Cookability and starch quality in faba bean (*Vicia faba*)

– a study of a diversity panel and effect of bean weevil damage (*Bruchinae rufimanus*)

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Swedish University of Agricultural Sciences, SLU
Faculty of Landscape Architecture, Horticulture and Crop Production Science
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Cookability and starch quality in faba bean (*Vicia faba*)

- a study of a diversity panel and effect of bean weevil (*Bruchinae rufimanus*)

Kokbarhet och stärkelsekvalitet i åkerböna (Vicia faba)
- en studie av en diversitetspanel och effekt av bönsmyg (Bruchinae rufimanus)

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Abstract

Increased consumption and cultivation of legumes are important for both health and environmental aspects. Faba bean is a legume crop that is on the rise again since 2020 in the field of plant breeding in Sweden after a long pause since 1990, therefore increased knowledge of its properties in cultivation and seed quality is important. Cookability is a seed quality parameter of importance for using legume seeds in food. However, very little knowledge is available on which factors determine cookability in faba beans. In this study, a diversity panel of 14 accessions with and without bean weevil damage were assessed for seed size, seed-coat hardness, cookability and starch quality. Examples of the most important instruments and methods used are Marvin seed-analyser, penetrometer analysis, Mattson Cooker and enzymatic starch assay. We found a variation in median cooking time from 20 to 62 minutes in the different accessions and seeds with bean weevil damage tended to cook slightly quicker than seeds without damage. There were not any large differences in starch content or quality (with regards to amylose/amylopectin) between accessions, or between seeds with and without bean weevil damage. Further, correlations between the different seed parameters assessed indicated that the best predictor for cooking time was seed size.

Keywords: Fava bean, horse bean, bell bean, broad bean, field-bean, tick-bean, Windsor bean, cooking time, amylose, amylopectin, seed hardness, seed quality

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Abbreviations

BW	Bean Weevil
Con A	Concanavalin A
CT	Cooking time
CV	Coefficient of Variation
DMSO	Dimethyl sulfoxide
DW	Dry Weight
GOPOD	Glucose oxidase peroxidase
MQ	Millipore quality
N	Newton
RAG	Relative Area Gain
TGW	Thousand grain weight
WAC	Water absorption capacity

1. Introduction

1.1 The role of legumes in the food system

For a more sustainable food system and a better health, our diets should include less red meat and more plant-based food, such as nuts, fruits, vegetables, and legumes (Willett, *et al.,* 2019) (Ferreira, *et al.,* 2021) (Willett, *et al.,* 2019). Legumes as beans, peas, and lentils are commonly known to be high in seed protein content compared to many other crops and can therefore replace protein from meat. Legumes typically have a symbiotic relationship with nitrogen fixating bacteria in the root nodules that can convert nitrogen from the air into forms that the plants can use, this mechanism minimizes the need for additional nitrogen fertilizer and lowers the nutrient leakage from the fields (Rubiales, *et al.*, 2021)

For every step up in the food chain approximately 90% of the energy invested in the organism is lost in form of heat and movement (National Geographic Society, 2024). Large amounts of energy and other resources are used to produce the animal products used for human food. If the areal used for animal feed production would instead be used for human food production, the area needed would decrease a lot. By switching from growing animal feed to more human grade food, Europe would be able to decrease the demand for imported feed and food and more easily supply the demand for quality food by growing more soy and other legumes within Europe.

Large amounts of soybean are imported to Europe and Sweden every year. Approximately 10 times more soy protein is used in Europe than protein from all other pulses combined, most of which is imported to keep up with the high demand for cheap and high-protein animal feed (European Parliament, 2018). Globally, around 85% of all soybean is grown today in the Americas (FAOSTAT, 2024). From an environmental perspective, both the transport and the issues with deforestation to grow more soybean in other parts of the world are problematic (Dreoni, *et al.*, 2022). Faba bean (*Vicia faba*) and pea (*Pisum sativum*) are the two major legume crops grown in Sweden today, but legumes are grown on a very small share, approximately 2%, of the total arable land (Jordbruksverket, 2022). Sweden has the capacity to increase the cultivation area of legumes by approximately 8000

hectares within a short period of time; however, it would not be enough to replace the entire import of soybean products, since this would need additionally 140 000 hectares of legume production (Jordbruksverket, 2022).

1.2 Faba bean

Faba bean is a cool season leguminous crop grown around the world with 2.4 million ha harvested globally 2016, both for human consumption and for feed (Maalouf, *et al.*, 2018). The species has many different names depending on the origin, use and context. Some of the names commonly used in English is bell bean, broad bean, faba bean, fava bean, field-bean, horse bean, horsebean tick-bean, and Windsor bean, just to mention a few (Smither-Kopperl, 2019). The plants are often erect (Figure 1), varying in length, flowering time, maturity time and the seeds have a large variation in both size and coloration, from light beige to brown, to red and almost black in some accessions (Ohm , *et al.*, 2024). The root system, just like many other members of the *Fabaceae* family, is often associated with the symbiotic nitrogen fixing bacteria, *Rhizobium leguminosarum* growing in the root nodules (Smither-Kopperl, 2019).

Increased cultivation of faba bean in Sweden and increased use of this crop for food production could be achieved if farmers had access to high-yielding varieties adapted to our climate conditions and that have a desired seed quality. However, breeding of faba bean in Sweden has been absent since the 1990s due to higher focus on grains and other crops; however, this is changing with the initiative SLU Grogrund — centre for plant breeding of food crops (Swedish University of Agricultural Sciences, 2022). A diversity panel of 220 different accessions has been studied for different characteristics both in field and seed properties (Ohm, *et al.*, 2024).

Figure 1 Illustration of faba bean plant morphology in Flora von Deutschland, Österreich und der Schweiz, 1885 (Thomé & Stüber, 2008).

1.3 Starch in faba bean

The major storage compounds in the seeds of most crop are starch, protein and fat. Faba beans contain large amounts of starch (28.1 - 47.5%) and protein (22.7 - 34.7%) of dry weight (Martineau-Côté, *et al.*, 2022). The seed content of these components seems to differ between publications, another study gives different results with lower starch content (20.9 – 37.8%) and slightly higher protein content (25.1 - 38.1%) (Ohm, *et al.*, 2024). Faba bean starch contains 33.5 - 39.9% amylose with unbranched chains of α-(1/4) D-glucopyranosyl units (Punia, *et al.*, 2019). The other main component of faba bean starch is amylopectin, which is constructed of shorter chains than in amylose that are connected to each other with α-(1/6) bonds to form a higher branched structure (Punia, *et al.*, 2019). The use of starches is

widely spread in both food and non-food purposes, and starches and their components properties are important to understand to increase the efficiency of the usage of the seeds (Punia, *et al.*, 2019).

1.4 Bean weevil in faba bean seeds

The bean weevil (*Bruchus rufimanus)* was first discovered in Sweden 2008, most likely arriving with imported seeds and has then spread prolifically since it is well adapted to the Nordic climate (Ryding, 2020). The weevils lay eggs in the faba bean pod, and the hatched larvae enters the developing seeds, consumes and develop inside the seed before emerging as an adult weevil from the mature seed; however, sometimes the weevil does not exit the seed; in most damaged seeds, only a minor part of the seed is lost (Ryding, 2020). In a field study of 220 different accessions of faba bean in the south of Sweden, bean weevil damage of seeds was significantly higher in early flowering accessions (Ohm, *et al.*, 2024). Resistance to bean weevils has been connected to early flowering, early pod setting and lower thousand grain weight (Carrillo-Perdomo, *et al.*, 2019). These two sources have opposite results, indicating that there might be large differences between the methods or the growing conditions, affecting the results of these studies. It can be considered a major problem when using faba bean for food purposes if the bean weevils are left in the seeds. Knowledge about if and how bean weevil affect seed quality in faba bean is still very scarce.

1.5 Cookability

Cooking can be an important processing step for legume seeds to reduce antinutritional factors (Geraldo, *et al.*, 2022). Hydration of dry legume seeds is a crucial step when cooking any type of pulse legume, as the hydration facilitates the cooking and secure a higher quality product (Perera, *et al.*, 2023). The seed coat has been found to be a significant barrier for absorbing water during imbibition (hydration) in faba bean (Rowland & Gusta, 1977). The water in beans is absorbed through the hilum, which is the point where the bean is attached to the pod before pod removal and sits right below the radicle, to pass the seed coat and then be distributed throughout the seed (Miano & Augusto, 2018).

Cookability, a portmanteau word of 'cooking' and 'ability' refers to the cooking time required for a batch of seeds to reach a desirable level of softness for consumption; however, no standardisation yet exists, and it is often measured differently across studies (Dueholm, *et al.*, 2023). A sufficient softness is obtained when the cotyledon cells, the main tissue of the legume seeds, separate sufficiently

(Dueholm, *et al.*, 2023). A common method of assessing cookability of legume seeds is by using a Mattson cooker. This apparatus is a brass tank holding a rack with plungers resting on top of the seeds placed in conical holes at the bottom plate. Boiling water in the bottom of the tank steams/boils the seeds, and a single seed is considered cooked when the plunger that rests on top of it penetrates the seed fully (Dueholm, *et al.*, 2023). Some authors use a definition of cooking time when 40% of the seeds are fully penetrated in the Mattsson cooker (Santos, *et al.*, 2018) and others use 80% as the definition (Bassett, *et al.*, 2021). In other legumes, cookability issues have been encountered such as the "hard shell" and "hard to cook" phenomena. The "hard to cook" behaviour is caused when the cotyledon cells do not separate properly, which could happen if an insufficient level of water is present in the cotyledon. On the other hand, the "hard shell" prevents proper hydration during the soaking process due to the hard shell that does not allow water to enter the seed. If a seed with a hard shell is peeled, the seed might hydrate properly during soaking in contrast to a peeled "hard to cook" seed that would remain hard when cooked (Perera, *et al.*, 2023).

In contrast to other legumes, knowledge on the cookability in faba bean is lacking. Addressing this knowledge gap can enable the breeding of faba bean varieties with more favourable traits for cooking and processing, and investigating the seed properties of faba bean is therefore important. Further, the effects of the bean weevil damage on the seeds' cooking properties have not been studied before, this would be interesting to reduce waste of damaged crops if it could be used for starch extraction or other types of processing.

1.6 Aim of study

The aim of this study was to characterize the seed size, seed-coat hardness, starch profile, and cookability in 14 accessions of faba bean (*Vicia faba*) with and without damage by bean weevil (*Bruchus rufimanus*). Further, pairwise correlation coefficients between these parameters were determined in an attempt to identify any easily measurable seed trait that could serve as a predictor of cookability. This knowledge can be useful when defining breeding targets for faba bean seed quality that are of importance for food production. The accessions used in this study are part of a diversity panel of 220 accessions previously characterized in field in the south of Sweden (Ohm, *et al.*, 2024).

2. Materials and Methods

2.1 Plant material

The plant material that was used in these experiments, was faba bean (*Vicia faba*) seeds. For the first experiment with water absorption, three accessions available in excess (Fuego, Fernando and Tiffany, which are all modern varieties) were used, however a morphologically diverse collection of 14 accessions were selected for all the following experiments. The accessions selected were the following, which had varying degree of breeding status, Kontu, Isabell, Banquise, Lövånger, Nadwislanski Nr.10, Svetlosemjannye, Grebo, COLUMBIA, Tiffany, Karmazyn, AKZENT, Gubbestad, TP 3930/SJOEDIN and Taifun (Figure 2). Seeds from all accessions in the panel came from the same field trial in Skara (Sweden) during 2022. The seeds were harvested and stored under the same conditions. The seeds of each accession were separated into two groups, being damaged by Bean weevils (BW) or not, i.e. BW+ and BW-, respectively. The infection rates in different accessions were largely varying. The seeds selected as BW- were the seeds with the lowest amount of signs of BW and the seeds selected as BW+ were with the highest amount of signs of BW. In some accessions this could mean that a BW- could have some signs of BW but much less than the BW+ and in other accessions, a BW+ seed could have only a black dot indicating some disturbance of the seed coat.

Figure 2 Collection of 14 accessions with and without bean weevil damage, rows 1 and 3 show accessions with bean weevil damage and rows 2 and 4 show the same accessions without bean weevil damage. Accessions from left to right Row 1 and 2; Kontu, Isabell, Banquise, Lövånger, Nadwislanski Nr.10, Svetlosemjannye and Grebo, Row 3 and 4; COLUMBIA, Tiffany, Karmazyn, AKZENT, Gubbestad, TP 3930/SJOEDIN and Taifun.

2.2 Water absorption assessment

A water absorption assessment was performed by weighing seeds before soaking, and then at seven different times during soaking (5 h, 10 h, 15 h, 20 h, 25 h, 30 h and 47 h). In this experiment the accessions Fuego, Fernando and Tiffany were used in two replicates of 50 seeds. Before weighing the seeds were dried with paper towels to remove excess moisture from their surface. This experiment was designed to show what soaking time to use in the following experiments and analyses.

2.3 Penetrometer analysis

For the penetrometer analysis, only seeds of BW- were used. Of 15 soaked seeds (20 h) only 10 seeds per accession were used for the penetrometer analysis. Figure 3A shows the setup of the penetrometer (STEP Systems GmbH, Nürnberg, Germany) and seed position (Figure 3B). The thickness of every individual seed was measured with callipers. The penetrometer measured the force pushing upon the penetrometer rod and the data was logged in Excel on a Windows computer every second. To ensure a stable determination of the hardness of the seed throughout the analysis, the rod was lowered by turning the wheel on the top of the drill stand, by pacing the laps manually aiming at a constant rhythm.

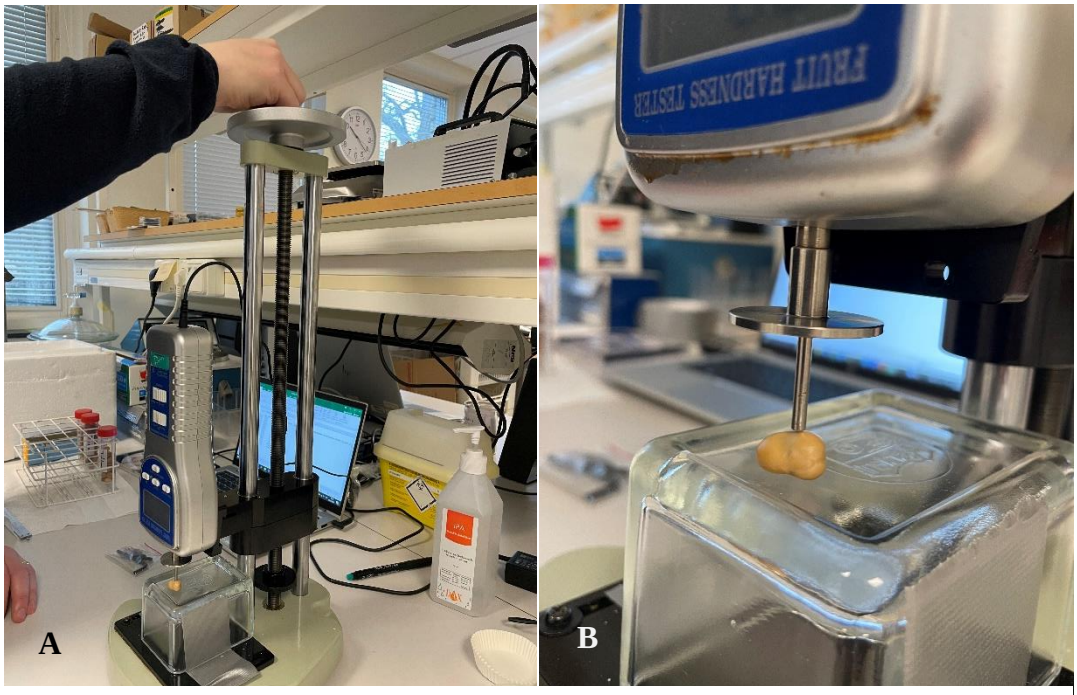

Figure 3 ***A****: Setup of penetrometer* ***B****: Positioning of seed under penetrometer rod before analysis.*

2.4 Seed weight and area determination

The 35 seeds of each accession BW±, that later were used in the cookability experiment, were analysed with a Marvin seed analyzer (MARViTECH GmbH, Wittenburg, Germany). The Marvin seed analyzer gives seed weight in grams (i.e. thousand grain weight, TGW) and seed area in mm^2 from image analysis combined with a scale. The area of all seeds was measured individually, to find the variance of size within each batch of 35 seeds. The TGW and seed area was measured before being soaked in Millipore quality water (MQ-water) and after 20h of soaking to get information of how much they had swelled during 20 h soaking.

Water-absorption capacity (WAC) was determined using the formula:

$$\frac{(weight_{soaked} - weight_{dry})}{weight_{dry}} \times 100$$

Relative area gained (RAG) was determined using the formula:

$$\frac{(area_{soaked} - area_{dry})}{area_{dry}} \times 100$$

2.5 Cookability

For the cookability assessment, a Mattson cooker (Lantmännen Agriculture, Svalöv, Sweden) was used. The 100 conical holes in the Mattson cooker were divided into four sections, one for each batch of 25 seeds. For each time the Mattson cooker was run, two accessions BW± were tested. 25 seeds (of 35 soaked for 20h) of each batch were placed in a Mattson cooker. Each seed was placed under a brass plunger (metal rod) with the surface contact being 2 mm in diameter and the mass ~89 g. The plunger was placed on the middle or on the thickest parts of the seeds (Figure 4). The tank of the Mattson cooker was placed on a stovetop and filled with 6 l deionized water. Some of the water was first boiled in an electric kettle to speed up the process of getting the water to boiling point. The stovetop was turned on to max effect (setting 6), when the water was poured into the cooking tank and later turned down to a middle effect (setting 3) and the Mattson cooker's own heating element were plugged in when the water was boiling. The rack with seeds and plungers, was carefully lifted into the cooking tank with boiling water at which time the cooking started. To track the cooking, a GoPro camera was put on a stand to film the top of the Mattson cooker to track when the plungers penetrated the seeds which is the measure of cooking time (Figure 5). The GoPro recorded a timelapse video with image interval of 0.5 s. After all seeds were penetrated or approximately 100-130 minutes of cooking, the cooking session was terminated and the timelapse recording was stopped. The time of penetration was later determined with the

timelapse footage and a converting formula to real cooking time, (Timelapse time [min] × 15 = Real cooking time [min]).

Figure 4 Placement of seeds under plungers in the seed rack of Mattsson Cooker.

Figure 5 Mattson cooker during cooking with timelapse recorded by GoPro cameras.

2.6 Starch quality analysis

For the starch analysis the Megazyme Amylose/Amylopectin Assay Kit (K-AMYL) was used with some revisions for efficiency, i.e. reduced volumes in some steps (with retained concentrations) and a filtering step replaced with a short centrifugation to remove large particles and debris after verification that it did not lower the end-product concentrations. The percentage of amylopectin in samples can be estimated from the determined amount of amylose and total starch.

2.6.1 Grinding and freeze drying

Five seeds of each accession BW± were ground in a 50 µl stainless-steel container with 1-2 stainless steel beads of diameter ~14 mm in a Retsch Mixer Mill 400 with a frequency of 30 Hz for 1 to 5 minutes, until the seeds became a relatively fine flour. In some cases, there were small pieces that could not be ground, and these pieces were discarded. The rest of the flour was transferred into a 15 ml Sarstedt tube. The tubes of all 28 ground samples were placed in a -18 °C freezer before being freeze-dried for 48 h to remove excess water. After freeze-drying, the flour was sifted through a mesh of ~1 mm to remove lumps and large debris.

2.6.2 Starch extraction

Two technical replicates were done for each sample, as well as a maize starch control that was run with every set of 7 samples. An amount of 20-25 mg flour was weighed into 15 ml glass centrifuge tubes and the mass of DW (dry weight) was noted. The sample was dissolved in 1 ml of DMSO (dimethyl sulfoxide) by rigorous vortexing. Then the tubes were put into a 100°C water bath with repeated vortexing and mixing to prevent sedimentation and gelatinous lumps in the solution. After 16 min the tubes were removed from the water bath and placed to rest for 5 min at room temperature. Then 6 ml 96% ethanol was added under vortex and the starch was allowed to precipitate for 30 min at room temperature. The tubes were then centrifuged at 2000 *g* for 5 min, the supernatant was discarded, and the tubes were then drained upside down onto a tissue paper for 10 min to remove excess ethanol. The pellet was then re-suspended in 2 ml DMSO by vortex and placed into a 100°C water bath for 15 min with repeated vortexing to prevent gelatinous lumps. When the tubes were removed from the water bath a volume of 4 ml of Con A (Concanavalin A) solvent was added and vortexed thoroughly. The tubes were then centrifuged shortly (600 *g* for 1 min) to remove large particles and debris. The supernatant was quantitively transferred to 50 ml Sarstedt tubes and the sample was diluted with Con A solvent to a total volume of 25 ml (this is called solution A and should be analysed within 2 h).

2.6.3 Amylose and amylopectin determination

Amylose

A volume of 1.0 ml solution A was transferred to a 2 ml Eppendorf microfuge tube together with 0.5 ml Con A solution (this contains Concanavalin A). The tube was inverted to mix the content and then left to rest for 1 h at room temperature to precipitate the amylopectin (for removal). After 1 h the tubes were centrifuged at 14 000 *g* for 10 min at room temperature. A volume of 1 ml of the supernatant was transferred to a 15 ml glass centrifuge tube and mixed with 3 ml of 100 mM sodium acetate buffer (pH 4.5) and then put into a 100°C water bath for 5 min to denaturate the Con A. The tubes were then transferred to a 40°C water bath for 5 min. A volume of 0.1 ml of amyloglucosidase/α-amylase enzyme mixture was added to the tubes and then incubated in the 40°C water bath for 30 min, after which the tubes were centrifuged at 2000 *g* for 5 min. Aliquots of 0.25 ml of the supernatant was transferred to 2 ml Eppendorf tubes and left till glucose analysis.

Total starch

From the supernatant 0.5 ml was transferred to a 15 ml glass centrifuge tube. The supernatant aliquots were then mixed with 4 ml of 100 mM sodium acetate buffer (pH 4.5) and 0.1 ml amyloglucosidase/α-amylase enzyme mixture was added to the

tubes. The tubes were incubated in a 40°C water bath for 10 min. Aliquots of 0.25 ml were then transferred to 2 ml Eppendorf tubes and left till glucose analysis.

D-Glucose control (duplicate) and blanks

A volume of 0.025 ml D-glucose standard solution (1 mg/ml) was pipetted into two 2 ml Eppendorf tubes and dissolved in 0.225 ml 100 mM sodium acetate buffer (pH 4.5) and left till glucose analysis.

For blanks, 0.250 ml 100 mM sodium acetate buffer (pH 4.5) was added to two 2 ml Eppendorf tubes and left till glucose analysis.

Glucose analysis

To each Eppendorf tube containing glucose (either coming from degradation of the total starch or amylose), 1 ml of GOPOD reagent was added and the tubes were incubated in a heating block at 40°C for 20 minutes for the GOPOD to react with the glucose molecules which gives a pink colour. Then 200 µl of the samples were transferred to a 96-well plate, the absorbance was read in a spectrophotometer at 510 nm to determine glucose concentration in the samples according to a standard curve. Values of the controls were then compared to the registered standards to confirm that there were no large differences. The absorbance values and the dry weight, as well as the dilutions backwards were used to calculate the percentage of amylose in the dried flour.

2.6.4 Calculations

The amylose content of the starch was calculated from the absorbance values obtained from the glucose analysis and the appropriate dilution factors according to the following equation:

$$Amylose\ \% = \frac{\text{absorbance amylose}}{\text{absorbance total starch}} \times \frac{6.15}{9.2} \times \frac{100}{1}$$

The amylopectin was estimated with the following equation:

$$Amylopectin\ \% = 100 - Amylose\ \%$$

The total starch content was estimated based on a glucose standard curve, giving the ‘m’ (intercept) and ‘k’ (slope) correlating the absorbance value to the glucose concentration. Appropriate dilution factors and measurements were taken in consideration according to the following equation:

$$Total\ starch\ content\ \% = \frac{\frac{absorbance - m}{k} \times 4.6 \times \left(\frac{25}{0.5}\right)}{flour\ weight} \times \frac{162}{180} \times \frac{100}{1}$$

3. Results

3.1 Water absorption assessment

To determine a suitable soaking time for seeds prior to cooking, a water absorption assessment was performed on three accessions. The water absorption assessment (Figure 6) showed that the rate of water uptake was highest between 5-15 h, and after around 15 h the absorption started to decrease. At around 20 h not much additional water was absorbed and the TGW seemed somewhat stable even though Fuego was still increasing a little bit after that. Based on this result, a 20 h soaking time of seeds was selected for further cooking analysis.

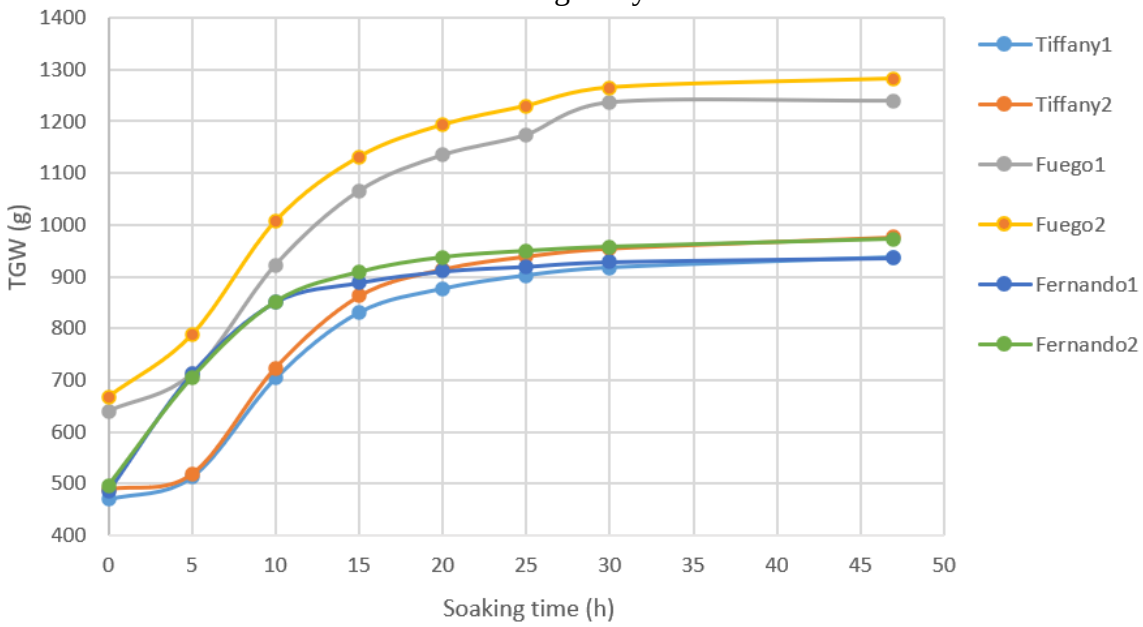

Figure 6 Water absorption of faba bean seeds in three different accessions over the timespan of 47 h after soaking in water. Dry mature seeds were used at the start of the experiment. Each curve in the diagram represents 50 seeds.

3.2 Penetrometer analysis

To determine seed-coat hardness a penetrometer analysis was performed. This resulted in different penetrometer profiles of seeds from different accessions (Figure 7). The value of the first point in the profiles where the pressure flattens out or momentarily dips, is an estimate of the hardness of the seed coat (marked with red dots in Figure 8). Generally, higher pressure-value indicates that the tissue is harder. Seed-coat hardness varied a lot between accessions, from ~13 N (Gubbestad) to ~24 N (Isabell and AKZENT).

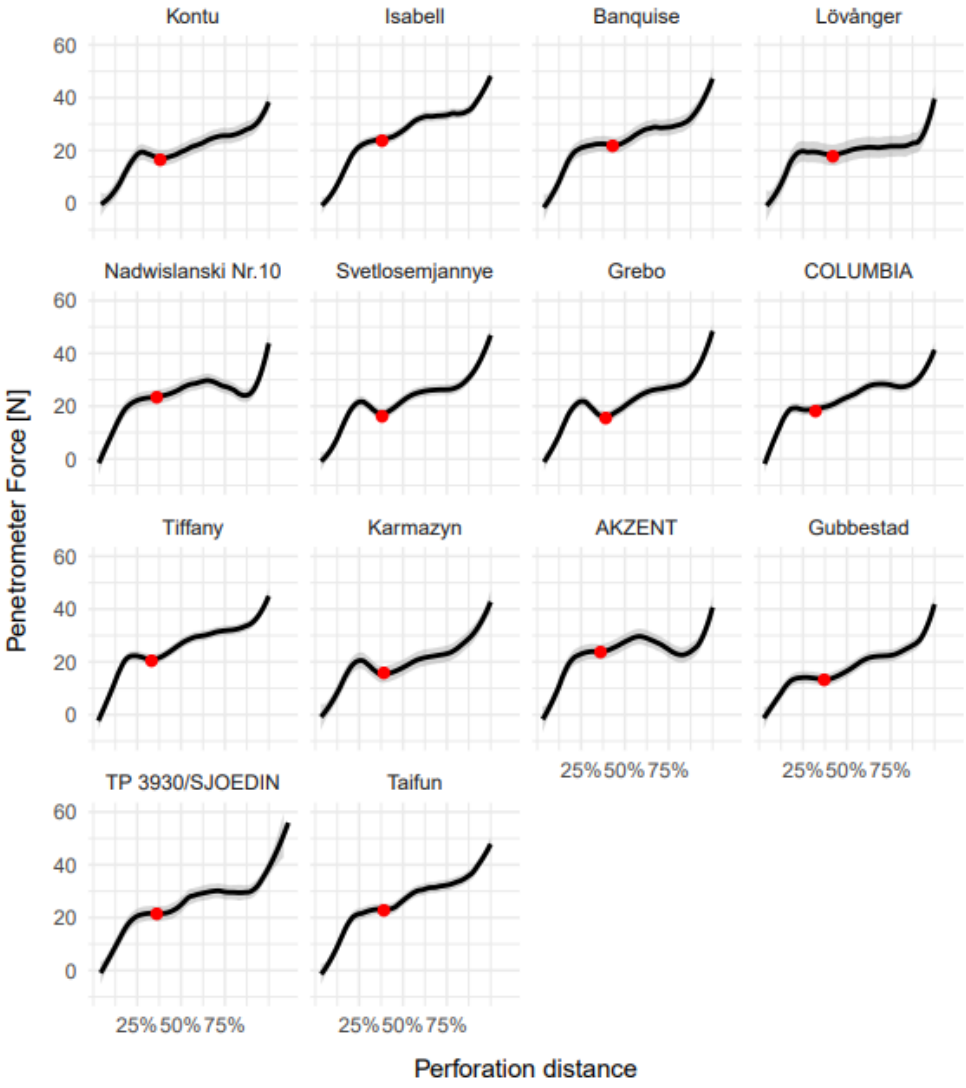

Figure 7 Pressure [Newton] applied with penetrometer at different perforation distances 0-100% in 14 different faba bean accessions (10 seeds of each). The shading around the graph indicates the variation of measurements from the average. Red dot indicate the values estimated for seed hardness

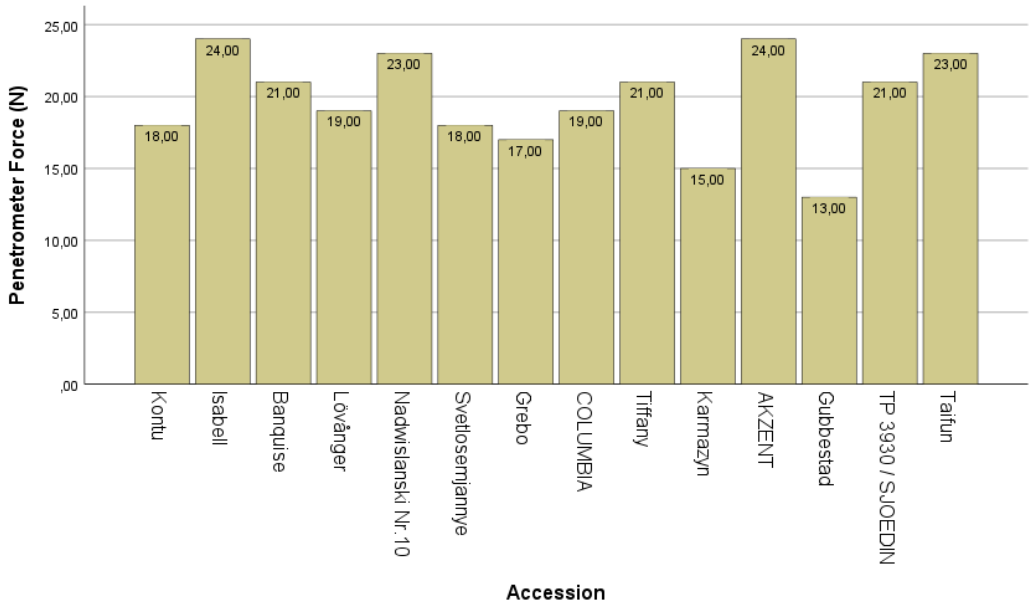

Figure 8 Estimated seedcoat hardness as average for 10 seeds each of the different faba bean accessions. Values represents the point of seed coat rapture marked in Figure 7 with red dots.

3.3 Seed weight and area determination

Seed morphology of the different accessions was different with regards to colour, size, and shape (Figure 2). Seed weight and area before and after soaking were determined as measures of seed morphology. The water uptake for the different accessions is presented in Figure 9 with the mean area of single seeds before and after soaking, as well as Figure 10 illustrating the TGW (thousand grain weight) before and after soaking. When looking at the general trends for the different accessions, the results for both seed area and seed weight look quite similar (with minor differences). There are clearly some accessions with smaller and some with larger seeds, there seems to be higher variation in size in the accessions with the larger seeds (e.g. Grebo and Karmazyn) compared to accessions with smaller seeds (e.g. Kontu, Isabell and Colombia) indicated by the larger error bars in the larger accessions. There seems not to be any large differences in seed area between BW+ and BW- (Figure 9). The TGW (Figure 10) assessment indicates a similar result as the seed area (Figure 9). Grebo seems to have larger seeds without BW damage than the seeds with BW damage.

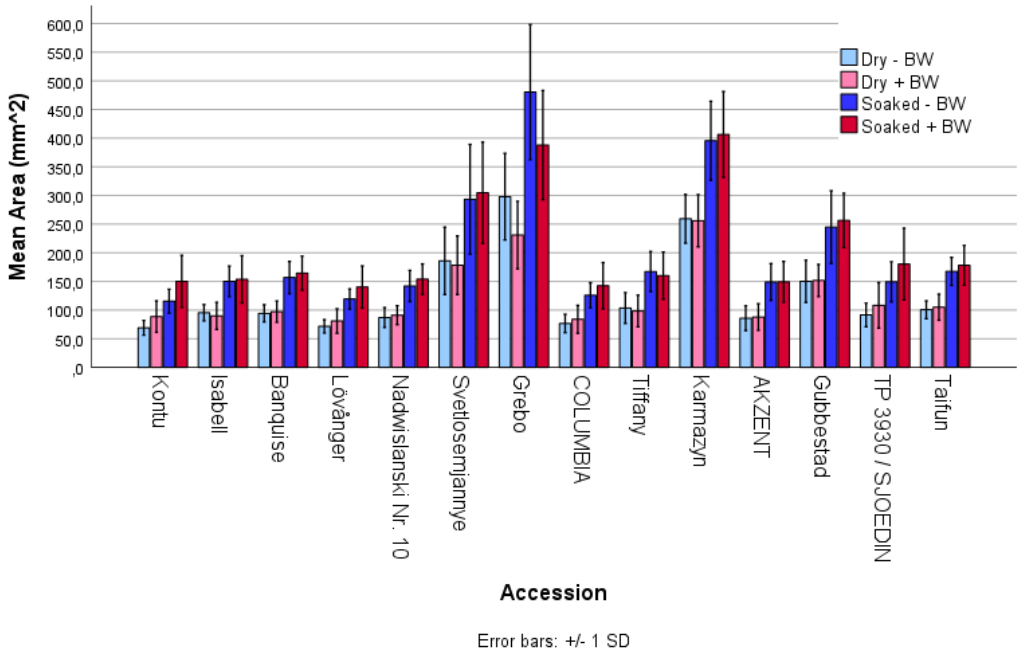

Figure 9 Seed area before and after soaking with or without bean weevil (BW) damage. Blue colour represents seeds without BW and red colour represents seeds with BW, the lighter colour represents seeds before soaking and darker colour represents seeds after 20 h of soaking. Data shows the mean value of 35 seeds ± standard deviation.

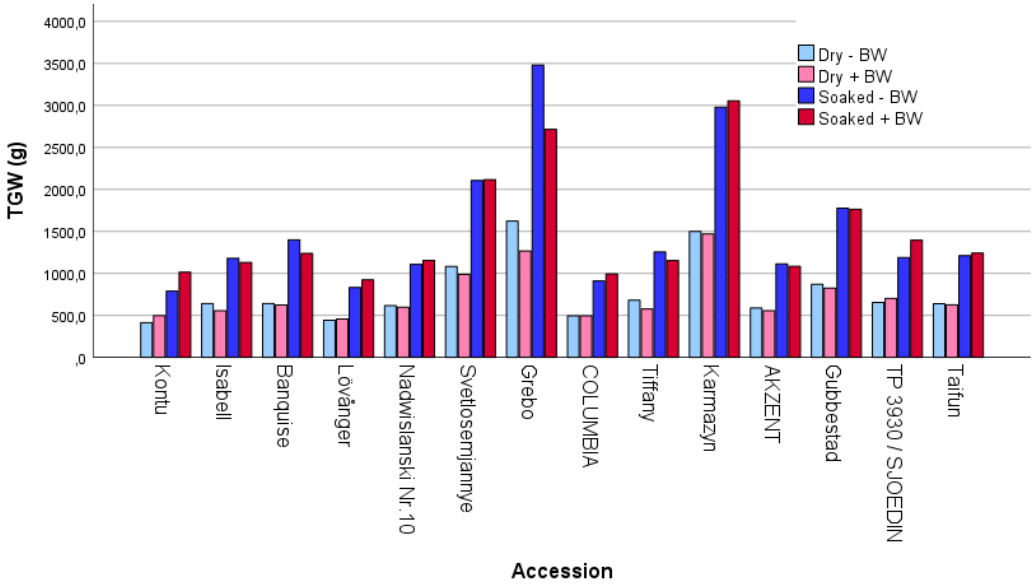

Figure 10 TGW (thousand grain weight) of accessions before and after soaking with or without bean weevil (BW) damage. Blue colours represent seeds without BW and red colours represent seeds with BW damage, the lighter colour represents seeds before soaking and darker colour represents after soaking. Standard deviation cannot be included since TGW is determined for a whole batch (35 seeds).

3.4 Cookability

To determine the cooking time for seeds of the different accessions with or without BW damage, a Mattson cooker analysis was performed. The results show that different accessions took varying amounts of time to cook, for example long time for Karmazyn (CT_median = 55 – 62 min) and short time for Nadwislanski (CT_median = 25 – 30 min) (Figure 11). For several accessions (e.g. Kontu, Lövånger, Taifun, Banquise, Gubbestad, TP3930, and Karmazyn) there is a trend showing that seeds without BW damage took a longer time to cook than the seeds with BW damage; however, in the other accessions there is no clear difference between BW damage absent and present. Accessions with a shorter interval between the times at which 40% and 80% seeds were cooked indicates a more homogenous cooking and could be seen in for example Nadwislanski Nr. 10, COLUMBIA and Lövånger, as compared to the accessions with a larger interval such as for Tiffany and Karmazyn (Figure 11). IQR (interquartile range) the range represents 50% of the cases inside the box of a boxplot. In this experiment that means the time from when seed nr 25 to seed 75 is fully cooked (Table 1).

Table 1 IQR (interquartile range) of cooking time [min] in different accessions of faba bean with and without bean weevil damage.

Accession	IQR [min] BW damage absent	IQR [min] BW damage present
Kontu	22,75	12
Isabell	28	20
Banquise	14	12
Lövånger	17	10
Nadwislanski Nr.10	12	10
Svetlosemjannye	29	18
Grebo	25,5	16,5
COLUMBIA	16	16
Tiffany	30	20,5
Karmazyn	30,25	22
AKZENT	14,25	12
Gubbestad	26,5	17
TP 3930/SJOEDIN	22	21
Taifun	11	14

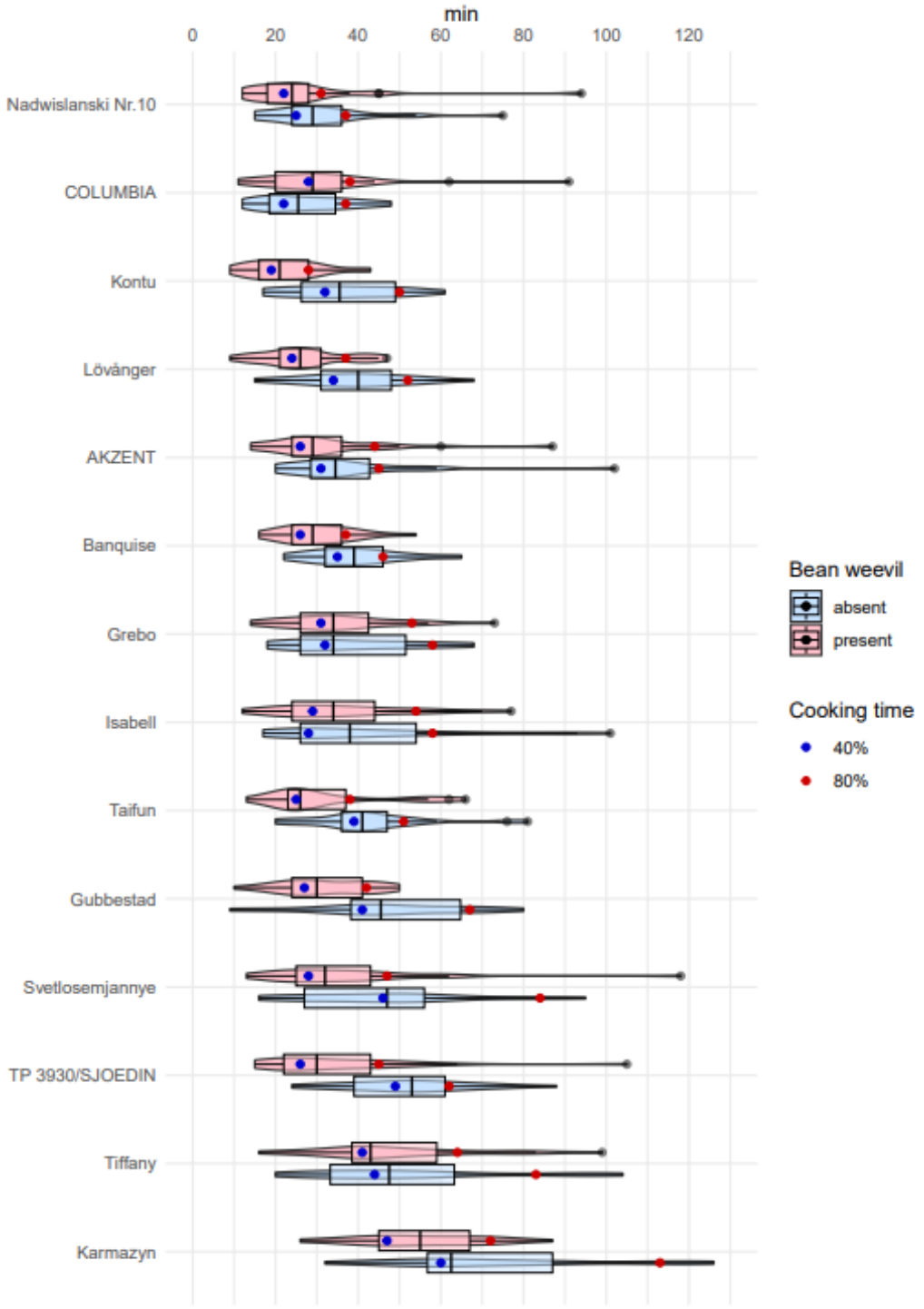

Figure 11 Violin/boxplots showing cooking time (min) of different accessions with and without bean weevil damage. Median cooking time is shown as lines within the boxes, while the blue and red dots represent two other definitions of cooking time, i.e. when 40% (blue) or 80% (red) of the beans are sufficiently soft to be penetrated by the plungers in the Mattson cooker. Each 'violin' represents the result from 25 seeds.

3.5 Starch quality

The amylose content in all accessions is relatively similar (from 9.8 – 12.5% by seed DW), but the amylopectin is more varying in the samples (from 15.4 – 21.8% by seed DW as well as the total starch content (from 25.9 to 33.6% by seed DW). In most of the accessions, there is a trend with lower starch content in the seeds with BW damage (Figure 12).

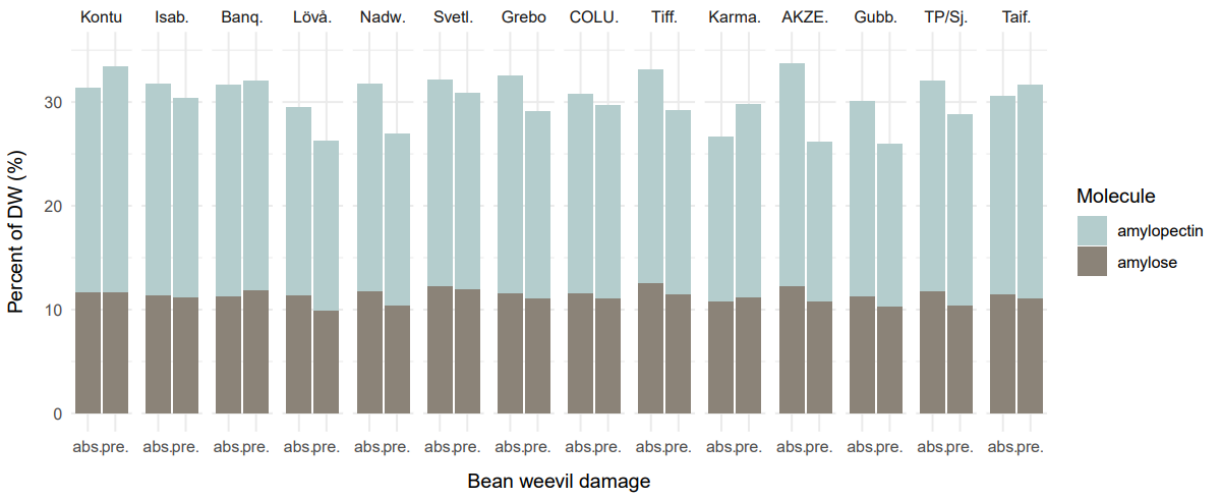

Figure 12 Starch content and profile in respect of amylopectin and amylose in full seed flour (DW). DW; Dry weight, abs.; BW damage absent, pre.; BW damage present.

3.6 Correlation analysis

To compare the different variables of this experiment and see if they were co-variating, a pairwise correlation analysis between traits was carried out. A strong positive correlation (a value close to 1) between two parameters means that a high value of one of these parameters is associated with a high value of the other parameter. A strong negative correlation (a value close to -1) between two parameters means that a high value of one of these parameters is associated with a low value of the other parameter. Here, only the strongest correlations between traits (i.e. values above 0.7 and below –0.7) are discussed in greater detail.

For the seeds without BW damage (Figure 13A), strongly positively correlated variables are the means of seed area (areaDry and areaSoak) and TGW before and after soaking (TGWDry and TGWSoak, respectively) meaning, not surprisingly, that accessions with larger seeds have larger seed area, and vice versa. A strong positive correlation is also seen between the variance in seed area before and after soaking (area_CV_dry and area_CV_soak respectively), reflecting those accessions with seeds having a large variation in seed size before soaking, also show a large variation in seed size after soaking.

Another strong but negative relationship is between the amylopectin and amylose content, which is natural since the sum of the two is 100%. However, it is also seen that a high total starch content in seeds is correlated to a high amylopectin content. It can be noted that starch parameters showed a very low correlation to seed size parameters.

With regards to cookability parameters, median cooking time (CT_med.), 40% cooking definition (CT_40) and 80% cooking definition (CT_80) are all strongly positively correlated to each other, which is natural since they are all different measures of cooking time. If focusing on CT_80 as a measure of cooking time, it was highly correlated to the starch quality i.e. content of amylose (positively) and amylopectin (negatively). Further, CT_80 was negatively correlated to RAG meaning that the more water a seed absorbs during the soaking period, the shorter cooking time it will have. There is indication of positive correlation of CT_80 and seed size (areaDry, areaSoak, TGWDry and TGWSoak) indicating that larger seeds take longer time to cook; however, the correlation coefficients are lower than 0.7. The negative correlation of CT_80 and penForce (seed-coat hardness) of – 0.5 suggests that a harder seed coat results in shorter cooking time.

For the seeds with BW damage (Figure 13B), the pattern of pairwise correlations looked similar as for the seeds without BW damage in most cases. However, the largest differences between the two correlation matrices are how they relate to starch factors such as total starch, amylopectin and amylose, and their correlation to the other factors. These correlations were much weaker in the seeds with BW damage than in the seeds without BW damage.

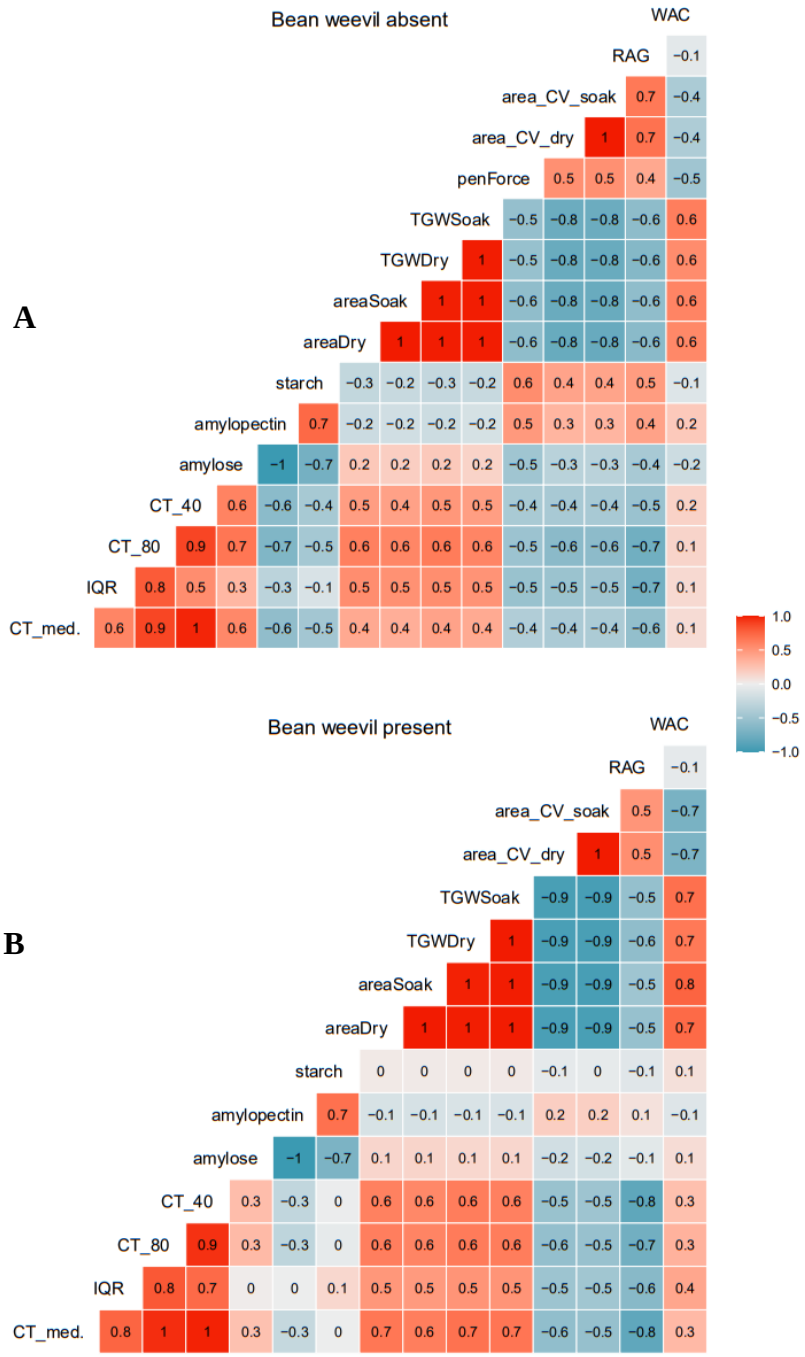

Figure 13 Correlation analysis of different factors measured during the experiment, red colour indicates a positive correlation and blue indicates a negative correlation, the strength of the correlation is indicated by the darkness of the colour (red or blue). ***A.*** *Bean weevil damage absent* ***B.*** *Bean weevil damage present. WAC; water absorption capacity (%), RAG; relative seed area gained during soaking (%), area_CV_soak; covariance of area of soaked seeds, area_CV-dry; covariance of area of dry seeds, penForce; Seed coat hardness, TGWSoak; thousand grain weight of soaked seeds (g), TGWDry; thousand grain weight of dry seeds (g), areaSoak; seed area of soaked seeds (mm^2), areaDry; seed area of dry seeds (mm^2), starch; Total starch content of dry weight flour (%), amylopectin; Amylopectin fraction of total starch (%), amylose; Amylose fraction of total starch (%), CT_40; Cooking time of 40%, CT_80; Cooking time of 80%, IQR; Interquartile range of cooking time for CT_med, CT_med; Median cooking time. Note that the parameter penForce is only available in A.*

4. Discussion

4.1 Seed size, water uptake and seed-coat hardness

The seed size was quite different between the 14 different accessions, both in aspect of seed area and TGW. One accession that stood out was Grebo (Figure 9 and 10) where the seeds without BW damage had both higher TGW and seed area than seeds with BW damage. This might be indicative an effect of BW damage in this accession or that larger seeds in this accession have lower rates of BW damage, however this would need more exploration to prove or dismiss. A study performed of a larger set of faba bean accessions (including the 14 accessions used in the present study) resulted in seeds with TGW (dry weight) from ~200 g to 1200 g and most of the accessions centred around 400 g (Ohm, *et al.*2024). The results from the present study show two accessions with a higher TGW (~1500 g) than in the study by Ohm *et al.* (2024), while the lowest TGW was around 400 g and most of the accessions had a TGW around 500 – 600 g. The TGW differences between these two studies could be due to that the plants producing the seeds grew in different places and different years, giving them different growth conditions (i.e. Skara 2022 in the present study vs Alnarp 2021 and 2022 in the study by Ohm *et al*.). In fact, the accessions in the present study with the largest seeds (Karmazyn and Grebo with TGW ~1500 g) were not the same accessions that had the largest seeds in Ohm *et al.* (TGW ~1200 g). It can be noted that the diversity panel of 14 accessions used in this study do not cover and represent the whole variation of the larger diversity panel of 220 accessions used in Ohm *et al.* (2024). Since most of the 14 accessions analysed had a seed size in the mid-range of available diversity within the species, it would have been interesting to include more accessions with small and large seed size.

The first step of cooking legume seeds is to soak them in order to hydrate properly, which will facilitate the cooking process of separating the cells in the seed. The seeds absorb different amounts of water at different times during soaking, reviewed by (Perera, *et al.*, 2023). Beans usually absorb water in a sigmodal (S-shaped) curve initially having slow absorption (called a lag phase) and then accelerating slowly until starting to flatten out closer to a saturated stage. This contrasts with cereals

that start to absorb water quickly without a lag phase. In the present study we found that two of the three tested accessions (Tiffany and Fuego) followed the sigmodal absorption curve while one accession (Fernando) followed the curve lacking a lag phase (Figure 6). It can be noted that Fernando is a white-flowering variety with low content of tannins in the seed coat. Low tannin content could influence water uptake (Martin, *et al.*, 1991), which could make low tannin accession start absorbing water more quickly and therefore have a less pronounced lag phase than accessions with a higher tannin content. However, further studies comparing more white-flowering with variegated-flowering varieties are needed to confirm such hypothesis.

The seed-coat hardness varied between different accessions, ranging 11 newtons between the softest (Gubbestad) and the hardest accessions (Isabell and AKZENT). This indicates a large variation. The values of the recorded seed hardness in the present study were close to those recorded in peas by Dueholm *et al*. (2023). In comparison to the study on pea, no clear peak in the penetrometer curve was observed in the present study, which in the pea study was indicative of the seed coat rupturing. This mean that seed hardness was estimated slightly differently in the present study on faba bean as compared to that on pea, since Dueholm *et al*. (2023) estimated the first peak as the seed-coat hardness and we estimated the dip after the first peak as the seed-coat hardness. This likely influenced the results since some of the faba beans had peaks higher than the following dip and others barely flattened out (Figure 7). Therefore, a direct one-to-one comparison between the two studies is difficult. Further, it can be noted that the seed shape of faba beans and peas are different. Peas are often more spherical in shape while faba bean seeds vary from small spherical, to mid-range sized ellipse shaped to large almost flat seeds. A spherical form is generally a stronger ‘construction’ when applying force to an area, compared to flat surfaces. Another factor can be the structural differences due to different amounts of tissues around the point of impact, helping larger seeds to keep together without breaking, where smaller seeds spread the pressure in another way. These differences in shape can probably affect the penetrometer results. Another source of error is the human influence as the penetrometer rod was lowered by turning a wheel manually, therefore the phasing of each lapse might affect the results, since these are related to the thickness of the seed and the time it took to penetrate the seed. The penetrometer profiles (Figure 7) might be different if the lowering of the penetrometer rod would be more consistent. Such results might be possible to obtain by using an automatic system that mechanically lowers the rod and applies pressure (such systems already exists).

4.2 Starch content and quality

The starch content of the 14 accessions varied between ~25 – 35%, similar to the bulk of the range of the diversity panel in Ohm et al. (2024). This indicates that the 14 accessions are quite representative of the variation available in the larger diversity panel. It is important to notice that the accessions analysed in the present study belong to the same diversity panel as analysed by Ohm et al. (2024); however, as mentioned above, the seeds in the two studies were grown in different locations and years. In both studies an enzymatic method was used, but not the exact same method, which might affect the results. Despite of this, the results seem to be quite consistent between the two studies. Regarding starch quality, we found the amylose content of the faba bean starch to be between 34 – 41% of total starch, this is quite close to the values reported in another study, that were in the range 33.6% to 39.9% (Punia, *et al.*, 2019). Dueholm et al. found that the pea seeds that had a wrinkly seed coat, had significantly lower starch content, but most importantly reduced amylopectin levels in the seeds, compared to non-wrinkled seeds. This phenomenon has been connected to a mutation in a gene coding the SBEI (starch branching enzyme) which normally catalyses the formation of the starch-branches with α-(1/6) bonds (Bhattacharyya, *et al.*, 1990). This has not been found in faba bean; however, looking at possible homologous genes, and searching for more accessions with possible indications of this phenomenon in faba bean would be interesting.

4.3 Cookability

The knowledge of the cooking properties in faba bean is very limited, especially compared to other legumes. When comparing different accessions and types of legumes with each other, it may be important to use the same definitions of cooking time and other parameters. However, whether different definitions of cooking time have a sizable impact on conclusions is a current knowledge gap. In the present study, we tested three definitions of cooking time: 40% of all seeds (Santos, *et al.*, 2018), 80% of all seeds (Bassett, *et al.*, 2021), and 50% of pierced seeds (median) (Dueholm, *et al.*, 2023). We found that when ranking the accessions based on cooking time, the order of accessions would be slightly different depending on the definition of cooking time used. This observation is most likely due to variation of cookability within the accessions. Therefore, a single measurement of cooking time might not be the best estimate for the cooking time of the whole batch, instead maybe an average or the positioning of the widest part of the violin plots (Figure 11) would potentially be a better estimate because it would represent a larger portion of the seeds, compared to a specific ranking of the seed.

When comparing the cooking times of the present study to the results of Dueholm *et al*. (2023), many of the faba bean and pea accessions had similar cooking times, falling within a range of 20 to 40 minutes. The study on pea had a wider range of cooking times, but the authors also used a larger panel, counting 24 accessions. What is noticeable is that there is a large variation in the number of uncooked seeds of pea remaining after cooking, ranging from 0 – 80% compared to faba bean where it was only in a few cases there were single uncooked seeds (0 – 8 %). The cooking sessions of the present study lasted longer (sometimes exceeding 120 min), while all the cooking sessions in the pea study were stopped after 90 min, which could lead to a higher number of uncooked seeds in the pea study. Some of the faba bean accessions had quite a long cooking time, for example Nadwislanski Nr 10 with an 80% cooking time (CT_80) of ~30 – 40 min and Karmazyn with a CT_80 of ~70 – 110 min; however, many of the other accessions had quite similar cooking times.

For some of the single seeds that did not cook properly, I tried adding extra pressure after finished cooking by pushing on the rod with my hand, which in some cases made the plunger go through the seed, in these cases it seems like it is the hard-shell phenomenon (Perera, *et al.*, 2023) in play. In some other cases, the centre of the seed was really hard, so the water did not absorb into it, resulting in that the seed did not cook and the plunger did not penetrate properly even after trying adding pressure after cooking. This can be referred back to the hard to cook phenomenon (Perera, *et al.*, 2023), where it is the inner tissues that are harder than normal.

4.4 Correlation analysis

From a breeding perspective, it is of great interest to determine if there are any easily measured seed quality parameters that strongly correlate to cookability in faba bean. Such correlations could suggest more convenient methods for predicting cooking time that are less laborious and time-consuming compared to a full cooking analysis. A parameter that predicted cooking time well according to this study was the seed area gained after soaking (RAG) which is a measure of hydration capacity. The higher the RAG, the shorter the cooking time. Another predictor would be the seed size, with a larger seed size (areaDry and areaSoak) taking longer to cook, which is reasonable since larger amounts of tissue would need more energy to be fully cooked. However, there were differences in this respect depending on whether bean weevil (BW) was present or absent as the correlation with CT_med. and seed size (areaDry, areaSoak, TGWDry and TGWSoak) was higher (0.7) for seeds with BW damage than seeds without BW damage (0.4) (Figure 13). Surprisingly, the parameters RAG and WAC were uncorrelated in opposition to the correlation found in pea (Dueholm, *et al.*, 2023). A possible explanation for this could be that large

and small seeds swell differently in the different 3-dimensional directions. Since the Marvin seed analyser only analyse a 2-dimentional view, the change in the third dimension is not recorded. When counting the WAC, the seed mass is used, which is a measurement that does not lack a dimension in the same way as in the RAG calculations. Larger seeds tend to be flatter than smaller seeds that tend to be rounder/egg shaped, this could mean that smaller seeds swell approximately the same amount in all directions, where the larger seeds might swell more in the 2-D-plane that is measured in the Marvin seed analyser.

The correlation results were quite similar to the results of Dueholm *et al*. (2023); however, our results had higher correlation values than those of the study in pea. In the study of pea, the seedcoat hardness was found to be a predictor for cooking time, but in the present study, seedcoat hardness had a negative correlation to cooking time, which is the opposite to pea. The differences could derive from the different method of determining the seedcoat hardness in the two studies as described above. The contrasting results indicate that seedcoat hardness is not a good predictor for cooking time in faba bean. The majority of legume seeds is made up of the cotyledons and not the seed coat. A better predictor might then be the hardness of the cotyledons, that might be possible to measure in a similar way to the seed coat hardness. If such support would be found, the structure of the inner parts of the seed could indicate the time needed to reach a certain cooking stage. Determining hardness and cooking time of cotyledons can also be of interest for food applications in which seed coats are not desired and therefore removed through dehulling prior to further processing such as cooking.

When comparing the different definitions of cooking time, they all (CT_40, CT_80 and CT_med.) correlated positively strongly with each other, indicating that the choice of definition does not have a great impact on the results, however it is still important to know the definition when comparing different results and to know that there can be slight differences when comparing individual cases.

4.5 Bean weevil damage

We found that in case of water absorption and cooking time, most accessions absorbed more water during soaking and showed a shorter cooking time in seeds with BW damage. Otherwise, the effect of bean weevil damage on faba bean seed quality was not very strong in most aspects this study focused on. In general BW damage seems to lower the total starch content; however, the low number of replicates as well as the large variation of damage level, could have impacted the result and therefore more replicates in analysis's would be desirable, both technical (same flour) and from different pools of seeds of the same accessions.

As mentioned earlier, the distinction between seeds with and without BW damage was very subjective and varied a lot between accessions, this is definitely a major source of error, which in this study could not be avoided, but might be able to take into consideration in future studies by scoring the level of damage instead of just two categories.

For the use of faba bean seeds for extracted pure starch, the bean weevil damage should not be a problem; however, for usage of the whole beans there could still be a cosmetic problem with people not wanting to consume the product with BW damage. Previous studies showed that the nutrient quality in faba bean seeds is not affected significantly by BW damage, although the BW damage had varying effects on the quality of beans for seeding (Ryding, 2020).

5. Conclusion

The results of this study is of interest for breeding of faba bean for increased use in food in the future. Cooking seeds is a simple but important processing method to decrease antinutrient factors in legumes. Through this work, the methods for determining cookability have been proven to work in faba bean in addition to peas (Dueholm, *et al.*, 2023). Another finding that is of interest for the plant breeding is the low variation in starch quality between accessions, indicating it is a quite a stable trait and does not seem to be connected to any of the other parameters we tested. For industrial purposes it can be of interest that the BW damage of seeds only seems to have a low effect on starch quality, while cooking time is slightly decreased. In the future it would be interesting to analyse cookability in a higher number of accessions covering a larger part of the diversity available in the species, and also to determine the cotyledon and seed hardness in addition to the seedcoat hardness.

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Popular science summary

Is faba bean the new Nordic soybean?

Faba beans can be grown in our Nordic climate as an alternative legume crop for food and industrial purposes. But more breeding and research needs to be done to be able to help reach the common goal of improving our personal and environmental health.

Today the world is somewhat unison in the need for increased consumption of beans and other legumes for health and environmental causes. If more legumes are grown in Europe, some of the large amounts of soybean that is imported every year to the EU can be replaced. Soybean is not a crop that can be grown in the northern parts of Europe, due to the climate, but other legumes could take its place, such as peas, common beans and faba beans. Faba beans have a large variation in size and shape, making them perfect for a variety of purposes, both in food and industry. However, it is important to increase the research and breeding of new varieties to produce better crops to help reach the Sustainable Development Goals.

In this study we found that larger seeds of faba beans tend to take longer time to be fully cooked. Another find was that damage of bean weevil do have an effect of shortening the cooking time, but it did not show any effects on the starch content or quality. The low variation of starch quality is an interesting find when it is compared to the results of studies performed in peas. They found that wrinkly seeds had quite a different starch profile (meaning the amounts of the different starch components such as amylose and amylopectin). So far this kind of wrinkly seeds have not been found in faba beans, but who knows what will be found in the future.

The findings of this study are a part of the foundation of the research that can influence future studies in the field, both in breeding and in food sciences.

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