

Cookability of 24 pea accessions—determining factors and potential predictors of cooking quality

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Abstract

BACKGROUND: Cooking time and cooking evenness are two critical quantities when determining the cooking quality (termed cookability) of pulses. Deciphering which factors contribute to pulse cookability is important for breeding new cultivars, and the identification of potential cookability predictors can facilitate breeding efforts. Seeds from 24 morphologically diverse pea accessions were tested to identify contributing factors and potential predictors of the observed cookability using a Mattson cooker. Size- and weight-based measures were recorded, and seed-coat hardness was obtained with a penetrometer. Content of protein, starch (amylose and amylopectin), and phytate was also determined.

RESULTS: Distinct differences were found between wrinkled and non-wrinkled seeds in terms of water-absorption capacity, seed-coat hardness, and plunger-perforation speed. Potential predictive indicators of cooking time and cooking evenness were seed-coat hardness ($r = 0.49$ and $r = 0.38$), relative area gained ($r = -0.59$ and $r = -0.8$), and percentage of swelled seeds after soaking ($r = -0.49$ and $r = -0.58$), but only for non-wrinkled seeds. Surprisingly, the coefficients of variation for the profile area of both dry and swelled seeds appeared to be potential cookability predictors of all pea types (correlation coefficients around $r = 0.5$ and supported by principal component analysis). However, no strong correlation was observed between cookability and protein, starch, or phytate levels.

CONCLUSION: Using three types of instruments together with chemical components enabled the identification of novel cookability predictors for both cooking time and cooking evenness in pea. This study unveils the diverse quantitative aspects influencing cookability in pea. Considering both cooking time and cooking evenness, as well as seed-coat hardness, underscores the multifaceted nature of pulse cookability and offers important insights for future breeding strategies to enhance pea cultivars.

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Supporting information may be found in the online version of this article.

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INTRODUCTION

With the rise in popularity of plant-based foods,¹ cooking quality, or cookability, of dry legume grain seeds (pulses) has gained increased importance. Cookability refers to the duration required for a batch of seeds to reach a desirable level of softness for consumption.^{2,3} Pea (*Pisum sativum* L., Fabaceae) provides multiple health benefits, including improved gastrointestinal function and reduced glycaemic index, making it an integral component in the transition from animal-based toward a predominant plant-based diet.^{4,5} Globally, pea is one of the most extensively cultivated pulses, after soybean, with approximately 15×10^6 t of dry peas produced annually (FAOSTAT 2021; <https://data.apps.fao.org/>), and is well adapted to temperate, agricultural regions.⁶

Cooking time of pulses can be defined as the duration required for the cotyledon cells to separate sufficiently.⁷ Many studies use a Mattson cooker for determining adequate softness, observing

when metal plungers pierce pre-soaked legume seeds during the boiling (or steaming) process in the cooker. However, cooking

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methodologies of legume seeds using the Mattson cooker lack standardization, and cooking time is often determined differently across studies, with some studies defining cooking time when 40%, 50%, 60%, 80%, or 92% of the seeds are pierced.^{8–12} Recent cookability studies have determined correlations between physico-chemical variables and cooking time and performed principal component analysis (PCA), exploring, for instance, the influence of soaking and seed-coat thickness in bean¹³ (cooking time defined at 80%) and hydration capacity and seed weight in faba bean, chickpea, lentil, and grass pea¹² (cooking time defined at 40%). An overlooked aspect of cookability is the evenness in the cooking of the legume seeds, although this characteristic is important to include as attaining a good cooking quality requires a consistently uniform result in the cooking process. Contributing factors to greater unevenness in the cooking could be both hard-shell (also called hardseededness) and hard-to-cook defects that are common among pulses.¹⁴ Hardseededness refers to the condition where certain seeds are unable to fully hydrate during the pre-soaking treatment, whereas hard-to-cook seeds are hydrated seeds that do not cook soft (i.e. never reach the desired softness). Both these defects can introduce variability in the cooking outcomes, affecting the texture and doneness of legume seeds within the same batch. Hence, understanding and addressing these factors is crucial in achieving more consistent and uniform cooking results, meeting the market demand for the desired level of softness, tenderness, or other textural attributes and doneness throughout the batch of cooked legume seeds.

Multiple factors interplay in the cooking behaviour of pulses, such as levels of different seed storage compounds, seed-coat structure, and field conditions during cultivation.¹⁵ These factors are reviewed elsewhere,^{7,14} but exactly how they interplay and which underpinning mechanisms are involved still need to be unravelled. In this study, this knowledge gap was addressed by investigating mature seeds of 24 diverse pea accessions across several variables to determine which variables measurably impact cooking time and cooking evenness. Peas can be grouped in many ways, but one way is to divide them into fresh peas and dry peas.¹⁶ Fresh peas are harvested when the pods and seeds are immature and include garden peas (GAP), snow peas, and sugar snap peas (SSP). Fresh peas are primarily bred and grown for human consumption. Dry peas are harvested at a mature stage and include marrow fat peas (MFP), fodder peas (FOP), dry yellow peas (DYP), and grey peas (GRP). They are used for both fodder and human consumption. We were especially interested in identifying potential predictors for cookability, in that both cooking time and cooking evenness can be used for defining target traits that contribute to crop improvement in pulses regarding cooking quality. A holistic approach was taken by including several types of instruments for the analysis of cookability (Mattson cooker and GoPro camera), seed hardness (penetrometer), and seed sizes (Marvin ProLine I seed analyser). By incorporating these diverse instruments, as well as chemical components in the seeds (protein, amylose, amylopectin, phytate), which could affect gelatinization and seed hardness,¹⁴ a more comprehensive understanding of the various factors influencing cookability in peas was obtained.

METHODS AND MATERIALS

Plant material

A diversity panel of 24 pea accessions was selected based on seed morphology and passport data. The criteria for selecting the accession included in the study was to compose a panel as diverse

as possible based on testa colour, seed shape, seed size and wrinkling, as well as different levels of material types and utility. Of the 24 accessions, 5 had distinctly wrinkled seeds, 7 had patterned testae, and 12 had darker coloured testae (i.e. dark green, brown, orange brown, or army green)¹⁷ (Supporting Information Table S1). The 24 pea accessions were classified based on their most common utility and germplasm type: cultivar (CV); breeding material (BR); landrace (LR); or wild type (WI). Accessions lacking information on utility were marked as GenBank accession (GBA). All seeds used in this study were multiplied under field conditions during 2021 in southern Sweden (55.90°N, 13.09°E). Planting was performed on 20 April, followed by irrigation before coverage with a fibre cloth until plant establishment. Owing to a persistent dry period, two additional watering sessions were carried out in June. Accessions with taller plants and vining growth habits were supported with metal trellises. Harvest of seeds was performed between 15 July and 20 August by manually picking the pods of mature plants. The pods were then threshed, weighed, and stored at 4 °C.

Imbibition analysis and absorption capacity

The water-absorption capacity (WAC), calculated as

$$WAC = \frac{\text{weight}_{\text{soaked}} - \text{weight}_{\text{dry}}}{\text{weight}_{\text{dry}}} \times 100$$

was determined by measuring the weight of 100 seeds for each tested accession before and after soaking in excess Milli-Q water for 20 h at room temperature. Percentage of hydrated seeds (i.e. swelled) was also determined for each accession by visual inspection. Seed sizes (profile area in square millimetres) were determined before and after soaking using a Marvin ProLine I seed analyser (MARVITECH GmbH, Wittenburg, Germany). Coefficients of variation (CV) for seed area (area_{CV} = SD/mean, SD is the standard deviation; for both dry and soaked) were calculated, as well as relative area gained (RAG), which was calculated as

$$RAG = \frac{\text{area}_{\text{soaked}} - \text{area}_{\text{dry}}}{\text{area}_{\text{dry}}} \times 100$$

Cooking time and evenness

A Mattson cooker (LM Agriculture, Svalöv, Sweden) – an enclosed brass box with boiling water in the bottom^{12,13,18} and accommodating 100 brass plungers (~89 g, 2 mm diameter for the piercing part; Supporting Information Fig. S1) – was used to investigate cooking quality parameters. After the seeds had been soaked for 20 h, 50 swelled peas per accession were randomly selected and placed in the holes of the Mattson cooker with the embryonic axis in the horizontal plane⁷ and a plunger resting on the top of each pea. The rack fitted two accessions at a time, and the loaded rack was placed on top of boiling deionized water with the water level just below the plate holding the peas.¹⁹ A time-lapse video for each 90 min cooking session with a GoPro HERO7 camera (GoPro, San Mateo, CA, USA) was recorded and the piercing time for each pea was determined as the minute number when a plunger fully perforated a cooked pea. Cooking time of each accession was determined by the median of pierced seeds, and cooking evenness by the interquartile range (IQR). By having video footage, qualitative perforation speed (slow, medium, or fast) could be determined for each accession; that is, the speed

by which the plungers pierced the peas: slow when most plungers took more than a minute for the piercing; fast when the piercing was instantaneous (< 5 s) for all pierced seeds; and medium when the piercing speed fell between slow and fast (60 s $>$ piercing time > 5 s).

Seed hardness

For each pea accession, hardness was tested with a penetrometer attached to a drill stand (STEP Systems GmbH, Nürnberg, Germany). Seeds were soaked in excess Milli-Q water for 20 h and half of the seeds were additionally boiled for 5 min at the surface of deionized water held in position by a rack. Preliminary tests with different boiling durations (5, 10, 15, and 30 min) showed that 5 min was a suitable duration that would provide a measure for seed-coat hardness after boiling (SCH5) for all accessions. For each treatment (soaked and soaked + boiled), ten seeds were analysed. The treated seeds were kept sealed in a small tube prior to analysis to prevent moisture loss. The width of each seed tested was measured from side to side with the embryonic axis in the middle, to an accuracy of 0.02 mm. A single seed was positioned with the embryonic axis in the horizontal plane and the penetrometer's rod was carefully placed just above the seed's surface without it exerting any force. The piercing rod was 3 mm wide, and the piercing speed was 0.2 mm s^{-1} . Care was taken to ensure a constant piercing speed for each seed. For each recorded data point through a single seed, the associated relative perforation distance was determined; that is, 0% at the top of the seed and 100% at the bottom. Single-seed data were then pooled for each accession and treatment and locally estimated scatterplot smoothing (LOESS) regression was performed using a span of 25%. As a measure for seed-coat hardness, the peak force from the LOESS regression within the first 50% of the displacement was estimated for each accession and treatment.

Total starch content and amylose/amylopectin ratios

Amylose and amylopectin content was determined enzymatically with an Amylose/Amylopectin Assay Kit (Megazyme, Bray, Ireland) on 20–25 mg freeze-dried flour according to manufacturer's protocol. The method is based on degradation of starch into glucose before and after separating amylopectin from total starch by a precipitation step using concanavalin A. The released glucose of both fractions (total starch and amylopectin) is then quantified colorimetrically by measuring the absorbance at 510 nm. The amylose content was calculated after corrections were made for dilution factors and a correction factor for conversion of molecular weights of glucose to starch:

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

Total starch was estimated based on the absorbance measurements of glucose in the total starch fraction and by reading against a standard glucose curve ($0\text{--}1 \text{ mg mL}^{-1}$), and by correcting for dilution factors and a correction factor for conversion of molecular weights of glucose to starch (k being the slope and m the intercept, of the standard curve):

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

All flours were tested in triplicate samples.

Protein and phytate content

Protein content of freeze-dried flour from each accession was analysed by the Dumas method^{20,21} using a Eurofins service (Linköping, Sweden). Phytate content was determined in duplicate samples on freeze-dried flour with a Phytic Acid Assay Kit (Megazyme).

Statistical analyses

All statistical analyses were performed using R v4.3.0.²² The data were checked for equal variances and normality using the Levene test and the Shapiro–Wilk test respectively. For the correlation analysis, Pearson correlation coefficients and their associated P -values (significance level at $\alpha = 0.05$) were calculated. For the PCA the data were zero centred and scaled to unit variance before analysis. Two-sample t -tests ($\alpha = 0.05$) on starch and amylopectin were performed separately for the comparison between accessions with wrinkled and non-wrinkled seeds.

RESULTS

Nutrient content, phytate levels, and water-absorption capacity

To obtain a better understanding of cooking behaviour in peas, several seed components were analysed in a diversity panel composed of 24 pea accessions. As can be seen in Fig. 1, the accessions are very diverse in seed morphology in regard to size (from 18 to 66 mm²), shape (smooth, dimpled, or wrinkled), and colour (yellow, green, dark green, or brown). Protein and starch (both amylose and amylopectin) content were quantified for each accession (Fig. 1). The five accessions with a wrinkled seed phenotype (CV_GbrMFP1, CV_GbrMFP2, CV_GbrGAP, BR_SweGBA2, and LR_SweMFP) had a much lower level of amylopectin ($P < 0.001$; two-sample t -test) and starch ($P < 0.001$; two-sample t -test) than accessions with non-wrinkled seeds. Starch content ranged from 20% to 53% and protein content ranged from 24% to 32% (Fig. 1; Supporting Information Table S1). Seed phytate levels ranged from approximately 0.8 to 1.3 g per 100 g dry weight. WAC varied from around 70% (CV_ArgGRP, CV_NorSSP, WI_DnkGBA, and LR_NplGBA) to around 150% (CV_GbrMFP1, CV_GbrMFP2, CV_GbrGAP, BR_SweGBA2, and LR_SweMFP) (Fig. 2).

Cookability

For each accession, swollen seeds ($n = 50$) were cooked in the Mattson cooker. Time-lapse footage was used to determine the time when a plunger completely pierced its corresponding seed, and violin plots and boxplots were constructed from the individual piercing times. The boxplot information of each accession based on pierced seeds was used for determining its cooking time (median) and cooking evenness (IQR) (Fig. 2; Supporting Information Fig. S2). Cooking time ranged from 12 min (CV_FinGBA) to more than an hour (CV_NorSSP and WI_DnkGBA), and evenness ranged from about 3 min (CV_FinGBA and CV_UkrGBA) to nearly 0.5 h (CV_GbrMFP1), thus also indicating a very large difference between the accessions analysed. The time-lapse footage also revealed six accessions with a slow perforation speed, and five of these accessions had the wrinkled-seed phenotype.

Seed hardness

Some accessions (CV_SweFOP, LR_NplGBA, CV_IndSSP, CV_ArgGRP, CV_NorSSP, and WI_DnkGBA) had a lower proportion

of swelled seeds after 20 h of soaking (indicative of hardseededness) compared with the other accessions, where more than 90% (and often close to 100%) of seeds became swollen. For some accessions, only little more than half of the peas were perforated after 90 min of cooking (LR_SweGRP1, BR_SweGBA2, and CV_NorSSP), whereas for a single accession (WI_DnkGBA) 80% of the peas remained unperforated (Fig. 2). Some accessions had thus both hardseededness and a hard-to-cook behaviour (CV_NorSSP and WI_DnkGBA). The penetrometer analysis showed that all five accessions with wrinkled seeds (CV_GbrMFP1, CV_GbrMFP2, CV_GbrGAP, BR_SweGBA2, and LR_SweMFP) had a much softer seed coat than all the other accessions (Fig. 3), and the force required for the seed-coat rupture only slightly exceeded 15 N (Fig. 2).

Correlation analyses

To examine the relationship between the different variables and to potentially infer which factors contributed to cookability

(cooking time and cooking evenness), we calculated the Pearson correlation coefficients (Fig. 4) and performed a PCA (Fig. 5; Supporting Information Fig. S3). Since wrinkled and non-wrinkled seeds behaved much differently, two sets of Pearson correlation coefficients were computed to potentially infer which factors contributed to cookability: one set with all 24 accessions (Fig. 4(A)) and a second set without the five accessions with wrinkled seeds, allowing for a more focused analysis (Fig. 4(B)). The PCA was also carried out on all accessions (Fig. 5) and non-wrinkled accessions (Supporting Information Fig. S3). A strong negative correlation between starch content and WAC was observed ($r = -0.5$; $P = 0.01$). The lower starch level in these five accessions was likely also due to a low level of amylopectin ($r = 0.94$; $P < 0.001$). When excluding the accessions with wrinkled seeds from the correlation analysis, the correlation coefficient between starch and WAC decreased to 0.03 ($P = 0.89$) (Fig. 4(B)). This suggests that the relationship between starch content and WAC is primarily driven by the accessions with wrinkled seeds. Phytate correlated positively

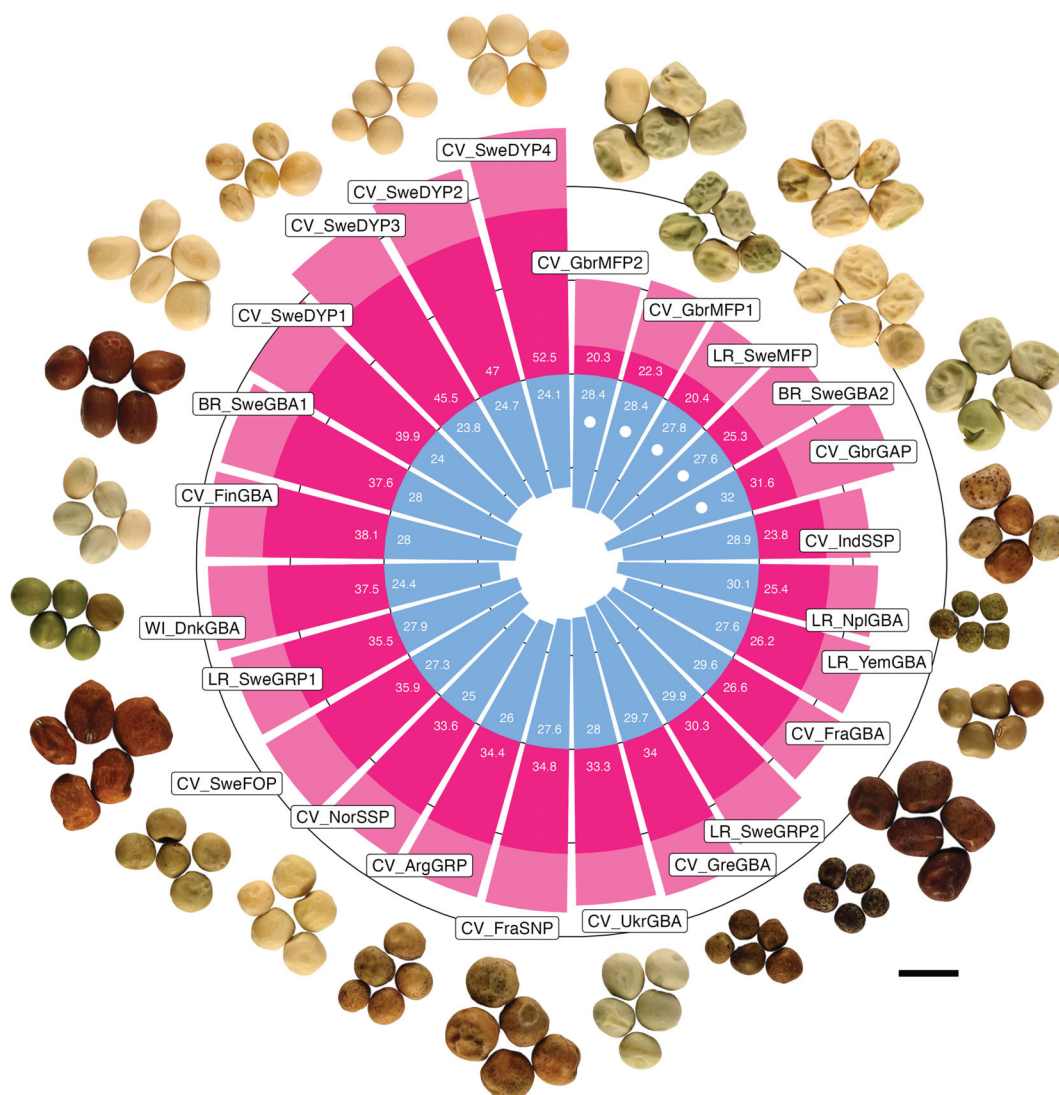


Figure 1. Morphological diversity of the 24 pea accessions used in this study. Scale bar: 1 cm. The figure shows relative levels of protein (blue) and starch (pink), with amylopectin in a darker shade and amylose in a lighter shade. Protein and starch content is shown in white text, and the black lines show 20% increments in content levels. The five accessions with wrinkled seeds are indicated by white dots. The accessions are labelled according to country of origin and specific categories. BR, breeding material; CV, cultivar; GBA, GenBank accession; LR, landrace; WI, wild; DYP, dry yellow pea; FOP, fodder pea; GAP, garden pea; GRP, grey pea; MFP, marrow fat pea; SNP, snow pea; SSP, sugar snap pea. Further information on seed content is found in Supporting Information Table S1.

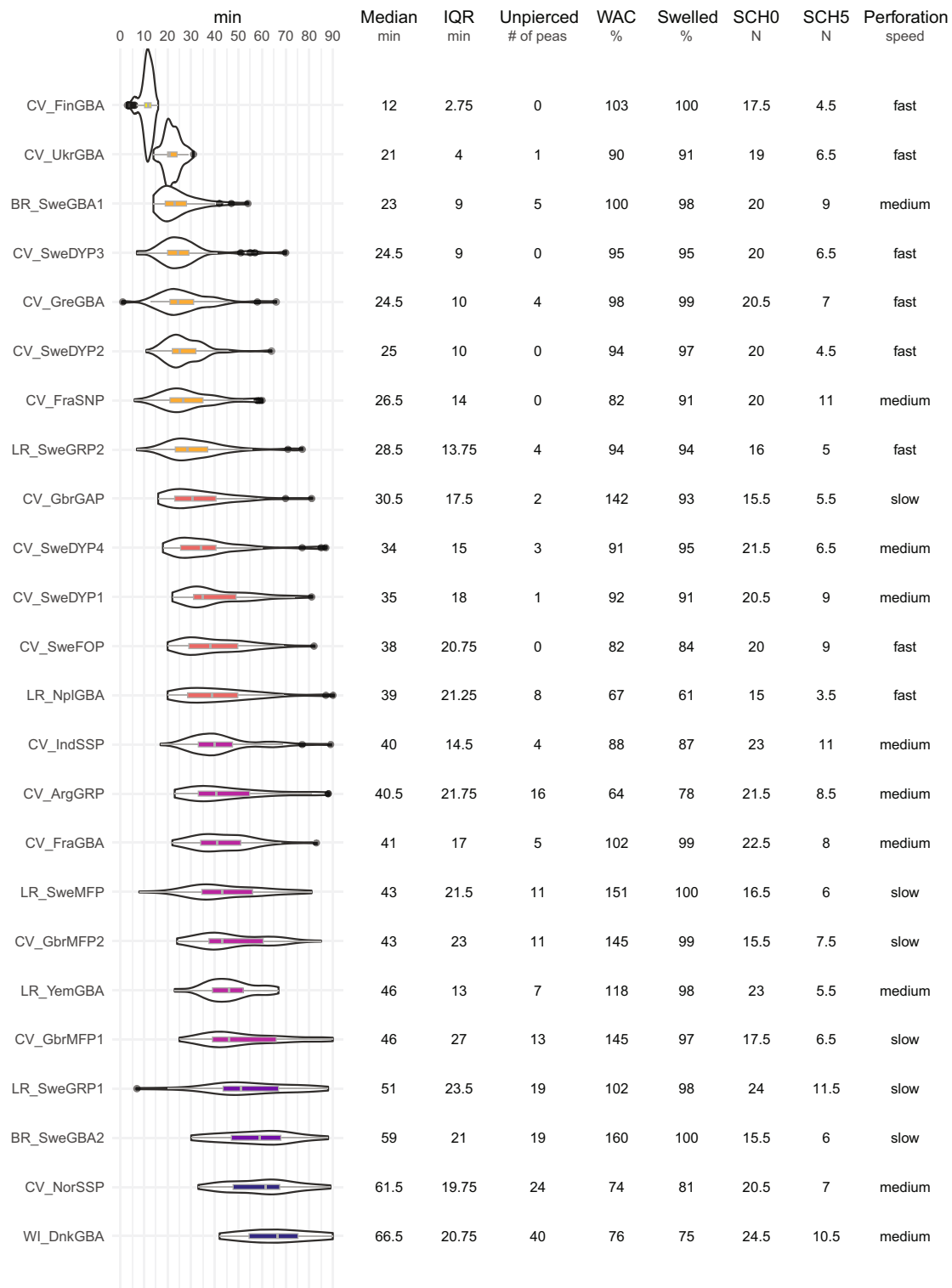


Figure 2. Cookability traits of the 24 pea accessions. Boxplot information from cooking 50 seeds in a Mattson cooker was used for determining cooking time (median) and cooking evenness (interquartile range (IQR)). Violin plots around the boxplots show the density distributions of pierced seeds. The number of unpierced seeds at the last time point analysed in the cooking analysis (90 min) was also recorded. The GoPro camera enabled classifying the perforation speed of the plungers in the Mattson cooker. Prior to cooking, the seeds were soaked for 20 h, which provided information on their water-absorption capacity (WAC) and percentage of swelled seeds. In addition, the hardness of soaked seeds was tested with a penetrometer and peak force (newtons) approximately 25% of the perforation distance before seed-coat rupture provided a measure for seed-coat hardness (SCH0, $n = 10$). Seeds that were additionally cooked for 5 min were also tested (SCH5, $n = 10$). Different intervals in cooking time (10 min intervals) are depicted with distinct boxplot colours: [10–20] in yellow; [20–30] in orange; [30–40] in red; [40–50] in magenta; [50–60] in purple; and [60–70] in dark blue.

with protein ($r = 0.49$; $P = 0.02$) but showed no strong correlation with other variables. Protein content was negatively correlated with starch content ($r = -0.65$; $P < 0.001$). This negative correlation became even stronger ($r = -0.76$; $P < 0.001$) when excluding the accessions with wrinkled seeds. When all accessions were included in the correlation analysis, WAC correlated poorly with both cooking time ($r = 0.13$; $P = 0.54$) and cooking evenness (IQR; $r = 0.24$; $P = 0.26$). When excluding the accessions with wrinkled seeds, stronger correlations were observed between WAC and cooking time ($r = -0.34$; $P = 0.16$) and between WAC and cooking evenness ($r = -0.52$; $P = 0.02$).

Swelled peas (indicative of a low hardseededness) and unpierced peas (indicative of a hard-to-cook behaviour) showed negative, non-significant correlation ($r = -0.34$; $P = 0.11$). However, when excluding the accessions with wrinkled seeds from the correlation analysis, this correlation became stronger and significant ($r = -0.47$; $P = 0.04$). The correlation analysis of seed-coat hardness, including all accessions, revealed a strong positive correlation between SCH0 and SCH5 ($r = 0.67$; $P < 0.001$). Upon removing the accessions with wrinkled seeds from the analysis, there was little change in the correlation coefficient ($r = 0.71$; $P < 0.001$) for seed-coat hardness. WAC showed significant negative correlation with SCH0 ($r = -0.50$; $P = 0.01$). When excluding the accessions with wrinkled seeds, the correlation between SCH0 and cooking time was 0.49 ($P = 0.03$). RAG exhibited weaker correlation with cooking time ($r = -0.34$; $P = 0.10$) and cooking evenness (IQR; $r = -0.36$; $P = 0.09$), but RAG showed a strong negative correlation with both cooking time ($r = -0.59$; $P < 0.01$) and cooking evenness ($r = -0.80$; $P < 0.001$) when excluding the accessions with wrinkled seeds.

The coefficients of variance for seed area both before and after soaking (area_CV_dry and area_CV_soak respectively) indicate how the seed size in a batch of seeds is dispersed around the mean. Both these parameters showed potential to be used as predictors of cooking time and cooking evenness (IQR). The Pearson correlation coefficient between area_CV_dry and cooking time was 0.46 ($P = 0.02$) and between area_CV_dry and IQR it was 0.51 ($P = 0.01$). The PCA also demonstrated a strong alignment of area_CV_dry with cooking time and cooking evenness (Fig. 5). The corresponding values for area_CV_soak were 0.42 ($P = 0.04$) and 0.44 ($P = 0.03$) respectively. Excluding the accessions with wrinkled seeds, the correlations became stronger: 0.51 ($P = 0.02$) for area_CV_dry and 0.64 ($P = 0.003$) for area_CV_soak with cooking time and 0.49 ($P = 0.03$) for area_CV_dry and 0.65 ($P = 0.003$) for area_CV_soak with cooking evenness.

DISCUSSION

In this study, we characterized seeds of 24 diverse pea accessions to decipher contributing factors of their cooking quality and whether potential predictors of cookability could be identified. Previous cookability studies often use either a Mattson cooker^{13,23} or a penetrometer^{2,24–26} when investigating the cooking behaviour of pulses, with limited studies utilizing both instruments.^{10,11} In this study, three different instruments were employed to determine cooking quality in terms of cooking time and cooking evenness (Mattson cooker), seed hardness (penetrometer), and seed size before and after soaking (Marvin seed analyser). The latter instrument is, to our knowledge, a novel application in cookability research.

The 24 accessions in this study show great diversity, representing all types mentioned in the Introduction across both fresh and

dry peas (Fig. 1). At one extreme, the five accessions with wrinkled seeds (CV_GbrMFP1, CV_GbrMFP2, BR_SweGBA2, LR_SweMFP, and CV_GbrGAP) have a much lower level of amylopectin than all other accessions. At the other extreme, in terms of amylopectin and starch content, are the dry yellow peas. The mature peas at both these extremes all have light-coloured testae, which has been a favourable trait when considering their use for human consumption.²⁷ Seeds with dark-coloured testae are typically mature peas used as fodder or consumed as fresh peas, and thus harvested when the seeds are tiny and immature, at which point they have a lighter colouration.²⁸ All seeds from the different accessions used in this study were harvested from the same field site under similar environmental conditions and were then uniformly stored under consistent cool and dry conditions. Hence, any variations in cooking quality observed among the accessions were unlikely to be attributed to storage conditions, but rather reflecting their inherent properties.

Seed components and water absorption

A pea seed can be divided into three main components: the embryonic axis; the seed coat; and the cotyledon. The embryonic axis includes the hilum and the micropyle, with the hilum serving as the connecting point between the seed and pod, and the micropyle being a small opening adjacent to the hilum. The seed coat (or testa) is mainly composed of cellulose, hemicellulose, lignin, pectin, and calcium,²⁹ whereas the cotyledon is mainly composed of starch, protein bodies, and phytate, with phytate residing in globoids within the protein matrix.³⁰ Water uptake in the seed can happen via entry points in the embryonic axis (micropyle and hilum) but also via the seed coat, depending on its permeability.^{14,31} For instance, high levels of phenolics in the seed coats of wild peas are associated with reduced permeability when compared with modern pea cultivars.³²

For a plunger to pierce a pea in the Mattson cooker the cotyledon needs to soften sufficiently. Upon heating, the starch granules in the cotyledon undergo gelatinization, by which the granules disintegrate. The gelatinization process requires sufficient moisture. Interestingly, our investigation revealed that the five accessions with a prominent wrinkled phenotype exhibited a higher WAC. This finding is congruent with other studies on wrinkled peas,^{33,34} further emphasizing the relationship between WAC and wrinkled pea characteristics. These wrinkled peas also had a significantly lower level of amylopectin than the other accessions tested. This property probably reflected the slower perforation speed observed for all five accessions with wrinkled seeds in our study (Fig. 2). The slow perforation speed for these five accessions resulted in excessively long cooking times and probably also led to larger unevenness in the cooking. The Mattson cooker thus has limitations when it comes to these types of seed.

A negative correlation was observed between protein content and starch content, which is consistent with other studies.^{35,36} The protein levels in the pea accession in this study match findings of other studies, ranging from 24% to 32%; however, starch levels were slightly lower than those reported elsewhere.³⁷ This discrepancy may be partly attributed to the enzymatic method used for starch determination in our study, as highlighted by Jezierny *et al.*³⁸ in their study on the methodological impact of starch determination, where a polarimetric method gave significantly higher starch levels compared to an enzymatic method. It should also be noted that, in the enzymatic kit used in our study,

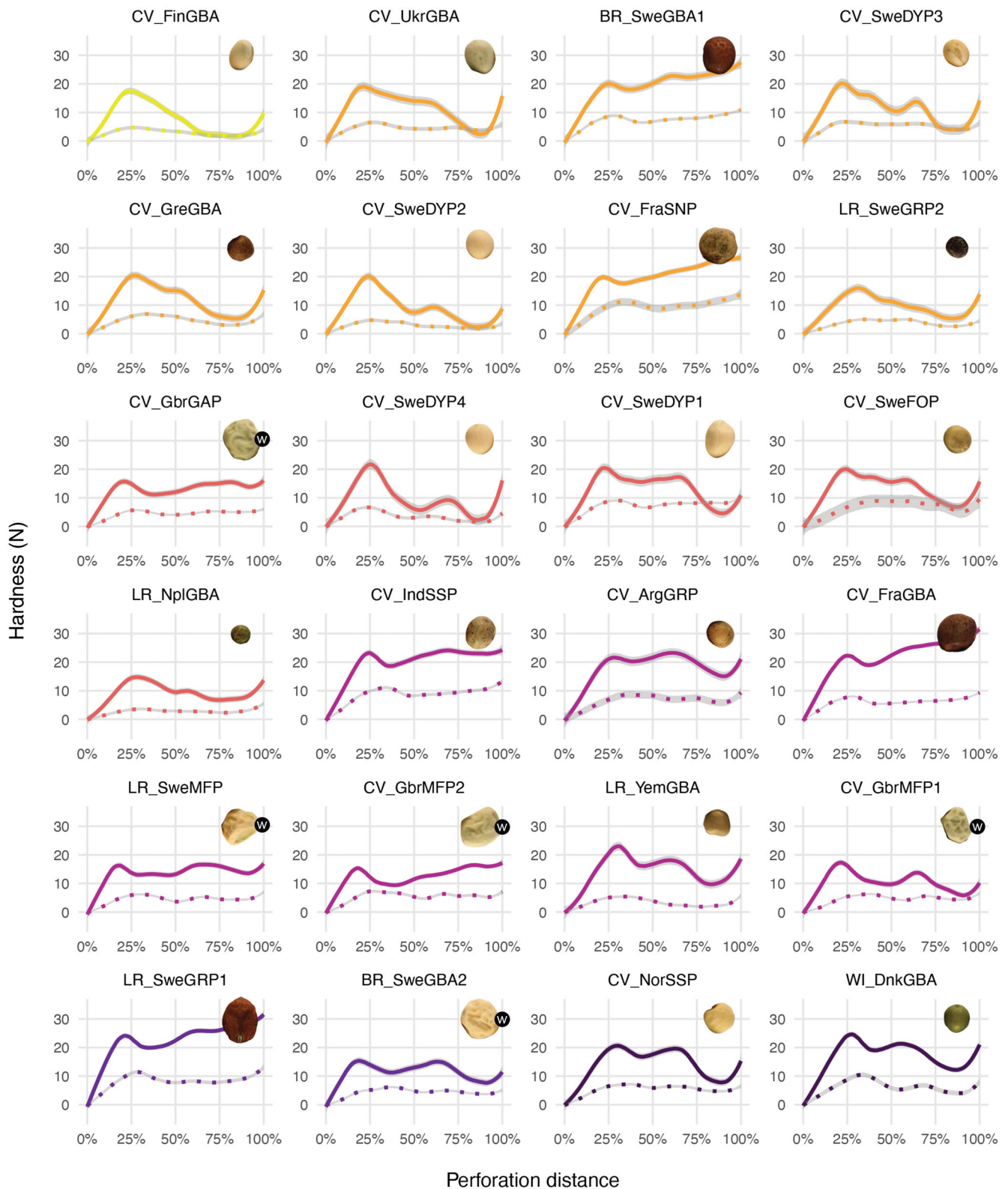


Figure 3. Seed hardness for all 24 pea accessions. Solid lines represent seeds soaked for 20 h, whereas dashed lines represent seeds soaked for 20 h and cooked for 5 min. Grey bars are 95% confidence intervals. The peak observed at approximately 25% of the perforation distance indicates the seed-coat hardness (newtons), since this force was required to rupture the seed coat. The graph colours correspond to those used in Figs 2 and 5, with each colour representing a 10 min interval in cooking time with the accession in the top left having the fastest cooking time (CV_FinGBA) and the accession in the bottom right having the slowest cooking time (WI_DnkGBA). Accessions with wrinkled seeds are indicated by 'w'.

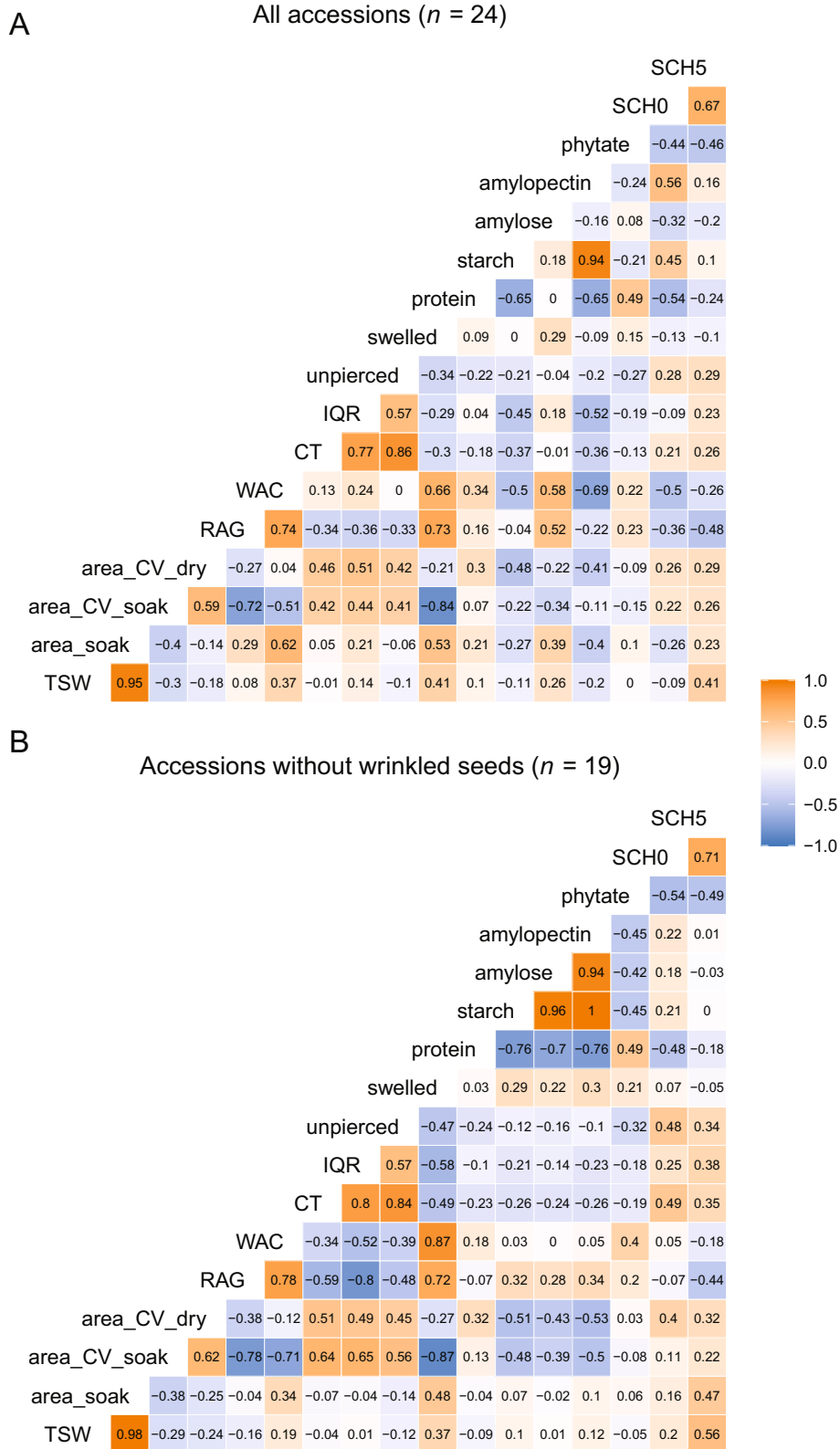


Figure 4. Pearson correlation coefficients. Two separate analyses with the same variables were carried out on all 24 accessions (A) and the 19 accessions without wrinkled seeds (B). area_CV_dry, coefficient of variation for dry seeds; area_CV_soak, coefficient of variation for soaked seeds; area_soak, mean profile area of soaked seeds; CT, cooking time; IQR, interquartile range; RAG, relative area gained; SCH0, seed-coat hardness of swelled (but nonboiled) seeds; SCH5, seed-coat hardness of swelled (and boiled) seeds; TSW, thousand-seed weight; WAC, water-absorption capacity.

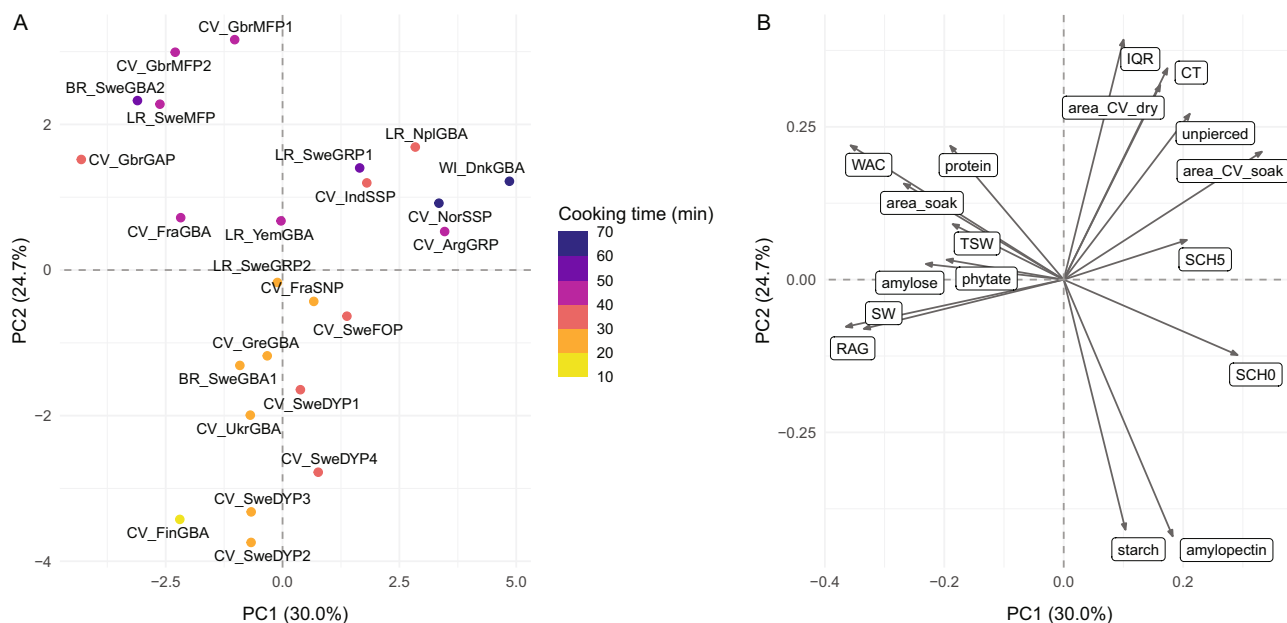


Figure 5. Principal component analysis on all accessions showing scores (A) and loadings (B). area_CV_dry, coefficient of variation for dry seeds; area_CV_soak, coefficient of variation for soaked seeds; area_soak, mean profile area of soaked seeds; CT, cooking time; IQR, interquartile range; RAG, relative area gained; SCH0, seed-coat hardness of swelled (but nonboiled) seeds; SCH5, seed-coat hardness of swelled (and boiled) seeds; SW, percentage of swelled seeds; TSW, thousand-seed weight; WAC, water-absorption capacity.

free sugars were removed prior to analysis so as not to be misinterpreted as sugars from degraded starch.

Seed hardness and cookability defects

Two types of textural defects have been reported in pulses, with pea being no exception: the hard-shell defect and the hard-to-cook defect.³⁹ In fact, the Mattson cooker was originally developed to investigate the hard-to-cook defect in pea.¹⁹ The hard-shell defect is due to hardseededness, in which the seed imbibes no water during the soaking period, whereas the hard-to-cook defect causes the seed to not cook soft within a sensible period, even though it has taken up water during soaking. By exclusively selecting swollen seeds for the Mattson cooker analysis, the observed prolonged cooking time (or failing to become pierced) from some of the seeds/accessions would therefore be attributed to the hard-to-cook defect and not to the hard-shell defect. Accession LR_NplGBA had a high proportion of seeds unable to absorb water during the soaking period, but the cooking time and cooking evenness for this accession were comparable to other accessions with much higher absorption percentages (e.g., CV_SweDYP1 and CV_FraGBA). Interestingly, seed-coat hardness (SCH0 and SCH5) of the LR_NplGBA accession was much lower than in other accessions of similar cooking time. Investigating the water entry points in this accession and the underlying mechanisms would provide valuable insights. Two accessions (WI_DnkGBA and CV_NorSSP) showed signs of having both the hard-shell and the hard-to-cook defects. The relationship between these two defects has received relatively limited attention, but existing studies suggest that the hard-to-cook defect primarily involves alterations in the cotyledon, whereas the hard-shell defect is associated with modifications in the seed coat.⁹ Since hard-shell seeds (seeds not taking up water during soaking) were excluded in our cooking tests, it remains to be deciphered how this factor could contribute to the unevenness observed in the cooking.

A prevalent explanation for the persistence of hardness in some seeds during cooking (i.e. the hard-to-cook defect), is the pectin–phytate hypothesis.^{14,19} Pectin binds together plant cells and exists usually in a water-soluble form that permits water uptake by the seed. However, when pectin undergoes cross-linking with magnesium and calcium cations, it transforms into insoluble pectates.²⁶ Phytate, which serves as a storage molecule of phosphate, possesses a stronger chelating capacity than the carboxyl groups of pectin do. Consequently, phytate impedes the cross-linking of divalent cations with pectin. However, in cases where phytate levels are reduced, magnesium and calcium cations more easily bind to the pectin, rendering the seed harder to cook, since the pectates prevent the uptake of the water needed for starch gelatinization during the cooking process. Decreased phytate levels can be attributed to various factors, including improper storage,⁴⁰ field conditions,^{9,41} and genetic factors.⁴² Seed phytate levels have been found to affect cooking quality in lentil,⁴³ red kidney bean,⁴⁴ pea,¹⁵ and common bean.²⁶ To explore the potential contribution of phytate levels to cookability, we determined the phytate levels in the 24 pea accessions. Given that the accessions were grown, harvested, and stored under the same conditions, differences in phytate levels were likely to be attributed to genotypic variation. We observed only a modest negative correlation between phytate levels and cookability (cooking time and cooking evenness). Notably, we did not determine levels of divalent cations (e.g. Ca²⁺) nor the level of pectin content within our accessions. The ratio between phytate and Ca²⁺ ions, for instance, has been found to correlate well with cooking time,^{45,46} highlighting the importance of investigating these factors in future research.

Seed hardness measurements were conducted using a penetrometer for all 24 accessions (Fig. 3), with seeds in the same orientation as in the Mattson cooker. The penetrometer pierces both the seed coat and the cotyledon, providing a measure for the force required for the perforation. The first peak in each

penetrometer graph, occurring at approximately 25% of the perforation distance, corresponds to rupture of the seed coat.¹⁰ Thus, the force recorded at this point is an indicator of seed-coat hardness (Supporting Information Fig. S2). When excluding the five accessions with a low amylopectin content and pronounced WAC (i.e. wrinkled seeds), a strong and positive correlation between cooking time and SCH0 emerged. Borowska *et al.*²⁵ reported no correlation between cookability and firmness in their study on eight pea accessions. However, their penetrometer tests were conducted on either raw seeds or cooked seeds and not on soaked seeds before cooking, as we did in our study. This difference in methodology could account for the disparity in findings. Previous research suggests that seed-coat thickness itself is implicated in cookability,¹⁰ but deciphering the connection between seed-coat thickness and seed-coat hardness in pea will require further research.

Chickpea, green gram, and horse gram seeds have cotyledons that are tightly adhered together.⁷ In Fig. 3, an adhesive cotyledon is visually represented by a continuously rising line in the graphs, starting from approximately 40% of the perforation distance that surpasses the SCH0 level (Supporting Information Fig. S2). Among our 24 pea accessions, including CV_FraGBA and LR_SweGRP1, we observed at least five accessions with tightly adhered cotyledons. However, there was no clear pattern between the presence of an adhesive cotyledon as indicated by the penetrometer graphs (Fig. 3) and cooking times (Fig. 2), since the accessions exhibited a wide range of cooking times ranging from 23 to 59 min.

Predictive indicators of cookability

Cooking pulses in a Mattson cooker for analysing cooking quality is a labour-intensive process, making the ability to predict cookability in breeding material without the need for actually cooking the seeds an asset for cultivar improvement of peas intended for human consumption. For example, previous research in dry bean revealed a strong negative correlation between cooking time and water absorption, with researchers proposing water absorption as an indirect selection method for cooking time.²³ However, contrasting findings in other studies find a weaker association between hydration and cooking time.^{47,48} In our study, water absorption was similarly found to be a weak predictor of cookability. Instead, RAG, which is similar to WAC but considers area instead of weight, showed a stronger correlation with cooking time and cooking evenness compared with WAC. When all accessions were included, RAG showed a negative, but non-significant, correlation with cooking time and cooking evenness. However, when excluding accessions with wrinkled seeds, RAG exhibited negative and significant correlation with both cooking time and cooking evenness. Another highly interesting factor we found correlating with cookability was seed-coat hardness of soaked seeds (SCH0), which showed a strong correlation with cooking time but, as in the case for RAG, only when the accessions with wrinkled seeds were excluded. Likewise, the percentage of swelled seeds (or unhydrated seeds) after 20 h of soaking could potentially serve as a predictive indicator of cookability, when the accessions with wrinkled seeds were excluded. The rate by which seeds imbibe water during soaking (i.e. hydration rate), although not included in the present study, would be of interest to investigate in terms of its predictability of cookability⁷ and the potential differences between wrinkled and non-wrinkled seeds.

Surprisingly, two variables that consistently demonstrated strong correlation with cookability encompassing all accessions and non-wrinkled seed accessions only, were the coefficients of variation of area for both dry seeds (area_CV_dry) and soaked seeds (area_CV_soak). To the best of our knowledge, this study is the first to report on these variables as potential predictors of cookability. The seed-area data used for determining RAG, area_CV_dry, and area_CV_soak were obtained using a Marvin seed analyser. When all accessions were included in the analysis, area_CV_dry exhibited a slightly better predictability for cookability than area_CV_soak did. Conversely, when excluding the accessions with wrinkled seeds, area_CV_soak displayed a stronger correlation with cooking time and cooking evenness than area_CV_dry did. The correlation between area_CV_dry and cookability remained relatively stable between the two analyses (Fig. 4). The PCA (Fig. 5; Supporting Information Fig. S3) supported the Pearson correlation coefficients, also indicating that the area_CV variables aligned with cooking time and cooking evenness, whereas the starch and protein variables negatively correlated to each other and did not align with cooking time and cooking evenness. The PCA also supported the Pearson correlation coefficients analysis on what has already been discussed regarding RAG, percentage of swelled seeds, and seed-coat hardness, with the exclusion of accessions with wrinkled seeds amplifying these variables as potential predictive indicators of cooking time and cooking evenness.

The approach of looking at pairwise correlations between cooking time and cooking evenness in legume seeds, *versus* several different characteristics associated with seed coat, size, and soaking ability, can be useful for formulating breeding targets as they are simpler to analyse than cooking analysis. However, it is important to note that correlation does not mean causation, and further studies are needed to identify the actual causes for differences found in the plant material for the parameters analysed.

Potential in breeding better-cooking cultivars

It is evident from our analysis that many, if not most, of the accessions that exhibited lower uptake of water and a longer cooking time were not specifically bred for desirable traits characterized by a cooking-type pea. These accessions often had darker coloured testae (typically linked to a higher level of phenolic compounds) and/or are categorized as fresh pea types consumed at an immature stage.

Cooking time and cooking evenness are both desirable traits in determining the quality of pulses. Cooking evenness is an overlooked aspect of cooking quality of legume grains. Among the accessions tested, LR_YemGBA stood out with a longer cooking time of 46 min, yet it exhibited a remarkably high level of cooking evenness (13 min). The penetrometer tests revealed that the cooked seeds from this accession had low seed-coat hardness, with a reduction of 17.5 N from the soaked state (SCH0, 23 N) to the cooked state (SCH5, 5.5 N). This reduction was greater than for any other accession. Whether this greater reduction of seed-coat hardness can serve as an indicator for selecting accessions with improved cooking evenness, as well as of the underlying factors causing the reduction in seed-coat hardness of LR_YemGBA, needs to be explored further. High protein content is a desirable trait, especially since pea and other legumes will provide an even larger part of the future green protein.⁵ Protein levels in our accessions were not significantly correlated with cookability. Therefore, selecting for offspring with higher protein levels would probably not affect cookability. It is important to note that the sensory

experience of cooked legumes, which includes factors such as taste, texture, aroma, and appearance, plays a significant role in consumer acceptance and preference.¹³ Thus, alongside information on cooking quality, sensory evaluation and consumer feedback needs to be incorporated into the breeding process, which enables breeders to make informed decisions for selection and developing new cultivars that meet consumer preferences. Once candidate cultivars with favourable cookability traits have been developed, it is important to assess their stability in cookability across multiple years and environments.¹⁵

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: C.H.; methodology: B.D., C.H., T.H., and Å.G.; investigation: B.D., J.F., S.C., M.H., and Å.G.; data analysis: B.D., J.F., S.C., M.H., and Å.G.; visualization: B.D.; writing: B.D., Å.G., and C.H. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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