was found that it was possible to produce protein-rich extrudates by extrusion-cooking from blue lupin, white lupin, and faba bean protein isolates, as well as with mixtures of legume protein isolates and pseudocereal flours - a combination of lupin protein isolate and buckwheat flour performed with best results.

[1] Blue lupin protein isolate, which is a commercial reference from Prolupin (SME partner), containing 89.8 % protein, and good functional and sensory properties (see D2.4), has been included in the application tests.

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- High moisture meat substitutes: Combinations of lentil, faba bean, white and blue lupin isolates/concentrates with buckwheat or amaranth flours were tested and fulfill human physiological requirements due to their well-balanced amino acid profile – combination of lentil isolate with amaranth flour was the best formulation.
- Plant-based milk substitute: Quinoa, legume flours (faba bean, chickpea, lentils and lupine) and lentil protein isolates were tested for their properties to produce plant-based milk substitutes. Products based on quinoa flour and lentil protein isolates performed best and were used to formulate prototypes.
- Wheat-based protein-rich bread: Wheat flour was partially replaced by a range of the new, protein-rich ingredients from legume and pseudo-cereal sources. The developed prototypes promise high potential due to their well-balanced amino acid profile, especially caused by the combination of legumes with cereals. The replacement of 15 % wheat flour by a mixture of blue lupin protein isolate with buckwheat flour and gluten was found to be the best formulation.
- Wheat-based protein-rich pasta: The developed lentil, blue lupin, faba bean, buckwheat, quinoa and amaranth ingredients, or combinations of them, have been used to partially replace wheat flour and impacts on pasta texture and cooking properties were assessed. The replacement of 23.1 % wheat flour by a mixture of blue lupin isolate, protein rich faba bean flour and buckwheat flour was found to be the best formulation.
- Infant formulae: Different ingredients (e.g., quinoa, amaranth and buckwheat protein rich flours and legume protein isolates) were studied in terms of nutritional composition, protein profile and functionality for its application in infant nutritional products. Overall, the lentil protein isolate obtained by isoelectric precipitation (IEP) performed the best, having exceptional functional properties (high solubility after homogenization, good emulsifying capacity and heat stability). Furthermore, the compatibility with the rest of the ingredients used in the infant formulation (e.g. maltodextrins and minerals) was optimal.

4. Conclusion and next steps

The information presented in the deliverable could be applied as a base for using the most optimal ingredients, thereby developing the most optimal plant-based protein products. The analytical data overview shows that the new protein rich flours and isolates developed in WP2 have excellent potential for a wide range of food applications. This is demonstrated by using them in the development of various food prototypes in WP3. Besides their partly high protein contents and highly functional properties, most of these new materials have good nutritional properties. Negative tastes like bitter or strong aromas of the single flours or isolates could be reduced by processing or influenced by mixing ingredients, which also contributes to a better amino acid composition of the developed foods.

Analytical data are also yielding information concerning side-streams with low or no protein contents. This data have been included in the assessment and important conclusions, including estimations for the commercial value of the side streams and their further processing and

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application potential, have been transferred to WP5 for Life Cycle Analyses (LCA) within the project. Overall, the presented information here can be applied as a base for using the best suitable ingredients, thereby securing the development of the most optimal plant based protein food product both within P2F and in a broader term.

Currently most of the analytical assessments of the protein ingredients are completed. The sensory evaluation of faba bean protein isolate and concentrate and the *in-vivo* nutritional analysis (rat studies) of the protein ingredients will be the next steps. The overview table (Table 1) presented in chapter 3 is considered as a living document and will be updated and distributed among partners as soon as new data is available.

5. Delays and difficulties

The newly developed protein ingredients have been produced and delivered for analytical characterisation at different time points, which means that some analyses have been slightly delayed. As D2.5 is a summary of previous reports, some delays in D2.4 have created some further delays in D2.5. Some of the missing data from D2.4 has already been included in D2.5; however it has not been possible to include all characterisation data for all protein ingredients. The missing analytical data will be included as soon as the results are available. Any of the delays in the deliverable has not affected the general progress of the overall project.

6. Impact and outreach

This report provides an overview of new protein ingredients developed within the scope of PROTEIN2FOOD and their properties with respect to food application, yields and main processing challenges. This knowledge base could be used for targeted food processing by the involved SMEs, but also be presented to the stakeholders of the project. The developed ingredients are not yet available on the market but represent highly potential alternatives to animal proteins or soy and pea proteins. As the developed raw materials are not yet available on larger scale, this could provide new market opportunities for producers of isolates, or the milling industry, as well as food producers. In the medium and long term, this is expected to help gain an increased market share of 10% for new plant protein based food products, which is one of the main targets of the project. The analytical results demonstrate that the new plant protein ingredients present an added value to the food ingredients and are suitable for the production of innovative protein-rich food products. This report can aid in increasing the development of plant based products, now and in the future, by providing the main results on the new protein ingredients in an accessible table.

The collaboration between partners of WP2 and WP3 has resulted in common published scientific papers and/or manuscripts under preparation, as has also the collaboration with partners of WP1 and WP5, thereby also communicating the results and findings to the academic audience.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 635727.