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D2.2 Report on dry milling methods

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Dissemination Level		
PU	Public	x
PP	Restricted to other programme participants (including the Commission Services)	
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1. Introduction and Objectives

Deliverable 2.2 relates to WP 2 “Protein extraction and fractionation”, which aims on the development of new protein ingredients with good functional and sensory properties suitable for the production of attractive and tasty food products with enhanced nutritional quality. For providing these ingredients two processing strategies are considered, dry milling and fractionation (Task 2.1) and aqueous protein extraction (Task 2.2).

For sustainability reasons and to focus on products assessed beneficial to human health, vegetable raw materials providing highly valuable protein were selected. Grain legumes such as lupin, faba bean and lentil as well as high-quality protein crops such as quinoa, amaranth, buckwheat feature high protein contents and high-quality amino acid composition. Besides, these crops belong to a traditional and increasing home-consumption and were therefore used to produce economically interesting protein flours.

The specific objective of Task 2.1 is a resource-efficient production of flours and protein-rich food ingredients by dry-fractionation and the provision of these products for the development of food prototypes in WP 3. Dry fractionation is known to present a sustainable method producing flours with significantly increased protein content. D2.2 summarizes the activities and results achieved by milling and dry fractionation of the selected legumes and high quality protein crops.

2. Activities for solving the task(s)

Since most seeds contain antinutrients such as saponins and tannins, which are primarily located in the seed hulls, the removal of seed hulls through special process parameters was investigated in order to enhance the potential utilization as food ingredients.

In order to design suitable methods for producing protein-rich flours, a variation of crushing methods in lab- and pilot-scale was tested. Rather coarse products were obtained by using impact mills without insertions. The refinement of the milling products could be adjusted through special milling insertions like tooth wheels, sieves or through jet milling. Additionally, different crushing intensities were obtained by the adjustment of wheel speed.

For further increasing the protein content of the flours, the milling products were fractionated using dry fractionation techniques such as sieve fractionation and air classification.

The fractionation efficiencies were evaluated by comparing the chemical composition (especially protein contents) of the flours before and after fractionation, by balancing the mass portions of the produced flour fractions and by verifying the separation of starch from protein particles via scanning electron microscopy or master sizer.

The applied milling and fractionation methods and corresponding results are detailed in section 4.

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3. Raw materials and analytical methods

3.1 Raw materials

For the milling and dry fractionation trials seeds of lupin, faba bean, lentil, quinoa, amaranth and buckwheat were used as specified and reported in Deliverable 2.1. Raw materials were obtained from project partners or purchased at local markets as follows:

Lupin			Faba bean		Lentil	Quinoa	Amaranth		Buckwheat	
Blue	White	Andean	Divine	Imposa	Itaka	Titicaca	Katia	*Amaranthus caudatus* L. from India	*Fagopyrum esculentum* Moench 'Kora'	*Fagopyrum esculentum* Moench
no flour planned	100 kg (from LBI, MFH)	25 kg (from UNALM)	100 kg (Naturland Bauern AG)	500 kg (ordered from LBI)	80 kg (from ISEA)	100 kg (from UC-PLEN)	500 g (from UC-PLEN)	125 kg (Teff-Shop)	100 kg (from PAS)	100 kg (Kümmel & Co. GmbH)

3.2 Optical analysis

3.2.1 Particle size analysis

Particle sizes were analyzed using approximately 10 g flour and determined in triplicate via laser diffraction using HELOS System Particle Technology (Sympatec GmbH). Data were evaluated using the computer programm Windox 5.

3.2.2 Scanning electron microscopy (SEM)

The microscopic examination was performed using a scanning electron microscope SEM ABT-55 (Akashi Beam Technology, Japan) with an acceleration voltage of 5 kV. The flours were examined at a 320-, 1100-, 1650- and 5500-fold magnification.

3.3 Determination of the chemical composition

3.3.1 Dry matter

For determination of the dry matter the samples were dried to weight constancy at 105 °C in a thermos gravimetrical system (TGA 601, Leco Corporation, St. Joseph, MI, USA) according to the AOAC method 925.10 (AOAC International, 2005).

3.3.2 Protein content

The protein content of the flours was calculated based on the nitrogen content determined according to the Dumas combustion method (AOAC International, 2005) using a Nitrogen Analyzer FP 528 (Leco Corporation, St. Joseph, MI, USA). For amaranth and quinoa the specific conversion factor of N x 5.85 was used (according to Valcárcel-et. al 2012), for faba bean and buckwheat N x 6.25 was used (De Santis et al. 2015 and Malgorzata et al. 2016) and N x 5,7 for lupin (e.g. reported by Berghout et al. 2014). The protein was reported on a dry matter basis.

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4. Results

4.1 Pre-processing of seeds (Dehulling – milling – flaking and optional defatting)

4.1.1 Dehulling and flaking (+ optional defatting)

Lupin (*Lupinus albus* 'Dieta'): Seeds were manually classified and contaminations (damaged seeds, impurities, pests) were separated (see Figure 1).

Figure 1: Classification of lupin seeds into ”appropriate”, ”darkly discoloured”, ”damaged”, ”contaminated by insects” and ”foreign seeds”.

Dehulling was approved in pilot-scale using an underrunner disc sheller with subsequent separation of the hull from the kernel with a zigzag classifier to reduce antinutrients such as low molecular weight polyphenols and tannins, associated with the husks and insoluble dietary fibers.

The removal of lupin-hulls was successful as evaluated optically and by measurement of the protein contents. The protein content before dehulling was 35% and increased to 40% after dehulling.

Next, the lupin kernels were flaked using a roller mill. Four batches of lupin flakes, 2.5 kg each, were furthermore defatted with supercritical CO_2 at 285 bar, 50 °C and a flow rate of 30 kg CO_2/h. Defatting the flakes led to a further increase in protein content to 45%.

The processing steps up to the defatted lupin flakes are shown in Figure 2 and Figure 3.

Figure 2: Preparation of flakes from *Lupinus albus* L. (var. Dieta) by dehuling, flaking and defatting of the seeds. From left to right: whole seeds, husks and kernels, full-fat flakes and defatted flakes.

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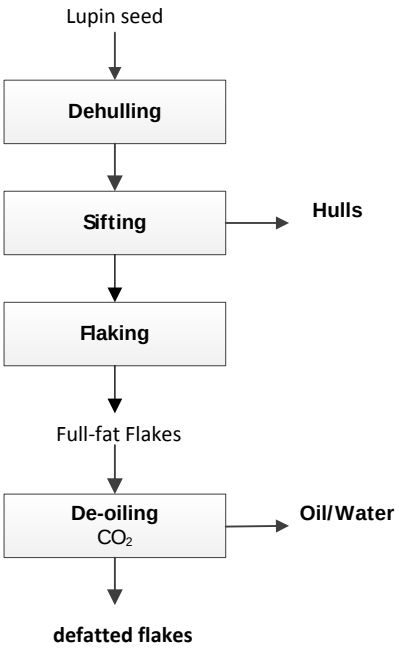

Figure 3: Process scheme of lupin seed dehulling (*Lupinus albus* L. Dieta), flaking and defatting with CO_2 in the Fraunhofer IVV pilot plant.

Faba bean (*Vicia faba* 'Divine'): **Dehulling** was approved using an underrunner disc sheller with subsequent separation of the hull from the kernel with a zigzag classifier. The observed fractions and the process scheme are displayed in Figure 4 and Figure 5.

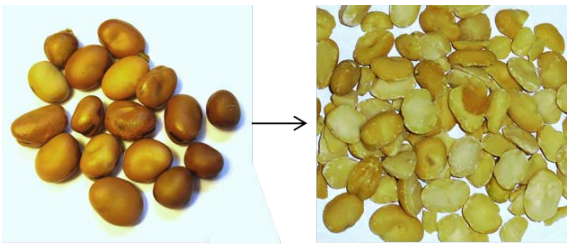

Figure 4: Whole (left) and dehulled (right) faba been seed (*Vicia faba* L. Divine).

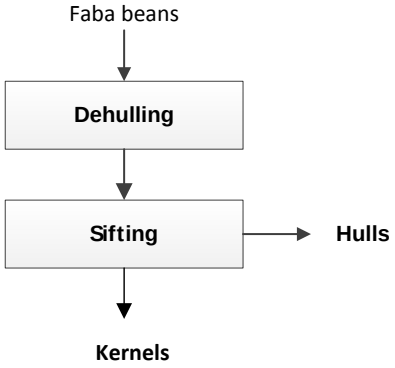

Figure 5: Process scheme of dehulling of faba been seed (*Vicia faba* L. Divine) in the Fraunhofer IVV pilot plant.

Dehulling of faba bean (*Vicia faba* L. Divine) was successful and provided kernels with slightly higher protein content (33%) compared to the original seeds (32%). The dehulled kernels were used as a basis for the preparation of flour for dry fractionation.

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Buckwheat (*Fagopyrum esculentum* M. Cora): Different pre-treatments for optimum dehulling were investigated varying the dry matter content of the seed through different drying times before hull removal. Best results (<10% residual hulls) were obtained after gentle heating for one hour at 40° prior to dehulling. The dry matter of the seeds was 98 % after this treatment. In addition, the protein contents of the fractions were determined to evaluate the protein loss during dehulling. Dehullig was performed using an underrunner disc sheller with subsequent separation of the hulls from the kernel with a zigzag classifier as presented in Figure 6 and Figure 7.

Figure 6: Buckwheat seed (*Fagopyrum esculentum* M. Cora) before and after dehulling. From left to right: Whole seed – dehulled kernels – hulls.

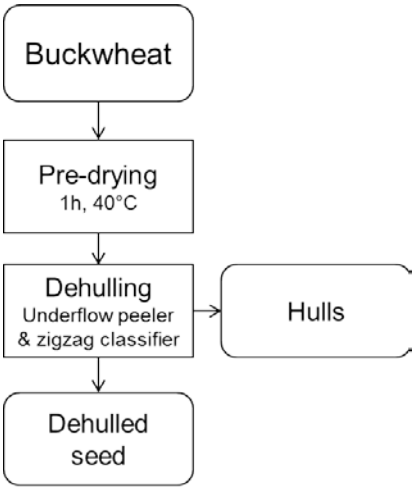

Figure 7: Dehulling of buckwheat (*Fagopyrum esculentum* M. Cora) at Fraunhofer IVV pilot plant.

Dehulling of buckwheat seemed promising using an underflow peeler. However, protein contents of the dehulled seeds were extremely low (12%) compared to industrially dehulled buckwheat (15%) and even compared to the whole seed flour (14%). The portion of hulls compared to the kernels was higher than described in literature (50% vs 20-30% in literature, Heyland et al., 2006). This designated losses of valuable seed kernel compounds. This was confirmed by the low protein content of the seed hulls (3%) compared to the whole seed (14%). If the 50% were pure hulls, the remaining 50% kernels should theoretically reach a protein content of 25% instead of 12% or would at least show a higher protein content than the whole seed.

To proceed in time, dehulled buckwheat kindly provided by project partner PAS was used for further experiments.

Quinoa (*Chenopodium quinoa* W. Titicaca): Combined pre-treatment and fractionation experiments (drying for 2 or 24 h, soaking & drying) were conducted for quinoa. The goal was to facilitate protein enrichment using an impact mill from Hosokawa Alpine AG (with or without screen insert). Subsequent fractionation via a sieve tower was performed to separate the seeds into the three fractions: 1.) protein-rich embryo, 2.) starch-rich endosperm and 3.) Saponin-containing hulls. Separation quality was determined microscopically by SEM and analytically on basis of the dry matter (DM) and protein contents.

The best results were obtained by drying of the seeds for 2 h at 40 °C in a drying oven and subsequent milling without screen insert. Dehulling degree was dependent on the wheel speed of the impact mill. However, the separation of hulls from quinoa using this process was not found to be sufficient regarding saponin contents and hull separation as the content of hulls was higher than 15%.

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Therefore it was decided to purchase industrially dehulled quinoa from QuinoaMarche srls in order to obtain sufficient amounts of effectively dehulled quinoa for the preparation of protein-rich fractions within the specified timeframe. This quinoa dehulled via an abrasive technique was used for pilot-scale preparation of protein-rich flour at IVV as described in section 4.2.3.

4.1.2 Milling

4.1.2.1 Preparation of flours and seed fragments

A laboratory and a pilot-plant impact mill, both from Hosokawa Alpine AG, were used to produce flours and seed fragments in lab-scale or pilot-plant.

Laboratory-scale impact mill. The universal impact mill, type 100 UPZ (Hosokawa Alpine AG) exhibits an eight-arm-plate useful for production of seed fragments. Optionally, screen inserts of different mesh sizes (e.g. 0.3 mm or 0.5 mm) can be inserted to mill the seeds to different flour levels. Figure 8 shows the laboratory impact with and without screen insert.

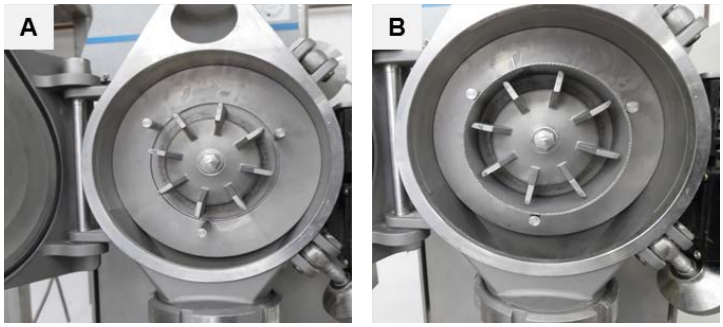

Figure 8: Universal impact mill, type 100 UPZ A) with and B) without screen insert.

Pilot-plant impact mill. A grinding gear based on the gear from the Ultraplex ® UPZ (Hosokawa Alpine AG) was constructed at Fraunhofer IVV to facilitate the production of seed fragments in pilot-plant. Both gears are presented in Figure 9.

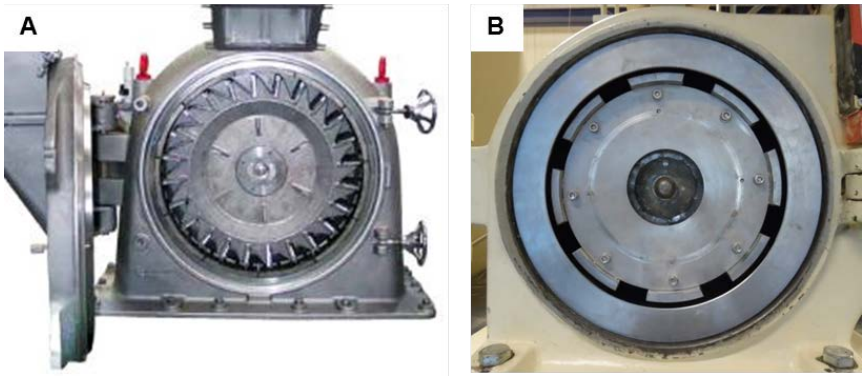

Figure 9: Grinding gear of A) Ultraplex ® (Saito, without J.) and B) Fraunhofer IVV grinding gear.

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To produce flours the grinding gear was substituted by a 0.5-mm-screen insert. Since the wheel speed adjustment (number of revolutions) at the pilot-plant impact mill was infinitely variable, wheel speed was labeled to define different wheel speed levels (Figure 10). “Level 1” designated the highest number of revolutions (maximum wheel speed) and “level 4” the lowest. Middle wheel speed was labeled by “level 3” and the one residing between highest and middle wheel speed by “level 2”.

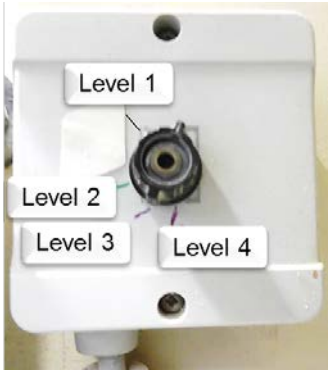

Figure 10: Speed governor at the pilot-plant impact mill with labeled wheel speed levels. The number of revolutions decreases from level 1 (highest wheel speed) to level 4 (lowest wheel speed).

First, experiments were approved in lab-scale and then scaled-up to pilot plant in order:

1) To investigate the best protein enrichment of **seed flours** by sieving, seeds were milled either in a lab-scale impact mill using 0.3-mm- or 0.5-mm-screen inserts or in a pilot-plant impact mill using a 0.5-mm-screen insert. The seeds milled in this way were amaranth, whole and dehulled buckwheat (dehulled at Fraunhofer IVV and commercially available dehulled), dehulled quinoa (industrially dehulled), whole and dehulled faba bean (dehulled at Fraunhofer IVV) and dehulled and defatted lupin flakes (preprocessed at Fraunhofer IVV).
2) To produce **seed fragments** in order to separate entire seed compartments (e.g. embryo, perisperm) from each other and to produce protein-rich flours, seeds were fragmented in the same mills, however by using a purpose-built collision insertion (pilot scale) or without any screen insert (impact mill: eight-arm-plate, 750 rpm). This process was applied to amaranth, dehulled buckwheat, dehulled quinoa and whole and dehulled faba bean.

An overview of the production of different seed flours or seed fragments in lab and in pilot scale is shown in Figure 11 and Figure 12.

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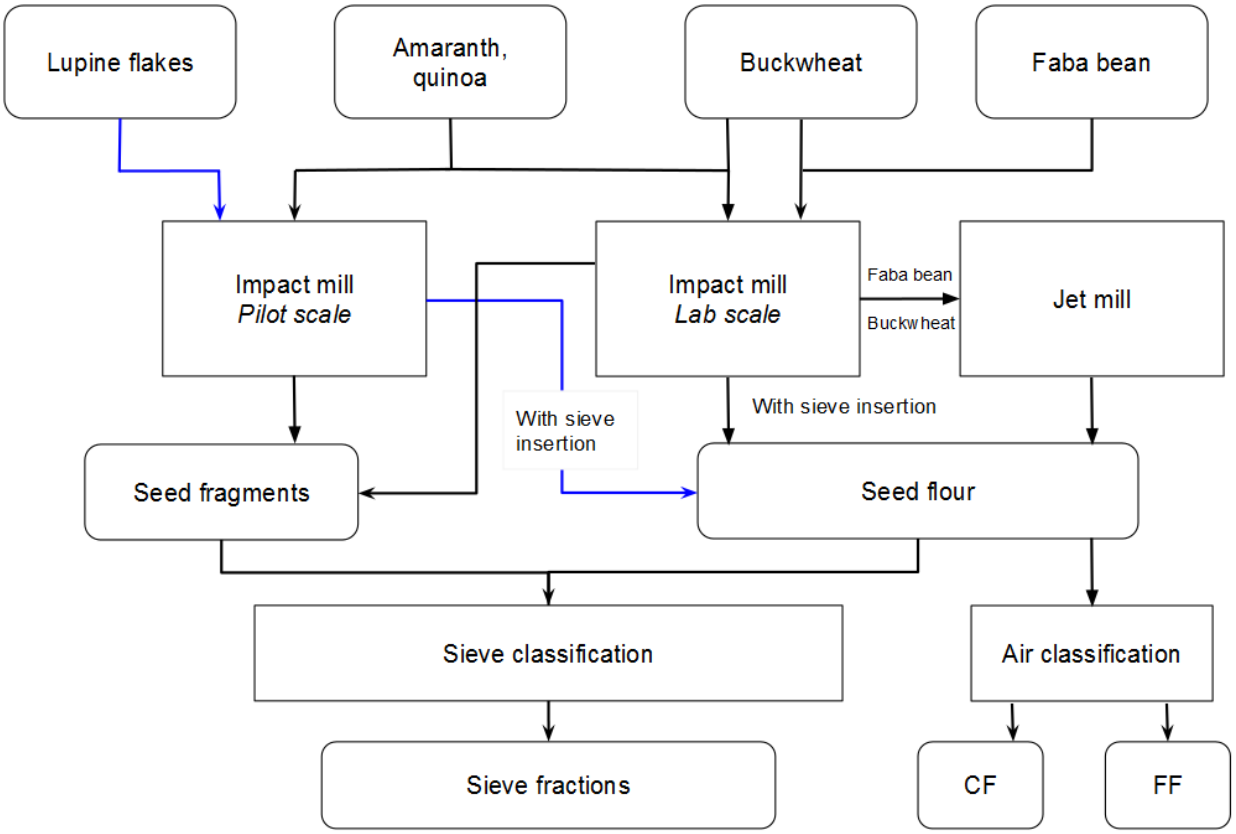

Figure 11: Production of flours or seed fragments to serve as protein-rich food ingredients or for further protein enrichment by sieving in lab- or pilot-scale. CF: coarse fraction, FF: fine fraction

Figure 12: Seeds before and after dehulling and milling. From left to right: whole seed – dehulled kernels – hulls – flour(s) (B and C: dehulled flour (left) and whole seed flour (right)). A) Lupin seed (*Lupinus albus* L. 'Dieta'), B) faba bean (*Vicia faba* L. 'Divine') and C) buckwheat (*Fagopyrum esculentum* M. 'Cora').

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4.1.2.2 Preparation of fine flours for air classification

Air classification requires extremely fine particle sizes to maximize separation of the proteins from the starch. Therefore, whole and dehulled flours from faba bean and buckwheat were first pre-milled by the lab-scale impact mill (0.5-mm-screen insert) and then finely milled using a jet mill

Figure 13 presents SEM-micrographs of the flours from dehulled faba bean as well as dehulled buckwheat produced by the different milling methods.

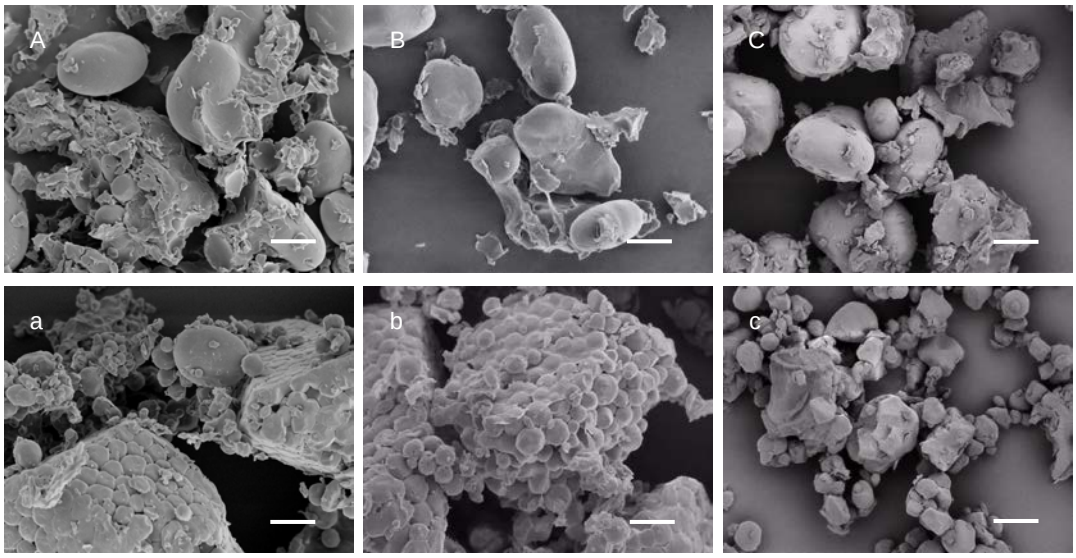

Figure 13: SEM micrographs of dehulled faba bean (A, B, C) and dehulled buckwheat (a, b, c). Seeds were milled using A) + a) pilot-plant impact mill (0.5 mm-screen insert), B) + b) lab-scale impact mill (0.3 mm-screen insert) or C) + c) jet mill. Scale bars present 10µm.

Both seeds showed notably different starch sizes. Whereas the starch size from faba bean was around 20 µm, the starch size of buckwheat ranged from 2 to 6 µm. After jet milling of faba bean, the starch particles were mostly dissociated from the protein which lay loosely on top of the starch granules. In contrast, after milling using screen inserts of 0.3- or 0.5 mm the protein particles still surrounded the starch particles of faba bean. For buckwheat, a separation of the individual starch particles was reached by jet milling, whereas after milling using a pilot-plant or a lab-scale impact mill bigger clusters of starch particles were found. The starch particles within these clusters seemed to be surrounded or connected by thin protein layers.

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4.2 Dry fractionation for protein enrichment

Dry fractionation of faba bean (*Vicia faba* L. Divine) and buckwheat (*Fagopyrum esculentum* M. Cora) was started using the whole and the dehulled flours to investigate whether dehulling prior to dry fractionation is required or whether hulls are automatically removed in a "waste fraction" (low in protein) during fractionation procedure. Three different fractionation strategies were investigated:

1) First, investigations of **air classification** were approved using both, whole seed fine flours and dehulled seed fine flours from faba bean and buckwheat which were previously prepared by jet milling (as described in 4.1.2).
2) Alternatively the **sieve fractionation** was tested using the same feedstock but **more coarse flours** (prepared with laboratory scale impact mill using the screen inserts 0.3 and 0.5 mm, respectively, see 4.1.2) and compared to the results of air classification.
3) As a third option, **seed fragments** obtained after impact milling (without screen insert) of the whole and the dehulled seeds were classified by **sieve fractionation**

The results are shown in chapters 4.2.1 to 4.2.3

4.2.1 Air classification

The goal of the air classification was to find the best wheel speed in order to obtain highest possible protein contents in the fine materials of faba bean and buckwheat. Results were presented by considering the protein contents of the fractions (coarse and fine fraction) relative to the sum of protein of both fractions. The factor of protein enrichment was calculated by dividing the protein content of the enriched fraction from the initial protein content of the flour. Only an extract of results is presented to concentrate on the most important findings.

Air classification was conducted using a deflector wheel air classifier ATP50 (Hosokawa Alpine AG) which was provided by the Technical University of Munich (chair: Lehrstuhl für Verfahrenstechnik disperser Systeme). Tests were carried out at wheel speeds from 0 – 24,000 min^{-1} for 15 to 25 min, a volume flow of 3 m^3/h and a clearance of 50 mbar. The air-mass flow varied depending on the process parameters and was individually noted. The coarse and the fine fractions from the corresponding collecting containers were weighed and analyzed for their dry matter and protein contents as described in section 3.3.

Air classification of faba bean

Best results for whole faba bean fine flour were obtained after air classification at wheel speed 7000 (1.1-fold protein enrichment), 9000 (1.2-fold protein enrichment) and 16000 min^{-1} (1.3-fold protein enrichment, Table 1). However, at 16000 min^{-1} only a mass portion of 4.1% and a protein portion of 5.5% was obtained. Best protein yields were achieved at 7000 (42.9%) and 9000 min^{-1}(34.9%).

At a wheel speed of 5000 min^{-1} protein portion of the fine fraction (FF) might be considered considerably high (86.4 %), however, this is a result of the abundant product mass. This means that hardly any protein enrichment was achieved which is shown by the low protein content of this fraction (20.9% vs. 23% in the feedstock).

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Table 1: Air classification of whole faba bean (”Divine”) fine flour.

Wheel speed [min^{-1}]	Fraction	Mass portion [%]	D_{50} [µm]	Dry matter [%]	Protein* [%]	Protein portion [%]
-	**Feedstock**	**-**	**n.d.**	**90.7**	**23**	**-**
5000	CF	12	104.3 ± 2.7	91.6	24.3	14
	FF	88	53.1 ± 1.5	92	20.9	86
7000	CF	68	35.2 ± 0.4	92.3	16.4	57
	FF	33	23.2 ± 1.2	92.3	25.7	43
9000	CF	73	32.6 ± 0.6	90.9	18.7	65
	FF	27	53.7 ± 1.2	91.9	26.8	35
10000	CF	85	34.4 ± 0.4	91.6	20.2	83
	FF	15	92.8 ± 2.3	92.2	23.7	17
12000	CF	81	29.0 ± 0.1	91.9	20.9	80
	FF	19	111.5 ± 1.6	92.6	21.5	20
14000	CF	78	29.3 ± 0.3	91.8	21.8	81
	FF	23	181.3 ± 1.6	92.8	17.7	19
15000	CF	82	28.5 ± 0.5	91.3	22.9	85
	FF	19	188.6 ± 2.8	92.1	17.5	15
16000	CF	96	32.3 ± 0.7	92.1	21.4	95
	FF	4	23.5 ± 6.7	91.8	29.1	6

CF: coarse fraction, FF: fine fraction, n.d.: not possible to be determined.

* Values are related to dry matter; determination of protein content using faba beans' specific N-factor of 6.25

However, an unintended finding was made, as particle sizes of the FF at wheel speed values from 9000 to 15000 min^{-1} were higher than particle sizes of the corresponding CF. An example of a particle size distribution (CF and FF at 10000 min^{-1}) is shown in Figure 14to facilitate possible explanation.

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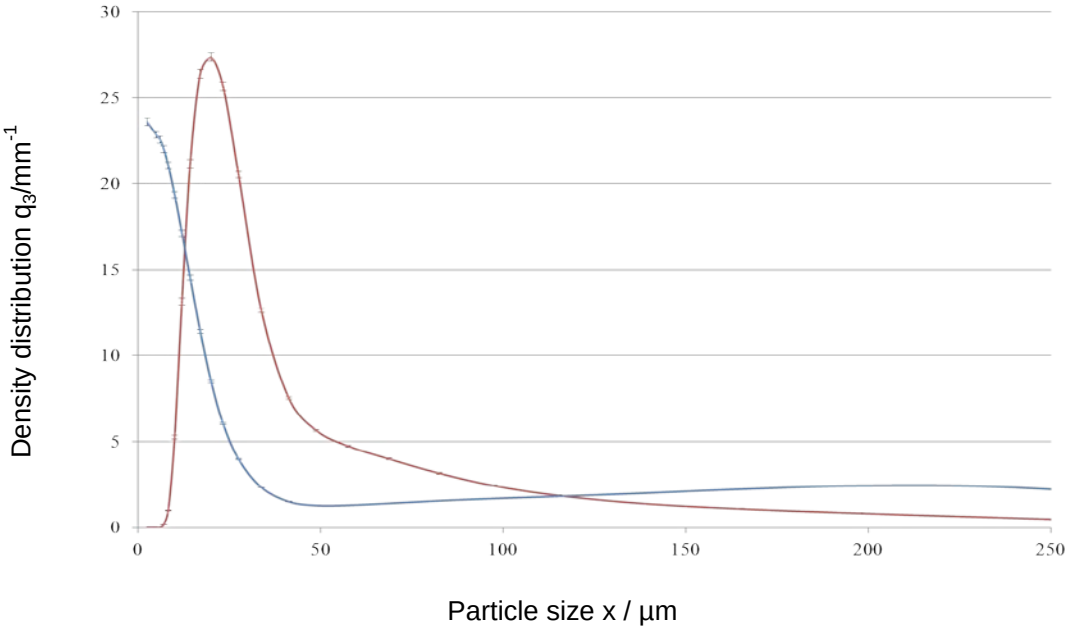

Figure 14: Density distribution of faba bean coarse fraction (CF, red) and fine fraction (FF, blue) at whel speed 10000 min^{-1}.

As expected, maximum particle sizes of the FF are lower, than maximum particle sizes of CF. Comparing the D_{10}-values of both fractions (4.8 ± 0.0 µm for FF and 15.8 ± 0.1 µm for CF), FF was proved to exhibit significantly more of the fine particles compared to CF. However, the particle size distribution of FF is modified by the presence of some significantly larger particles exhibiting diameters above 280 µm and occurring to lower amounts in the CF. Accordingly, the calculated D_{50} and D_{90} values of FF are higher than those of CF. The reason for this phenomenon was most probable the presence of hulls rather in the FF than in the CF, which might be explained by the low density of the hull fragments. Therefore, if hulls are unrequested in a protein-rich product (possibly due to antinutrients of the seed variety), dehulling prior to air fractionation was shown to be indispensable for faba bean.

Air classification of dehulled faba bean fine flour is presented in Table 2.

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Table 2: Air classification of dehulled faba bean (”Divine”) fine flour.

Wheel speed [min^{-1}]	Fraction	Mass portion [%]	D_{50} [µm]	Dry matter [%]	Protein* [%]	Protein portion [%]
-	**Feedstock**	**-**	**17.9 ± 0.0**	**90.8**	**23**	**-**
9000	CF	75	24.2 ± 0.1	90.2	18.1	63
	FF	25	19.1 ± 0.2	90.5	31.1	37
10000	CF	90	23.9 ± 0.1	90.6	20.1	83
	FF	10	12.5 ± 0.1	90.7	37.8	17
12000	CF	92	23.4 ± 0.1	90.2	20.9	88
	FF	8	13.0 ± 0.1	90.4	34.4	12
14000	CF	90	22.7 ± 0.1	90.8	22.7	88
	FF	11	28.6 ± 0.2	90.5	27.1	12
15000	CF	90	21.8 ± 0.3	90.6	23.2	86
	FF	10	10.3 ± 0.9	90.9	35.2	14
16000	CF	92	21.7 ± 0.2	91.0	25.0	89
	FF	8	n.d.	91.0	33.4	11
20000	CF	98	n.d.	91.0	25.6	98
	FF	2	n.d.	90.0	34.8	2

CF: coarse fraction, FF: fine fraction, n.d.: not possible to be determined.
* Values are related to dry matter; determination of protein content using faba beans' specific N-factor of 6.25

Maximal 1.6-fold protein enrichment was achieved in the FF at wheel speed 10000 min^{-1}. However, due to the low protein portion of 17%, wheel speed 9000 is to be preferred reaching 1.4-fold protein enrichment and a protein portion of 37%.

Air classification of buckwheat

Air classification of whole buckwheat fine flour is presented in Table 3. The best result was reached at wheel speed 15000 min^{-1}. However, only marginal protein enrichment (1.1-fold) was achieved. Generally, protein contents of the FF and corresponding CF are very similar reflecting insufficient protein shifting for whole buckwheat fine flour. Similar to the observations made for faba bean, the hulls are enriched in the FF at 12000, 14000, 16000 and 17000 min^{-1}, showing the necessity of dehulling prior to air classification due to tannins in the buckwheat hulls.

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Table 3: Air classification of whole buckwheat (”Cora”) fine flour.

Wheel speed [min^{-1}]	Fraction	Mass portion [%]	D_{50} [µm]	Dry matter [%]	Protein* [%]	Protein portion [%]
-	**Feedstock**	-	**n.d.**	**92.9**	**11.1**	-
7000	CF	40	72.7 ± 0.7	92.7	7.0	32
	FF	60	39.9 ± 3.8	92.5	9.7	68
10000	CF	65	51.0 ± 0.2	92.3	8.2	57
	FF	35	14.2 ± 0.7	92.3	11.2	43
12000	CF	72	43.6 ± 0.7	92.1	8.8	70
	FF	28	61.3 ± 3.2	92.1	9.6	30
14000	CF	79	33.7 ± 0.2	92.6	9.4	79
	FF	21	69.5 ± 1.3	92.4	9.4	21
15000	CF	86	39.3 ± 0.6	92.6	9.3	82
	FF	14	11.1 ± 0.3	92.2	12.8	18
16000	CF	77	36.4 ± 0.3	92.3	9.8	76
	FF	23	77.9 ± 1.7	92.2	10.1	24
17000	CF	85	39.9 ± 0.1	92.6	9.0	85
	FF	15	79.5 ± 4.6	92.2	9.2	15

CF: coarse fraction, FF: fine fraction, n.d.: not possible to be determined.
* Values are related to dry matter; determination of protein content using buckwheats' specific N-factor of 6.25

Air classification results of dehulled buckwheat are presented in Table 4. Protein contents of the FF increased with increasing wheel speed and the highest values were reached at 12000 to 16000 min^{-1}. Maximum 1.5-fold protein enrichment was achieved at the highest wheel speed with only a small protein portion of 16.7%. Protein enrichments in the FF as well as differences of protein contents in the FF compared to the CF were not as pronounced as for faba bean.

Table 4: Air classification of dehulled buckwheat (”Cora”) fine flour.

Wheel speed [min^{-1}]	Fraction	Mass portion [%]	D_{50} [µm]	Dry matter [%]	Protein* [%]	Protein portion [%]
-	**Feedstock**	-	**23.1 ± 0.4**	**91.7**	**8.6**	-
7000	CF	62	42.8 ± 0.1	91.4	6.7	56
	FF	38	13.6 ± 0.1	92.3	8.9	45
10000	CF	74	40.7 ± 0.4	91.8	7.0	71
	FF	26	9.9 ± 0.0	92.2	8.4	29
12000	CF	83	28.5 ± 0.3	92.4	7.0	77
	FF	17	6.6 ± 0.1	92.3	10.3	23
14000	CF	90	26.2 ± 0.3	92.4	7.0	86
	FF	11	8.6 ± 0.1	92.4	9.6	14
15000	CF	92	29.0 ± 0.3	92.4	6.9	87
	FF	8	n.d.	91.9	11.8	13
16000	CF	90	28.3 ± 0.1	92.3	7.0	83
	FF	10	6.0 ± 0.1	92.2	13.1	17

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

CF: coarse fraction, FF: fine fraction, n.d.: not possible to be determined.
* Values are related to dry matter; determination of protein content using buckwheats' specific N-factor of 6.25

As shown by scanning electron microscopy (Figure 13) using jet milling some parts of the aleurone layer were still connected to the small starch particles which might lead to an enhanced protein content of FF. Furthermore, the separation of the starch particles from the protein particles is difficult because of the extremely small particle size of buckwheat starch (2-6 µm). By finer milling of the seed to optimize separation of the starch from protein, the starch particle would show similar particle sizes than the protein particles, impeding effective separation through densitometric separation techniques such as air classification.

Air classification was expedient for faba bean. However, due to the small starch sizes of the high quality protein crops this method shows inappropriate for protein enrichment of buckwheat, amaranth and quinoa. But, as the protein-rich embryo is morphologically dislocated from the starch-rich endosperm, as demonstrated in Figure 15, a separation technique by separating bigger seed parts (e.g. embryo and endosperm) from each other seems promising.

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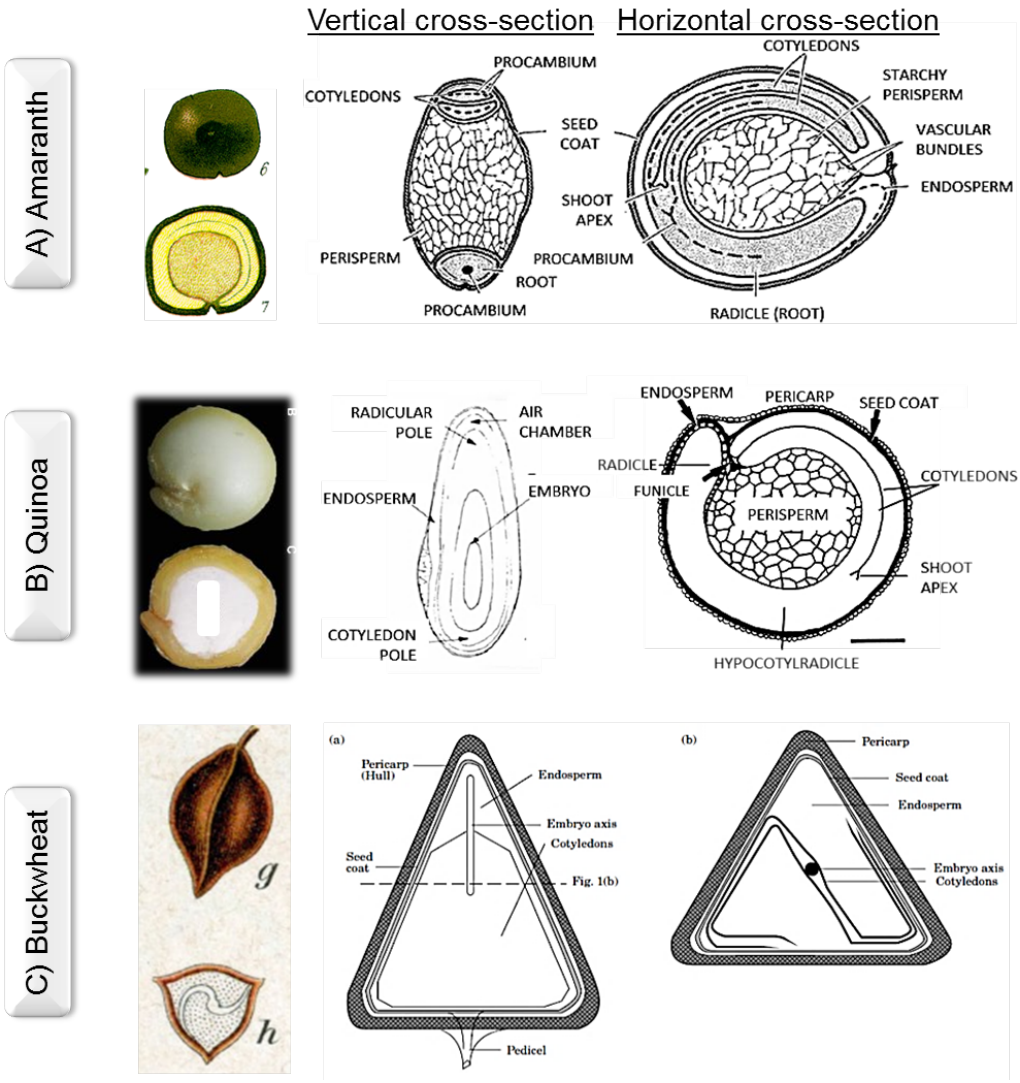

Figure 15: Schematic morphology of A) amaranth, B) quinoa (Irving et al., 1981) and C) buckwheat (Steadman et al., 2001).

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

4.2.2 Sieve classification

4.2.2.1 Lab-scale sieve classification of flours

Sieving of faba bean and buckwheat (whole and dehulled seeds) served to investigate an alternative method to air classification to enrich the protein content of seeds. Mass portions for the individual sieve fractions were calculated and dry matter and protein contents determined analytically. The individual protein contents of the sieve fractions were calculated on the basis of the total amount of protein of all sieve fractions. The protein enrichment was calculated by dividing the protein content of the protein-enriched sieve fraction from the initial protein content of the flour.

At first, sieving was conducted at laboratory scale (AS200, Retsch GmbH). Later, the results were transferred to the pilot-plant for scale-up. Both sieving techniques were evaluated using the same 0.5-mm-buckwheat flour (prepared by the lab-scale impact mill). Similar results were obtained in laboratory- and in pilot-scale. Therefore, both sifting methods were comparable.

To avoid clustering of fine flour particles due to adhesive forces Retsch™ Polyurethane Sieving Aids Cubes were used for experiments.

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

Faba bean

The sifting results of whole faba bean flour and dehulled faba bean flour produced using different screen inserts for grinding (0.5 mm and 0.3 mm) are presented in Table 5.

Table 5: Sieve classification of whole and dehulled faba bean (”Divine”) flours (produced using the lab-scale impact mill with 0.5- and 0.3-mm screen inserts during milling).

Faba bean flour		Sieve fraction [µm]	Mass portion [%]	Dry matter [%]	Protein* [%]	Protein portion [%]
Whole seed		**Feedstock**	**-**	**89.5**	**31.6**	**-**
	0.5mm	> 160	30.1 ± 2.1	89.9 ± 0.5	23.2 ± 0.4	24.9 ± 2.5
		160 – 90	23.4 ± 3.4	89.9 ± 0.4	33.1 ± 0.3	27.7 ± 4.3
		90 – 40	38.9 ± 5.9	89.7 ± 0.3	31.7 ± 0.1	43.8 ± 5.9
		< 40	3.2 ± 0.6	90.6 ± 0.6	30.2 ± 0.6	3.5 ± 0.6
		Losses	3.7 ± 0.8	-	-	-
	0.3mm	> 160	17.8 ± 3.0	91.5 ± 0.3	15.6 ± 1.7	10.2 ± 1.9
		160 – 90	38.9 ± 5.4	91.1 ± 0.1	31.4 ± 0.2	44.9 ± 8.5
		90 – 40	37.1 ± 6.9	90.9 ± 0.2	31.3 ± 0.3	43.2 ± 8.6
		< 40	1.9 ± 0.4	90.7 ± 0.2	28.5 ± 0.2	1.7 ± 0.5
		Losses	4.3 ± 1.1	-	-	-
Dehulled seed		**Feedstock**	**-**	**88.9**	**32.6**	**-**
	0.5mm	> 160	24.5 ± 0.4	87.5 ± 0.4	33.7 ± 0.1	26.7 ± 0.5
		160 – 90	10.7 ± 0.1	88.0 ± 0.3	35.1 ± 0.4	12.2 ± 0.2
		90 – 40	27.7 ± 4.2	88.1 ± 0.4	33.2 ± 0.8	29.8 ± 3.7
		< 40	34.9 ± 4.1	87.8 ± 0.2	27.6 ± 0.3	31.3 ± 3.5
		Losses	2.2 ± 0.2	-	-	-
	0.3mm	> 160	5.9 ± 0.7	88.9 ± 1.0	34.0 ± 0.3	6.5 ± 0.8
		160 – 90	8.8 ± 0.4	89.3 ± 1.0	35.8 ± 0.4	10.3 ± 0.6
		90 – 40	38.6 ± 4.5	88.5 ± 0.1	33.6 ± 0.9	42.3 ± 4.3
		< 40	44.3 ± 4.5	88.4 ± 0.2	28.4 ± 0.9	40.9 ± 4.2
		Losses	2.5 ± 0.6	-	-	-

* Values are related to dry matter; determination of protein content using faba beans' specific N-factor of 6.25

Only marginal protein enrichments were obtained after sifting of the whole seed flours. The low protein contents in the biggest sieve fractions (> 160 µm) might reflect important amounts of hulls in these fractions.

The results obtained after dehulling of the seeds were insufficient for an appreciable protein-enrichment (maximum 1.1-fold protein enrichment in sieve fraction 90-160 µm for both, 0.5 mm- and 0.3 mm-flours). Sieve fraction < 40 µm might be rich in starch according to its low protein content.

As postulated before, faba bean is not suitable for sifting of seed fragment as it showed insufficient protein enrichments. The reason is the regular distribution of protein and starch inside the entire seed. .

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

Buckwheat

Table 6: Sieve classification of whole and dehulled buckwheat (”Cora”) flours (produced using the lab-scale impact mill with 0.5- and 0.3-mm screen inserts during milling).

Buckwheat flour		Sieve fraction [µm]	Mass portion [%]	Dry matter [%]	Protein* [%]	Protein portion [%]
Whole seed		**Feedstock**	**-**	**91.6**	**14.3**	**-**
	0.5mm	> 160	25.0 ± 0.6	92.4 ± 0.3	14.9 ± 0.1	26.8 ± 1.1
		160 – 90	18.5 ± 1.3	91.4 ± 0.2	19.7 ± 0.1	25.3 ± 1.0
		90 – 40	47.2 ± 0.8	91.2 ± 0.4	11.2 ± 0.0	37.5 ± 0.6
		< 40	7.3 ± 0.7	91.4 ± 0.1	19.9 ± 0.1	10.3 ± 0.9
		Losses	2.0 ± 0.1	-	-	-
	0.3mm	> 160	18.6 ± 0.6	91.5 ± 0.3	10.7 ± 0.5	17.2 ± 0.3
		160 – 90	24.5 ± 4.7	90.6 ± 0.2	14.9 ± 0.7	31.4 ± 5.7
		90 – 40	50.5 ± 2.9	90.5 ± 0.3	10.2 ± 0.3	44.7 ± 2.9
		< 40	4.4 ± 1.7	90.8 ± 0.2	15.6 ± 0.1	6.7 ± 2.8
		Losses	2.0 ± 0.2	-	-	-
Dehulled seed		**Feedstock**	**-**	**90**	**11.5**	**-**
	0.5mm	> 160	9.6 ± 0.2	91.5 ± 0.3	24.1 ± 0.1	21.1 ± 0.5
		160 – 90	16.2 ± 0.4	90.4 ± 0.3	17.7 ± 0.2	25.9 ± 0.3
		90 – 40	57.3 ± 0.4	90.1 ± 0.3	6.2 ± 0.1	31.7 ± 0.3
		< 40	14.7 ± 0.4	90.5 ± 0.3	16.1 ± 0.1	21.3 ± 0.6
		Losses	2.2 ± 0.3	-	-	-
	0.3mm	> 160	13.8 ± 2.7	90.3 ± 0.3	19.1 ± 0.6	25.1 ± 3.2
		160 – 90	33.8 ± 5.1	90.0 ± 0.3	12.0 ± 0.2	37.1 ± 3.2
		90 – 40	47.0 ± 6.1	89.5 ± 0.4	8.7 ± 0.3	34.6 ± 1.7
		< 40	2.7 ± 0.8	90.5 ± 0.6	15.0 ± 0.1	3.1 ± 0.8
		Losses	2.8 ± 0.4	-	-	-

* Values are related to dry matter; determination of protein content using buckwheats' specific N-factor of 6.25

Indeed, protein enrichment was achieved using seed flours of buckwheat for sieve classification. Results were dependent on the dehulling, as with hull highest protein contents in sieve fractions 90-160 µm as well as < 40 µm (1.4-fold protein enrichment), whereas for dehulled buckwheat flour sieve fraction > 160 µm showed most appropriate (up to 2.1-fold protein enrichment).

The coarser 0.5 mm-flour was even better suitable than the 0.3 mm-flour. This might corroborate the assumption, that due to the specific buckwheat morphology, the protein-rich embryo and the aleurone layer were separated best from the starch-rich endosperm by using the 0.5 mm screen insert.

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4.2.2.2 Lab-scale sieve classification of seed fragments

Faba bean

Table 7 presents the results of the protein shifts caused by faba bean seed fragmentation followed by sifting in a sieve tower.

Table 7: Sieve classification of whole and dehulled faba bean (”Divine”) seed fragments (produced using the lab-scale impact mill at 1000 min^{-1} without screen insert).

Faba bean seed fragments	Sieve fraction [µm]	Mass portion [%]	Dry matter [%]	Protein* [%]	Protein portion [%]
Whole seed	**Feedstock**	-	**89.5**	**31.6**	-
	> 2 mm	91.0 ± 2.4	88.4 ± 0.1	27.3 ± 1.5	89.6 ± 3.6
	2 – 1 mm	6.5 ± 1.8	89.2 ± 0.4	31.9 ± 0.7	7.8 ± 2.7
	1 mm – 800 µm	0.7 ± 0.2	90.8 ± 0.7	33.1 ± 0.1	0.8 ± 0.3
	800 – 180 µm	1.3 ± 0.4	89.1 ± 1.1	37.0 ± 0.1	1.5 ± 0.5
	< 180 µm	0.3 ± 0.1	91.1 ± 1.1	33.5 ± 0.1	0.3 ± 0.1
	Losses	0.3 ± 0.1	-	-	-
Dehulled seed	**Feedstock**	-	**88.9**	**32.6**	-
	> 2 mm	79.2 ± 4.1	88.3 ± 1.8	31.2 ± 1.0	78.8 ± 4.1
	2 – 1 mm	13.3 ± 2.6	88.5 ± 1.7	32.0 ± 1.0	13.6 ± 2.6
	1 mm – 800 µm	2.1 ± 0.5	89.1 ± 0.4	32.0 ± 0.4	2.2 ± 0.5
	800 – 180 µm	4.1 ± 1.2	88.6 ± 1.5	33.4 ± 0.5	4.4 ± 1.3
	< 180 µm	1.0 ± 0.2	92.0 ± 1.1	31.4 ± 0.5	1.0 ± 0.2
	Losses	0.3 ± 0.1	-	-	-

* Values are related to dry matter; determination of protein content using faba beans' specific N-factor of 6.25

For the whole seed flour protein contents of the sieve fractions < 2 mm are not significantly different from each other and range within 32 and 37%. Maximum protein enrichment was achieved in sieve fraction 180-800 µm, however, exhibiting only a 1.2-fold protein enrichment compared to the initial protein content.

Due to major amounts of hull in the biggest sieve fraction (> 2 mm, Figure 16-A), protein content was lowest.

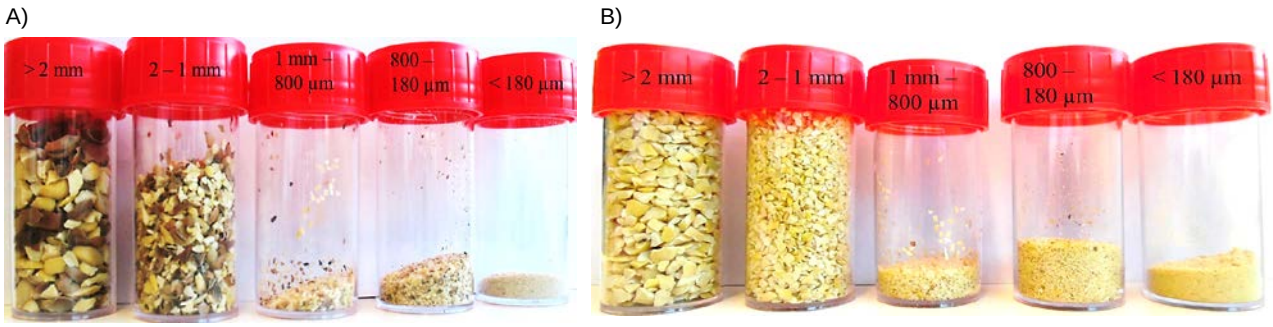

Figure 16: Sieve fractions of (A) whole faba bean seed fragments and of (B) dehulled faba bean seed fragments.

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Dehulling faba bean prior to fragmentation showed generally slightly smaller particles, than without dehulling (Figure 16-B). The reason might be, that during dehulling the seed is partially grinded. As a consequence of the enlarged particle surface, further fragmentation is facilitated.

Additionally, after dehulling prior to fragmentation the sifting of faba bean showed no significant protein enrichment in any sieve fraction. This might be due to the inadequate seed morphology of fragmentation, as the starch and the protein particles are distributed regularly throughout the entire cotyledon (Figure 17). Consequently, in all sieve fractions the same portion of starch and protein arise.

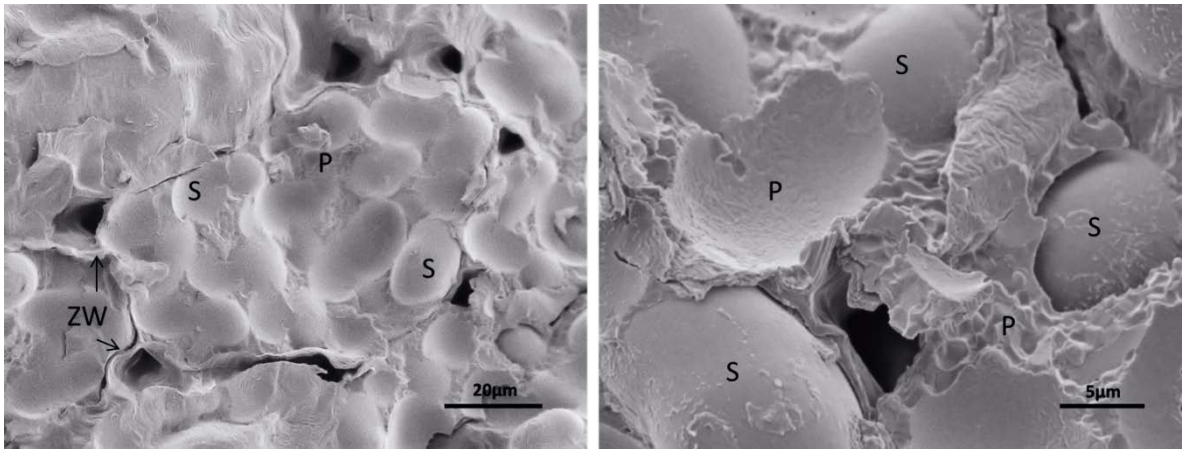

Figure 17: Cross-section of faba bean seed (*Vicia faba* L. Divine); P: protein, S: starch, ZW: cell wall [Horvat, 2016].

Buckwheat

Sifting results of whole buckwheat seed fragments as well as dehulled buckwheat seed fragments are shown in Table 8.

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

Table 8: Sieve classification of whole and dehulled buckwheat ("Cora") seed fragments (produced using the lab-scale impact mill at 1000 min^{-1} without screen insert).

Buckwheat seed fragments	Sieve fraction [µm]	Mass portion [%]	Dry matter [%]	Protein* [%]	Protein portion [%]
Whole seed	**Feedstock**	**-**	**91.6**	**14.3**	**-**
	> 2 mm	44.5 ± 3.7	91.1 ± 1.2	10.8 ± 0.3	39.9 ± 4.4
	2 – 1 mm	33.4 ± 2.1	90.8 ± 1.3	12.3 ± 0.6	34.0 ± 2.4
	1 mm – 800 µm	4.6 ± 0.1	90.5 ± 0.9	15.7 ± 0.1	6.0 ± 0.2
	800 – 180 µm	11.4 ± 1.1	90.3 ± 0.9	18.8 ± 0.7	17.7 ± 2.3
	< 180 µm	5.5 ± 0.5	90.0 ± 1.3	5.6 ± 0.1	2.5 ± 0.2
	Losses	0.6 ± 0.1	-	-	-
Dehulled seed	**Feedstock**	**-**	**87.9**	**9.9**	**-**
	> 2 mm	3.6 ± 0.5	90.0 ± 2.4	14.4 ± 0.7	4.8 ± 0.9
	2 – 1 mm	43.5 ± 1.9	88.9 ± 2.2	9.5 ± 1.0	37.8 ± 2.2
	1 mm – 800 µm	13.1 ± 0.7	89.2 ± 1.9	10.7 ± 1.5	12.9 ± 1.1
	800 – 180 µm	25.7 ± 2.6	89.1 ± 2.3	16.1 ± 0.8	37.9 ± 2.1
	< 180 µm	13.3 ± 1.7	88.4 ± 1.9	5.4 ± 0.1	6.6 ± 1.1
	Losses	0.8 ± 0.5	-	-	-

* Values are related to dry matter; determination of protein content using buckwheats' specific N-factor of 6.25

For the whole seed fragments two sieve fraction were identified best, the 180µm-800µm one (18.8 ± 0.7 %) and the 800µm-1mm one (15.7 ± 0.1 %). However, only 1.3-fold or 1.1-fold protein enrichments were achieved.

Due to its white color (Figure 18) and a low protein content of 5.6 ± 0.1 % fraction < 180µm is assumed to show considerable starch contents. To prove this assumption, an analytical determination of the starch contents would be appropriate.

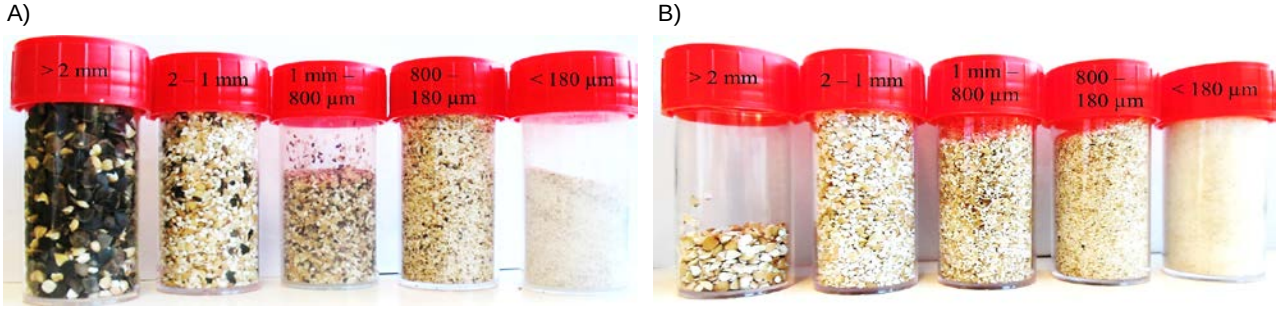

Figure 18: Sieve fractions of (A) whole buckwheat seed fragments and of (B) dehulled buckwheat seed fragments.

Most of the seed hulls arise in sieve fraction > 2 mm. However, some hulls were grinded and were distributed also throughout the other sieve fractions. To avoid sensory and nutritional deterioration due to tannins present in the seeds, it was decided to dehull them before sieve fractionation.

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With 1.6-fold protein enrichment sifting of dehulled and fragmented buckwheat showed again the highest protein content in sieve fraction 180µm-800µm. The protein content was raised from 10 to 16%. Furthermore, this fraction showed the highest protein portion (38%) among all investigated sieve fractions.

Similar to the results obtained using the whole buckwheat seed, fraction < 180 µm showed the lowest protein content.

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

Amaranth

Sieve classification results of fragmented amaranth seed in a **lab-scale** impact mill without screen insert are shown in Table 9.

Table 9: Sieve classification of amaranth seed fragments (produced using the lab-scale impact mill at 1000 min^{-1} without screen insert).

Sieve fraction	Mass portion [%]	Dry matter [%]	Protein* [%]	Protein portion [%]
Feedstock	**100**	**93.02**	**15.94 ± 0.1**	**100**
>500 µm	85.3	94.9	12.2 ± 0.2	65.3
500–250 µm	12.0	94.1	38.4 ± 1.8	28.6
250–125 µm	2.0	93.3	36.0 ± 0.9	4.5
<125 µm	0.7	93.8	35.0 ± 1.1	1.6

* Values are related to dry matter; determination of protein content using amaranths' specific N-factor of 5.85

Combined sieve fractions	Mass portion [%]	Dry matter [%]	Protein* [%]	Protein portion [%]
>500 µm	85.3	94.5	12.2	65.3
<500 µm	14.7	93.7	37.9	34.8

* Values are related to dry matter; determination of protein content using amaranths' specific N-factor of 5.85

The > 500 µm-fraction showed maximum mass- and maximum protein portion, while the protein content was lowest (12.17%). The 500-250 µm fraction featured the highest protein enrichment (2.4-fold) and exhibited the highest protein content, which decreased with decreasing mesh sizes. Summing up all sieves < 500 µm to one protein-rich amaranth fraction increased the mass portion to 15% and showed a protein content of 38%.

As verified by scanning electron microscopy, the sieve fraction < 500 µm consisted mainly of the protein-rich embryo (Figure 19).

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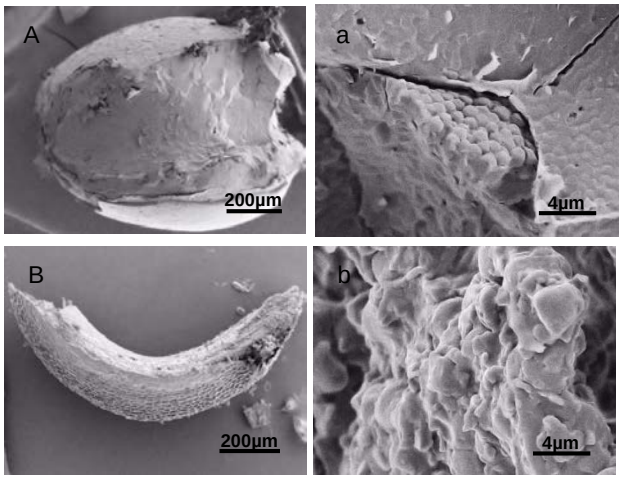

Figure 19: SEM micrographs of fractionated amaranth (*Amaranthus caudatus* L.) at different magnifications after sieve classification. A) and a): starch-rich endosperm found in sieve fraction >500µm, B) and b): protein-rich embryo found in sieve fraction 280µm.

Despite the high protein content, the protein separation needs further optimization because of the low total protein yield: The protein-rich fraction amounted only to 15% of total amaranth seed mass, whereas the low-protein fraction amounted to 85% of total amaranth seed. Screen insert (2 mm) during amaranth milling showed to be inappropriate for dry separation. Therefore, impact milling was scaled-up to pilot-plant scale at Fraunhofer IVV to produce sufficient amounts of protein-rich flours for WP 3.

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4.2.3 Process scale-up of sieve classification

Investigations to increase the protein contents using sieve classification in pilot-plant were approved for amaranth, quinoa as well as commercially dehulled buckwheat similarly to the sifting methods described before.

Presentation of the results were done by summing up different sieve fraction with similar protein contents to one sieve fraction to get as close as possible to the pilot-scale.

Sifting results obtained after lab-scale milling and after pilot-plant milling varied significantly which was ascribed to differing mill geometries. As identified by particle size analysis, flour particles were significantly smaller after pilot-plant milling than after lab-scale milling, even though the same screen inserts (0.5 mm) were used. Furthermore, a bimodal particle size distribution was found after pilot-plant milling compared to a unimodal distribution obtained after lab-scale milling indicating the release of individual flour compounds such as starch and protein after intense impact milling. Figure 20 presents the particle size comparison distribution of buckwheat flours obtained after both impact mills.

Therefore, to scale up the protein enrichment investigated in lab-scale, feed rate and number of revolutions were varied in the pilot-plant impact mill, to identify individually the best fractionation procedures. However, as the feed rate showed uninfluential, only results obtained by varying the number of revolutions (wheel speed) were further investigated. The following results were obtained:

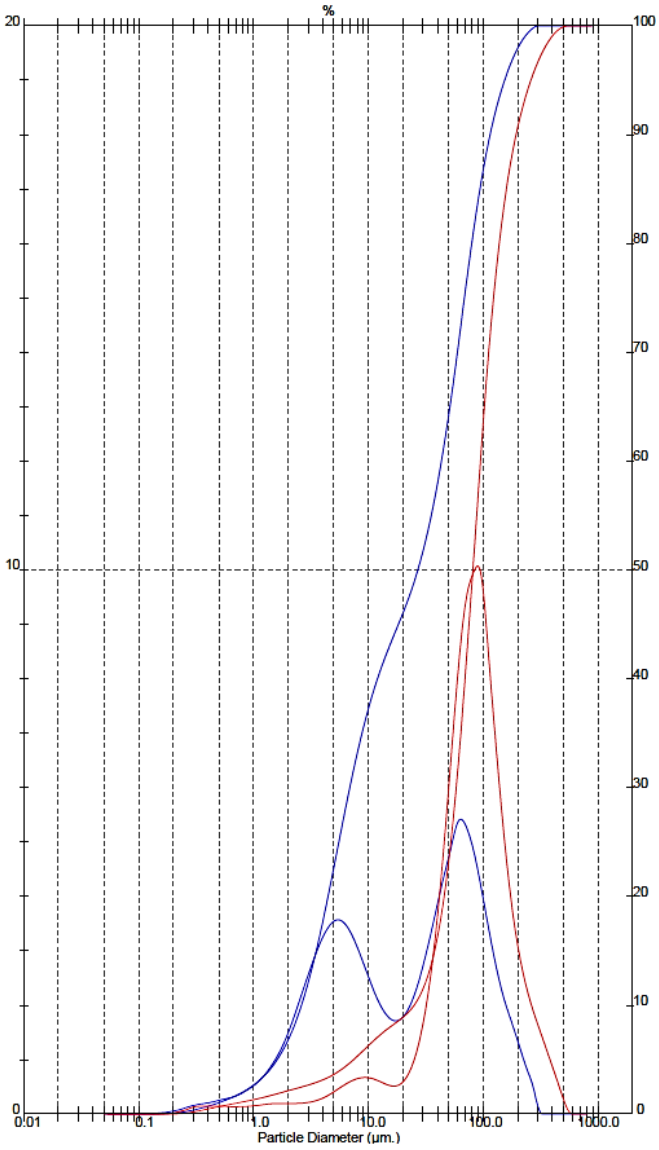

Figure 20: Volume distribution of commercially dehulled buckwheat (each with density and sum curves) milled in an impact mill in lab-scale (red) and in pilot-plant (blue) using 0.5-mm-screen inserts.

For **quinoa,** the best protein enrichment was achieved by fragmenting the dehulled seeds in a pilot-plant impact mill at Level 2 (number of revolution between high and middle) prior to sieving (< 710µm-sieve).

Similarly, **amaranth** was fragmented in a pilot-plant impact mill at Level 2 prior to sieving (< 500µm-sieve).

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

For dehulled **buckwheat** best results were obtained after fragmentation in a pilot-plant impact mill at Level 1 (high wheel speed) prior to collecting the product between two sieve mesh sizes (> 180µm and < 710µm).

All results are summarized with the corresponding protein contents in Table 10.

4.3 Production of flours and seed fragments for WP 3

Based on the parameter settings approved in pilot-scale processing flours and protein-rich flours were produced in amounts of 10-30 kg and provided to WP 3 partners for food development (see below). Small amounts of all products were also sent to the respective partners for analytical purposes.

Buckwheat: Commercially dehulled buckwheat was used for the preparation of flours for application in WP 3 and sufficient amounts for a first series of experiments were provided. The flours were prepared by milling the dehulled buckwheat using a pilot-plant impact mill with a 0.5 mm screen insert. In addition protein-rich flour (24.2% protein) was prepared by impact milling and sieve fractionation of the fragments and subsequent milling of the protein-rich fraction with a 0.5 mm screen insert.

Amaranth: Flour from whole amaranth was prepared using a pilot-plant impact mill with 0.5-mm-screen insert and was provided for analytical purposes and for product development (WP 3).

Quinoa: Flour from quinoa was also provided in sufficient amounts for product development and analytical purposes. Thereby, commercially available dehulled quinoa was used as staring material and milled to fine flour using a pilot-plant impact mill with 0.5-mm-screen insert. The protein-rich quinoa flour was prepared by impact milling followed by sieve classification as described in section 4.2.3

Lupin: Flour of full-fat lupin kernels as well as defatted lupin flakes (see section 4.1.1) were prepared in a pilot-plant impact mill using 0.5-mm-screen insert to provide flours and protein-rich flours for food application (WP 3).

Lentil: For application trials in WP 3 whole lentil flour (lentil with hulls) and dehulled lentil flour (=protein-rich flour) will be provided. The lentils will be milled using a pilot-plant impact mill with 0.5-mm-screen insert. However, the provision of this material is delayed as the amount and quality of lentils (i.e. mixture of different lentils varying in morphology and properties) received up to now is not sufficient for use in WP 3 (see section 6).

Faba bean: Flour from dehulled faba bean cultivar “Imposa” will be prepared in autumn 2016 using a pilot-plant impact mill with 0.5-mm-screen insert. A part of the flour will be treated by air classification with modified technical equipment in order to provide protein-rich flour for food

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application. The provision of these flours for WP 3 is delayed because of problem with the raw material supply (see also section 6).

Table 10 summarizes all protein-rich flours achieved after application of the best settings for protein enrichment developed until now.

Table 10: Dry matter [%] and protein contents [% based on dm] of the seed flours before (always in first row) and after (second, third and fourth rows) dry fractionation. Protein enrichment was achieved for quinoa by fragmentation (Level 2) prior to sieving (<710µm-sieve), for amaranth by fragmentation (Level 2) prior to sieving (<500µm-sieve), for buckwheat by fragmentation (Level 1, high wheel speed) prior to sieving (>180µm and <710µm), for faba bean by dehulling and for lupin by dehulling and defatting.

Raw material	Flour type	Dry matter [%]	Protein* [%]
Quinoa	dehulled	90.8	15.7
Chenopodium quinoa WILLD. 'Titicaca'	protein-rich (<710µm)	94.9	35.7
	protein-low (>710µm)	93.4	7.9
Amaranth	whole	90.6	15.3
Amaranthus caudatus L. (from India)	protein-rich (<500µm)	93.1	37.5
	protein-low (>500µm)	93.3	12.6
Buckwheat	dehulled	89.5	15.5
Fagopyrum esculentum Mönch.	protein-rich I	92.5	24.2
	protein-rich II (>710µm)	92.3	20.3
	protein-low (<180µm)	89.6	8.0
Faba bean	whole	89.5	31.6
Vicia faba L. 'Divine'	dehulled	88.9	32.6
Lupin	whole	90.5	34.8
Lupinus albus L. 'Dieta'	dehulled	88.8	40.3
	dehulled and deoiled	91.2	45.1

* Protein contents were related to dry matter and determined using N-protein conversion factor 5.85, except for buckwheat and faba bean where N x 6.25 was used and lupin where N x 5.7 was used.

5. Conclusion and next steps

The trials performed within Task 2.1 showed that impact milling combined with sieve classification or air classification is suitable for providing protein-rich flours of legume seeds and of high quality protein crops. The best suited technology thereby depended on the raw material.

Because of the different seed morphologies, protein-enrichment using air classification showed best expedient for starch containing legumes (Faba bean), whereas for the high quality protein crop buckwheat, amaranth and quinoa sieve classification was more appropriate. Lupin protein-rich flour was produced by dehulling, flaking and oil extraction of the flakes prior to milling.

All processes for the production of protein-rich flours were successfully upscaled from lab- to pilot scale. Protein contents of the protein-rich flours were in a range from 24% to 45 %. The protein

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contents compared to the initial protein contents were at least doubled. Corresponding flours were successfully provided to partners for analytics and for use in food development (WP 3).

In addition pre-processing of the seeds (dehulling, milling) provided whole flours or flours from dehulled seeds for use in WP 3 as required for milestone 9 (MS 9).

Next steps:

Improvement of the air classification: Even though significant protein enrichments could be shown with air classification, the protein contents of the final products were considered insufficient. In fact only medium enriched faba bean flours were achieved. This was due to significant protein losses during jet milling prior to air classification which could be attributed specifically to the experimental equipment used. Therefore, further trials with modified processing facilities will be carried out in October 2016 using faba bean variety “Imposa”.

Processing of lentil flours for WP 3: The trials to process lentil flours for WP 3 are pending, as single-variety lentils were not available in sufficient quantity so far. The trials are planned for the last quarter of 2016, as soon as lentil seeds are available.

6. Delays and difficulties

- Fraunhofer IVV shows a wide knowledge in the dehulling of legumes such as lupin, faba bean and lentils. One goal within WP 2.1 was to extend this knowledge to the high quality protein crops buckwheat and quinoa. However, even though detailed and intense investigations were carried out, results were not as satisfying as expected. The existing technical equipment at Fraunhofer IVV was well suited for the dehulling of legume seeds but of limited suitability for the proper dehulling of the fine-grained quinoa and buckwheat seeds. Therefore commercially dehulled seeds of these crops were used for the dry fractionation trials. If further investigations are required they could probably be carried out within a future adapted research project.
- Delay of lentil provision and difficulties to purchase any pure lentil variety in sufficient amount (> 300 kg). To date it remains unclear, when exactly the according investigations of protein enrichment can be started. Therefore the consortium agreed to switch to commercially available lentils (a variety mixture). The search for a supplier is in progress and the investigations will start as soon as the requested amount of lentils is available (currently projected to the end of 2016).
- The provision of faba bean flour for WP 3 is delayed, as in the first series of experiments different cultivars (“Divine” and “Colombo”) were characterized. At the first annual meeting the consortium decided to select the cultivar “Imposa” for further trials. Sufficient amounts of “Imposa” (up to 700 kg) needed proper cleaning at LBI and therefore are not available before September 2016. Milling trials will take place in autumn 2016.

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7. Impact and dissemination activities

The work performed within Task 2.1 generated in-depth knowledge about the effects of different milling and dry fractionation processes and parameters on the composition and (after analytical evaluation) on techno-functionality and sensory properties of the resulting fractions.
Different dry fractionation processes for selected crops were developed which enable to produce protein-rich fractions (ingredients) for food purposes.
The processes work under environmentally friendly conditions since also the by-products (protein-low fractions) could potentially be used for food development.

After analytical evaluation these new ingredients can be used in WP 3 for the development of innovative and tasty food prototypes with enhanced nutritional quality that are attractive for the consumers.

The results in this report have not been disseminated so far. In a first step this report will be disseminated within the consortium and results will be presented at project meetings. External dissemination, e.g. publication of the results in scientific papers or the website will be discussed and decided considering potential patentable intellectual property rights (IPR).

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