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D3.3 – Optimised processing conditions for protein-rich bakery products and extruded cereals and snacks

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4 – University College Cork – UCC

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Dissemination Level		
PU	Public	X
PP	Restricted to other programme participants (including the Commission Services)	
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0. Summary

This deliverable describes the development of several prototypes for protein-rich (PR) bakery products, pasta, breakfast cereals, and snacks (cf. GA WP3 – subtask 3.3.3. and 3.3.5), using new plant protein sources developed in WP2. The food prototype development was focused on wheat-based and gluten-free (GF) breads, wheat-based pasta, wheat-based and GF cookies, extruded snacks and breakfast cereal products. A large number of trials were conducted to investigate the influence of the developed protein ingredients (WP2) on the macro-structure of products. In combination with fundamental experiments, predictive models were established to develop prototypes for these product categories.

The results indicated that the protein isolate from blue lupin, as well as protein-rich (PR) fractions of faba bean and buckwheat (developed as part of WP2 of this project),were well suited to produce wheat-based bread and pasta of adequate quality. The developed prototype products have been thoroughly characterised regarding their technological properties and a preliminary sensory evaluation has been undertaken. Furthermore, optimal extrusion conditions, to improve the nutritional value of bean flour for porridge-type product development, were determined. A sensory evaluation of the developed porridge, cookies, extruded snacks and breakfast cereal prototypes was also performed. Nutritional profiling has been finalised, while market testing of the products is still ongoing.

Protein-rich extrudate prototypes from blue lupin, white lupin, and faba bean protein isolates (WP2) by extrusion-cooking have been also developed. The prototype based on a combination of lupin protein isolate and buckwheat food products resulted in highest popularity of sensory analyses (taste and texture). Mixtures of legumes and pseudocereals were found particularly promising in fulfilling human nutritional requirements, due to their well-balanced amino acid profile. Significant investigations were also undertaken to optimise formulations and extrusion conditions for gluten-free breads and extruded products by using Andean flours (quinoa and kichiwa), respectively, in combination with network-forming hydrocolloids (gums). These formulations represent also promising matrices for the incorporation of tarwi (Andean lupine) flour.

1. Introduction and objectives

In order to accelerate the transition of main human nutrition from animal-based protein to plant-proteins, available food products with high quality plant protein content and with high consumer acceptance have to be provided. The application of protein-rich (PR) ingredients from new, EU-grown sources in staple foods like (gluten-free) bread, pasta, cookies, breakfast cereals and extruded snacks, offer a way to supply customers with plant-based PR alternatives for commonly consumed products. UCC, FRAUNHOFER, MAKERERE and UNALM performed screening trials of PR ingredients for gluten-free and wheat-based target food products. To allow comparison of the products, the screenings included the P2F ingredients, which were provided by the project partner FRAUNHOFER (lupine protein isolate, protein-rich flours of amaranth, buckwheat, faba bean and quinoa, and lentil flour from dehulled grains), as well as traditional products. These included commercially available protein isolates (soy, carob germ, potatoes, pea, zein, gluten, lupine, chickpea flour), and locally sourced flours (bean, soy, amaranth quinoa and kiwicha flours). Simple control recipes containing wheat flour (baker's flour)were used as reference products and base formulations.

For PR pasta and bread formulations, wheat flour was partly replaced by either protein isolates or PR flours, in order to obtain a PR product in accordance with(EC) regulation No 1924/2006. Based on the screening trials and the fundamental investigations onboth gluten-free and wheat-dough as well as the product properties, predictive models were acquired to develop prototypes with broad consumer acceptability. These were based on technological and sensory quality characteristics (e.g., texture profile, appearance, taste, and flavour).The development of protein-rich breakfast cereals by legume and pseudocereal proteins done by extrusion cooking required systematic investigation of the raw material-process interactions. Low moisture extrusion-cooking of legumes (lupin, lentil, faba bean) and pseudocereals (amaranth, buckwheat, quinoa) was investigated and the results of the extruded products could potentially be used as breakfast cereals or snacks.

Furthermore, the conducted preliminary sensory tests have underlined the high potential of the prototypes meeting the consumer expectations on plant protein products, and is likely to help narrow down the number of food prototypes, for each food category, which should be further investigated for their sensory and nutritional properties (cf. GA WP3 – subtasks 3.5 and 3.6).

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2. Activities for solving the task(s)

The activities undertaken in this study in order to solve the tasks has been divided according to the different prototypes, due to the differences in activities that these products entailed.

2.1 Wheat-based bread and pasta (UCC)

a) Selection of recipe and processing conditions for wheat based reference product

Standard bread and pasta formulations based on wheat flour, which are known to exhibit high consumer acceptance, were selected as reference products. The chosen formulations contain few ingredients to keep their influences and interactions with the PR ingredients to a minimum. Reference formulations, an overview of processing conditions and procedures are provided below for wheat-based bread (Figure 1 and Table 1) and pasta (Figure 2 and Table 2).

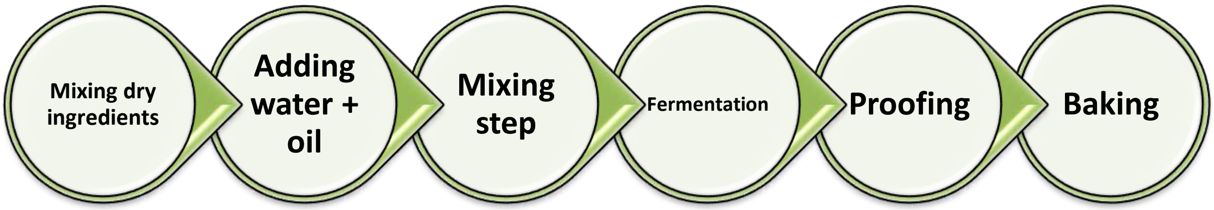

Figure 1. Baking procedure for wheat-based breads.

Table 1. Formulation and processing conditions of reference wheat bread.

Ingredient	% Flour weight	% Dough weight	Processing conditions	
Baker's flour	100.0	59.7	Mixing	1 min; speed 1
Baker's yeast	2.0	1.2		2 min; speed 2
Water	62.5	37.3	Proofing	75 min
NaCl	2.0	1.19		85 % rel. humidity; 30 °C
Oil	1.0	0.6	Baking	14 min; 210 °C; 700 ml steam
Total amount	167.5	100.0		before loading, draft open

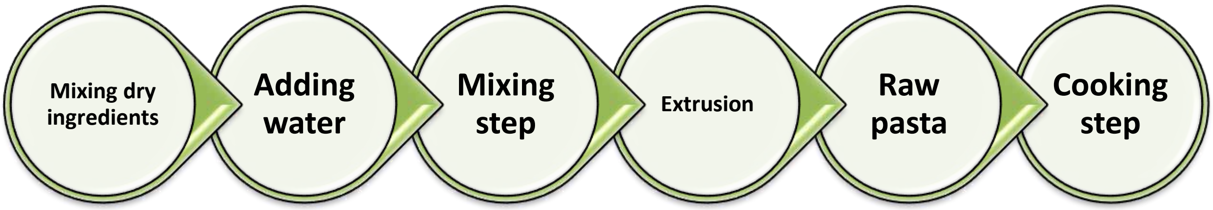

Figure 2. Production procedure for wheat-based pasta.

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Table 2. Formulation of reference wheat pasta.

Ingredient	% Flour weight	% Dough weight	Processing conditions	
Baker's flour	99.5	76.5	Water temperature	50 °C
Water	30.0	23.1	Mixing	10 min
NaCl	0.5	0.4	Pasta matrix	Diameter of holes 2 mm
Total amount	167.5	100.0		

b) Selection of protein-rich (PR) ingredients for incorporation in reference recipes

A broad range of PR ingredients, commercially available as well as developed in WP2, were incorporated in the reference recipes. A detailed compositional analysis was performed for all ingredients and used to calculatethe protein content of each formulation.

c) Establishment of recipes for wheat based PR bread and pasta

Recipes where established on the basis of the reference formulations. The replacement of wheat flour by PR ingredients increases the protein content in the formulations. In order to meet regulatory requirements, recipes were developed in order to reach a minimum of 20 % of calories provided by protein (requirement for products claim to be 'high in protein' according to regulation (EC) No 1924/2006).

d) Adjustment of processing conditions (water addition)

For both wheat bread and pasta, the amount of water incorporated in the formulation is a key parameter. The water absorption of dry ingredients substantially changes when wheat flour is replaced by PR ingredients. Therefore, prior to baking or pasta production trials, the optimum water amount for the formulation was determined. The standard method to determine water absorption of wheat flour in baking is a Farinograph measurement. For pasta, water absorption indices (WAI) were obtained using a centrifugation method. Optimum water amounts for pasta formulations were calculated based on the WAIsdescribed below.

Operation principle: The pasta formulation mix (2.5 g) was dispersed in 10 mL distilled water (30 °C) using an Ultra-Turrax equipped with a S10N-5G dispersing element (Ika-Labortechnik, Janke and Kunkel GmbH, Staufen) for 15 s and then shaken for 30 min at 1000 rpm using a platform shaker (UNI MAX 1010, Heidolph, Schwabach, Germany). After the mixture was thoroughly wetted, the sample was centrifuged at 3000 g for 10 min (Centrifuge 5417 r, Eppendorf, Hamburg, Germany). WAI was calculated as percentage of water retained per sample:

$$WAI\ [\%] = \frac{Weight\ of\ sediment}{Weight\ of\ dry\ sample} \cdot 100$$

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The corrected level of water added to the mixed formulations was calculated using the following equation:

$$Water[\%] = \frac{WAI\ of mixed\ formulation}{30\ (water\ level\ reference\ pasta)} \cdot WAI\ (reference\ pasta)$$

e) Experimental baking and pasta production (macro-structure investigations)

According to the optimised recipes (optimum water amount), baking and pasta production trials were performed to screen the selected PR ingredients for the most promising results. Regarding bread formulations, technological analyses were undertaken after baking and during shelf life assessment by analysing the staling behaviour of the products. Furthermore, the impact of the PR ingredients on the dough properties was determined in order to investigate the network/structure-forming capabilities of the investigated PR ingredients, particularly when incorporated in a wheat-based foodsystem (Table 3). GlutoPeak tests were conducted to gain information about gluten-aggregation in wheat flour doughs with the presence of PR ingredients. The pasting behaviour of wheat flour, when partially replaced by PR ingredients, was evaluated by rapid visco analysis (RVA). Further rheological characterisation of the doughs was performed by rheometer measurements applying an oscillation method. The quality of PR pasta was assessed by analysing technological characteristics of raw and cooked pasta. Table 3 displays a list of performed measurements. All screening trials were conducted in triplicate to ascertain reproducibility of results.

Table 3.Analyses performed for macro-structure investigations of PR bread and pasta.

Bread	*Composite flour/dough*	*Baked breads*	
	Farinograph GlutoPeak RVA Rheometer	Bake loss Specific volume Crumb structure (C-Cell) Crumb texture and staling behavior (TPA; day 0, day 2, day 5) Water activity (day 0, day2, day5) Colour (crust and crumb) Pictures Brief sensory evaluation	
Pasta	*Composite flour*	*Raw pasta*	*Cooked pasta*
	WAIs	Colour Pictures Optimal cooking time	Colour Pictures Cooking loss Firmness Stickiness Tensile strength

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f) Combination of different PR ingredients and development of prototype formulations

Formulations for prototypes were developed by combining PR ingredients that showed the most promising results in the screening stage. For PR wheat bread, dough properties and bread quality characteristics were evaluated using principal component analysis (PCA). Based on this, three bread prototypes, combining project ingredients (WP2) and commercial PR ingredients, were developed. A more complex approach was chosen for PR wheat pasta. Response surface methodology was applied and a total of 17 additional trials were performed to obtain a formulation with optimum product characteristics.

g) Production and analysis of prototypes

Three bread prototypes and one pasta prototype were produced. Prior to production, optimum water amounts and protein influence on food structure and rheology were determined as previously described.

2.2 Porridge, cookies and extruded snacks (MAKERERE)

In Subtask 3.3.5 the project partners (MAKERERE and FRAUNHOFER) aim to develop laboratory-scale PR extrudates, such as breakfast cereals and snacks. Below, the prototyping activities performed by MAKERERE are summarised, and the final production protocols and optimised recipes for the developed food prototypes are described. The following protein ingredients were used for the development of PR products of porridge flours, cookies and extruded snacks (Table 4).

Table 4. PR ingredients utilised for development of PR prototypes.

Ingredient	Protein content [%]
Dry beans	**22.53**
Soy beans	**36.49**
Grain amaranth	**13.56**

Performed activities

Porridge flours

i. Nutritional and physical characterisation of the developed protein source porridge flours (both instant and fast-cooking).

ii. Market testing of both the instant and fast cooking porridge flours.

iii. Contributed towards the development of Uganda food nutritional standard to cater for the developed instant porridge flour by availing supporting data. A draft of national standard (DUS 1852) on pulses and pulse products is available. Specification is ready for approval by the Uganda National Bureau of Standards (UNBS).

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Cookies

iv. Development of cookies with high protein, gluten-free and reduced wheat content.

v. Assessment of the sensory acceptability of the developed cookies.

vi. Characterisation of the nutritional composition of the developed cookies.

Extruded snack

vii. Development of extruded snack and breakfast cereal products with iron-enhanced beans; for children aged 1-5 years and women of reproductive age.

viii. Optimizing extrusion conditions for iron-enhanced beans flour, used for extruded snack and breakfast cereal products.

ix. Characterisation of optimised extruded snack and breakfast cereal products.

x. Determination of the acceptability of the breakfast cereal and snack products.

2.3 Wheat-based extruded cereals (FRAUNHOFER)

a) Selection of raw materials based on their chemical, functional and physical properties

As part of this deliverable, a screening of the application potential of the new ingredients was crucial for the selection of the most suitable flours and isolates developed in WP2. Basic data of the different flour fractions and isolates like protein, starch and fat content as well as protein solubility, particle sizes and emulsifying properties were provided by WP2. Temperature-dependent thermal properties and viscosity behaviour were analysed within the scope of this deliverable, as these properties were considered crucial for the development progress of the breakfast cereal prototypes.

b) Characterization of protein ingredients and functional properties

Protein content

As the aim of this deliverable was to develop products of which a high percentage of the total calories comes from proteins, high protein content in the raw materials were therefore one of the most important characteristics. For the extrusion trials, the highest possible protein content in the formulations needed to be investigated, since proteins can have a major impact on the expansion of the products and therefore on textural and sensory quality.

Starch content

The starch content, as well as the type of starch, can influence the firmness, mouthfeel and stability of cereals to a high extent and is therefore a very important parameter. Starch contents and characteristics influence the extrusion process because starch is known to contribute favourably to

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expansion. Too low or too high starch contents in extrusion mixtures can therefore lead to low expansion or can influence the extrusion process by e.g. clogging the extruder.

Thermal properties

Thermal properties of untreated legume protein isolates and legume protein isolates preheated at 120 °C were analysed by differential scanning calorimetry (DSC). The samples were diluted with water in a ratio of 15:85 and dispersed with a spatula until all flour particles were dissolved. The sample and the reference were heated from 40 °C to 120 °C at a rate of 2 K/min. Each sample was reheated one time to verify that there was no reversibility of denaturation. The denaturing behaviour of proteins can be characterised by the enthalpy of denaturation (ΔH) and the denaturation temperature (Td). The denaturation temperature and enthalpy of denaturation were computed from the thermograms. The peak area corresponds to ΔH and the peak tip corresponds to Td.

Viscosity behaviour

In addition to the starch content of the new ingredients, the quality and thermal dependent viscosity of the different starch types and proteins is an important evaluation criteria for the suitability in food applications. For that reason, the viscosity of the raw materials and mixtures were measured with a Rapid Visco Analyser (RVA 4500, Perten Instruments GmbH, Hamburg, Germany) in relation to the temperature. The samples were diluted with water in a ratio of 20:80 and dispersed with a spatula until all flour particles were dissolved. The diluted samples were stirred with a speed of 960 rpm in the first 20 seconds and then with a speed of 160 rpm in the first two minutes of the experiment. Afterwards, they were heated up to 95 °C and held at 95 °C for three minutes. After that, the samples were finally cooled to 50 °C and held there for one minute.

Protein solubility

The protein solubility is an important criteria for the development of cereals. It can on one hand have a great impact on extrusion properties and it is also related to the emulsifying ability of proteins. The protein solubility is defined as the determined amount of protein that has dissolved after mixing into a 0.1M sodium chloride solution at an appropriate pH value.

Emulsifying properties

The emulsifying capacity (EC) was determined according to the Fraunhofer IVV standard method by continuous addition of oil to an oil-in-water emulsion, to the point of phase inversion of the emulsion.

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Phase inversion is detected by an abrupt collapse of the electrical conductivity. The volume of oil needed for phase inversion was used to calculate the EC (ml oil/g protein product).

Water binding capacity

Water binding capacity of the raw materials was determined. 2 g sample was weighed into a centrifuge glass and diluted with 40 ml demineralised water. The sample was mixed for one minute with a test tube vibrator (VF 2, Janke & Kunkel, IKA-Labortechnik GmbH, Staufen, Germany) and then kept at room temperature. The sample was dispersed again after 5 and 10 minutes for 30 seconds, respectively. After 10 minutes, the sample was centrifuged for 15 min at 1000 RZB and 20 °C (centrifuge 6K15, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). After centrifugation, the supernatant was decanted and the sediment was weighed back. The water-binding capacity refers to the dry mass of the sample.

Particle size

The particle size can influence the mouthfeel of products, as insoluble particles with a diameter of over 8 µm can contribute to a coarse perception of smooth textures. Too small particles can be problematic for the dosage system of the laboratory extruder because of the low pourability of the powders. Volume diameter D (0.53) was calculated from the particle volume distributions of the pseudocereal protein flours.

c) Development of extrudates in laboratory

The production of high protein breakfast cereals from legume proteins by extrusion cooking can be divided into two steps: extrusion and drying.

Extrusion: Extrusion trials were performed using a laboratory, co-rotating twin screw extruder (HaakeRheocord, Thermo Fisher Scientific, Inc., UK). The data acquisition and system control was performed with the PolyLab Software (Version 1.0, Thermo Fisher Scientisfic, Inc, Japan). Both extruder screws have a length of 40 cm, a diameter of 1.6 cm and a length/diameter-ratio of 25:1. The screw configuration is constructed with different screw elements, which ensure constant mixing and conveying of the feed. The screw configuration applied has been designed according to Osen et al. (2014). Products were extruded through a 3.5 mm circular die. The extruder barrel is segmented into five heating zones that can be heated by an electric cartridge heating system and cooled with water. A twin-screw gravimetric feeder type KCM (K-tron, Niederlenz, Switzerland) was used to feed the dry protein mix into the extruder. Water at ambient temperature was pumped with a detachable HPLC pump (Alpha 50 Plus, ECOM, Prague, Czech Rep.) into the top of the extruder barrel 130 mm

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downstream from the centre of the feed port. Before starting the extrusion, the water pump was controlled to a steady outflow and the feeder was calibrated. During the initial heating phase, both moisture content and temperature were varied. The moisture content was reduced stepwise to 33 %, while the barrel temperatures were increased stepwise to a temperature profile reaching from 40 to 140 °C from the first to the fifth zone. After these temperature settings were reached, the water content was kept constant at 33 % and only the fourth temperature zone was varied. A constant screw speed of 300 rpm was used.

Drying: After extrusion, the samples were dried in a drying oven (Thermo Scientific Heraeus UT 6760, Thermo Electron LED GmbH, Langenselbold, Germany) at 65 °C to a dry mass of 95 ± 1 %. The samples were stored in welded vacuum plastic bags.

d) Physical and sensory analysis of the extrudates

The physical and sensory characteristics for protein-rich extrudates were analysed to evaluate the influence of different extrusion settings and formulations on breakfast cereal quality.

Expansion: After leaving the die, the extrudate expands so that the volume of the extrudate increases. Expansion is influenced by material parameters (composition), as well as process parameters (mass flow rate, shear stress, moisture content, temperature and pressure at the die outlet) and machine parameters (die geometry). The expansion that occurs is characterised by dimensionless figures. The sectional expansion index (SEI) describes the area expansion in radial direction. SEI was calculated from the ratio of extrudate diameter d_E and die diameter d_D. The diameter of ten pellets was measured with a digital caliper (Holex, Hoffmann GmbH, Munich, Germany).

Product texture properties: Texture properties for each extrudate were evaluated using a TA.XT plus Texture Analyser (Stable Micro System, UK) equipped with a 50 kg load cell and a 25 mm diameter specimen. The extrudates were placed horizontally under the test probe and compressed to fracture. The force required was recorded with the Exponent TEE 32 software. To calculate the specific hardness, the maximum force was related to the lateral surface area of the pellets. For this purpose, the length and the diameter of five extrudates were determined with a digital caliper before the measurement. Commercially available oat flakes (KöllnVollkornHaferfleksKnusper-Klassik, Peter Kölln GmbH & Co. KGaA, Elmshorn, Germany) were used as a reference.

Sensory evaluation: To characterise and evaluate the sensory properties of the different samples, descriptive sensory combined with popularity sensory was performed. In two sensory analyses, the extrudates were tasted by 10-15 trained and untrained people and evaluated according to smell, taste and texture. The legumes with 30 and 50 % protein and the legume-pseudocereal-mixtures were

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sensory analysed. The extrudates were stored in separated WECK-glasses with encrypted three-digit code. The panel evaluated each sample with tablet computers. In general, the properties were judged on a visual analog scale, ranging from 0 to 100, with 0 being defined as not present at all and with 100 being defined as maximum intensity. The results were evaluated in the Fraunhofer IVV sensory department.

2.4 Gluten-free breads and extruded products (UNALM)

In the first part of this study, with the aim to establish the optimum control gluten-free bread, the influence of the starch (corn starch and potato starch) and gum (xanthan gum and tara gum) on rheological characteristics (G' and G") and quality characteristics of gluten-free breads (volume, texture and alveolar structure) were evaluated. Four formulations were developed containing corn starch with xanthan gum (CS-XG), corn starch with tara gum (CS-TG), potato starch with xanthan gum (PS-XG) and potato starch with tara gum (PS-TG). Leavening properties of the four doughs were examined by image analysis. Viscoelastic properties of the dough were measured at 25 °C, using a controlled strain rheometer Haake Mars 60 (Thermo Fisher Scientific, Germany) equipped with a system of parallel plates (diameter 35 mm, gap 2 mm), and the fermentation kinetic was modelled using the Gompertz equation. The quality-related properties of the breads of the four formulations were then evaluated.

In the second part of the study, the proportion of quinoa and amaranth flour was determined preparing gluten-free masses with Andean grain flours that imitate the textural properties of the selected control dough. A D-Optimal mixture design with three variables (quinoa/amaranth flour, tara gum, and water) was proposed. Sixteen formulations were experimented and characterised in their textural properties. In the same way, a mixture design was carried out with three variables: quinoa/amaranth flour, gum mixture (50% tara gum and 50 % xanthan gum) and water. A mathematical model of prediction of textural properties was obtained in order to determine the optimal proportions of quinoa/amaranth flour, gum, and water that could imitate the control dough.

Extrusion tests: The extrusion tests were carried out in a double screw laboratory extruder (JINAN DINGRUN MACHINERY CO; LTD, China). The extrusion chamber has four zones with independent electrical heating elements. The following conditions were established by preliminary experiments: 2.95 mm exit diameter, screw speed 125 RPM, and feed rate 280 g/min. The feed was made by a pipeline with screw, by which the amount of flour was controlled. The treatments were defined by response surface methodology using the central composite design with a centered face and five central points. The independent variables were the moisture of the kiwicha flour and the

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temperature of the extrusion chamber. The levels of the variables were determined with previous tests.

3. Results

3.1 Wheat-based bread and pasta (UCC)

a) Selection of recipe and processing conditions for wheat based reference product

Appearance, sensory characteristics and main technological quality characteristics of the reference products are presented below for bread (Figure 3 and Table 5) and for pasta (Figure 4 and Table 6).

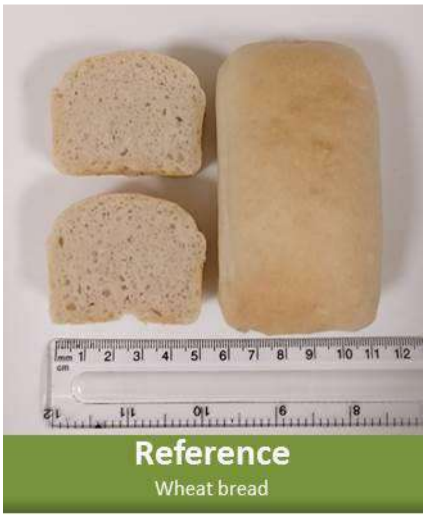

Figure 3 – Appearance of reference wheat bread

Smell: dried yeast
Taste: neutral, starchy, salty
Colour: light brownish, quite pale

Table 5– Technological quality characteristics of reference wheat bread

Bake loss [%] ± conf	**Spec. Volume** [mL/g] ± conf	**Hardness day 0** [N] ± conf	**Hardness day 2** [N] ± conf	**Hardness day 5** [N] ± conf
10.6 ± 0.5	2.61 ± 0.17	10.87 ± 0.69	20.98 ± 0.83	32.44 ± 1.59

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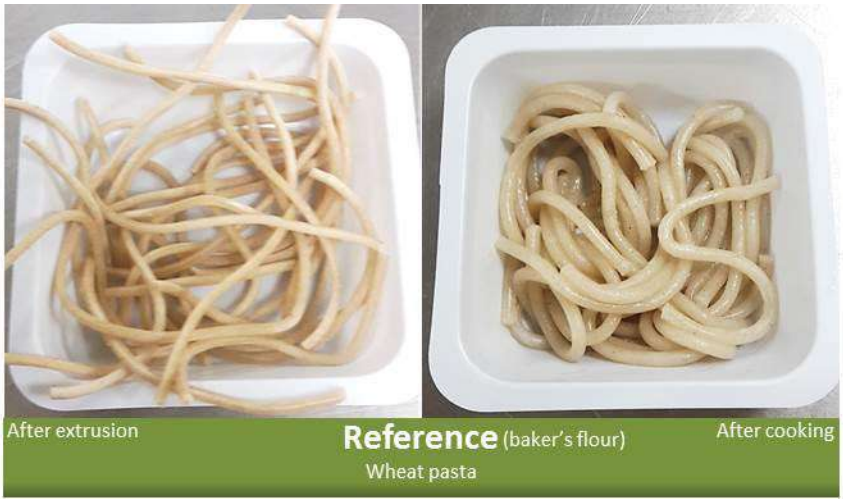

Taste: neutral, starchy
Colour: pale
Smell: neutral

Figure 4 – Appearance of reference wheat pasta

Table 6 - Technological quality characteristics of reference wheat pasta

Cooking loss [g/100 g] ± conf	**Optimum cooking time** [min]	**Firmness** [N] ± conf	**Tensile strength** [N] ± conf	**Stickiness** [N] ± conf	
5.57 ± 0,08	7.5	2.21 ± 0.02	0.21 ± 0.00	2.28	0.12

b) Selection of protein-rich (PR) ingredients for incorporation in reference recipes

A list of PR ingredients selected for screening can be found in table 7 (including ingredients for the reference recipes).

Table 7– Selected screened ingredients

PR ingredient, commercial (name as referred to below)
Soy protein isolate ('Soy')
Carob germ flour ('Carob')
Potato protein isolate 1 ('Potato 1')
Potato protein isolate 2 ('Potato 2')
Pea protein isolate ('Pea')
Corn protein isolate, zein ('Zein')
Lupine protein isolate ('Lupine, commercial')
Wheat protein isolate, gluten ('Gluten')
Chickpea flour ('Chickpea')
PR ingredient, developed in WP2(name as referred to below)
Lupine protein isolate ('Lupine, IVV')
PR-Quinoa flour ('Quinoa')
PR-Amaranth flour ('Amaranth')
PR-Buckwheat flour ('Buckwheat')
PR-Faba bean flour ('Faba bean')
Lentil flour, dehulled ('Lentil')
Other ingredients
Baker's flour

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Baker's yeast	

c) Establishment of recipes for wheat based PR bread and pasta

The protein content of the screened PR ingredients varied between 20.5 % for buckwheat flour and 92.4 % for soy protein isolate. The difficulty herein was to decide whether all products should have the same protein content or whether a constant level of replacement should be used. For PR bread, a constant level of wheat flour replacement of 15 % (protein isolates) or 50 % (PR flours) was chosen. For pasta prototype development, the required level of wheat flour replacement for each PR ingredient was calculated in order to reach the threshold of 20 % of calories provided by protein. 7 to 11 % of wheat flour were replaced with protein isolates and 27 to 50 % in the case of the PR flours. An overview of protein levels reached and the level of baker’s flour replacement for pasta and bread is presented in Table 8.

Table 8 – Levels of wheat flour replacement and calories provided by protein for PR formulations (screening stage)

PR formulation	Baker’s flour replacement [%]		Calories provided by protein [%]	
	Bread	Pasta	Bread	Pasta
Baker‘s flour, control	-	-	15.56	15.2
Soy	7	15	28.74	21.8
Carob	11	15	23.21	21.0
Potato 1	7	15	28.30	21.5
Potato 2	7	15	27.68	21.2
Pea	8	15	25.85	21.0
Zein	7	15	28.12	21.4
Lupine, commercial	20	15	20.04	21.5
Lupine, IVV	7	15	27.19	20.9
Gluten	8	15	25.93	21.0
Chickpea	40	50	21.80	20.4
Quinoa	30	50	25.63	21.7
Amaranth	20	50	30.58	21.5
Buckwheat	57	50	19.62	20.0
Faba bean	9	15	25.44	21.4
Lentil	27	50	25.82	21.4

d) Adjustment of processing conditions (water addition)

Optimum water levels determined for the screening trials and bread dough characteristics are presented in Table 9 and Table 10. The partial replacement of wheat flour by PR ingredients led to substantial changes in both water absorptions determined for bread doughs, and water indices

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determined for pasta recipes. This underlines the importance of the determination of optimum water levels for both bread and pasta formulations prior to production.

Table 9 –Results of Farinograph trials performed to determine optimum water content for PR bread formulations

Composition	**Water absorption [%]**	**Dough development [min]**	**Stability [min]**	**Degree of softening [FU]**
Baker's flour (BF)	62.5	2.5	>8.3	5
BF + soy (85:15)	74.5	11	>5.5	0
BF + carob (80:15)	70.5	6.5	7.5	10
BF + potato306 (85:15)	65.5	5	2.5	140
BF + potato201 (85:15)	66	8	7	5
BF + pea (85:15)	71	9	>5	0
BF + zein (85:15)	61	2.75	2.5	100
BF + lupine (commercial) (85:15)	73	6	4.5	20
BF + lupine (IVV) (85:15)	66	8.5	4	5
BF + gluten (85:15)	70	4	>10	0
BF + chickpea (50:50)	58	6.25	3	45
BF + quinoa (50:50)	56	4.3	2	130
BF + amaranth (50:50)	68	5	2	100
BF + buckwheat (50:50)	59.5	4.5	3	80
BF + faba bean (85:15)	62.5	7.5	3.5	20
BF + lentil (50:50)	56	8	3.25	10

Table 10 – Results for water absorption indices (WAIs) to determine optimum water content for PR pasta formulations

Pasta formulation (level of BF replacement)	**WAI [%]**	**Water [%]; solid based**
Bakers flour, control	189.6±0.9	30.0
Soy (7 %)	174.8±0.9	27.7
Carob (11 %)	182.0±1.2	28.8
Potato 1 (7 %)	176.1±1.9	27.9
Potato 2 (7 %)	165.9±1.7	26.2
Pea (8 %)	176.6±0.6	27.9
Zein (7 %)	185.7±0.2	29.4
Lupine (commercial) (20 %)	190.4±1.1	30.1
Lupine (IVV) (7 %)	183.4±2.5	29.0
Gluten (8 %)	179.8±3.9	28.4
Chickpea (40 %)	168.8±0.3	26.7
Quinoa (30 %)	177.1±0.3	28.0
Amaranth (20 %)	176.7±0.9	28.0
Buckwheat (57 %)	173.6±0.5	27.5
Faba bean (9 %)	179.3±1.0	28.4
Lentil (27 %)	177.4±0.7	28.1

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e) Experimental baking and pasta production (macro-structure investigations)

The PR products were significantly different in their appearance and technological quality. The results of the screening provided an in-depth understanding of the effects of the PR ingredients in a wheat-based bread and pasta product systems. Interestingly, and very important for the project, were the findings indicating that wheat breads and pasta containing the protein-rich faba bean flour had the best overall quality results, followed by lupin and buckwheat products. This shows the high potential and suitability of these P2F ingredients (provided by the project partner Fraunhofer - WP2) for further industrial validation and demonstration activities.

f) Combination of different PR ingredients and development of prototype formulations

Bread prototypes

The results for bread quality characteristics of PR formulations and the reference product were subjected to a principal component analysis (PCA). This provided an overview of the overall quality of the products and emphasised the different functionalities of the PR ingredients, resulting in improved or inferior overall quality compared to the reference products. A total of six groups of PR breads,characterised by different overall qualities, were identified (by means of hierarchical classification of PCA results).The suitability of formulations with faba bean, lupin and buckwheat were confirmedfor the production of adequate quality breads, due to many bread characteristics similar to the reference product.

Pasta prototype

Response surface methodology (RSM) was used to evaluate the effect of the independent variables (level of protein-rich ingredient) on the dependent variables (firmness, stickiness, elasticity and cooking loss). Hereupon, optimum ingredient levels could be determined. A circumscribed, two-dimensional central composite design was developed featuring variations in the addition levels of protein-rich ingredients, whereas the percentage of calories provided by protein ranged from 0 to 5 %. A total of 17 trials were carried out comprising eight for the factorial (Run 3, 4, 7, 9, 10, 13, 16, 17), six for the axial (Run 1, 5, 8, 11, 12, 15) and three as central points (Run 2, 6, 14). Again, WAI were determined prior to pasta extrusion to ascertain the right level of water addition to the formulations.

The response of each of the investigated parameters was analysed by fitting cubic models to the data. Based on this, significant effects of variations in parameter levels on the responses were identified ($p < 0.05$). Design Expert Version 7 (Stat-Ease Inc., Minneapolis, MN, USA) was used for experimental design and to generate surface response plots that permitted evaluation of the linear, quadratic and

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interactive effects of independent variables on the selected dependent variables and to optimise the pasta formulation. The best fitting model, which evaluated the effect of the independent variables on the response, was chosen. The significance of the lack of fit error term, coefficient R2, coefficient of variation (CV) and model significance were used to judge the adequacy of the model fit. The predictive models developed for firmness, stickiness and tensile strength of pasta were all considered adequate. Furthermore, threshold values and ranges for qualitatively acceptable pasta were defined (T).

After evaluation of all data, maximum firmness, minimum stickiness, maximum tensile strength and minimum cooking loss were the main quality criteria for the pasta optimisation. One combination of PR ingredients (5 % of protein calories provided by faba bean and buckwheat and 3 % by lupin protein isolate) was chosen for experimentally testing the optimised process. The reason for the lower amount of lupin protein isolate was due to its allergen potential and higher cooking loss. The results were compared to those predicted by the mathematical RSM model (Table 11). The measured values for firmness and tensile strength of the PR pasta corresponded well to the predictions (RSM), while the predicted (RSM) value for stickiness was slightly overestimated.

Table 11 – Optimum ranges for independent parameters and predicted (by Response Surface Methodology) and measured values for the responses of high-protein pasta

	Settings for optimisation			**Pasta control** Protein-calories = 15.2 %	**High-protein pasta** Protein-calories = 21.8 %		
Independent variables	**Target**	**Lower limit**	**Upper limit**	**Standard dough**	**Predicted parameters**	**Chosen parameters**	
Faba bean	maximise	0	5	0	4.8-5.0	5.0 (4.8)*	
Buckwheat	maximise	0	5	0	3.8-5.0	5.0 (15.9)*	
Lupin IVV	maximise	0	5	0	3.1-5.0	3.0 (2.4)*	
Dependent variables				**Measured values**	**Predicted values**	**Measured values** – **Wheat based**	**Measured values** – **Semolina based**
Firmness [N]	maximise	2.2	3.7	2.2±0.1	2.9	2.7±0.1	2.8±0.0
Stickiness [N]	minimise	1.2	2.4	2.3±0.8	1.5	1.9±0.6	0.7±0.1

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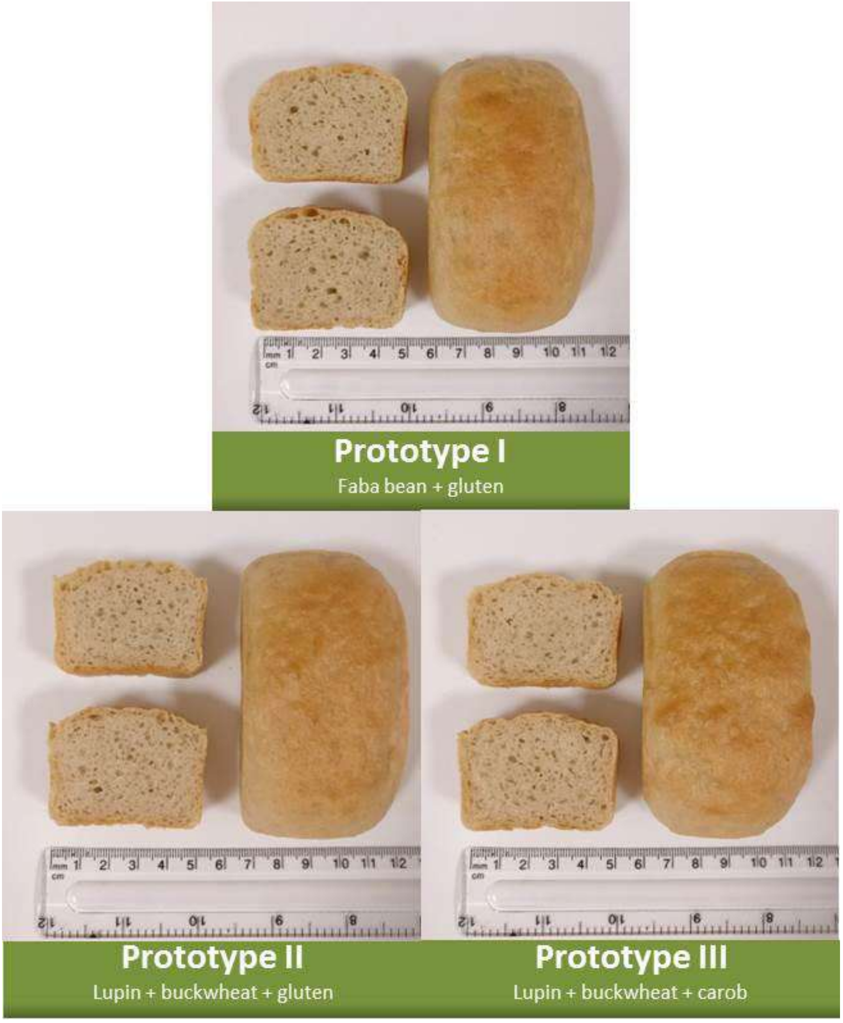
Tensile strength [N]	maximise	0.2	0.4	0.2±0.0	0.4	0.4±0.0	0.4±0.0
Cooking loss [%]	minimise	2.8	6.2	5.6±0.2	5.4	5.0±0.1	5.3±0.1

*Values in brackets show the percentage of protein-rich ingredient based on solids used in the recipe, whereas 100 % represents the sum of solids considering wheat flour, protein-rich ingredient and salt (0.5 %), water level 30.5 %; solid based.

g) Production and analysis of prototypes

Appearance, sensory characteristics and main technological quality characteristics of the developed prototypes are presented below (Figure 5 and Figure 6; Table 12, Table 13 and Table 14).



<u>Prototype I</u>

Smell: slightly starchy, roasty, slightly beany
Taste: quite plain, savoury, slight green/bitter note in after taste

<u>Prototype II</u>

Smell: nutty, slightly beany
Taste: quite plain, slightly nutty, mouthfeel slightly floury, slight green/sweet after taste

<u>Prototype III</u>

Smell: slightly nutty/roasty
Taste: quite plain, slightly nutty, slight green/sweet after taste, mouthfeel less floury than with gluten

Figure 5 – Appearance of PR wheat bread prototypes

Table 12 – Technological quality characteristics of PR wheat bread prototypes

Prototype	**Bake loss** [%] ± conf	**Spec. Volume** [mL/g] ± conf	**Hardness day 0** [N] ± conf	**Hardness day 2** [N] ± conf	**Hardness day 5** [N] ± conf

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I	11.8 ± 0.4	2.19 ± 0.03	15.69 ± 0.70	26.22 ± 0.92	32.26 ± 1.60
II	12.6 ± 0.2	2.46 ± 0.09	12.74 ± 0.45	23.49 ± 0.99	32.50 ± 0.67
III	11.6 ± 0.2	2.29 ± 0.05	12.68 ± 0.66	23.90 ± 1.14	31.67 ± 1.53

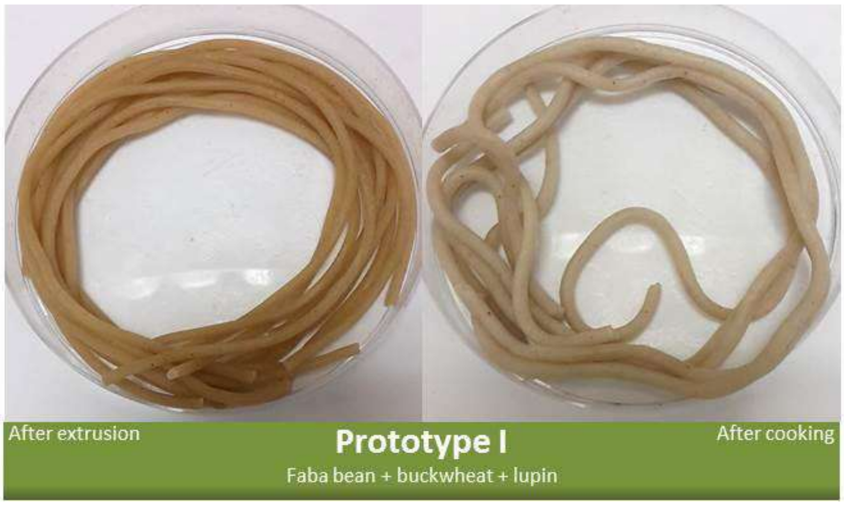

Figure 6 – Appearance of PR wheat pasta prototype

Table 13– Technological quality characteristics of PR wheat pasta prototype

Prototype	**Cooking loss** [g/100 g] ± conf	**Optimum cooking time** [min]	**Firmness** [N] ± conf	**Tensile strength** [N] ± conf	**Stickiness** [N] ± conf
I	5.0 ± 0.1	8.0	2.7 ± 0.0	0.4 ± 0.0	1.9 ± 0.2

Table 14–Sensory evaluation of reference wheat pasta, selected screened PR pasta and PR wheat pasta prototypes. The samples were rated from 1 (least intense) to 6 (most intense) with regard to the listed properties.

	Colour intensity	Thickness	Firmness	Floury	Overall intensity	Off-Flavour	Bitterness
Reference	2	2	1	1	1	0	1
Buckwheat (57 %)	6	3	2	3	5	0	3
Faba bean (9 %)	3	3	3	2	2	0	1
Lupin IVV (7 %)	1	4	3	1	2	0	1
Prototype I	4	4	4	1	3	0	2

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Bread

The bread prototypes exhibit similar or slightly lower specific volumes compared to the reference. Also crumb hardness is in a similar range as for the control or slightly higher. Interestingly, measurements of crumb hardness on day 5 after baking reveal a similar staling behaviour of the prototypes as for the reference. A similar overall quality regarding technological characteristics and staling behaviour was reached. All prototypes were described as plain in taste. Only slight beany or green notes were perceived in after taste.

Pasta

Interestingly, the reference pasta was characterised by a softer and stickier texture than the prototype. As an “al dente” like firmness is desired for pasta, the prototype exhibits a better quality than the reference. The addition of the PR ingredients also increased tensile strength compared to the reference. Considering consumer’s perspective, a low cooking loss is desirable. The prototype’s cooking loss is in the same range as the reference pasta.Sensory evaluation of the prototype reveals a higher colour intensity and thickness compared to the reference, but also a better firmness. No off-flavour was perceived and the floury mouthfeel was not more pronounced than for the reference.

3.2 Porridge, cookies and extruded snacks (MAKERERE)

a) Composite porridge flours

Characterisation of the developed composite porridge flours

The proximate composition of the cooking and instant porridge flours was determined on dry matter basis (AOAC, 2012). Moisture content was determined by oven drying overnight at 100 °C. Protein content was determined using the Kjeldahl method, % fat using the Soxhlet method, and % fiber content was determined by the fibertecmethod using a fiber analyser. Total ash content was determined by igniting a dry ground sample at 550 °C for 8 hours and gross energy using the oxygen bomb calorimetry (Gallenkamp Auto bomb, UK) (Table 15).

Table 15– Proximate composition of porridge flours

Parameter	Composite porridge flour type	
	Raw (fast cooking)	Extruded (instant)
Moisture (%)	9.59 ± 0.19[b]	5.32 ± 0.11[c]
Gross energy (kcal/100 g)	391.14 ± 27.04[a]	390.19 ± 8.53[a]
Crude Protein (%)	17.60 ± 0.08[b]	17.50 ± 0.58[b]

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Crude Fat (%)	4.90 ± 0.05^{c}	2.24 ± 0.03^{d}
Crude Fiber (%)	5.27 ± 0.03^{a}	4.29 ± 0.20^{b}
Total Ash (%)	2.53 ± 0.10^{b}	2.56 ± 0.13^{b}

In vitro protein digestibility was determined using a method described by Axtell *et al*. (1981) with modifications by Mertz *et al*. (1984) (table 16).

Table 16 – Protein digestibility of porridge flours

Flour type	Protein content (%)	Digestibility (%)
Raw (for cooking)	17.60 ± 0.08^{b}	60.54 ± 2.08^{a}
Extruded (instant)	17.50 ± 0.58^{b}	74.38 ± 1.20^{b}

Extrusion significantly ($P < 0.05$) increased protein digestibility of the flours; from 60.54 % in raw flour to 74.38 % in extruded flour. The increase in protein digestibility could be attributed to reduction in anti-nutrient factors, which are sensitive to high temperatures. Increase in protein digestibility may also be attributed to breakdown of structural proteins, including enzyme inhibitors and lectins, which are protein in nature.Total iron and zinc contents of raw and extruded composite flours were analysed following a method described by Okalebo*et al*. (2002) (table 17).

Table 17 – Total iron and zinc contents of raw and extruded composite flours

Mineral	Flour type	
	Raw flour (mg/Kg)	Extruded flour (mg/Kg)
Zinc	13.47 ± 0.99^{a}	18.37 ± 0.51^{b}
Iron	53.30 ± 3.60^{a}	114.59 ± 2.56^{b}

Values are means ± SD (**n** = 3). Values in the same row with different superscripts are significantly ($P \leq 0.05$) different.

After extrusion, both the zinc and iron contents of the raw flour significantly ($P < .05$) increased. As much as the extrusion increased mineral extractability, the increase in iron content was far more than expected. Iron and zinc extractability were determined using a method described by Duhan*et al*. (2002), with slight modifications (table 18). Zinc and iron extractabilities of extruded flour were significantly ($P < 0.05$) higher than that of raw flour.

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Table 18 – Iron and Zinc extractability of porridge flours

Flour type	Mineral (%)	
	Zinc (%)	Iron (%)
Raw (for cooking)	36.95 ± 2.85[a]	31.21 ± 3.13[c]
Extruded (instant)	64.95 ± 2.97[b]	78.50 ± 0.82[d]

Values are means ± SD (**n** = 3). Values in the same column with different superscripts are significantly (**P** ≤ 05) different.

Physical and functional properties of porridge flours

Bulk density, water absorption capacity and oil absorption capacities were determined using methods described by Onwuka (2005). Dispersibility was determined using a method described by Kulkarni and Ingle (1991). Swelling capacity was determined using a method described by Bamidele *et al*. (2015). Significant ($P < 0.05$) differences were observed in all of the functional properties that were studied (table 19). Extruded flour had a significantly higher (71 %) dispersibility compared to that of raw flour (40.67 %).Water absorption capacity was significantly higher in extruded flour (328.78 %) compared to raw flour (151.61 %) (Table 19). During extrusion, the high temperature treatment caused starch, a major component of the composite flour, to gelatinise and increasing the swelling capacity. This may explain the high water absorption capacity observed in extruded flours. Oil absorption capacities of the flours were generally high. Extruded flour however had a significantly higher oil absorption capacity (209.27 %) compared to raw flour (142.57 %). Oil absorption capacity (OAC) measures the ability of food material to absorb oil. The relatively higher OAC observed in extruded flour may be attributed to the hydrophobic character of protein in the flour after extrusion.

Table 19– Bulk density, disperisibility, water absorption capacity, swelling capacity, solubility and oil absorption capacities of porridge flours.

Functional property	Flour type	
	Raw (for cooking)	Extruded (instant)
Bulk density (g/ml)	0.73 ± 0.02[a]	0.51 ± 0.01[b]
Dispersibility (%)	40.67 ± 1.53[c]	71.00 ± 3.00[d]
Water Absorption Capacity (%)	151.61 ± 12.13[e]	328.78 ± 15.88[f]
Swelling Capacity	2.54 ± 0.01[c]	2.04±0.07[a]
Solubility (%)	0.11 ± 0.01[e]	0.40 ± 0.03[b]
Oil absorption capacity (%)	142.57 ± 15.02[d]	209.27 ± 6.32[c]

Mean values with different superscripts within a row are significantly different (P< .05).

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Pasting properties of porridge flour samples

Pasting properties of the samples were assessed using a Rapid Visco-Analyser (Model RVA series 4; Newport Scientific Pty Ltd., Warriewood, Australia) with Thermocline for Windows software. Significant differences ($P < 0.05$) were observed in the different pasting characteristics of the flours (table 20).

Pasting temperature of the flours ranged from 91.07 °C to 53.20 °C, being significantly lower for extruded flour compared to raw flour. Raw flour has a relatively higher content of complex carbohydrates and resistant starch (than extruded flour), which requires much higher temperatures to breakdown.

Table 20 – Pasting properties of porridge flour samples

Pasting properties	**Flour type**	
	Raw (for cooking)	**Extruded (instant)**
Pasting Temperature (°C)	91.07 ± 0.45^{c}	53.20 ± 0.37^{d}
Peak Time (mins)	6.24 ± 0.10^{a}	1.07 ± 0.00^{d}
Peak Viscosity (cP)	886 ± 42.23^{a}	543 ± 43.27^{b}
Trough Viscosity (cP)	826 ± 9.54^{c}	56.33 ± 3.21^{d}
Breakdown (cP)	60 ± 33.6^{a}	486.67 ± 40.38^{c}
Setback Viscosity (cP)	1304 ± 20.66^{c}	26.67 ± 0.58^{d}
Final Viscosity (cP)	2130 ± 11.53^{b}	83 ± 3.61^{c}

Values are means ± SD (**n** = 3). Values in the same row with different superscripts are significantly (**P**≤.05) different.

On the other hand, the high extrusion temperatures aid in hydrolysis and breakdown of these complex sugars to simpler sugars could possibly explain the low pasting temperature observed in extruded flour. Extruded flour had a significantly ($P < 0.05$) lower peak time (1.07 min) compared to that of raw flour (6.24 min) (Table 20). During extrusion processing, starch granules are pre-gelatinised and when these granules are heated in water, they rapidly absorb moisture, swell and rapture losing their crystalline structure with amylose molecules leaching out. The extruded flour thus absorbed water, instantly became viscous achieving its peak viscosity in a shorter time. This could possibly explain the relatively shorter peak time that was observed in the pasting behaviour for the extruded flour. The peak viscosity was significantly ($P < 0.05$) higher in raw flour (886 cP) than in extruded flour (543 cP). Trough viscosity was significantly ($P < 0.05$) higher in raw flour (826 cP) than in extruded flour (56.33 cP) (Table 20).

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Extruded flour exhibited a significantly ($P < .05$) higher breakdown viscosity (486.67 cP) compared to raw flour (60 cP). Setback viscosity was significantly ($P < 0.05$) higher in raw flour (1304 cP) than in extruded flour (26.67 cP). Furthermore, the final viscosity of raw flour was significantly higher (2130 cP) than that of extruded flour (83 cP). The final viscosity is the ability of starch to form a viscous paste. Final viscosities of both the raw and extruded flours were higher than all the other pasting viscosities determined. The instant porridge flour was analysed by the Uganda National Bureaux of Standards (UNBS) and met the Uganda standard specifications for composite porridges (table 21).

Table 21 - Quality parameters of the instant porridge flour

Parameter	Result	Specification	Interpretation
Moisture content (%m/m)	5.4	13.5 (Maximum)	Pass
Total ash on dry basis (%m/m)	2.8	4.0 (Maximum)	Pass
Acid insoluble ash (%m/m)	0.13	0.4 (Maximum)	Pass
Fatty acidity (mg KOH/100g)	30	50 (Maximum)	Pass

Effect of extrusion processing on the phytochemical compounds in the composite flours

The effect of extrusion processing on the phytochemical compounds in the composite flours was assessed. Results revealed that total phenolics, total flavonoids and vitamin A contents were significantly higher in extruded porridge flours compared to cooking porridge flours. However, total antioxidant activity was higher in the cooking porridge flours, compared to the extruded porridge flours (Table 22).

Table 22 – Phytochemical properties of porridge flours

Flour type	**Total Phenolics Content** (mg·GAE/100 g dry flour)	**Total Flavonoids Content** (mg·QE/100 g dry flour)	**Vitamin A** (RAE)	**Total Antioxidant Activity** (%)
Raw (for cooking)	317.50 ± 10.31[a]	835.07 ± 35.57[a]	297.02 ± 8.54[a]	55.63 ± 2.05[a]
Extruded (instant)	393.33 ± 7.10[b]	1116.23 ± 39.02[b]	101.91 ± 1.50[b]	1.69[b]

Microbial quality of instant porridge flour

Microbial quality, safety and stability of the instant porridge flour was assessed (table 23). It was found to meet the Uganda's standard specification for composite flours and remain stable for two months so far (shelf-stability studies are still on-going and longer shelf stability is being investigated).

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Table 23 – Microbial quality of instant porridge flour

Test parameter	Results	Specification	Interpretation
Total coliforms (cfu/g)	ND	NA	Passed
E. coli (cfu/g)	ND	Shall not be detected	Passed
Yeasts and moulds (cfu/g)	ND	1,000 (Max)	Passed
Total plate count (cfu/g)	1.5×10^2	100,000 (Max)	Passed
Staphylococcus aureus (cfu/g)	ND	Shall not be detected	Passed
Salmonella	Absent	Shall not be detected	Passed

b) High protein cookies

High-protein gluten-free cookies

Three prototypes namely: A (62 % bean & 38 % amaranth), B (62 % Faba bean & 38 % protein enriched amaranth), C (35 % soy, 25 % millet, 25 % maize, 8 % beans and 7 % amaranth) were developed. To determine the acceptability of high protein cookies, a sensory evaluation session was held in the sensory laboratory at the School of Food Technology, Nutrition and Bio-engineering in Makerere University. The cookies were evaluated by a panel of 30 panelists, with a range of both male and female aged 18-50 years, and familiar with cookie characteristics. The panelists evaluated the products' sensory characteristics including: appearance, smell, taste, texture, color, aftertaste, flavor and overall acceptability. A 9-point hedonic scale was used to determine acceptability scores of the attributes tested (1 = dislike extremely, 9 = Like extremely) (table 24).

Table 24 - Sensory properties of high protein gluten free cookies.

Attribute	Sample sensory scores		
	A	B	C
Appearance	$6.8{\pm}1.49^{a}$	$7.3{\pm}1.20^{a}$	$5.9{\pm}1.75^{b}$
Color	$7.0{\pm}1.21^{a}$	$7.2{\pm}1.38^{a}$	$6.3{\pm}1.80^{b}$
Texture	$6.5{\pm}1.52^{a}$	$5.7{\pm}2.00^{b}$	$6.4{\pm}1.58^{a}$
Aroma	$6.5{\pm}1.38^{a}$	$5.9{\pm}1.87^{a}$	$6.7{\pm}1.59^{a}$
Taste	$6.7{\pm}1.61^{ab}$	$6.0{\pm}1.75^{a}$	$7.2{\pm}1.51^{b}$
Mouth feel	$6.5{\pm}1.27^{a}$	$5.8{\pm}1.75^{b}$	$7.1{\pm}1.30^{a}$
Aftertaste	$6.5{\pm}1.49^{a}$	$5.5{\pm}2.00^{b}$	$7.1{\pm}1.35^{a}$
Overall acceptability	$6.8{\pm}1.34^{a}$	$6.2{\pm}1.59^{b}$	$7.2{\pm}1.32^{a}$

Values are means ± SD (n= 3). Values in the same row with differentsuperscripts are significantly (P≤.05) different.

Cookie C was generally most liked (52.4 % of the 30 panellists) with an overall acceptability of 7.2 ± 1.32, corresponding to like moderately on the hedonic sensory scale. The preference for this

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cookie can be attributed to a more appealing taste and mouthfeel, which were on average scored at 7.2 ± 1.51 and 7.1 ± 1.30, respectively, on the hedonic scale. Cookie A was liked moderately (35.7 % of the panellists) while the cookie B was liked least with a mean overall acceptability of 6.2 ± 1.59, corresponding to like slightly on the hedonic scale. The low acceptability of cookie B (11.9 % of the panellists) could be attributed to the relatively undesired after-taste and mouthfeel properties of the raw flours.

High protein wheat-based cookies

Three prototypes A, B and C incorporating faba bean and protein enriched amaranth flour or ordinary bean and amaranth flours were developed. A mini sensory evaluation test (using 10 panellists) revealed that utilisation of the protein enriched flours slightly improved the aroma, flavour, colour and taste but made the cookies hard, thereby reducing their textural acceptability. Replacement of the protein enriched flours as evidenced in B and C improved their texture (to softer and crunchier) thus increased their acceptability. Cookie B tasted better and was preferred compared to A and C in both cases. The nutritional composition of the developed gluten-free and wheat-based cookies are reported in Table 25.

Table 25– Nutritional composition of gluten-free and wheat cookies

	Sample	Fiber	Protein	Fat	Moisture
Gluten-free	A	3.84±0.42[a]	11.14±0.67[a]	21.70±0.29[a]	7.22±0.12[a]
	B	3.85±0.19[ab]	23.75±0.13[b]	22.41±0.39[b]	7.33±0.29[ab]
	C	3.15±0.24[c]	12.27±0.37[c]	17.93±0.03[c]	6.56±0.03[c]
Wheat-based	A	1.90±0.48[d]	18.08±0.38[d]	14.43±0.19[d]	4.85±0.06[d]
	B	0.82±0.23[e]	24.83±0.99[e]	16.77±0.53[e]	5.71±0.07[e]
	C	3.00±0.47[cf]	18.45±0.44[f]	19.48±0.22[f]	2.72±0.34[f]

Product certification

A draft standard (DUS 1852 – Pulses and pulse products – specification), to accommodate the developed cookies and other similar products, was developed in collaboration with the Uganda National Bureau of Standards (UNBS). Availability of a standard of identity assures consumer safety and confidence, thus, facilitates commercialisation of the products.

c) Extruded Snacks

Raw materials and their pre-processing

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Bio-fortified beans (ROBA1 variety), rice, grain amaranth, millet, maize, peanuts and soybean were procured from local markets. The produce were first cleaned by manual sorting and then milled by using a commercial hammer mill and developed into fine flour.

Processing of extruded products (cereal and snack)

The breakfast cereal and snack products were processed by following the protocols described below in Figure 8 and 9.

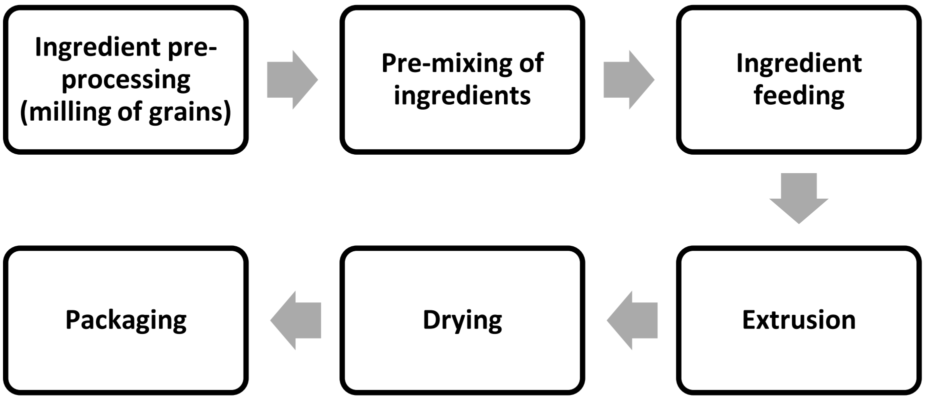

Figure 7 – Processing Protocol for breakfast cereal products

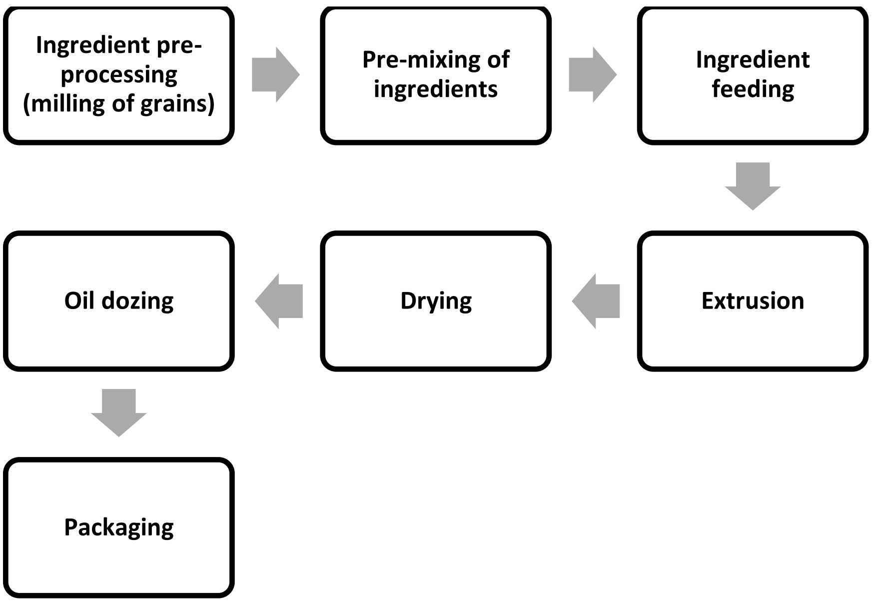

Figure 8 - Processing Protocol for snack products

Extruder parameters during extruded product processing

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AMC series Double-Screw Extruder (Jinan Americhi Machinery & Equipment Co; Ltd, Jinan, Shandong, China) was used. To obtain nutritionally balanced extruded products, mild processing conditions (described in the table 26) were used.

Table 26. Extruder settings used for extrusion of breakfast cereal and snack products.

Specification	Settings and Units
1st chamber Heater	60 ^{0}C
2nd chamber Heater	130 ^{0}C
3rd chamber Heater	150 ^{0}C
Speeds	
Filler speed	30 HZ
Main Extruder speed	35 HZ
Cutter speed	30 HZ
Die diameter	4 Cm

Research design for Optimisation of bean flour

Since beans naturally have a high content of anti-nutrients such as total phenolics and phytic acid, it was necessary to optimize bean flour extrusion conditions, with the aim of reducing anti nutrient content, which would otherwise reduce mineral extractability and protein digestibility. Sample moisture content, 3rd extruder chamber heater temperature and screw speed were varied at three levels to accommodate extreme conditions as shown in Table 27.

Table 27 – Processing variables and their levels in the D-Optimal design

Variables	Coded variables			
	Symbol	**-1**	**0**	**1**
Bean flour moisture content (%)	X_1	10	15	20
Screw speed (Hz)	X_2	30	35	40
3rd chamber extruder heater temperature (° C)	X_3	130	150	170

Effect of extrusion conditions on nutritional quality of the extruded bean flour

The extruded bean flour's responses (dependent variables: protein digestibility (PD), iron extractability (Fe Ex.), zinc extractability (Zn Ex.), total phenolics (TP) and phytic acid (PA)) varied

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with the extrusion conditions applied, i.e. moisture content, screw speed and 3rd heater temperature (table 28). Anti-nutrient content reduced with increasing moisture content and increasing 3rd extruder heater temperature while increased with increasing screw speed. Increasing moisture content and screw speed increased mineral extractability, while reduced with increasing 3rd extruder chamber heater temperature.

Table 28 – D- Optimal design arrangement and responses

RUN	MC (%)	SS (Hz)	Temp (°C)	PD (%)	Fe Ex. (%)	Zn Ex. (%)	TP (%)	PA (%)
1	10	30	130	60.31	63.45	58.4	0.338	0.0360
2	10	30	130	59.93	62.79	57.04	0.343	0.0365
3	10	30	170	48.53	40.67	40.09	0.153	0.0391
4	10	40	130	57.5	56.78	53.54	0.368	0.0373
5	10	40	170	50.11	41.11	48.09	0.148	0.0387
6	20	30	130	63.6	68.01	63.66	0.293	0.0359
7	20	30	170	58.09	55.61	52.02	0.356	0.0358
8	20	40	130	61.87	65.76	60.24	0.303	0.0362
9	20	40	170	64.23	72.18	64.77	0.287	0.0346
10	20	40	170	64.81	71.97	65.51	0.282	0.0345
11	20	35	150	82.21	80.41	87.73	0.074	0.0306
12	10	35	150	66.79	72.31	68.18	0.238	0.0306
13	15	30	150	75.09	77.92	84.19	0.123	0.0327
14	15	40	150	69.67	76.48	78.64	0.187	0.0335
15	15	35	130	67.33	74.48	71.72	0.222	0.0359
16	15	35	170	72.81	76.62	82.1	0.154	0.0334
17	15	35	150	79.77	84.27	87.86	0.078	0.0273
18	15	35	150	81.08	83.53	88.18	0.081	0.0291
19				36.06	38.22	24.57	0.683	0.0436
20				64.36	61.29	67.33	0.402	0.0366

MC= Moisture Content, SS= Screw Speed, Temp =3rd heater temperature, PD = Protein digestibility, Fe Ex. = Iron extractability, Zn Ex. = Zinc extractability, TP = Total Polyphenols, PA = Phytic acid

Predictive models

The variables were expressed individually as a function of the independent variables. The data were fitted to the following second-order approximation model (Equation 1):

$$Y = B_O + \sum_{i-1}^{k} B_i X_i + \sum_{i-1}^{k} B_{ii} X_i^2 + \sum_{\substack{i-1 \\ i<j}}^{k} B_{ij} X_i X_{j+} \, \varepsilon \text{ ...... (Equation 1)}$$

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Extrusion parameters were found to influence nutritional properties and the relationships could be represented by the predictive models shown below.

Protein digestibility

Moisture content (X_1) had positive linear effect while screw speed (X_2) and 3rd heater temperature (X_3) has negative linear effect on protein digestibility (equation 2). Moisture content with screw speed, moisture content with 3rd heater temperature and screw speed with 3rd heater temperature had positive interactive effects on protein digestibility. All parameters had negative quadratic effects on protein digestibility. The quadratic effect by screw speed and 3rd heater temperature was significant ($p < 0.05$) and the model was significant ($p = 0.000433$).

$$Protein\ digestiblity = 79.89 + 4.64X_1 - 0.27X_2 - 1.73X_3 + 0.73X_1X_2 + 2.02X_1X_3 + 1.55X_2X_3 - 5.11X_1^2 - 7.24X_2^2 - 9.55X_3^2$$

$R^2 = 0.94$ … … …. *Equation 2*

Iron extractability

While moisture content and screw speed had positive linear effects on iron extractability, 3rd heater temperature had negative linear effect on iron extractability (equation 3). Linear effects by moisture content and 3rd heater temperature were significant at $p = 0.00275$ and $p = 0.007$, respectively. Moisture content with screw speed, moisture content with 3rd heater temperature and screw speed with 3rd heater temperature had positive interactive effects on iron extractability. All parameters had significant ($p < 0.05$) quadratic effect on iron extractability. The model was significant at $p = 0.00135$.

$$Fe\ extractability = 84.85 + 6.94X_1 + 0.84X_2 - 4.06X_3 + 2.49X_1X_2 + 3.99X_1X_3 + 3.17X_2X_3 - 8.96X_1^2 - 8.12X_2^2 - 9.77X_3^3$$

$R^2 = 0.96$ … … …. *Equation 3*

Zinc extractability

Increasing moisture content and screw speed increased zinc extractability while reduced with increasing 3rd heater temperature. Increase in moisture content significantly ($p = 0.004$) increased

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zinc extractability (equation 4). Moisture content with screw speed, moisture content with 3rd heater temperature and screw speed with 3rd heater temperature had positive interactive effects on zinc extractability. All parameters had significant ($p < 0.05$) quadratic effect on zinc extractability. The model was significant at $p = 0.0004$.

$$Zn\ extractability = 89.45 + 5.96X_1 + 0.64X_2 - 2.1X_3 + 0.71X_1X_2 + 2.02X_1X_3 + 3.57X_2X_3 - 12.22X_1^2 - 8.76X_2^2 - 13.26X_3^2$$

$$R^2 = 0.94 \ldots \ldots \ldots .Equation\ 4$$

Total polyphenols

The total polyphenol content of extruded bean flour reduced with increasing moisture content and increasing 3rd heater temperature, while it increased with increasing screw speed (equation 5). Moisture content with screw speed, moisture content with 3rd heater temperature and screw speed with 3rd heater temperature had positive interactive effects on total polyphenol content of the extruded bean flour. All parameters had positive quadratic effect on total polyphenol content.

$$Total\ polyphenols = 0.097 - 0.018X_1 + 0.026X_2 - 0.099X_3 + 0.0074X_1X_2 + 0.023X_1X_3 + 0.0079X_2X_3 + 0.049X_1^2 + 0.026X_2^2 + 0.045X_3^2$$

$$R^2 = 0.92 \ldots \ldots \ldots .Equation\ 5$$

Phytic acid

Increasing bean moisture content and increasing 3rd heater temperature reduced phytic acid content of the extruded bean flour while increasing screw speed increased phytic acid content (equation 6). Significant reduction ($p = 1.18\text{x}10^{-5}$) in phytic acid content was realised with increasing 3rd heater temperature. Moisture content and screw speed had a negative interactive effect on phytic acid content while moisture content with 3rd heater temperature and screw speed with 3rd heater temperature had positive interactive effects on phytic acid content of the extruded bean flour. All parameters had positive quadratic effect on phytic acid. The model was significant at $p = 0.000677$.

$$Phytic\ acid = 0.0298 - 0.0002X_1 + 0.000796X_2 - 0.0037X_3 - 5.1 \times 10^{-5}X_1X_2 + 2.14 \times 10^{-5}X_1X_3 + 0.00048X_2X_3 + 9.38 \times 10^{-6}X_1^2 + 0.0025X_2^2 + 0.00073X_3^2$$

$$R^2 = 0.94 \ldots \ldots \ldots .Equation\ 6$$

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Optimum extrusion parameters for ROBA1 bean flour

Solution 1 (highlighted) was selected as the optimum extrusion condition for bean flour from a number of optimal solutions (table 29) as these conditions resulted into the highest desirability (0.94) and most desired levels of responses. These were; 17 % moisture content, 34.8 Hz screw speed and 156 °C third extruder chamber heater temperature (Table 27). These extrusion parameters resulted in bean flour with 79.7 % digestible protein, 84.4 % extractable iron, 88.2 % extractable zinc, 0.073 % total polyphenol content and 0.029 % phytic acid content.

Table 29 – Optimal solutions for extrusion of nutritious bean flour

Soln. No.	MC (%)	SS (Hz)	Temp (°C)	PD (%)	Fe Ex. (%)	Zn Ex. (%)	TP (%)	PA (%)	Desirability
1	17.015	34.791	156.028	79.748	84.431	88.17991	0.073606	0.028635	0.946209
2	17.002	34.789	156.044	79.740	84.421	88.17997	0.073475	0.028632	0.946209
3	17.009	34.796	156.043	79.743	84.427	88.17939	0.073558	0.028633	0.946209
4	17.012	34.784	156.025	79.747	84.427	88.17955	0.073562	0.028634	0.946209
5	17.019	34.800	156.034	79.749	84.436	88.17994	0.073664	0.028635	0.946209
6	17.002	34.800	156.057	79.738	84.423	88.17964	0.073498	0.028632	0.946209
7	17.022	34.788	156.015	79.754	84.436	88.17953	0.073672	0.028636	0.946208
8	16.998	34.783	156.044	79.738	84.416	88.17937	0.07342	0.028632	0.946208

MC= Moisture Content, SS= Screw Speed, Temp =3rd heater temperature, PD = Protein digestibility, Fe Ex. = Iron extractability, Zn Ex. = Zinc extractability, TP = Total Polyphenols, PA = Phytic acid

Sensory acceptability of the products processed under the optimised extrusion parameters

To determine the acceptability of breakfast cereal and snack products, a sensory evaluation session was held in the sensory laboratory at the School of Food Technology, Nutrition and Bio-engineering in Makerere University. The breakfast cereals and snacks were evaluated in two separate sessions with 51 and 55 panellists, respectively, including both male and female, aged 18-50 years, and people familiar with cereal and snack characteristics. The panellists evaluated the products' sensory characteristics of the products including: appearance, smell, taste, texture, color, aftertaste, flavor and overall acceptability, using 9-point Hedonic scales on which a score of 1 = dislike extremely while a score of 9 = like extremely. The cereal products were evaluated after addition of milk.

Overall, acceptability for Robasnacksalty (50 % bean, 43 % maize, 2 % salt, 5 % soy oil) was rated more favorably than Robasnack1 (45 % bean, 40 % maize, 10 % sugar and 5 % soy oil) and Robasnack6 (35 % bean, 30 % grain amaranth, 20 % maize, 10 % sugar and 5 % soy oil) (Table 30). Acceptability of Robaceral2 (50 % maize, 20 % beans, 20 % soybean, 10 % sugar) was rated more favourably by panellists than for Robacereal1 (40 % beans, 30 % grain amaranth, 20 % rice and 10 % sugar), after adding milk to the cereal. On this basis, therefore, that the snack Robasnacksalty (with

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formulation of 50 % bean, 43 % maize, 2 % salt, 5 % soy oil) and the cereal Robacereal2 (constituting 50 % maize, 20 % beans, 20 % soybean, 10 % sugar) were selected as final products (table 30).

Table 30– Frequency of overall acceptability scores of snack products and of breakfast cereal products after addition of milk.

Product code	Frequency for overall acceptability scores								
	1	2	3	4	5	6	7	8	9
Robasnack1	0	0	0	3	8	16	15	7	6
Robasnacksalty	0	0	0	0	2	13	11	19	10
Robasnack6	0	0	0	5	10	26	10	4	0
Robacereal1	0	0	0	2	8	23	16	1	1
Robacereal2	0	0	0	0	6	7	20	11	7

Iron, zinc and phytate content of the selected products

The selected products Robasnacksalty and Robacereal2 had relatively high contents of iron and zinc and reduced content of phytate (table 31). The high iron and zinc content makes the products suitable to supplementing the diets of children below five years old and women of reproductive age, who are very vulnerable to their deficiencies. The reduced levels of phytate in the products facilitate the bioavailability of the minerals, which would otherwise be complexed, thus, decreasing their bioavailability.

Table 31 – Iron, Zinc and Phytate content of the selected products

Sample code	Iron content (mg)	Zinc content (mg)	Phytate content (%)
Robasnacksalty	7.1	4.23	0.027
Robacereal2	6.3	3.9	0.028

3.3 Extruded cereals (FRAUNHOFER)

a) Thermal Properties

The thermal properties of legume proteins were analysed by differential scanning calorimetry. The denaturing behaviour of proteins can be characterised by the enthalpy of denaturation (ΔH) and the denaturation temperature (Td). Samples were either untreated or pre-heated to evaluate the effect of temperature on denaturation of the proteins. Both the untreated samples and the samples heated dry at 120 °C were analysed (data not shown). The thermal properties of the differently preheated samples show whether the protein conformation changed due to pre-heating. Numerous studies have shown that specific hardness of the extrudates is highly affected by protein structure and degree of

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denaturation (Wood, 1987; Dahl and Villota, 1991). The endothermic peaks obtained for the untreated legume protein isolates may represent the thermal transition of the partly native protein fraction. The absence of any peaks in the thermograms of preheated blue and white lupin protein isolates may indicate the denaturation of the respective protein fractions. However, the smaller peaks in the thermograms of the preheated faba bean and lentil protein isolates indicate that proteins were only partially denatured by preheating. However, the presence of native proteins in dry heated lentil and faba bean samples requires further studies focusing on protein composition. Heat treatment may also have been too short, as lentil and faba bean contain a higher amount of native proteins than lupin protein isolates (Figure 10).

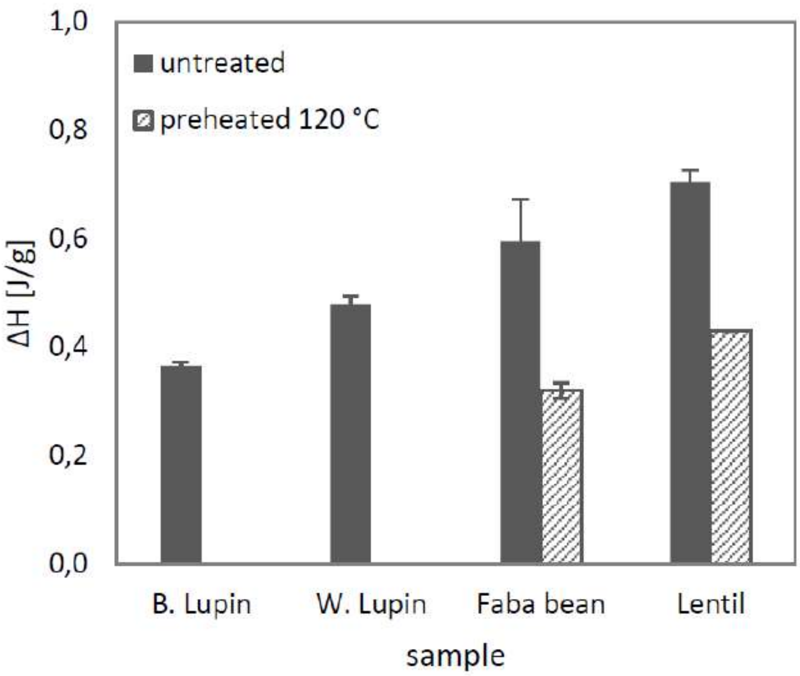

Figure 9 – Denaturation behavior of proteins; ΔH (enthalpy of denaturation) of the untreated samples and the samples dry heated at 120 °C (15 % w/w). The untreated samples had a small ΔH, which was even reduced by pre-heating. Among the four dry heated samples, only faba bean and lentil had a small endothermic peak. Data are shown as means ± SEM.

b) Functional properties

Functional properties and particle size of the protein powders play a large role in extrusion cooking as they influence the processing and texture properties of the extrudates. The functional properties of proteins in food products are strongly affected by their interactions with food ingredients (e.g. water, ions, proteins, fats, carbohydrates) and the environment (e.g. pH value temperature, ionic strength), and therefore reflect their intrinsic properties (composition, amino acid sequence, conformation,

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structure). The analysed functional properties (protein solubility, emulsifying capacity and water binding capacity) and the particle size of legumes and pseudocereals are shown in (table 32).

Table 32 – Functional properties (protein solubility; emulsifying capacity (EC); water binding capacity (WBC)) and particle size (D(0.5)) of legumes protein isolates/concentrates and pseudocereal protein flours. Plant protein samples had a relatively high protein solubility and EC and a small WBC.

Sample	**Protein solubility** [%]	**EC** [ml/g]	**WBC** [g/g]	**D(0.5)** [µm]
Blue Lupin	71.1	530	1.42	n.a.
White Lupin	65.1	245	n.a.	n.a.
Lentil UF	45.9	505	2.50	n.a.
Faba bean	76.1	530	1.06	8.0
Amaranth	38.8	165	1.58	204.0
Buckwheat	60.4	155	4.16	98.5
Quinoa	67.8	185	1.61	210.8

Protein solubility ranged from 60.4 to 76.1 % for all raw materials except for amaranth and lentil with only 38.8 and 45.9 % protein solubility, respectively. The protein solubility depends on the intact secondary and tertiary structure and the pH value. Furthermore, the differences in protein solubility of the protein samples may have resulted in the different processing conditions to enrich the protein. According to the Osborne classification, proteins can be divided into four solubility subtypes: hydrophile albumins, salt-soluble globulins, alcohol-soluble prolamins and insoluble glutelins. (Osbone, 1924) In most cereals, the alcohol-soluble prolamins represent the major storage proteins, but in legumes and pseudocereals the main storage proteins are globulins and albumins (Gorinstein et al., 2002; Haros and Schoenlechner, 2017).

The emulsifying capacity (EC) indicates the volume of oil that can be emulsified by one gram of protein in the solvent (Melde, 2017). Legumes had an EC of around 522 ± 14 ml/g except for white lupin, where only an EC of 245 ml/g was determined. Pseudocereals had an EC of 168 ± 15 ml/g. The EC depends on the particle size of the protein powders. For example, smaller particles can stabilize the interface less strongly than larger particles. In general, legumes have a very high protein solubility and EC. The lower EC for white lupin may be attributed to the manufacturing process, since this raw material has been purchased and not been produced by wet fractionation at the Fraunhofer IVV.

The water binding capacity (WBC) depends on the number of polar sites interacting with H2O (Osen et al., 2014). The amount of exposed polar sites varies due to the basic conditions of the production process that strongly affects the protein structure (Rodríguez-Ambriz et al., 2005; Melde, 2017). Lentil and buckwheat had the highest WBC compared to the other legumes and pseudocereals

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(2.50 and 4.16 %, respectively). The different WBCs can be explained by the different particle sizes. It takes longer for larger particles to get fully wet by a liquid (Crosbie and Ross, 2007). Compared to the other pseudocereals, buckwheat had the smallest particle size with 98.5 μm and the highest WBC. The average particle size of buckwheat was the lowest, followed by amaranth and quinoa. The different particle size distribution of the raw materials can be traced back to different manufacturing processes. The particle size distribution of legumes was not analysed.

c) Rheological and gelatinizing properties

The rheological and gelatinizing properties of the raw materials and selected mixtures of protein isolates with wheat starch (30 % and 50 % protein content) were determined using RVA. With RVA, thermal and mechanical stress can be applied to the samples and simplified conclusions can be drawn about the behaviour of the mixed materials during the extrusion process.

Raw materials***:*** The results of the viscosity analysis for the pseudocereal protein flours are presented in (figure 11). All samples showed a low initial viscosity of around 30 mPas. The viscosities of the samples increased at temperatures around 75 °C. The peak viscosity of the pseudocereals amaranth and quinoa were similar and ranged from 1613 to 2511 mPas, respectively. Buckwheat had a significantly higher peak viscosity of 7588 mPas than the other pseudocereals. The final viscosity of each sample was lower than the related peak viscosity. Furthermore, the viscosity curves of buckwheat and quinoa showed irregularities. In summary, comparison of the protein ingredients revealed that the rheological and gelatinization properties of pseudocereals depends on their functional properties. There is a correlation between the measured peak viscosity and the WBC of the raw materials. The peak viscosity increases with increasing WBC. The correlation is also evident in the viscosity analyses of legumes. The results of the viscosity analysis for the legumes protein isolates/concentrates are presented in figure 11.

The high initial viscosity of lentil refers to the functional properties of proteins, especially to a high protein solubility. The protein solubility of lentil (45.9 %) was lower than it was for the other samples that ranged from 66.1 to 76.1 % (Figure 11). The low peak viscosity of blue lupin, white lupin and lentil can be explained by their low overall starch content. Protein isolates of blue/white lupin and lentil had a protein content of 90 %. Faba bean extract had a protein content of only 67.3 % but had a higher starch content of 5 %. Due to these low starch contents, the viscosities were much lower compared to the viscosities of the pseudocereals. Furthermore, the peak viscosity decreased with decreasing WBC. The surprisingly high viscosity value of white lupin may be attributed to the manufacturing process, since this raw material has been purchased and not been produced by wet

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fractionation at the Fraunhofer IVV. Both the high peak viscosity and the low EC of white lupin protein isolate indicate a small particle size, which is determined by the manufacturing process.

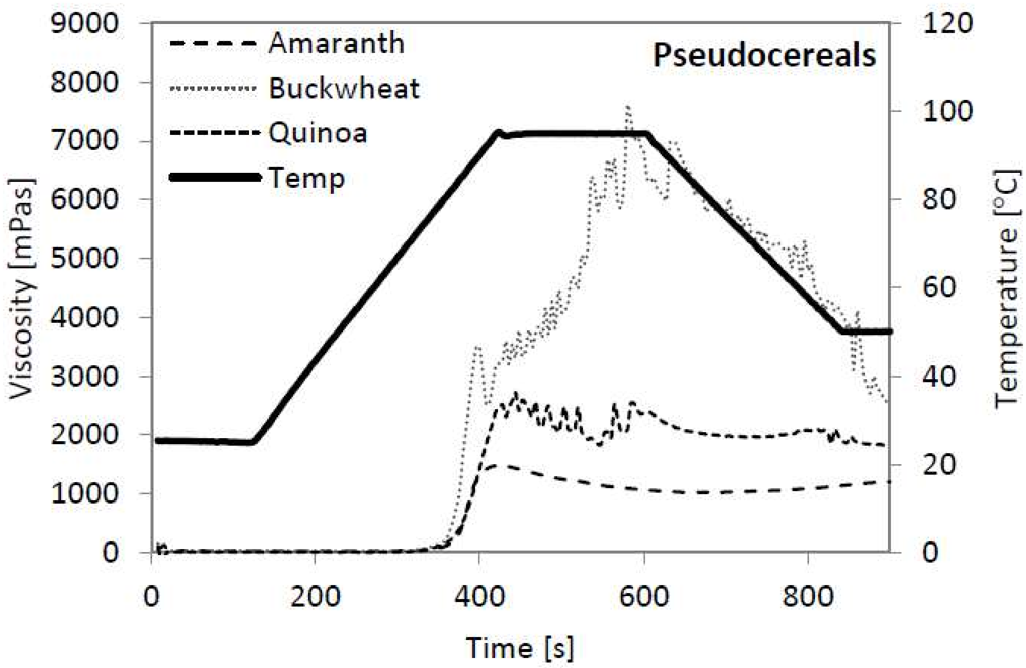

Figure 10 – RVA viscosity curves of pseudocereals (protein flour/ water-mixture in ratio of 20:80)

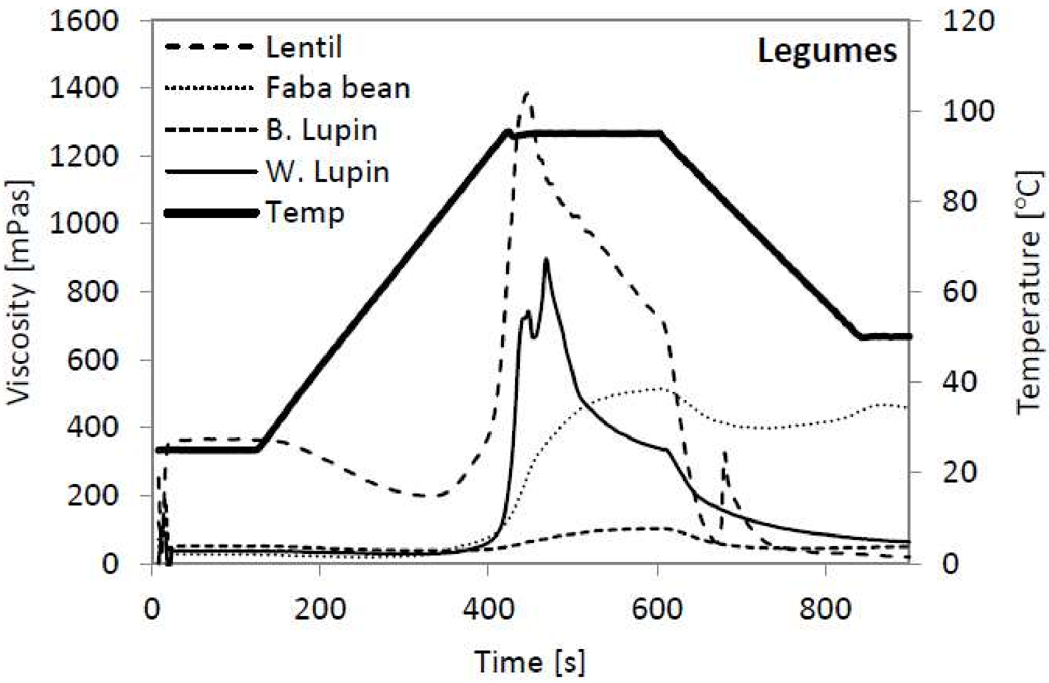

Figure 11 – RVA viscosity curves of legumes (protein isolate/ water-mixture in ratio of 20:80)

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Mixtures: To investigate the effects of protein enrichment on the extrudate characteristics, varying protein concentrations were added to a starch matrix. Wheat starch showed a peak viscosity of 6234 mPa s (data not shown). The viscosity patterns of the legume-starch mixtures with different protein contents are presented in Figure 12. The viscosity curves of the protein isolates/concentrates follow the typical curve of starch-containing products. The samples showed a low initial viscosity of 400 mPa s which increased during heating to 95 °C. The peak viscosities of the samples with 50 % protein content ranged from 2851 mPa s to 1510 mPa s. The peak viscosities of the sample with 30 % protein content were higher and ranged from 4668 mPa s to 2487 mPa s. The peak viscosity of the samples decreased from lentil to white lupin to blue lupin and then to faba bean. After the peak, the viscosity decreased again for a short time period until the final viscosity was reached. Furthermore, the final viscosity of each sample was lower than the related peak viscosity. The viscosity curves of lentil and faba bean exhibit variations or fluctuations and can thus be considered irregular. The rheological results of the mixtures correlate with the results of the raw materials. All samples had the same protein content but differed in starch content. As already mentioned, a higher starch content is expected to result in a higher viscosity during the cooking process.

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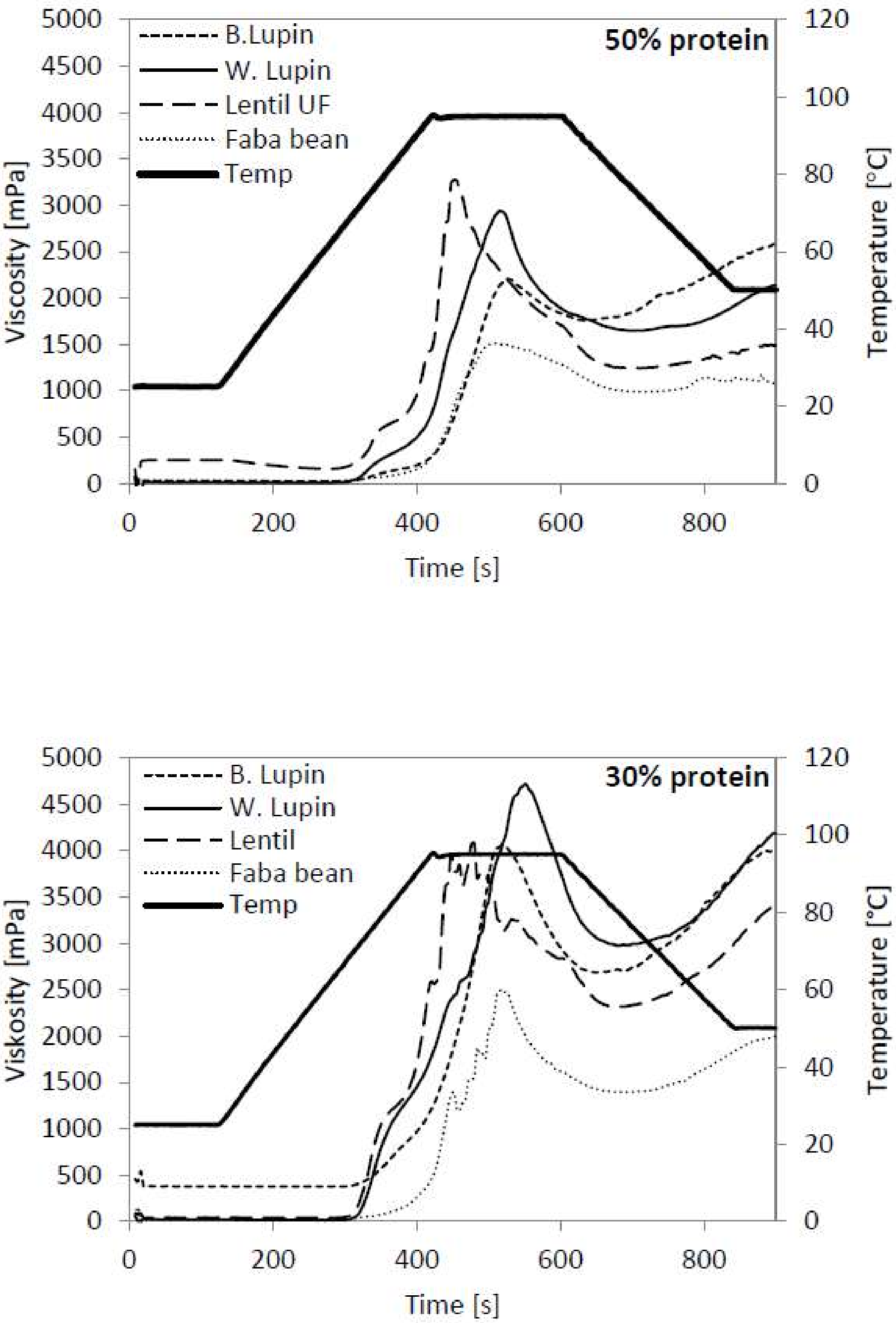

Figure 12 – RVA viscosity curves of legumes-wheat-starch mixtures with a protein content of 50 and 30 % (20 % w/w). Peak viscosity of the raw materials decreased with increasing protein content and decreasing starch content.

d) Expansion

The impact of protein enrichment on extrudate expansion was investigated by adding increasing amounts of blue lupin protein isolate to a wheat starch matrix. The sectional expansion index (SEI) describes the expansion area of the extrudate in the radial direction and is defined as the ratio of the cross-sectional area of the extrudate to the cross-sectional area of the die. Figure 13 shows the expansion for blue lupin extrudates at different barrel temperatures as a function of protein content.

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The SEI for blue lupin starch extrudates decreased with increasing protein content and increased with increasing barrel temperature. Interestingly, SEI for blue lupin extrudates with a 70 and 89% protein content were almost equal for all three barrel-temperatures, whereas SEI for samples with lower protein content varied depending on the barrel temperature.In extrusion cooking, a direct expansion of the product takes place at the die outlet. The product is pressed through the die and it accelerates. During expansion, the boiling point is exceeded so that a part of the water content of the product quickly evaporates and dissolved gases are released. This leads to spontaneous blistering. Due to the thermal and mechanical energy input during extrusion the starch undergoes several changes. Over a critical temperature the starch granule imbibes water and swells, the internal crystalline structure melts (gelatinization), the granule itself breaks down and a continuous gel is formed. The gas bubbles formed during expansion are enclosed and fixed by the viscoelastic melt. Therefore, SEI increased with increasing starch content. The increase in SEI with increasing barrel temperature is due to the evaporation of superheated water and thus the formation of bubbles at the die is increased with rising temperature.

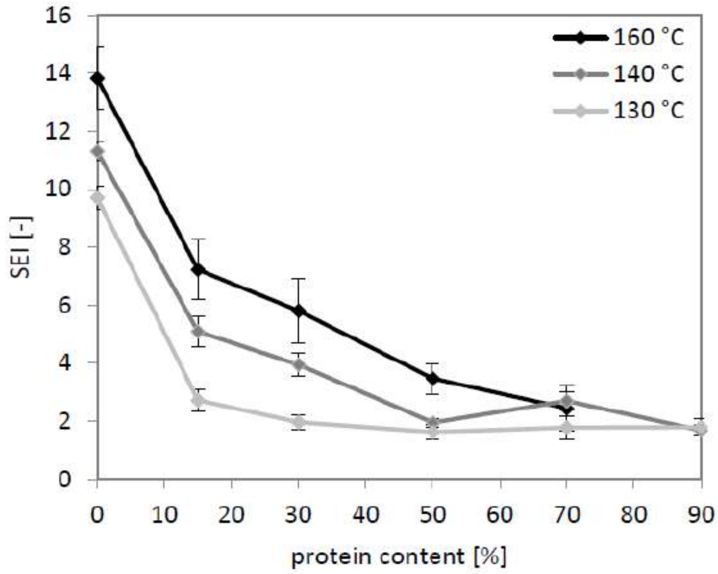

Figure 13 - Influence of protein content on expansion. SEI of blue lupin protein isolates as a function of protein content (0 %; 15 %; 30 %; 50 %; 70 % and 89 %) at 130, 140 and 160 °C and 33% moisture content. The SEI of blue lupin isolates decreased with increasing protein content and increased with increasing barrel temperature. SME for the mixture with 90 % protein content at 160 °C couldn´t be calculated due to technical limitations. Data is shown as means ± SEM. Lines between measurement points are just to guide the eye.

The impact of protein enrichment on specific hardness was investigated by adding increasing amounts of blue lupin protein isolate to wheat starch. Specific hardness describes the maximum force required to break the extrudate and is related to the surface area of the extrudates. Figure 14 shows the specific

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hardness for blue lupin extrudates at different barrel temperatures (130, 140, 160 °C) as a function of protein content.

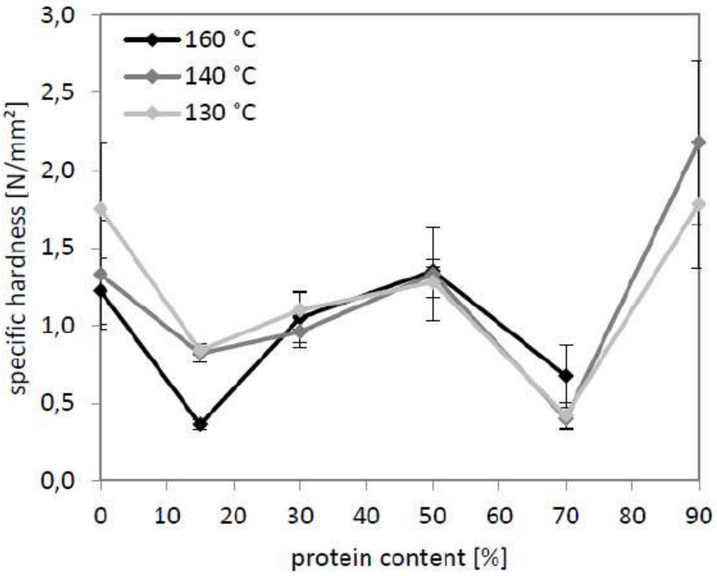

Figure 14 – The effect of blue lupin protein enrichment and cooking temperature on specific hardness at 33 % moisture content (w/w). Specific hardness of blue lupin extrudates shows no specific trend. SME for the mixture with 90 % protein content at 160 °C couldn´t be calculated due to technical limitations. Data is shown as means ± SEM. Lines between measurement points are just to guide the eye.

Specific hardness of blue lupin samples show no specific trend. The addition of 15 % protein to wheat starch reduced the specific hardness from 1.22 ± 0.21 N/mm^{2}to 0.36 ± 0.03 N/mm^2(T = 160 °C). With increasing protein content, the specific hardness increased again to 2.18 ± 0.53 N/mm^2(89 % protein; T = 140 °C), except for a protein content of 70 % where a significant decrease in specific hardness was measured. In addition to protein content, barrel temperature also affected the specific hardness of the extrudates.

Figure 15 shows a visual comparison of the different extrudates and reports the expansion rate with either 30 or 50 % protein content. It is evident that extrudates with higher protein contents were smaller in diameter (Figure 15).

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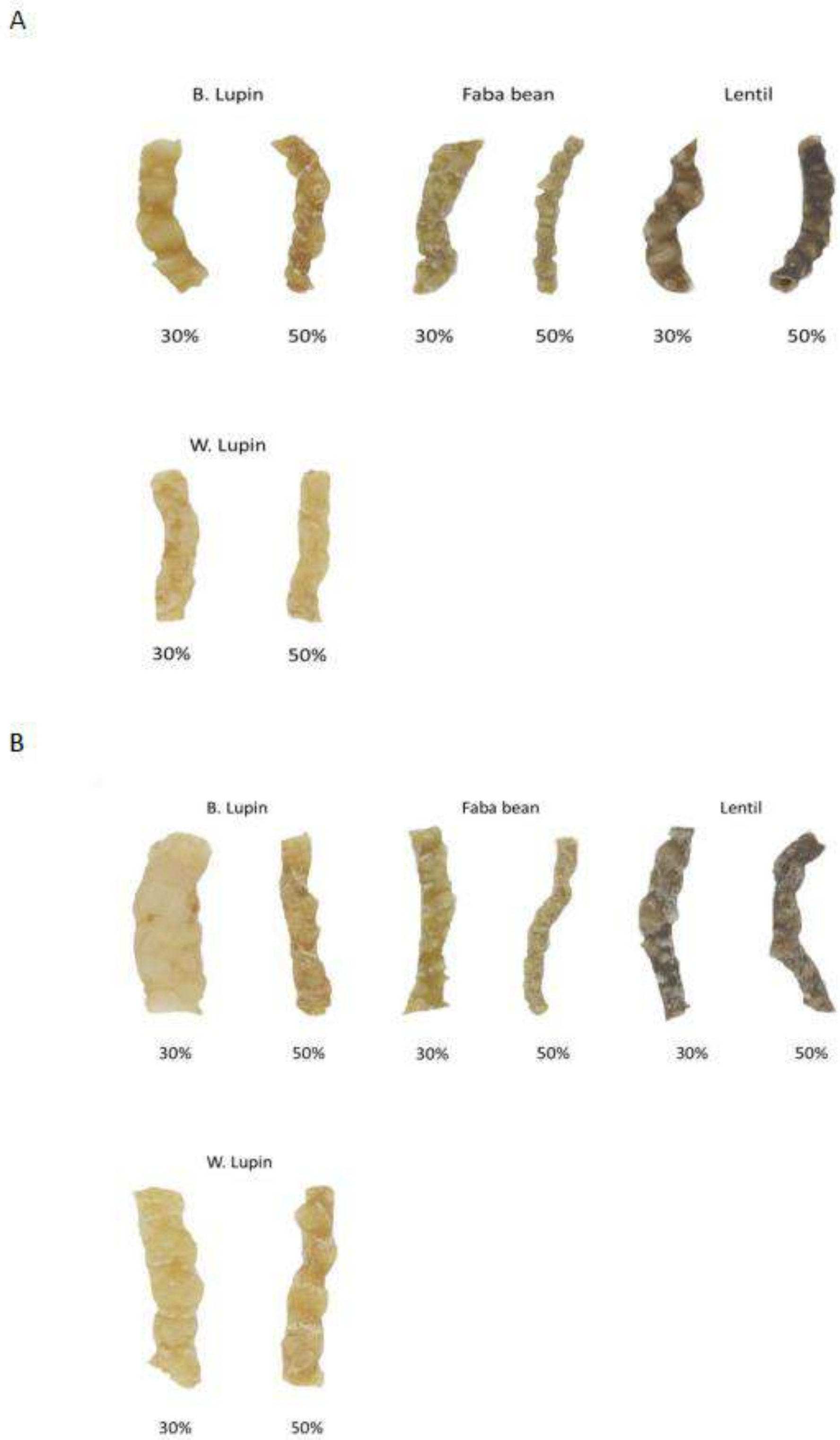

Figure 15 - Extrudate samples: A) Legumes with 30 and 50 % protein; B) Legumes + preheated (30 min at 80 °C and 10 min at 110 °C) +addition (2.0 % lipid and 0.5 % NaCl) with 30 and 50 % protein content (T = 160 °C; w = 33 %). Extrudates with higher protein contents were smaller in diameter.

Figure 16 shows the product texture properties of the samples analysed with the TA. The specific hardness of the samples with 30% protein content increased in the order white lupin, blue lupin, lentil,

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and faba bean. Furthermore, specific hardness for all samples increased with increasing protein content. Texture differences of raw materials can be explained by the different proportion of native proteins, compared to the other raw materials. Furthermore, the lipid content in lentil and faba bean mixtures was higher than in lupin mixtures.

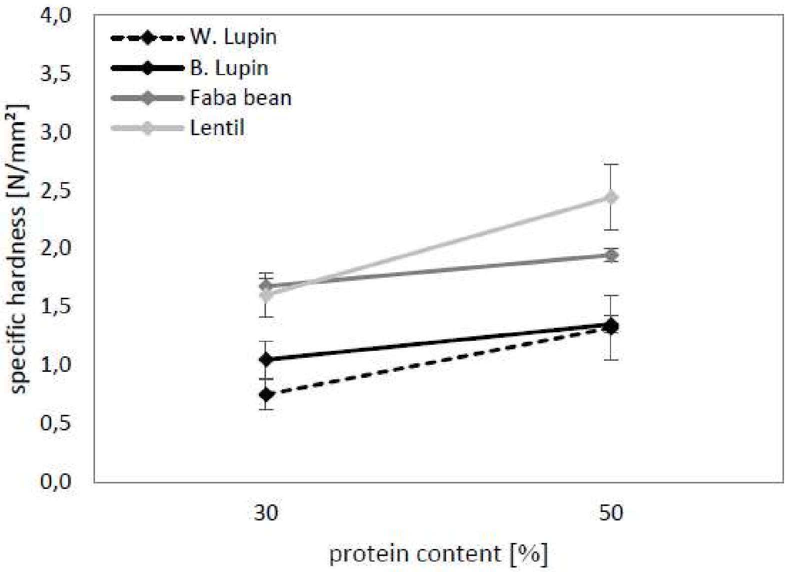

Figure 16 – Specific hardness as a function of protein content for legumes (T = 160 °C; w= 3 %). Data is shown as means ± SEM. Lines between measurement points are just to guide the eye.

e) Sensory properties

To characterise and evaluate the sensory properties of the different samples, descriptive sensory combined with popularity sensory was carried out. For the sensory analysis, extrudates from legume mixtures with 30 and 50 % protein content were used. The following figures (Figure 17, 18, 19 and figure 20) show the results of the sensory evaluation of odour, retronasal odour, taste and texture, respectively.

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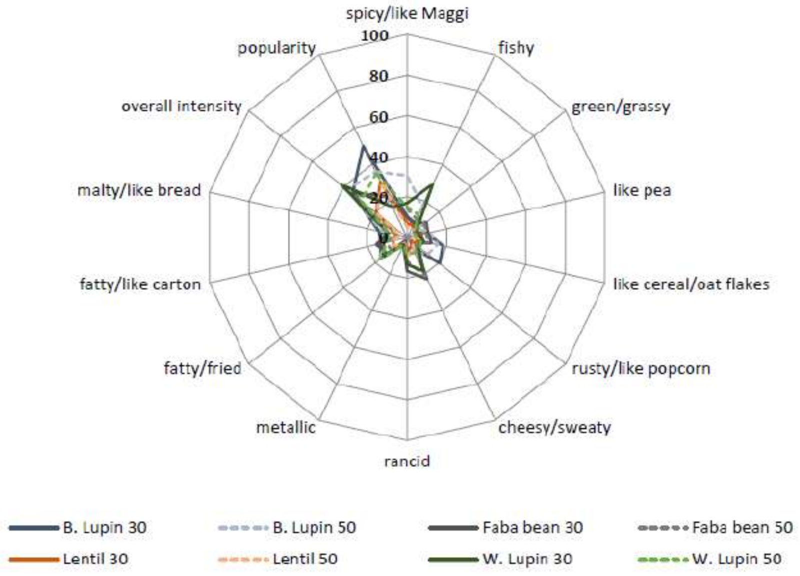

Figure 17 – Odour properties of legume-wheat starch-mixtures with additives (2.0 % lipid and 0.5 % NaCl) (T = 160 °C; w= 33 %).

Figure 17 hows the attributes of odour for the different legume extrudates. All samples showed a low overall intensity (less than 40 scale points (SP). This parameter did not show specific difference between the extrudates with different legume content. A difference can be seen between white and blue lupin. Blue lupin shows a higher intensity in the attributes of rusty/like popcorn and like cereal/oat flakes. White lupin shows a higher intensity in the attributes fishy, rancid, cheesy/sweaty. Faba bean also shows a high intensity in the attributes rancid, cheesy/sweaty. Lipids themselves may not be oxidised during the short residence time in the extruder but can become rancid during storage (Schuchmann and Schuchmann, 2005). However, the attributes fishy and cheesy/sweaty were not detected during the attribute finding for retronasal properties and played a minor role in taste properties. The most popular samples were blue lupin (50 SP), followed by white lupin (35 SP), and closely followed by lentil and faba bean.

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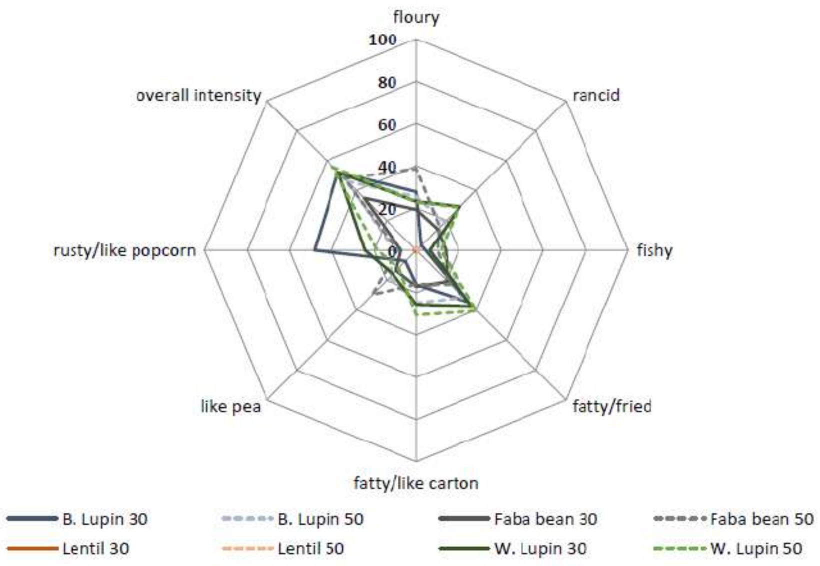

Figure 18 – Retronasal odour properties of legume-wheat starch-mixtures with additives (2.0 % lipid and 0.5 % NaCl) (T = 160 °C; w= 33 %).

Figure 18 shows retronasal odour properties of the different extrudate samples. The overall intensity ranged from 50 to 60 SP and can therefore be considered as relatively strong. This parameter did not show specific difference between the different legume content. A difference can be seen between white and blue lupin. Blue lupin shows a higher intensity in the attributes rusty/like popcorn and white lupin in the attribute rancid. All samples show a high intensity in the attribute fatty/fried.

Figure 19 shows the evaluation of the five taste properties. All extrudates are rated low with less than 30 SP. As one can see the different samples are overall very similar. However, faba bean showed a higher intensity in the attribute bitter (30 SP) (Figure 20). A potential reason for the high rating in bitter taste of faba bean could be the higher content of saponins in faba bean seeds. These bitter substances should have been removed during the raw material preparation process. However, our results show that some bitterness was still apparent.

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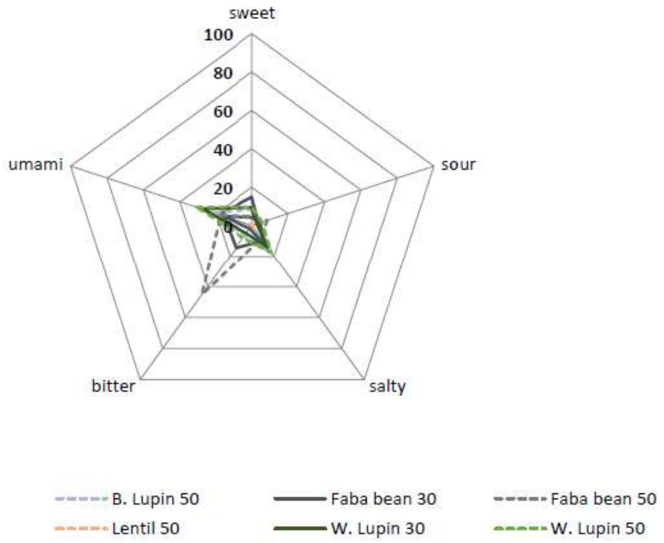

Figure 19 – Taste properties of legume-wheat starch-mixtures with additives (2.0 % lipid and 0.5 %NaCl) (T = 160 °C; w= 33 %).

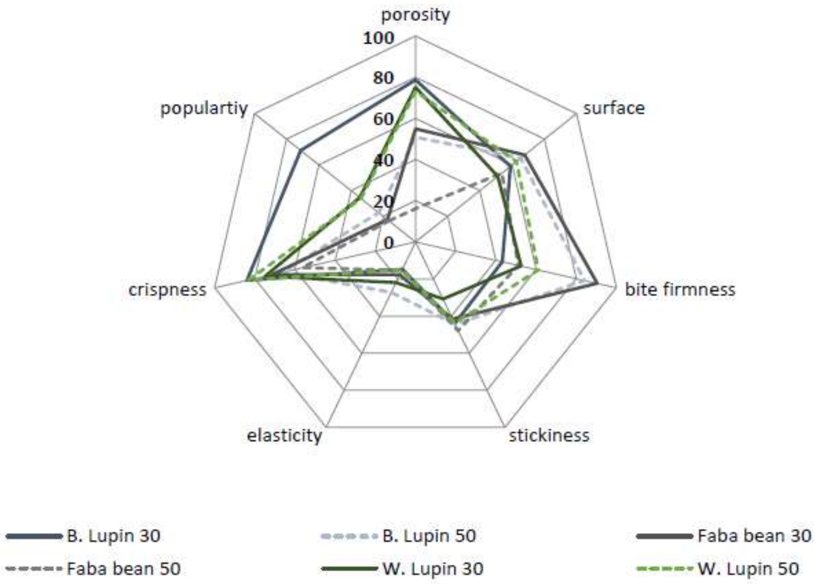

Figure 20 – Texture properties of legume-wheat starch-mixtures with additives (2.0 % lipid and 0.5 % NaCl) (T = 160 °C; w= 33 %).

Figure 20 shows the texture properties of legumes. The textural properties of extruded snacks play an important role in terms of quality and consumer acceptance (Szczesniak, 2002). Bite firmness and porosity show the biggest variances with values ranging from 15 to 90 SP. These results go along with the results of the texture analysis. The specific hardness of the samples with 30 % protein content increased in the order of blue lupin, white lupin, faba bean and lentil. Popularity values were also rated relatively low, with values from 15 to 35 SP. Only the samples with blue lupin and 30 % protein content were very popular (71 SP). Nevertheless, it is noticeable, that a high popularity of the

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extruded cereals is apparently linked to a low bite firmness. For example, blue lupin has the highest popularity value and the lowest bite firmness value concurrently. An important point to improve the popularity of the samples would be to reduce the bite firmness.

f) Mixtures of legumes and pseudocereals

As a next step, mixtures of legumes and pseudocereals were extruded. Regarding the sensory analysis, white and blue lupine were combined with either amaranth, buckwheat or quinoa. Additionally, to improve texture properties, three starches were evaluated as a further ingredient, where maize starch seemed the most promising one (especially, because then the final product can be claimed as" gluten-free"). Based on the texture and sensory results, extrudates with 70% protein were produced (Figure 21).

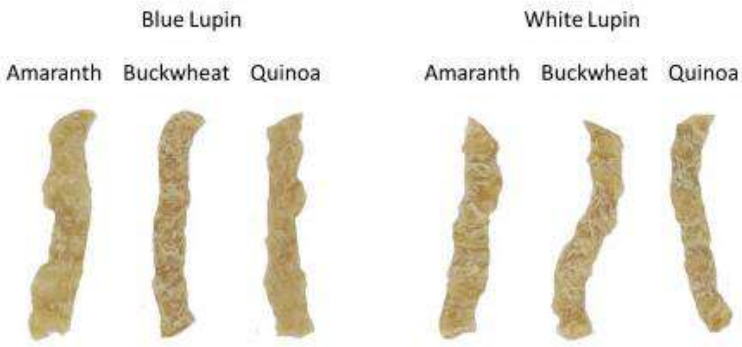

Figure 21 – Extruded legume-pseudocereal samples with 70 % protein content (T = 140 °C; w = 33 %). Mixtures with lupin protein isolate and additives (0.5 % NaCl).

Mixtures with blue lupin have a higher SEI than mixtures with white lupin (figure 22). The minimal decrease in expansion for the legume-pseudocereal-samples compared to blue lupin samples can be explained by a lower starch and higher fat content. Since the proportion of pseudocereals is only 15 %, there was not much difference between amaranth, buckwheat, and quinoa (Figure 22).

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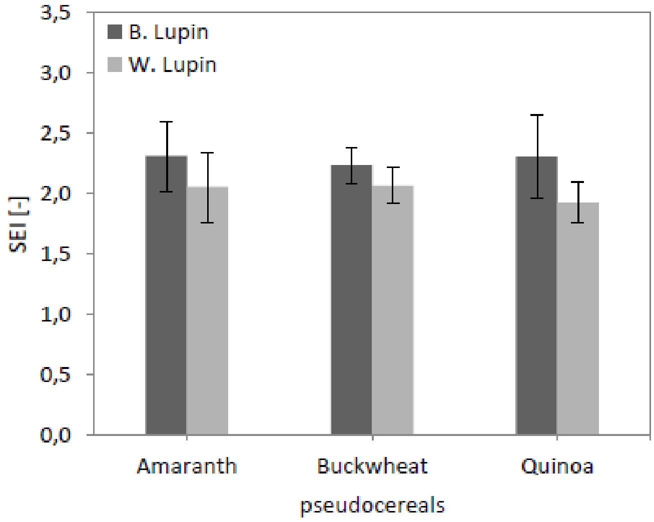

Figure 22 – SEI of legume-pseudocereal mixtures with 70 % protein content (T = 140 °C; w = 33 %). Mixtures with lupin protein isolate and additives (0.5 % NaCl). Data is shown as means ± SEM.

Figure 23 shows specific hardness values for different legume-pseudocereal mixtures. Extrudates of white lupin exhibited the highest specific hardness. The results of the specific hardness correlate with the results of the extrudate expansion (cf. Chapter 3.4). The analysis of the extrudates shows that white lupin extrudates were smaller in diameter, denser and harder.

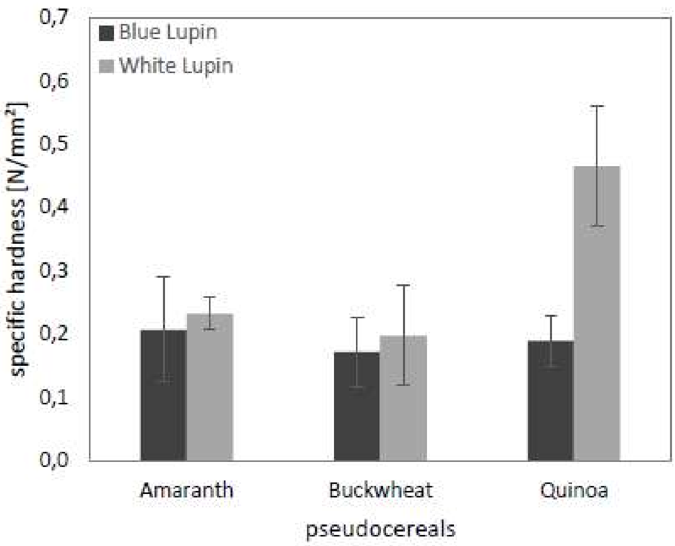

Figure 23 – Specific hardness of legume-pseudocereal mixtures with 70% protein (T = 140 °C; w = 33 %). Mixtures with lupin protein isolate and additives (0.5 % NaCl). Data are shown as means ± SEM.

Figure 24 shows retronasal odour properties of the different legume-pseudocereal extrudate samples. Samples with white lupin show higher intensity in the attributes fishy, cheesy/sweaty, rancid and

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fatty/like carton. Furthermore, the intense rusty/like popcorn odour of blue lupin-buckwheat was noticeable. Blue lupin-buckwheat samples reached highest popularity with values closed to 60 SP. The other samples reached lower popularity with values close to 40 SP (Figure 24).

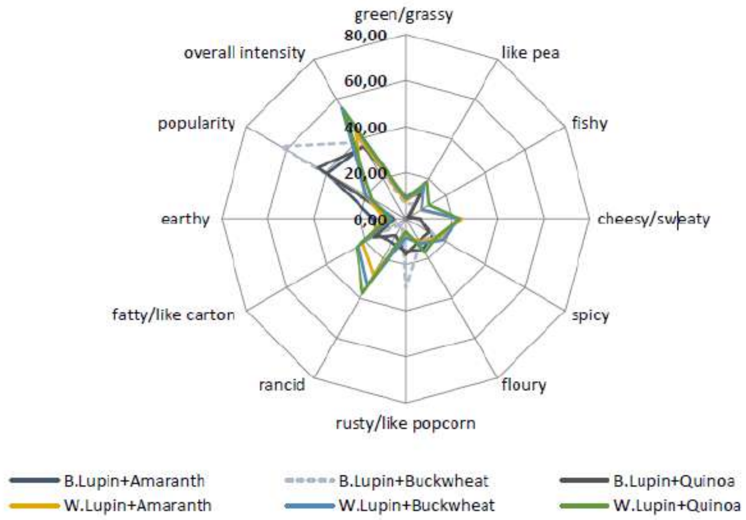

Figure 24 – Retronasal odour properties of pseudocereal-legume-maize starch-mixtures with additives (0.5 % NaCl) (T = 140 °C; w= 33 %).

Figure 25 shows the texture properties of the different legume-pseudocereal samples. In general, texture properties show the broadest variations amongst all properties tested. The samples with white lupin had a higher bite firmness than the samples with blue lupin. In summary, the white lupin-quinoa samples had the highest bite firmness. Popularity values were also rated relatively low, with values from 20 to 45 SP.

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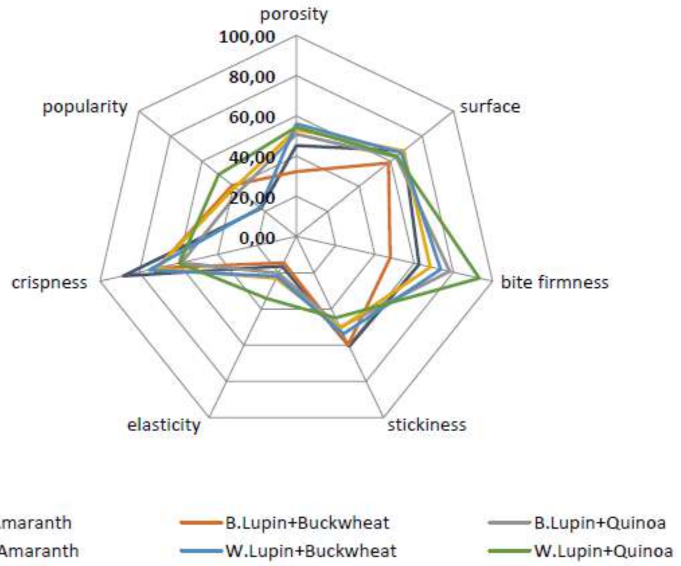

Figure 25 – Texture properties of pseudocereal-legume-maize starch-mixtures with additives (0.5 % NaCl) (T = 140 °C; w= 33 %).

3.4 Gluten-free breads and extruded products (UNALM)

a) Development of gluten-free breads using Andean grain flours

Control bread made with potato starch with xanthan gum had the highest specific volume, more cells/cm^2 and less firmness (table 33).

Table 33 – Quality characteristics of gluten-free breads based on starch

Quality parameters	CS-XG	CS-TG	PS-XG	PS-TG
Baking loss (%)	16.64 ± 2.5^a	18.77 ± 1.5^b	15.85 ± 0.7^a	17.67 ± 0.7^{ab}
Specific volume (mL/g)	2.24 ± 0.04^b	2.41 ± 0.01^c	**2.77 ± 0.05^d**	1.93 ± 0.03^a
N° Cells/cm^2	52.48 ± 5.3^a	46.40 ± 10.9^a	**83.50 ± 8.5^b**	-
% Area of cells	31.04 ± 2.8^{ab}	28.11 ± 3.9^a	33.20 ± 3.0^b	-
Average cell area (mm^2)	2.83 ± 0.3^a	2.69 ± 0.5^a	2.87 ± 0.3^a	-
Crumb hardness (g-f)	241.73 ± 25.88^b	246.89 ± 3.95^b	**193.78 ± 0.31^a**	1289.27 ± 17.21^c
Cohesiveness	0.73 ± 0.23^a	0.60 ± 0.01^a	3.93 ± 0.01^b	0.56 ± 0.06^a
Springiness	0.98 ± 0.02^b	0.93 ± 0.02^a	0.99 ± 0.01^b	0.96 ± 0.01^b
Gumminess (g-f)	143.01 ± 12.59^a	146.51 ± 0.65^a	764.79 ± 30.60^c	653.78 ± 24.72^b
Chewiness (g-f)	139.95 ± 13.39^a	135.74 ± 3.39^a	752.73 ± 32.88^c	657.84 ± 29.22^b

CS= corn starch, PS = potato starch, XG = xanthan gum, TG = tara gum

In the case of quinoa bread, a mixture design with restrictions was carried out, using as variables: Quinoa flour: 10 – 50 %; water: 70 – 110 % and tara gum: 0.5 – 2 %. In the same way, a mixture

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design was performed using: Quinoa flour: 10 – 50 %; water: 70 – 110 % and gum mixture (Tara gum: Xanthan gum): 0.5 – 2 %. Sixteen formulations for each experiment were analysed, texturally using the Back Extrusion technique (table 34 and table 35).

Table 34 – Textural properties of gluten-free doughs with quinoa flour and tara gum

Mixture design - Formulations of gluten-free doughs with quinoa flour and tara gum				Textural results using the Back-Extrusion technique							
F	**Quinoa flour**	**Water**	**Tara gum**	Firmness	± SD	Consistency	± SD	Cohesiveness	± SD	Viscosity index	± SD
1	**50.00**	**71.50**	**0.50**	8.09	0.42	118.13	4.20	-7.19	0.62	-71.62	3.67
2	**50.00**	**70.00**	**2.00**	59.13	2.73	848.38	43.96	-51.45	2.10	-510.63	63.85
3	**20.94**	**100.19**	**0.88**	2.65	0.39	39.15	3.64	-1.88	0.10	-26.51	1.68
4	**40.19**	**80.94**	**0.88**	9.72	0.26	145.17	4.94	-8.71	0.28	-99.13	5.99
5	**30.38**	**90.38**	**1.25**	10.36	0.44	150.45	2.42	-9.29	0.17	-97.04	4.12
6	**36.67**	**83.83**	**1.50**	14.65	0.22	218.21	1.97	-13.76	0.15	-144.07	8.43
7	**10.00**	**110.00**	**2.00**	7.91	0.38	118.24	4.26	-6.70	0.41	-70.01	3.02
8	**50.00**	**70.00**	**2.00**	60.96	0.67	878.29	7.85	-52.94	0.49	-533.69	41.15
9	**10.00**	**110.00**	**2.00**	8.46	0.60	119.60	1.93	-6.77	0.33	-69.40	3.59
10	**37.17**	**84.33**	**0.50**	2.71	0.03	42.15	0.36	-2.15	0.04	-28.75	1.16
11	**30.38**	**90.38**	**1.25**	10.04	0.51	148.12	3.06	-9.00	0.42	-95.07	4.67
12	**30.00**	**90.00**	**2.00**	17.39	0.48	250.80	1.75	-16.01	0.23	-159.46	7.40
13	**30.38**	**90.38**	**1.25**	10.31	0.50	149.64	3.81	-9.11	0.49	-96.98	4.23
14	**30.00**	**90.00**	**2.00**	16.70	0.85	248.24	5.67	-15.73	0.51	-160.62	5.72
15	**24.33**	**97.17**	**0.50**	1.05	0.11	15.32	1.74	-0.53	0.10	-7.48	1.10
16	**11.50**	**110.00**	**0.50**	0.44	0.02	6.84	0.14	-0.17	0.01	-1.68	0.18

Table 35 – Textural properties of gluten-free doughs with quinoa flour and gum mixture (tara gum + xanthan gum, 1:1 ratio)

Mixture design - Formulations of gluten-free doughs with quinoa flour and tara gum + xhantan gum				Textural results using the Back-Extrusion technique							
F	**Quinoa flour**	**Water**	**Gum mixture**	Firmness	± SD	Consistency	± SD	Cohesiveness	± SD	Viscosity index	± SD
1	**40.19**	**80.19**	**1.63**	24.59	0.92	355.71	22.81	19.65	0.55	194.22	9.46
2	**30.00**	**90.00**	**2.00**	20.79	0.75	301.17	17.97	17.45	2.20	159.06	9.22
3	**50.00**	**70.00**	**2.00**	64.64	1.81	922.77	33.84	50.89	5.41	425.46	53.61
4	**50.00**	**70.75**	**1.25**	30.26	0.52	433.52	11.36	25.65	2.37	238.70	11.68
5	**20.19**	**100.19**	**1.63**	8.88	0.43	126.97	2.06	7.01	0.50	71.28	4.20
6	**40.19**	**80.94**	**0.88**	9.27	0.27	135.56	4.21	7.64	0.33	84.63	4.08
7	**50.00**	**70.00**	**2.00**	62.52	1.90	899.68	28.57	47.46	1.26	413.28	38.47
8	**10.00**	**110.00**	**2.00**	8.71	0.15	128.19	3.30	6.69	0.14	76.15	1.49
9	**10.75**	**110.00**	**1.25**	3.71	0.30	56.13	4.42	2.70	0.24	35.31	3.28
10	**10.00**	**110.00**	**2.00**	8.39	0.21	123.95	8.56	6.53	0.42	76.67	6.38
11	**11.50**	**110.00**	**0.50**	0.90	0.03	14.19	0.80	0.41	0.01	6.32	0.05
12	**30.00**	**90.00**	**2.00**	22.95	0.55	333.82	9.63	17.28	0.80	168.70	10.18

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13	**50.00**	**71.50**	**0.50**	8.57	0.09	121.13	0.67	7.19	0.24	79.14	1.83
14	**30.38**	**90.38**	**1.25**	9.41	0.43	135.03	9.06	7.45	0.26	82.53	6.06
15	**11.50**	**110.00**	**0.50**	1.00	0.08	15.45	0.48	0.45	0.04	6.76	0.47

For kiwicha bread, a mixture design with restrictions was carried out, using as variables: kiwicha flour: 10 – 50 %; water: 70 – 110 % and tara gum: 0.5 – 2 %. In the same way, a mixture design was performed using: kiwicha flour: 10 – 50 %; water: 70 – 110 % and gum mixture (Tara gum: Xanthan gum): 0.5 – 2 %. Sixteen formulations for each experiment were analysed texturally using the Back Extrusion technique (table 36 and table 37).

Table 36 – Textural properties of gluten-free doughs with kiwicha flour and tara gum

Mixture design - Formulations of gluten-free doughs with kiwicha flour and tara gum				**Textural results using the Back-Extrusion technique**							
F	**Kiwicha flour**	**Water**	**Tara gum**	Firmness	± SD	Consistency	± SD	Cohesiveness	± SD	Viscosity index	± SD
1	**30.00**	**90.00**	**2.00**	12.92	1.0	187.53	0.4	11.52	0.4	128.44	7.6
2	**10.75**	**110.00**	**1.25**	1.79	0.1	27.26	1.5	1.29	0.1	18.99	0.9
3	**10.00**	**110.00**	**2.00**	5.57	0.1	81.50	1.8	4.74	0.1	56.95	1.8
4	**50.00**	**70.00**	**2.00**	49.01	0.9	710.04	18.6	41.61	0.3	413.39	35.4
5	**50.00**	**71.50**	**0.50**	8.82	0.4	127.79	3.3	7.64	0.3	83.82	3.2
6	**40.19**	**80.94**	**0.88**	6.29	0.2	94.89	2.1	5.54	0.1	63.61	1.4
7	**30.00**	**90.00**	**2.00**	13.18	0.7	190.94	3.2	11.90	0.4	132.79	3.3
8	**30.38**	**90.38**	**1.25**	4.96	0.2	75.00	1.3	4.38	0.1	51.71	0.6
9	**20.19**	**100.19**	**1.63**	4.96	0.1	75.72	0.3	4.28	0.1	51.69	1.4
10	**50.00**	**71.50**	**0.50**	7.99	0.3	119.80	3.0	6.92	0.2	80.52	0.7
11	**11.50**	**110.00**	**0.50**	0.36	0.0	6.34	0.1	0.13	0.0	0.65	0.1
12	**50.00**	**70.75**	**1.25**	22.60	0.3	323.62	14.4	19.68	0.6	200.37	7.2
13	**11.50**	**110.00**	**0.50**	0.39	0.0	6.67	0.3	0.12	0.0	0.54	0.0
14	**50.00**	**70.00**	**2.00**	48.23	0.5	700.60	5.4	41.30	0.5	423.42	47.4
15	**10.00**	**110.00**	**2.00**	6.00	0.3	83.65	1.9	4.86	0.1	57.19	1.9
16	**40.19**	**80.19**	**1.63**	15.82	0.1	228.71	4.1	14.55	0.4	159.23	5.1

Table 37 – Textural properties of gluten-free doughs with kiwicha flour and gum mixture (tara gum + xhantan gum, 1:1 ratio)

Mixture design - Formulations of gluten-free doughs with kiwicha flour and tara gum				**Textural results using the Back-Extrusion technique**							
F	**Kiwicha flour**	**Water**	**Gum mixture**	Firmness	± SD	Consistency	± SD	Cohesiveness	± SD	Viscosity index	± SD
1	**30.00**	**90.00**	**2.00**	19.17	1.8	280.76	1.2	15.31	1.2	159.06	8.1
2	**10.75**	**110.00**	**1.25**	4.05	0.3	59.37	5.5	2.65	0.2	32.53	2.0
3	**10.00**	**110.00**	**2.00**	10.15	0.1	148.05	5.3	7.21	0.3	70.73	3.4
4	**50.00**	**70.00**	**2.00**	52.46	1.9	757.88	26.9	39.80	1.8	436.39	7.9
5	**50.00**	**71.50**	**0.50**	8.86	0.4	130.89	5.6	7.33	0.3	79.62	2.6

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6	**40.19**	**80.94**	**0.88**	8.77	0.5	130.26	4.5	6.90	0.0	75.28	1.5
7	**30.00**	**90.00**	**2.00**	20.20	0.6	300.68	10.5	15.91	0.4	170.87	5.4
8	**30.38**	**90.38**	**1.25**	9.23	0.1	139.54	1.3	7.15	0.2	85.52	1.7
9	**20.19**	**100.19**	**1.63**	10.09	0.6	142.92	5.4	8.03	0.3	81.24	5.4
10	**50.00**	**71.50**	**0.50**	8.06	0.1	122.85	0.5	6.78	0.1	77.08	4.4
11	**11.50**	**110.00**	**0.50**	0.97	0.0	15.37	0.6	0.43	0.0	6.53	0.3
12	**50.00**	**70.75**	**1.25**	26.87	0.2	388.77	13.9	22.50	0.7	243.26	3.1
13	**11.50**	**110.00**	**0.50**	0.93	0.0	14.75	0.7	0.43	0.0	6.17	0.1
14	**50.00**	**70.00**	**2.00**	60.60	1.0	871.57	18.0	46.72	1.0	473.88	9.3
15	**10.00**	**110.00**	**2.00**	10.00	0.4	147.96	7.8	7.28	0.3	78.76	7.4
16	**40.19**	**80.19**	**1.63**	23.48	0.3	349.63	2.7	18.89	0.1	218.18	11.1

In the flowing flow charts (Figure 27 and 28) the processing condition and production steps of the gluten free quinoa and kiwicha bread are reported.

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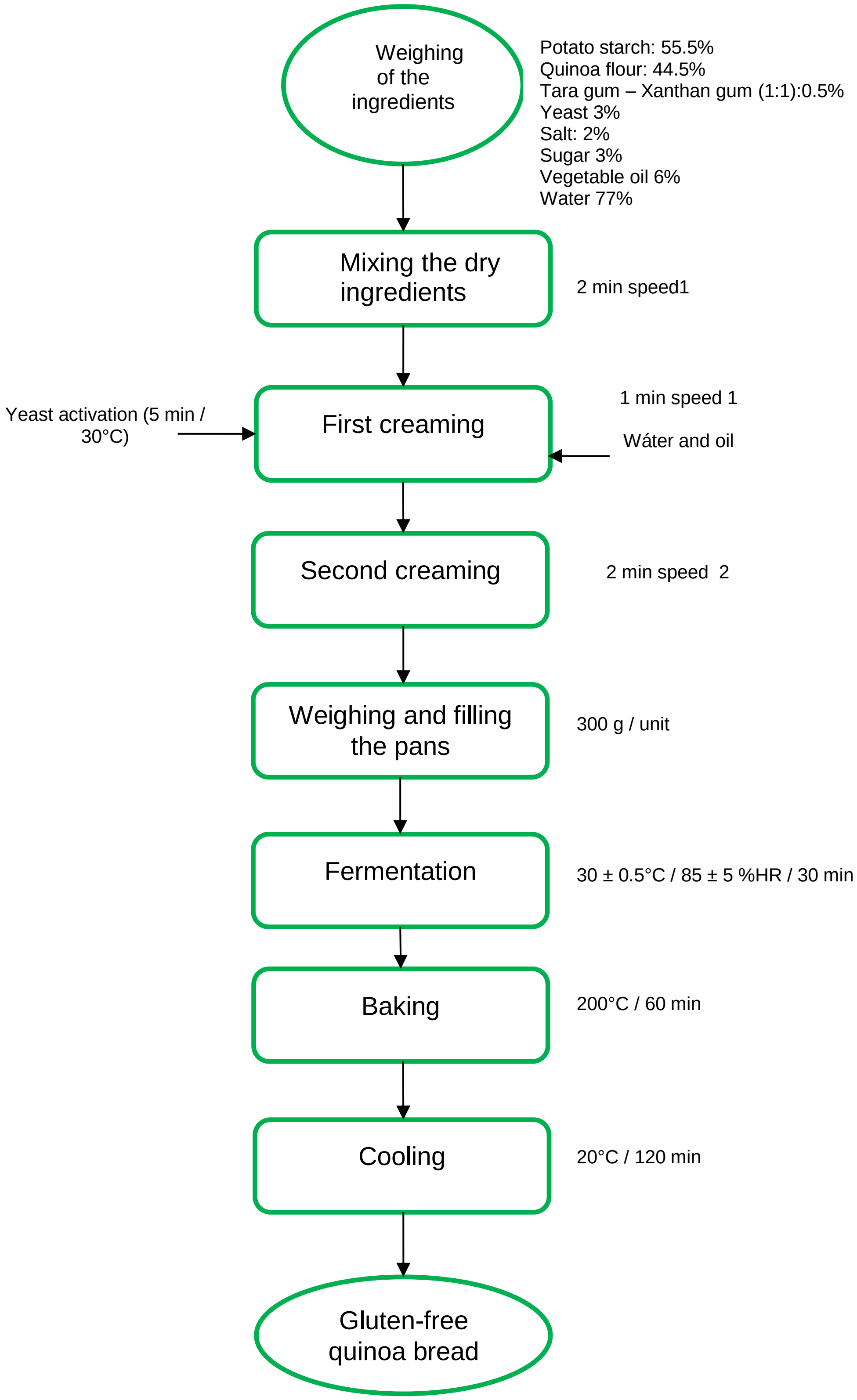

Figure 26: Processing conditions for gluten-free quinoa bread.

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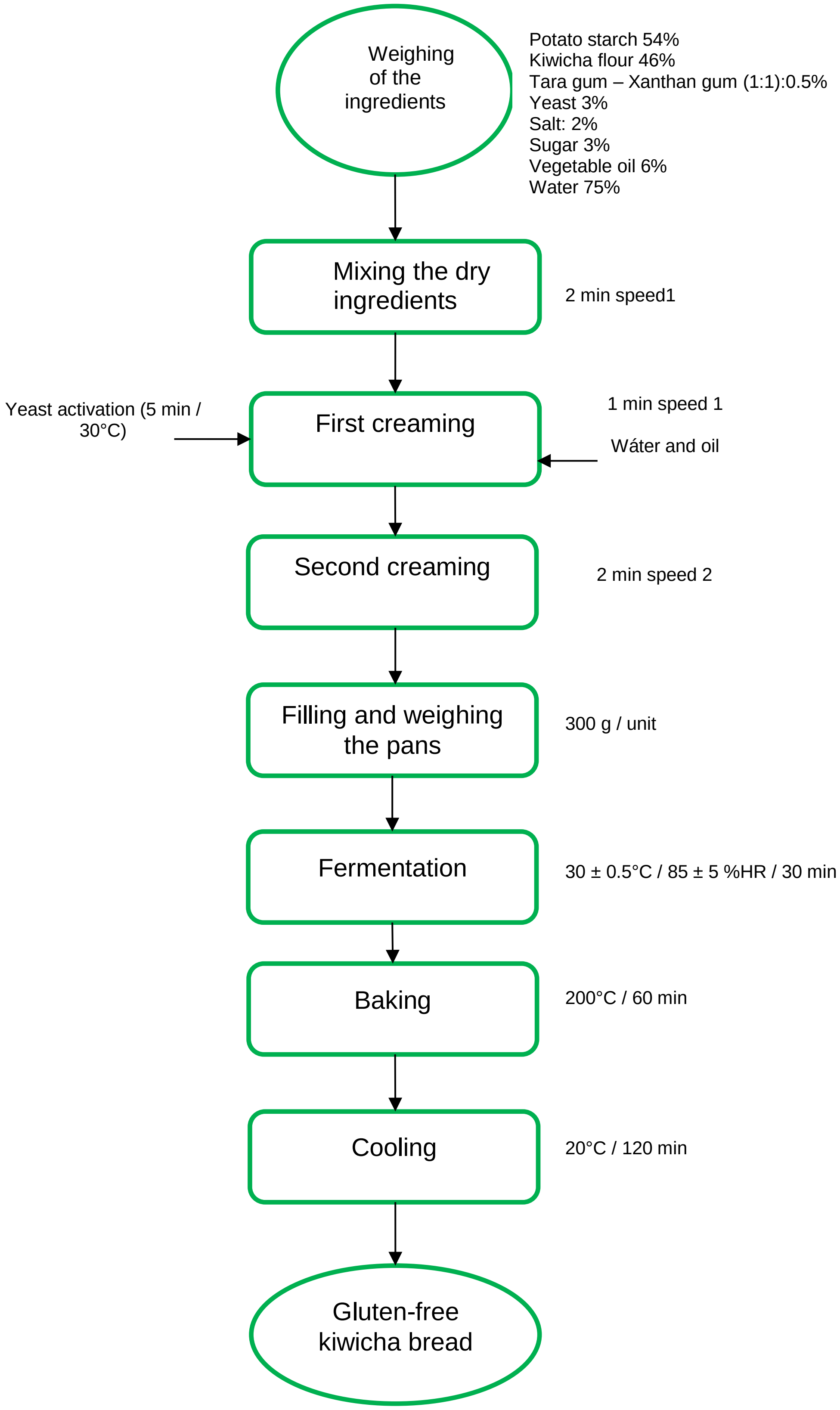

Figure 27: Processing conditions for gluten-free kiwicha bread

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b) Development of extruded products with Andean grains

The effect of the two independent variables (moisture content and temperature) in the responses (sectional expansion index SEI and bulk density) were analysed by response surface methodology which is shown in (figure 28).

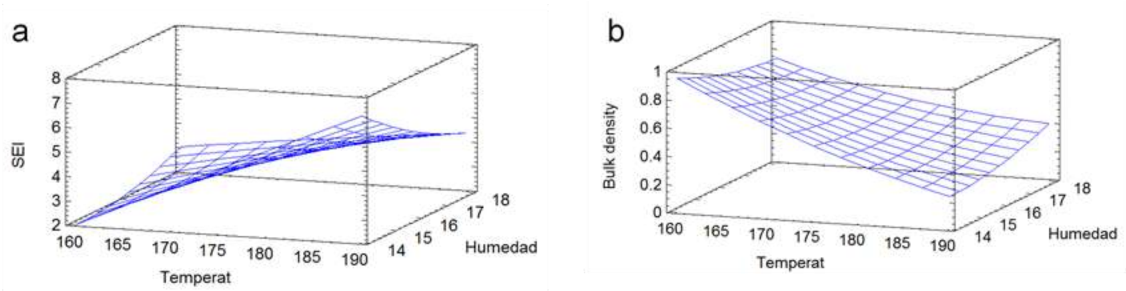

Figure 28- 3D surface of the moisture effect of the kiwicha flour and the extrusion temperature in a) Sectional expansion index of the extrudates. b) bulk density.

The ANOVA of the result of the sectional expansion index shows that temperature and moisture have a highly significant effect $p < 0.01$. Temperature has a positive effect on the expansion index, in other words, when the temperature increases also the rate of expansion increases. Regarding moisture, at low temperature (160 °C) the rate of expansion increases when moisture increases; while at high temperature, the index of expansion decreases when moisture increases. The sectional expansion index has as optimal value of 7.17 at 190 °C final temperature and 14 % moisture of the kiwicha flour. The ANOVA of results shows that the temperature has a highly significant effect $p < 0.01$ and moisture has no significant effect $p \geq 0.05$ on the bulk density. Temperature has a negative effect on bulk density: when temperature increases, bulk density decreases (Figure 28). The optimum parameters are 190 °C final temperature and 14% moisture of the kiwicha flour, with bulk density of 0.233 g/cm^3. In figure 29, the photographs of extruded kiwicha samples are shown. Preliminary results for quinoa flour are shown in figure 30.

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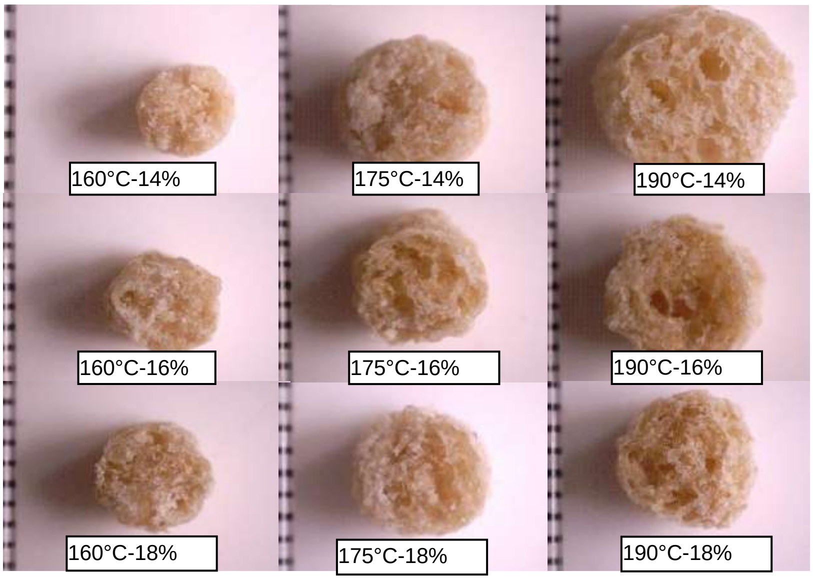

Figure29 – Sectional cut of kiwicha samples extruded in different temperatures and moistures

Figure 30 – Photograph of extruded quinoa extruded at different temperatures and moisture

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4. Conclusion and next steps

a) Wheat-based breads and pasta (UCC)

Conclusion: The protein-enriched flours (faba bean, buckwheat) and lupin protein isolate, which were produced by the project partner Fraunhofer (WP2), appeared to be well suited for the preparation of PR wheat-based breads and pasta. They resulted in products with similar or higher quality when compared to the reference products of bread and pasta. High protein contents (meeting requirements for 'high in protein' claim according to regulation (EC) No 1924/2006) can be attained by incorporation of these PR ingredients while maintaining a high consumer acceptance due to their good texture and sensory properties.

Next steps: Screening trials showed that products made with amaranth, lentil and quinoa resulted in goods of inferior quality and intensive bitter off-flavours. This can potentially be improved by means of enzyme technology (cf. GA WP3 – subtask 3.3.4). A final descriptive sensory evaluation of all prototypes will be performed as well as a characterisation of their nutritional profile (cf. GA WP3 – subtask 3.5).

b) Porridge flours, cookies and extruded snack (MAKERERE)

Conclusion**:** The formulated products showed high nutritional quality and high sensory acceptability. Extrusion conditions leading to the development of nutritious extruded snack. Extrusion processing significantly improved the nutritional quality of ROBA1 beans. It induced important desirable nutritional modifications making it more suitable for feeding infants and young children. Based on product acceptability, anti-nutrients, iron and zinc extractability, as well as on protein digestibility, the optimum extrusion conditions for the bean-based food prototypes designed for infant and young child as well as feeding women of reproductive age, were found to be 17 % moisture content, 35 Hz screw speed and 156 °C third heater temperature.

Next steps: MAKERERE will perform the following extra activities to validate the developed food products:

- Shelf-stability studies of the porridge flours
- Certification process by UNBS will be completed
- Optimal extrusion conditions for the instant flours for nutritional quality, sensory acceptability and phyto-chemicals will be determined
- Work with the gluten-free and wheat cookies will continue, to improve sensory

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acceptability and further characterise the products (nutritional, sensory, safety, shelf stability, phyto-chemicals, etc.).

- Determining the textural properties of extruded products and cookies will be done
- Shelf-life studies of the extruded products and cookies will commence
- Marketing testing to assess consumer acceptability

c) Extruded Cereals (FRAUNHOFER)

Conclusion**:** The development of extrudates from blue lupin, white lupin, and faba bean protein isolates by extrusion cooking has been successfully finalized. Mixtures of legumes and pseudocereals can be used for the production of protein-rich cereals via extrusion cooking in order to fulfil human physiological requirements due to the cereals´ well-balanced amino acid profile. In summary, legume protein isolates had a high protein solubility and emulsifying capacity. RVA results of protein slurries showed that if temperature increased above 75°C, denaturation of the native proteins caused the protein solubility to decrease, which resulted in an increase in viscosity. Sensory analysis showed only average results in popularity for all raw material extrudates. The low popularity can be attributed to the suboptimal odour/taste and the hard texture of the extrudates. The most disrupting attributes in odour were rancid, fishy and cheesy/sweaty for faba bean and white lupin. However, when protein isolate from white lupin at IVV was used instead, these off flavours were not persistent. Hence, we analyse that white lupin is a valuable source of plant protein for the development of protein-rich cereals and snacks that need further research regarding the occurrence of off-flavors during production of protein isolate.

Next step: For further investigations, various flavours could be added to the extruded products to enhance consumer acceptance. Coating technologies can be tried out to achieve this goal with various ingredients. By sensory screening of the large number of legume extruded products legumes with the most acceptance could be preselected and extruded with pseudocereals. The experiments together with the sensory analysis showed that it is possible to produce extruded products with pseudocereals in combination with legume protein isolates. Pseudocereals had only a minor influence on the appearance of the extrudates.

d) Gluten-free breads and extruded pro-ducts (UNALM)

Conclusion: The validation of the surface response model gave the following optimum proportions of the main ingredients for quinoa bread: 55.5 % of potato starch, 44.5 % of quinoa flour, 0.5 % of

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gum mixture and 77 % of water. For kiwicha bread the optimum proportions were: 54 % potato starch, 46% kiwicha flour, 0.5 % gum mixture and 75 % water. The optimum extrusion conditions for kiwicha were: temperature of 190 °C and 14 % of moisture, and for quinoa temperature of 190 °C and 15.3 % of moisture.

The next step: Further activities will be focus on the optimization of the gluten-free breads formulations made with a mixture of quinoa and tarwi (Andean lupin) flour, and also breads with a mixture of kiwicha flour and tarwi flour. In the following months, the nutritional properties of these breads will be determined. Nutritional analysis of extruded products will be carried out. The final product, a flour mixture for breakfast, will be formulated taking into consideration the protein content and chemical score.

5) Delays and difficulties

For the development of extruded cereals at FRAUNHOFER, the major challenge was the limited availability of some of the raw materials. Therefore, it was necessary to perform the majority of experiments in lab-scale (1kg/h). Although this approach results in important data on the processing behaviour of the biopolymers, the expansion of the extrudates is limited compared to extrudates from small pilot-scale (10kg/h). Therefore, starch was added as an additional ingredient to enhance expansion properties. For that reason, trials without additional starch should be tested in further trials.

Regarding UNALM activities, the lab-scale extruder has had technical issues and this is why it has not been possible to continue the formulation of flour mixture of quinoa and kiwicha as originally planned.

6) Impact and outreach

The protein-rich prototypes provides high-quality alternatives to traditional breads, pasta, cookies and extruded products and supplies a broad range of consumers with plant-based protein from new, locally produced sources. This contributes to a healthy diet and a wider product portfolio for vegetarians, vegans, coeliac patients, athletes, and elderly.

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New knowledge regarding recipes and processing conditions have been investigated and optimized at a laboratory and/or pilot-scale,by using new and alternative plant-based protein sources. These new protein sources are not yet available on the market and have been developed in WP2 as part of PROTEIN2FOOD. The prototyped food products are characterized by an improved sensory and nutritional properties when compared to their commercial counterpart products. This could potentially boost a more proactive shift towards diets from more-sustainable protein ingredients that are also domestically sourced (EU-zone).Appropriate targeted consumers could be especially meat lovers and flexitarians, but the prototypes could provide a wider variety of plant-based food products for also vegetarian, vegan, health-conscious and life-style consumers.

As an example of a successful outreach activity, a prototype of protein-rich pasta, developed at University College Cork, within the PROTEIN2FOOD project, was displayed at the Bioeconomy Village at the info week on 'Horizon 2020 Societal Challenge 2', the biggest EU research and innovation programme. PROTEIN2FOOD received much interest from policy makers, scientists and other visitors present at the event. They marvelled at the different showcased samples of seed groups (e.g. quinoa) or grain legumes (e.g. faba beans and lupins) and their protein-rich flours and isolates.

Finally, PROTEIN2FOOD project applied processing technology, processing conditions and prototypes formulations have been validated on lab-environment (TRL 4) and represents a valuable background for SMEs and food industries for moving towards a further validation and demonstration of the developed food prototypes at the industrial level. At such regards, Makerere University promotes their developed prototypes at several meetings, conferences and exhibitions (Figure 31 and Figure 32) and stocks the products at a sales outlet of the University's incubation centre.

Figure 31 – Exhibition of prototypes at the 'First Extra-ordinary Summit of the Committee of Ten Heads of State and Goverment (C10) Championing Education, Science and Technology in Africa' (Lilongwe, Malawi; Nov 2018)

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Nutreal (U) Ltd (www.nutreal.ug), a food company from Uganda, is specialised in developing nutrient-enhanced foods using pulse grains. This company commenced industrial production and is currently promoting the PR porridge flours developed by MAKERERE University in supermarkets, at exhibitions and trade fairs around Kampala, Uganda (Figure 33). Nutreal Ltd sources raw materials including beans, grain amaranth, soy, maize and millet from farmers' groups, thus providing a stable market and timely payments to the farmers. The company builds capacity of the farmers' groups in good agricultural and post-harvest management practices, to ensure supply of high-quality raw materials.

Figure 32 – left: Reaching BoP (base of the pyramide) children with developed porridge flour (Sep 2018); right: Explaining developed products to Uganda's Dep. Prime Minister (2nd right), Minister of State for Agriculture (3rd right) accompanied by University and Ministry officials (Nov 2018).

Figure 33 – Nutreal Ltd exhibiting the PR products developed by Makerere University as part of PROTEIN2FOOD WP3 at a public fair and exhibition (Sep 2018).

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The developed gluten-free (GF) breads using Andean flours will provide a more nutritious and tastier alternative for the Peruvian market of existing gluten-free products. Currently, commercially available GF breads are expensive and of very low nutritional and organoleptic value. Therefore, the final extruded product, a flour mixture with a high nutritional value, could be used in the food aid programs of the Peruvian national health authorities. Product samples of the PR breakfast cereals developed by Fraunhofer can be presented to the stakeholders of the project. These ready-to-use recipes can be up-scaled and produced in industrial scale and represent new market opportunities following the current consumer trends towards products that contain high-quality protein crops. Since the developed raw materials are not yet available on a larger scale, the increasing popularity could influence the production capacities of producers of isolates, the milling industry and also legume and pseudocereal producing farmers.

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