Screening of pea varieties of Sweden

- for physical, chemical & functional properties

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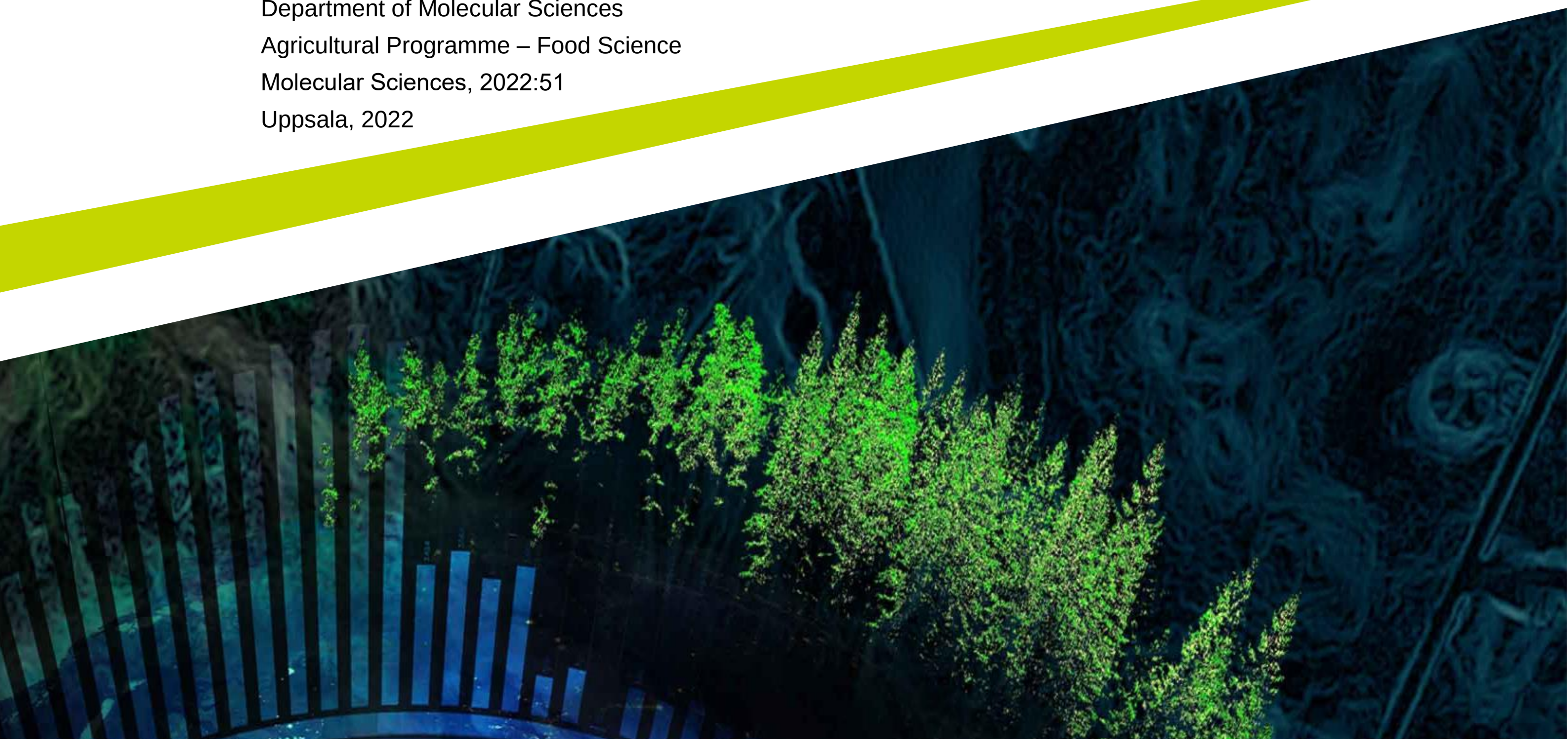

Screening of pea varieties of Sweden - for physical, chemical & functional properties.

Undersökning av svenska ärtsorter och deras fysikaliska, kemiska och funktionella egenskaper

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Abstract

There is a need for improved resource efficiency in the food chain, while still producing nutritious foods with a low environmental impact. A crop with a favourable nutritional profile, while being well suited for cultivation in Swedish environmental condition is the field pea (*Pisum Sativum L.*). The purpose of this study was to provide information about the genetic and environmental effect on the physical, chemical and functional properties of peas.

Physical, chemical and functional properties were analysed of pea seeds of three different varieties cultivated at three different years. Moreover, an additional pea variety was studied, with samples being provided from one year only. Pasting properties, oil absorption capacity, water absorption capacity and starch content were also analysed for some pea flour fractions. Additionally, scanning electron microscopy were performed on a few whole flour samples as well as the flour fractions of selected varieties.

The results showed differences between varieties as well as by cultivation years in several of the different variables studied. For instance, there was a significant difference in the average size of seed between years as well as between varieties. The analyses of the pea flour fractions showed differences in composition and functional properties compared to the whole pea flour, although these results need to be confirmed in further studies with more samples. In conclusion, variety and year appear to affect the physical, functional and chemical properties of peas. However, the results need to be confirmed by further studies including more samples and preferably, also considering effects of different environments and storage.

Keywords: Pisum Sativum L., pea, physical properties, chemical properties, functional properties

Sammanfattning

Det finns ett behov av förbättrad resurseffektivitet i livsmedelskedjan, samtidigt som det krävs fokus på produktion av näringsrika livsmedel med låg miljöpåverkan. En gröda med gynnsam näringsprofil samtidigt som den lämpar sig väl för odling i svenskt miljötillstånd är ärtan (*Pisum Sativum L.*). Syftet med denna studie var att ge information om de genetiska och miljömässiga effekterna på ärtornas fysikaliska, kemiska och funktionella egenskaper.

Fysikaliska, kemiska och funktionella egenskaper analyserades hos ärtor av tre olika sorter från tre olika odlings-år. Dessutom studerades ytterligare en ärtsort, som endast var tillgänglig från ett odlings-år. Funktionella egenskaper och stärkelsehalt analyserades också för några ärtmjölsfraktioner. Dessutom utfördes svepelektronmikoskopi på ett fåtal mjölprover såväl som mjölfraktionerna från utvalda sorter.

Resultaten visade att genetiska och miljömässiga faktorer påverkade flera av de analyserade egenskaperna. Signifikant skillnad i storlek mellan sorter samt år kunde till exempel visas. Analyserna av ärtmjölsfraktionerna visade skillnader i sammansättning och funktionella egenskaper jämfört med hela ärtmjölet, även om dessa resultat måste bekräftas i ytterligare studier med ett större antal prov. Sammanfattningsvis påverkar sort och odlings-år de fysikaliska, funktionella och kemiska egenskaperna hos ärter. Resultaten behöver dock bekräftas genom ytterligare studier inklusive fler prover och helst även med hänsyn till effekter av olika miljöer och lagring.

Nyckelord: Pisum Sativum L., ärta, fysikaliska egenskaper, kemiska egenskaper, funktionella egenskaper

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Abbreviations

ANOVA	Analysis of Variance
ara	Arabinose
B	Breakdown
BD	Bulk Density
cP	Centipoise
FV	Final Viscosity
gal	Galactose
glc	Glucose
HGW	100 Grain Weight
HS	Holding Strength
KL	Klason Ligning
L	Length
man	Mannose
OAC	Oil Absorption Capacity
P	Porosity
PCA	Principle Component Analysis
PeakT	Peak Time
PFF	Pea Flour Fractions
Ptemp	Pasting Temperature
PV	Peak Viscosity
rha	Rhamnose
RVA	Rapid Visco Analyser
RVU	Rapid Viscosity Units
S	Sphericity
S	Setback
SEM	Scanning Electron Microscopy
T	Thickness
TD	True Density
TDF	Total Dietary Fibre

UA	Uronic Acids
V	Volume
W	Width
WAC	Water Absorption Capacity
WPF	Whole Pea Flour
xyl	Xylose

1. Introduction

A growing world population, with a growing demand for nutritious food stuff, is continuously increasing the pressing need of having an efficient and sustainable food chain. Currently, our food systems need to become more resource efficient to meet the growing demand for food products while simultaneously reducing the environmental impact. Further, these changes need to be made while also keeping the food value chain economically and socially sustainable.

To improve resource efficiency in the food chain, from a Swedish perspective, there is a need for resource efficient production and processing of crops with a favourable nutritional profile, that also are suitable for the Swedish environment. A good alternative for such a crop is the field pea which has been cultivated in the Nordic region for a long time, probably since the bronze age (Kirleis 2019). The field pea has a cold tolerance during the growth and germination period, making it a common and favourable alternative protein crop in cooler climates, as compared to, for instance, soybean (Lu et al. 2020). Further, there has been an increase in the demand for protein from peas, along with the interest for plant-based alternatives to meat. Especially in relation to the discussion of the environmental impacts of soybean production (Martinez & Boukid 2021). The field pea has a favourable nutritional composition, high in protein, starch and dietary fibre (Mondor 2020), making it a perfect candidate as a nutritious food source that is well suited for production in the cooler climate of Sweden.

This project is part of a collaboration between Lantmännen and researchers at the Swedish University of Agriculture (SLU) and Chalmers University, called 100% PEA. This project aims to upgrade the Swedish value chain for pea production, through a highly efficient pea biorefinery. To accomplish this, it is necessary to screen the Swedish pea varieties to determine which are the most suitable to cultivate for starch, protein and dietary fibre extraction. Additionally, information about the effect on field peas by different environmental conditions and storage is valuable.

1.1 Aim and objective

The aim of this project was to study and give an overview of some physical, chemical and functional properties of different pea varieties of Sweden, cultivated in different years. Furthermore, the aim was to include information of chemical and functional properties of pea flour fractions. Thus, the purpose was to provide information about the genetic and environmental effect on the physical (average size, 100 grain weight, bulk density, true density, porosity and particle size), chemical (dietary fibre and starch content) and functional properties (water and oil absorption capacity as well as pasting properties) of peas. This could provide information helpful in determining suitable Swedish pea varieties to include in a future highly efficient pea biorefinery.

1.2 Delimitations

The study was limited to include four field pea varieties, cultivated in three years, from 2018 to 2020. These varieties were chosen by Lantmännen. However, one of the varieties were obtained from only one cultivation year (see section 3.2). Therefore, the ability to conduct detailed statistical calculations for all varieties became limited. The chemical properties were limited to include only dietary fibre composition and starch content. Furthermore, the samples were grown in the same location, thus excluding the effect of cultivation site on the different pea varieties. Moreover, fractions were obtained and analysed from only two different pea samples (see section 3.2), due to lack of time. Therefore, it was not possible to conduct statistical analyses on the obtained results from the flour fractions.

2. Background

2.1 Pea

2.1.1 History

Throughout the world legumes, dicotyledon seeds belonging to plants of the Leguminosae family (Ratnayake et al. 2001), are an important part of the diet. They are a great source of nutrients as they are high in protein, starch and dietary fibre (Nikolopoulou et al. 2007). The field pea is one of the major legumes and one of the most widespread legume crops in the world, especially in temperate regions (Ljuština & Miki 2010).

Peas, along with other European legumes such as chickpeas and lentils, originate from the Near Eastern centre of diversity, or centre of origin. Mediterranean and African centres of diversity are considered secondary centres of origin (Ljuština & Miki 2010). The Near Eastern region still has many wild taxa of pea in its flora, such as red-yellow pea (*Pisum fulvum* Sm.) (Ljuština & Miki 2010). There are three signs of domestication of legumes that are regularly used, non-dehiscent pods, smoothness of seeds and seed size (Tanno & Willcox 2006). However, these signs are difficult to interpret and do not always survive archeologically, therefore, there is not much information on the early stages of pulse domestication (Ljuština & Miki 2010). Peas are, however, considered one of the founder crops of Old World Neolithic agriculture (Zohary & Hopf 1973).

In Sweden peas have also been grown for a long time. They are probably, along with cereals such as wheat and barley, one of the crops that have been cultivated for the longest time in this area (Leino & Nygårds, 2008). Although it is not the case anymore, up until the 19th century, mainly Swedish landrace peas that were adapted to the local environment were used. Some examples of yellow pea varieties are Östgötaärt, Skånsk gulärt and Upplandsärt (Leino & Nygårds, 2008).

2.1.2 Production

The total production of dry peas in the world was approximately 14 600 000 metric tonnes in 2020 (FAO, 2022). There are several potential uses for the field pea, including green vegetables that are either fresh or frozen, it could be used fresh for grazing animals as well as being further treated to serve as hay or silage. Additionally, it is commonly produced for the mature seed, which has several uses for feed as well as food production (French 2016).

According to the Swedish Board of Agriculture (2021a) dry peas are the most predominantly produced legumes in Sweden. Approximately 72 800 metric tonnes were harvested in Sweden in 2021, compared to the second largest legume in Sweden, the field bean, with a total harvest of 58 400 metric tonnes in 2021 (Swedish Board of Agriculture 2021a). In 2021, the area of production of the category dry pea, field bean, etc. was 44 181 hectares, of which the dry pea cultivation constituted 52% (Swedish Board of Agriculture 2021b). As seen in table 1 below, showing the production of pea seeds of different Swedish varieties and their usage, most of the produced peas are used as animal feed. Ingrid is the variety with the highest production with 4947 metric tonnes in 2020, followed by Bagoo and Eso with 4800 and 2442 metric tonnes respectively. The food production of peas is substantially smaller, where the variety that is produced for this purpose, to the largest extent is Clara with 340 metric tonnes produced in 2020.

Table 1. Production, in tons, and application of Swedish pea varieties in 2020 (Data obtained from Lantmännen 2021)

Variety	Clara	Rokka	Ingrid	Rocket	Balder & Greenway	Nitouche	Eso	Bruno	Bagoo	Alvesta
Production (tons)	340	6	4847	101	14	24	2442	1100	4800	92
Application	Food		Feed							

2.1.3 Seed anatomy & composition

The pea core contains two embryonic leaves (defining it as di-cotyledonous) that makes up the spherical shape of the seed. Moreover, it has an outer layer called the testa, also known as the hull. The two cotyledons are the storage units of the pea, containing protein bodies and starch granules, that lie within the storage tissues. Protein bodies of the field pea have a diameter of approximately 1-3 μm (Möller et al. 2021a). Starch granules of pea cells range in size between 2 and 40 μm, with a round or oval shape (Ratnayake et al. 2001). Further, they generally divide in to two types of granules, large oval granules with a size of 23-30 μm and smaller granules that are either oval or spherical with a size of 5-20 μm. The starch granules exist in the pea cell embedded in a matrix of protein bodies (Pernollet 1978).

2.1.4 Pea flour composition and functional properties

The composition of the field pea varies based on variety, growing conditions and year of cultivation. However, it is generally reported in the ranges of 33-52% for starch, 22-32% for protein, 19-31% for fibre and 0.4-4% for fat (Mondor 2020). The starch contains on average 36% amylose, where 9.5% is made up of amylose-lipid complexes (Ratnayake et al. 2002). Pea starch has a high amylose:amylopectin ratio. Furthermore, compared to cereal and tuber starches, there is a large relative amount of short amylose chains (Martinez & Boukid 2021). These characteristics makes pea starches less suitable for uses such as thickening agents in food products. However, they have other potential uses, for example in edible coatings (Martinez & Boukid 2021).

Compared to pea starch, the proteins of peas are more desired for food production, especially in plant based meat alternatives (Martinez & Boukid 2021). Pea proteins are classified in four major groups: globulin, albumin, prolamin and glutelin, where globulin and albumin constitutes about 55-65% and 18-25% respectively, and prolamin and glutelin constitutes only minor amounts (Lu et al. 2020). Pea proteins have good functional properties that lends them favourable to use in meat substitutes. Furthermore, there are opportunities in improving the functional properties through chemical and enzymatic modifications (Ge et al. 2020).

Dietary fibre has been defined chemically as the sum of non-starch polysaccharide residues, amylase-resistant starch and Klason lignin (Theander et al. 1995). Pulse cotyledons are the main source of soluble fibre in pulses, mainly consisting of arabinose residues and galacturonic acid rich pectins. The hulls are the primary source of insoluble fibre in pulses, mainly consisting of cellulose, xylans and arabinans (Brummer et al. 2015).

2.1.5 Extraction of pea components

There are several fractionation processes, wet and dry, available that are possible alternatives for purifying the main components of field peas, namely starch, protein and dietary fibre. However, wet fractionation results in purer protein fractions with higher yield (Möller et al. 2022). Different wet fractionation processes include alkaline extraction followed by isoelectric precipitation (AE/IEP), salt extraction (SE), micellar precipitation (MP) and mild wet fractionation (Lam et al. 2018; Möller et al. 2022).

2.2 Environmental and genetic effects on dry pea composition

The composition of field peas varies based on several factors, including variety, environmental factors and storage. The impact of environment and variety on the composition of peas have been studied and reviewed (Black et al. 1998; Nikolopoulou et al. 2007; Lam et al. 2018; Mohammed et al. 2018; Daba & Morris 2022).

Mohammed et al. (2018) studied the proximate composition of six dry pea varieties in a randomized block design with four replications in a total of 22 environments. The authors found that differences in environmental means were larger compared to variety means. Further, they found significant environment x variety effects on protein, starch and ash concentrations.

Three different pea varieties, grown in three locations over two subsequent years were evaluated for chemical composition in a study by Nikolopoulou et al. (2007). The authors found significant effects of cultivation area on the chemical composition. Further, the study also showed effects of the cultivation year on all traits, except for starch, indicating that starch content was less impacted by the climatic conditions.

In a literature review by Daba & Morris (2022), which focused on pea proteins, their variation, composition and genetics, the authors concluded that the regulation of pea proteins is controlled by complex genetic mechanisms and that environmental variation is a major contributor to protein variation in peas. Further, they found that protein content of peas is negatively correlated to the starch content. Therefore, they conclude that it could be problematic breeding for peas with optimal protein and starch content. Moreover, they suggest that the possibility of breeding for protein- and starch-optimized versions separately might be the best course of action while focusing on peas as a functional ingredient of foods.

A literature review by Lam et al. (2018) discussed the effects of environment and variety on pea protein content. The authors mention associations between the environmental factors high temperatures and low rainfall with a higher protein content in peas. Moreover, a negative correlation between protein content and seed yield was found. Furthermore, the authors discuss that pea varieties of interest should be cultivated and tested at different locations over several years to determine the effects of environment on the specific genotype.

A study by Black et al. (1998) studying 61 field peas of different categories, over two seasons showed somewhat contradictory results. The authors found wide variations in the physico-chemical properties of the field peas based on genotypes. However, significant differences based on the cultivation year was not detected in the study, with the exception of dehulling quality.

3. Method and material

3.1 Literature review

A literature review was conducted by using databases such as Primo and Google Scholar. Examples of search words that were used in different combinations are: “Pisum Sativum”, “Pea”, “Dietary Fibre”, “Starch”, “Pea varieties”, “Environmental Factors”.

3.2 Material

Mature dried peas from four different pea varieties, were kindly provided by Lantmännen Lantbruk (Svalöv, Sweden). In three out of the four varieties, samples were provided from three different years, as explained in Table 2 below. Furthermore, information regarding sowing date, location of cultivation, fertilization and treatments, with Fenix and Centium, were also provided by Lantmännen Lantbruk (Svalöv, Sweden) and is presented in Table 3 below.

Table 2. Pea varieties, years of harvest as well as type of pea

Variety	**Year**	**Colour**	**Usage**	**Code**
Ingrid	2018	Yellow	Feed	I18
Ingrid	2019	Yellow	Feed	I19
Ingrid	2020	Yellow	Feed	I20
Clara	2018	Yellow	Food/Feed	C18
Clara	2019	Yellow	Food/Feed	C19
Clara	2020	Yellow	Food/Feed	C20
Balder	2018	Yellow	Feed	B18
Balder	2019	Yellow	Feed	B19
Balder	2020	Yellow	Feed	B20
Rokka	2019	Green	Food	R19

Table 3. Sowing date (SD), year of cultivation, location of cultivation, fertilization level and type before sowing (F1), treatment levels and type (T1 and T2), and date of first (DT1) and second (DT2) treatments of the pea varieties

SD	Year	Location	F1	T1	DT1	T2	DT2
21/4	2018	Banvaktsvången	200 kg PK*/ha	Fenix 0.9 L/ha	29/4	-	-
13/4	2019	Almdala V	75 kg N**/ha	2 L/ha Fenix	25/4	Basagran 0,6 L/ha; Fenix 0,35 L/ha	22/5
7/4	2020	Felestads kyrka	75 kg N/ha	Fenix 2 L/ha; Centium 0,4 L/ha	20/4	-	-

*) PK = Phosphor and potassium fertilizer

**) N = Nitrogen fertilizer

The weather data, from the years the pea samples were cultivated, including rainfall, hours of sunshine and average temperature of the months April-September as well as for the entire year are shown in Table 4 below. Weather data recordings were obtained from Swedish Meteorological and Hydrological Institute (SMHI) from their observation station in Lund, as the closest available observation station to the site of cultivation.

Table 4. Weather data in Lund from 2018-2020 (SMHI 2022). Rainfall (mm), hours of sunshine and average temperature (°C) in Lund from 2018-2020 when the pea samples were cultivated

Time period	Rainfall (mm)			Sunshine (h)			Average temperature (°C)		
	2018	2019	2020	2018	2019	2020	2018	2019	2020
April	34	10	17	227	298	279	9.7	8.6	8.6
May	45	39	40	368	219	252	16.4	11.5	11.4
June	18	66	49	311	307	290	18.8	18.9	18.9
July	3	45	60	354	256	201	21.5	18.6	16.8
Aug	91	64	66	216	237	281	19.6	19.0	19.9
Sept	32	111	53	174	118	192	15.2	14.2	15.2
Year	477	707	633	2057	1823	1873	10.2	10.1	10.5

All pea samples were analysed for average size of seed, 100 grain weight (HGW), bulk density, true density and porosity. Moreover, whole pea flour (WPF) from all pea samples were analysed for particle size analysis, water absorption capacity (WAC), oil absorption capacity (OAC), pasting properties by rapid visco analyser (RVA), total dietary fibre and starch content. Furthermore, scanning electron microscopy (SEM) of WPF samples from 2020 (I20, C20 and B20) as well as three pea flour fractions (PFF) of the sizes <50 µm (PFF50), <150 µm - 50 µm (PFF150) and >1mm – 250 µm (PFF250) from I20 and C20, were performed. Analysis of WAC, OAC, pasting properties by RVA and starch content were also conducted for PFF50 and PFF150 from I20 and C20. The PFF50 and PFF150 of I20 and C20 was referred to as I20F50 and I20F150 as well as C20F50 and C20F150, respectively. Chemicals used in the analyses were purchased from Sigma Aldrich (St Louis, United States).

3.3 Physical properties of the seeds

3.3.1 Average size of seed & 100 grain weight

From each pea sample 100 seeds were randomly picked and measured by a digital vernier calliper with 0.03 mm accuracy (Cocraft, Sweden) to acquire the average length (L), width (W) and thickness (T) of the seeds. Further, the sphericity (S) was calculated according to the equation below (Yalçın et al. 2007). The volume of the peas was also calculated based on L, W and T. Furthermore, an additional 100 seeds were randomly chosen and weighed to acquire the HGW.

$$S = \frac{(LWT)^{1/3}}{L}$$

3.3.2 Bulk density, true density & porosity

The standard test weight procedure (Singh & Goswami 1996) were used to measure the bulk density of the peas. A 500 ml container of known weight was filled with peas at a constant rate from approximately 15 cm height. The contents were then weighed, and the bulk density was calculated from the mass of seeds in relation to the volume of the container.

True density was determined by the water displacement method (Karababa 2006), with some modifications. A 100 ml graduated measuring cylinder was filled with 50 ml of distilled water and 25 g of pea was added. The change in volume was noted and the seed density was calculated as the ratio of the weight of the seeds and the volume change. Moreover, the porosity (P_f) of the seeds were calculated in percentage from the bulk density (P_b) and true density (P_t) (Yalçın et al. 2007) according to the equation described below.

$$\mathrm{Pf} = \left(1 - \frac{\mathrm{Pb}}{\mathrm{Pt}}\right) x\ 100$$

3.4 Milling & particle size analysis

Approximately 250 g seeds from each pea variety were initially ground by a Cemotec™ 1090 sample mill (Foss, Hillerod, Denmark) as a first step of disintegration. Centrifugal milling with a ZM-200 mill (Retsch, Haan, Germany) was then used to further disintegrate the samples at 18000 rpm. All the samples obtained from Cemotec™ sample mill were milled in the Retsch mill for achieving finer particle size of flour.

Sieve fractionation was used to analyse the particle size of the flours. An AS 200 Shaker (Retsch, Haan, Germany) equipped with five different sieves (1 mm, 600 µm, 250 µm, 150 µm and 50 µm) was used. Sieving was conducted for ten minutes, at 1.5 mm/g amplitude, with a ten second interval and three second pause. PFF of two pea samples were saved for further analysis, Clara and Ingrid from the year 2020 (C20 and I20). Fractions saved were <50 µm, <150 µm – 50 µm and >1mm – 250 µm.

3.5 Microstructural study of the WPF & PFF

SEM images for the WPF (I20, C20 and B20) and PFF50, PFF150 and PFF250 from C20 and I20 were performed using a HITACHI TM-1000 tabletop scanning electron microscope (SEM). The samples were mounted on a SEM stub by carbon tape, subsequently, the samples were coated with gold before the analysis.

3.6 Functional properties of the WPF & PFF

3.6.1 WAC & OAC

WAC of all WPF as well as PFF50 and PFF150 from I20 and C20 were determined by a modified version of the AACC method no. 51-56 as described by Edwards et al. (2020). Three grams of flour were measured in a pre-weighed tube and 15ml of distilled water was added. The tube was then centrifuged for 15 minutes at 1000×g, the excess water was removed at the tube was weighed. The WAC was then calculated as the amount of water absorbed divided by the amount of flour.

OAC of the WPF as well as PFF50 and PFF150 from I20 and C20 were determined according to the method described by Lin et al. (1974) with some modifications. One gram of flour was measured in a pre-weighed centrifugal tube and 10 ml of rapeseed oil (ICA, Solna, Sweden) was added. The tubes were then vortexed every five minutes, for 30 minutes, to ensure proper mixing of the samples. Thereafter, centrifugation was carried out at 3000×g for 25 minutes. After centrifugation, the oil was discarded, and the tubes were inverted for ten minutes to drain the excess oil. Finally, the tubes were weighed and the OAC was calculated as the amount of oil absorbed by the amount of flour.

3.6.2 Pasting properties RVA

An RVA (Newport Scientific Works, Warriewood, Australia) was used to measure the pasting properties of the WPF as well as PFF50 and PFF150 from I20 and C20 according to the standard 1 method of the manufacturer. Three grams of pea flour were measured and mixed with 25 ml of distilled water in an aluminium canister. A paddle rotation of 160 rpm was used throughout the experiment, apart from an initial ten s at 960 rpm to properly mix the suspension. The suspension was held at 50°C for 1 min, heated to 95°C and held there for 3.5 min before cooling to 50°C. The program had a total run time of 13 min.

3.7 Chemical properties of the WPF & PFF

The WPF samples, PFF50 and PFF150 from I20 and C20 were analysed for starch content, and the results are reported as average values as percentage of dry matter (DM). The WPF samples were analysed for dietary fibre content and the results are reported as average values as percentage of DM. DM was determined according to the AACC method 44-15A (2000) by drying the samples for 16 h at 105°C.

3.7.1 Starch content

The starch content of the WPF as well as PFF50 and PFF150 from I20 and C20 were analysed according to Åman et al. (1994) with thermostable α-amylase (Megazyme, Ireland). In short, approximately 25 mg of each sample was extracted with 80% ethanol. Thereafter, hydrolysis to glucose was commenced. After completion of the hydrolysis, the starch content was measured by colorimetry.

3.7.2 Dietary fibre analysis

The Uppsala method (AOAC - NMKL methods no. 162, 1998) was used to determine the dietary fibre contents of the WPF. In the Uppsala method, starch is removed through addition of heat stable α-amylase and amyloglucosidase (Megazyme, Ireland) in an acetate buffer. Ethanol (80%) is used to precipitate soluble polysaccharides. Both soluble and insoluble polysaccharides are thereafter hydrolysed with sulphuric acid. Uronic acids are then determined by colorimetry, neutral monosaccharide residues are quantified by gas chromatography and Klason lignin is determined gravimetrically. The total amylase resistant polysaccharides along with Klason lignin are defined as the total dietary fibre content. Further, fructan and fructooligosaccharides are not included in this analysis since the acid hydrolysis will lead to their breakdown (Theander et al. 1995).

Calculations of the relative cell wall content of sugar residues were conducted based on the results of the dietary fibre analysis. Each sugar unit, including uronic acids, were expressed as a percentage of the total content of sugar residues and uronic acids.

3.8 Statistical analysis

Two statistical techniques were used to analyse the data obtained in the experiments. A principal component analysis (PCA) was conducted, using SIMCA 17 (Sartorius Stedim Data Analytics AB). A two-way analysis of variance (ANOVA), using Minitab® 19, with Tukey pairwise comparisons was conducted with a significance level of 95% to obtain mean values and significant differences between varieties and years of cultivation. Furthermore, a PCA was conducted on the results of the PFF of I20 and C20, together with the results from their whole flour counterparts.

4. Results & Discussion

4.1 Physical properties of the pea seeds

4.1.1 Average size of seed & 100 grain weight

It was shown in the statistical analysis that the average size of the pea seeds varied significantly depending on the variety (Table 5). However, only samples of the varieties Balder, Ingrid and Clara were included in the ANOVA, since the data of Rokka was only available from one year of cultivation.

The HGW ranged from 21.8 to 29.6 g, with Clara being the lightest and Ingrid being the heaviest. Moreover, the length varied from 7.17 (Clara) to 8.07 mm (Ingrid). Ingrid was significantly longer compared to Clara and Balder, where no statistical difference was detected. The width of the pea varieties varied from 6.52 (Clara) to 7.18 mm (Rokka). Moreover, Clara had a significantly smaller measurement of width compared to Ingrid and Balder. Thickness varied between 5.95 (Clara) and 6.75 mm (Rokka). There was a significant difference between Ingrid, Clara and Balder, where Balder was the widest and Clara was the least wide. Sphericity was calculated based on the length, width and thickness (calculation described in the methods section). Moreover, a value closer to one indicates a rounder shape. The sphericity ranged from 0.87 (Ingrid) to 0.94 (Rokka). A significant difference was shown between Ingrid, Balder and Clara, where Balder was the roundest, followed by Clara, while Ingrid was the least round variety. Volume varied from 147 (Clara) to 195 μl (Rokka). Clara had a significantly smaller volume compared to Balder and Ingrid.

The variance of size depending on year was also considered. There was a significant difference for the size of the different peas between 2018 and 2020, where peas from 2018 was consistently smaller in all categories, although the shape remained similar.

Table 5. Average size of the pea seeds, values expressed as means by variety and means by year of cultivation. 100 grain weight (HGW), length (L), width (W), thickness (T), volume (V) and sphericity (Sp)

	Variety				**Year**		
Parameter	Ingrid	Clara	Balder	Rokka[1]	2018	2019	2020
HGW (g)	29.6[a]	21.8[c]	25.0[b]	27.8	23.5[b]	26.2[ab]	26.7[a]
L (mm)	8.07[a]	7.17[b]	7.36[b]	7.64	7.28[b]	7.61[ab]	7.71[a]
W (mm)	7.07[a]	6.52[b]	6.87[a]	7.18	6.62[b]	6.88[a]	6.95[a]
T (mm)	6.12[b]	5.95[c]	6.30[a]	6.75	5.95[b]	6.18[a]	6.25[a]
V (µl)	184[a]	147[b]	168[a]	195	151[b]	171[a]	177[a]
Sp	0.87[c]	0.91[b]	0.93[a]	0.94	0.91[a]	0.90[a]	0.90[a]

Values in the same row with different letters represent a significant difference, although, by variety only and year only.

[1]Data of Rokka was only available from 2019

A principal component analysis depicts the correlation between loadings (parameters analyzed), pea varieties and year of cultivation. Figure 1 and 2 originates from the PCA and illustrates these correlations. A total of 91.1% of the variance were contributed to the first and second principal component (PC), depicted in the biplot below. PC1 (along the x-axis) explained 59.8% of the variance and PC2 (along the y-axis) explained 31.3% of the variance.

In Figure 1 below a biplot originating from the PCA is shown. Loadings (sphericity, thickness, width, volume and length) are combined with the scores of each individual pea, coloured by variety. Variables further out in the plot has more of a contribution to the variance, thus, it appears as if all the variables are contributing to the variance to a large extent. Variables that are located closely together, further out on the plot, are positively correlated, while variables on the opposite side are negatively correlated. Moreover, variables that are positioned with a 90̊ angle implies no correlation. Length, width, volume and thickness seems to be the parameters influencing PC1 and were closely related to each other, whereas sphericity, length and thickness appears to be influencing PC2 the most. Furthermore, there seems to be no correlation between volume and sphericity, a positive correlation between width and thickness, a negative correlation between length and sphericity, moreover, volume of the seed was strongly linked to seed width. Sphericity appears the least linked with the other dimensional parameters like length, width and thickness.

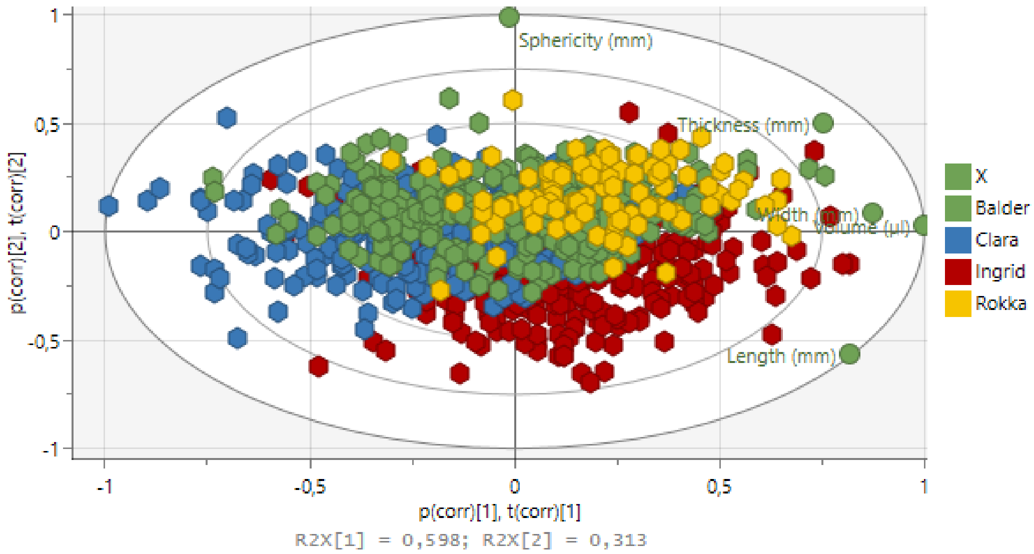


Figure 1. Biplot, containing loadings (X), in green (round circles) with labels of each variable, as well as scores of each pea, colored by variety. A total of 91.1% of the variance was covered by the first (along the x-axis) and second PC (along the y-axis), depicted in the plot.

The individual scores are overlapping to a large extent. However, it is still possible to see the general patterns of each variety. These patterns appear consistent with the means discussed and presented in Table 5 above. For instance, as seen in Table 5 Clara has lower mean values for length, width, thickness and volume, compared to the other varieties. However, Clara has a rounder shape compared to Ingrid. This pattern can also be observed in Figure 1, where Clara, in blue, is situated further away from the loadings thickness, width, volume and length, compared to the other varieties. However, Clara is situated closer to the sphericity loading compared to Ingrid.

Figure 2 below is a score-plot, also originating from the PCA, where the pea samples are coloured by year and distributed in a scattered manner. Although, the scores are overlapping, it seems as if the scores from 2018 are situated more on the left side, which is further away from the loadings thickness, width, length and volume (as can be observed in Figure 1 above). This is indicating that the pea seeds cultivated in 2018 are smaller compared to 2019 and 2020, which is confirmed by the means presented in table 5.

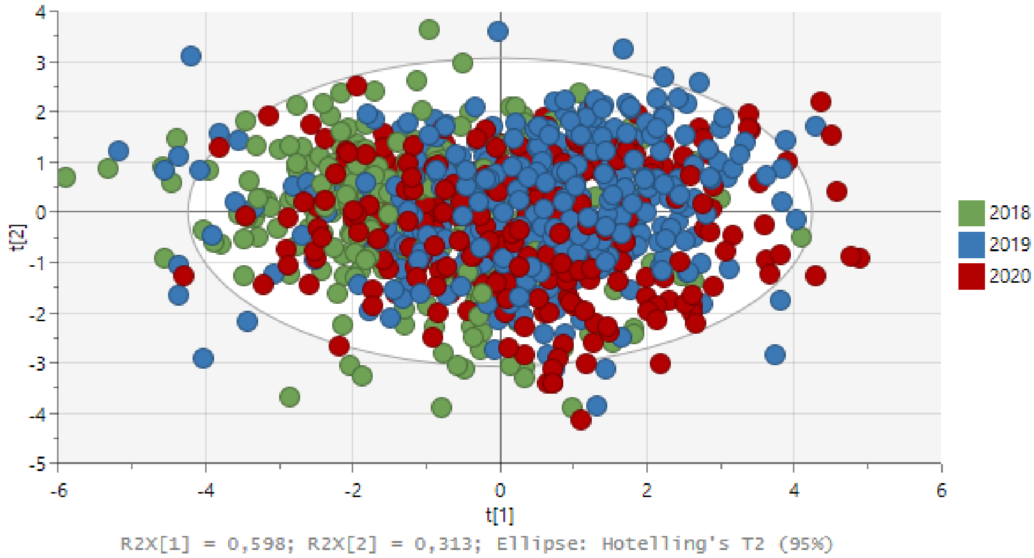


Figure 2. Scoreplot containing the individual scores of the peas, coloured according to the year of cultivation.

Based on these results it can be shown that Clara was the smallest variety analysed in this study and also round. Ingrid could be considered the largest, with high values for HGW, length, and width, while being low in thickness (Table 5). This disproportion probably also led to Ingrid being the least round variety. Balder mainly resided between Clara and Ingrid in regards of size, except when considering thickness. Further, as mentioned, Balder is rounder in shape compared to Clara and Ingrid. Rokka could also be considered one of the larger varieties, while also having the roundest shape out of the varieties analysed.

Regarding the extreme weather conditions of 2018 (Table 4) with very little rainfall, this could have impacted the growth of the peas. In a study by Tao et. al. (2017), looking at the effects of environment on different green and yellow dry peas, the authors concluded that environmental conditions were the major factor influencing yield of the different pea varieties. Further, the study showed a positive correlation between yield and average seed size. The authors could also see differences in the effects of environmental conditions, such as drought conditions, on different varieties.

4.1.2 Bulk density, true density & porosity

The mean values for bulk density, true density and porosity, by variety as well as year, are presented in Table 6 below. Considering the parameters by variety, bulk density ranged from 0.86 to 0.90 g/cm^3 in the pea varieties. ANOVA showed that Balder has a significantly lower bulk density compared to Ingrid, while no statistically significant difference could be detected for Clara. True density ranged from 1.39 to 1.46 g/cm^3, with Ingrid, Balder and Rokka showing the same true density, while Clara showed a higher value. Although, there was no significant difference. Porosity ranged from 35.6 for Ingrid to 39.4% for Clara.

When analysing the parameters by year, there was no statistically significant difference for true density or porosity. However, the ANOVA showed that the bulk density of samples from 2018 was significantly lower compared to 2019 and 2020. This correlates to the results of average size and HGW, where the samples from 2018 were the lightest and smallest group.

Table 6. Functional properties of the pea seeds, values expressed as means by variety and means by year of cultivation. Bulk density (BD), true density (TD) and porosity (P)

	Variety				**Year**		
Parameter	Ingrid	Clara	Balder	Rokka[1]	2018	2019	2020
BD (g/cm^3)	0.90^a	0.89^{ab}	0.88^b	0.86	0.88^b	0.89^a	0.89^a
TD (g/cm^3)	1.39^a	1.46^a	1.39^a	1.39	1.41^a	1.42^a	1.41^a
P (%)	35.6^b	39.4^a	36.7^{ab}	37.1	37.8^a	37.1^a	36.8^a

Values in the same row with subscript different letters represent a significant difference, although, by variety only and year only.

[1]Data of Rokka was only available from 2019 and is not included in the means of the years

4.2 Particle size analysis

The particle size analysis is reported in Table 7 below. There were large discrepancies in the results, especially considering the percentage of particles in the <50 and <150 µm fraction, indicating that the method chosen for the analysis could have been improved. Therefore, the distribution of particles in the <50 and <150 µm fraction is difficult to discuss since there were contradicting results. However, it is possible to see that, with the milling technique used, most of the particles are smaller than 150 µm, for all the varieties.

Table 7. Particle size distribution of whole pea flours based on varieties as well as years of cultivation, values expressed as means. The particle size distribution is presented as the percentage of flour in each fraction

	Variety				**Year**		
Particle Size	Ingrid	Clara	Balder	Rokka[1]	2018	2019	2020
<50µm	40.27^{ab}	32.64^b	42.94^a	37.91	34.21^b	34.87^b	46.76^a
<150µm	36.01^{ab}	40.83^a	31.47^b	37.60	37.94^{ab}	39.48^a	30.88^b
<250µm	14.27^a	17.20^a	15.98^a	15.32	18.2^a	15.93^{ab}	13.31^b
<600µm	8.55^a	8.25^a	8.53^a	8.63	8.54^a	8.73^a	8.06^a
<1mm	0.70^a	0.85^a	0.82^a	0.48	0.84^a	0.76^a	0.78^a
>1mm	0.20^a	0.22^a	0.27^a	0.06	0.26^a	0.22^a	0.21^a

Values in the same row with different letters represent a significant difference, although, by variety only and year only.

[1]Data of Rokka was only available from 2019 and is not included in the means of the years

4.3 Microstructural study of the WPF & PFF

Figure 3 below shows three SEM pictures of the WPF from B20, C20 and I20. The WPF of the different varieties appear to be quite similar. All WPF contain a cluster of what appears to be starch granules based on their size (<40 μm) and oval shape (Ratnayake et al. 2002). On the surface of the starch granules smaller structures of irregular shapes resides. They are probably protein bodies based on their placement on the starch granules and their size of about 3 μm, which correlates to the reported size of protein bodies in pea storage cells (Pernollet 1978). Moreover, all flours also contain particles larger than the supposed starch granules, with a different structure. They contain indentations, rounded holes with a smooth structure, that corresponds to the size of the starch granules. Furthermore, these larger structures, which appears to be made up of several smaller particles that are connected together, correspond to what has been identified as fragments of the intracellular matric (Möller et al. 2021b). Starch granules, protein bodies and fragments of the intracellular matrix are visible in the SEM images in Figure 3 below.

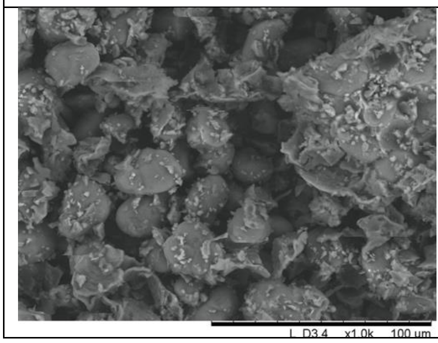
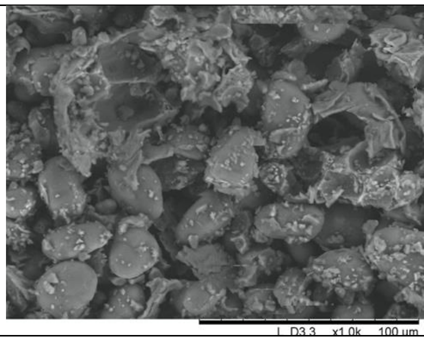
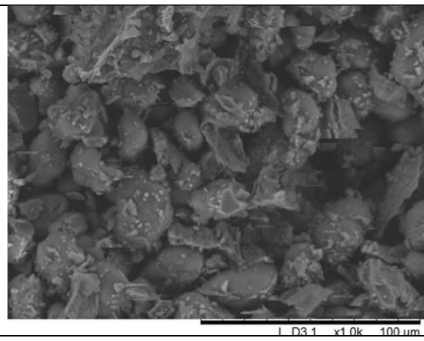
B20 WPF x 1000	C20 WPF x 1000	I20 WPF x 1000
		

Figure 3. SEM pictures of whole flours from Balder (B20), Ingrid (I20) and Clara (C20) cultivated in year 2020, in magnification x1000. The bar in the lower right corner of the image is 100 μm for size reference.

In Figure 4 below SEM images of the PFF250 of sample C20 in different magnifications are shown. It becomes apparent that the flour fractions containing only particles >250 μm are vastly different compared to the whole pea flour. There is no cluster of starch granules, protein bodies and intracellular matrix to be observed. Instead, the fraction seems to be consisting of large particles with differing geometrical shapes. Further, the surface of the particles contains what seems to be a uniform pattern of several smaller indents and raises. These patterns corresponds to the visual appearance of the pea testa surface during SEM analysis (Stolárik et al. 2015), indicating that what can be observed in the PFF250 of C20 could be fragments of the seed coat. Images of the same fraction from sample I20 were also obtained and showed similar structures (Appendix 1- SEM-images).

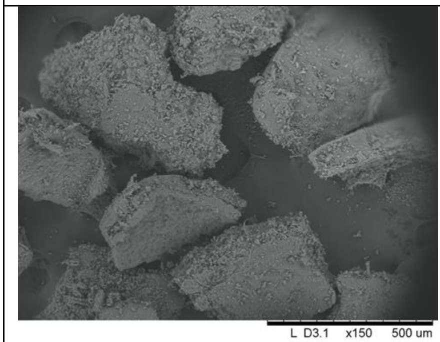
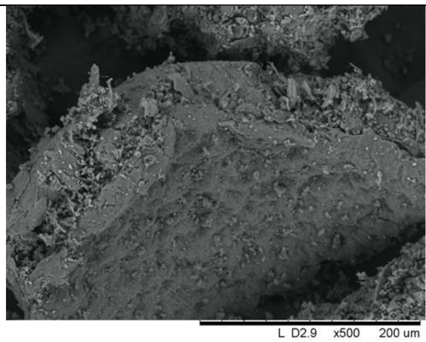
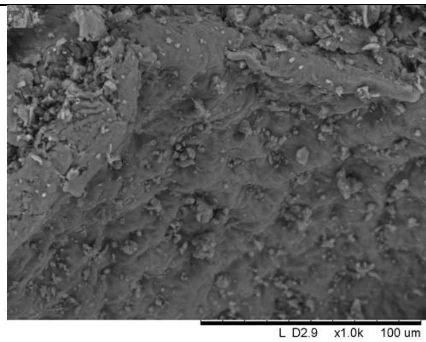
C20 PFF250 x150 a	C20 PFF250 x 500 b	C20 PFF250 x 1000 c
		

Figure 4. SEM-pictures of PFF20 of pea variety Clara harvested in year 2020, in magnifications x150 (a), x 500 (b) and x 1000 (c). The bar in the lower right corner represents 500 μm (a), 200 μm (b) and 100 μm (c).

Figure 5 shows PFF50 and PFF150 of the flour sample C20 in magnifications x500 and x2000. The PFF50 appear to contain mainly starch granules of varying sizes, in oval and round shapes, protein bodies and cell wall fragments. While the PFF150 also contain these elements, there appears to also be several larger fragments of the pea storage cell, like the fragments also visible in the whole flours. A close-up of what appears to the fragments of a pea storage cell is seen in figure 5 (d). It is possible to distinguish oval and round structures which corresponds to the appearance of starch granules as well as smaller round particles that seems to be protein bodies, which seems to make up the larger particle. Further, in figure 5 (d), the larger particle appears to be surrounded by a sheet-like structure, which could represent the cell wall (Möller et al. 2021b).

In Figure 5 (b) it is possible to observe two the types of starch granules of peas, two oval granules (36.4 μm and 22.8 μm) as well as one round granule (14.9 μm), previously described. Also, a smooth surface of the starch granules can be observed, with no visible pores.

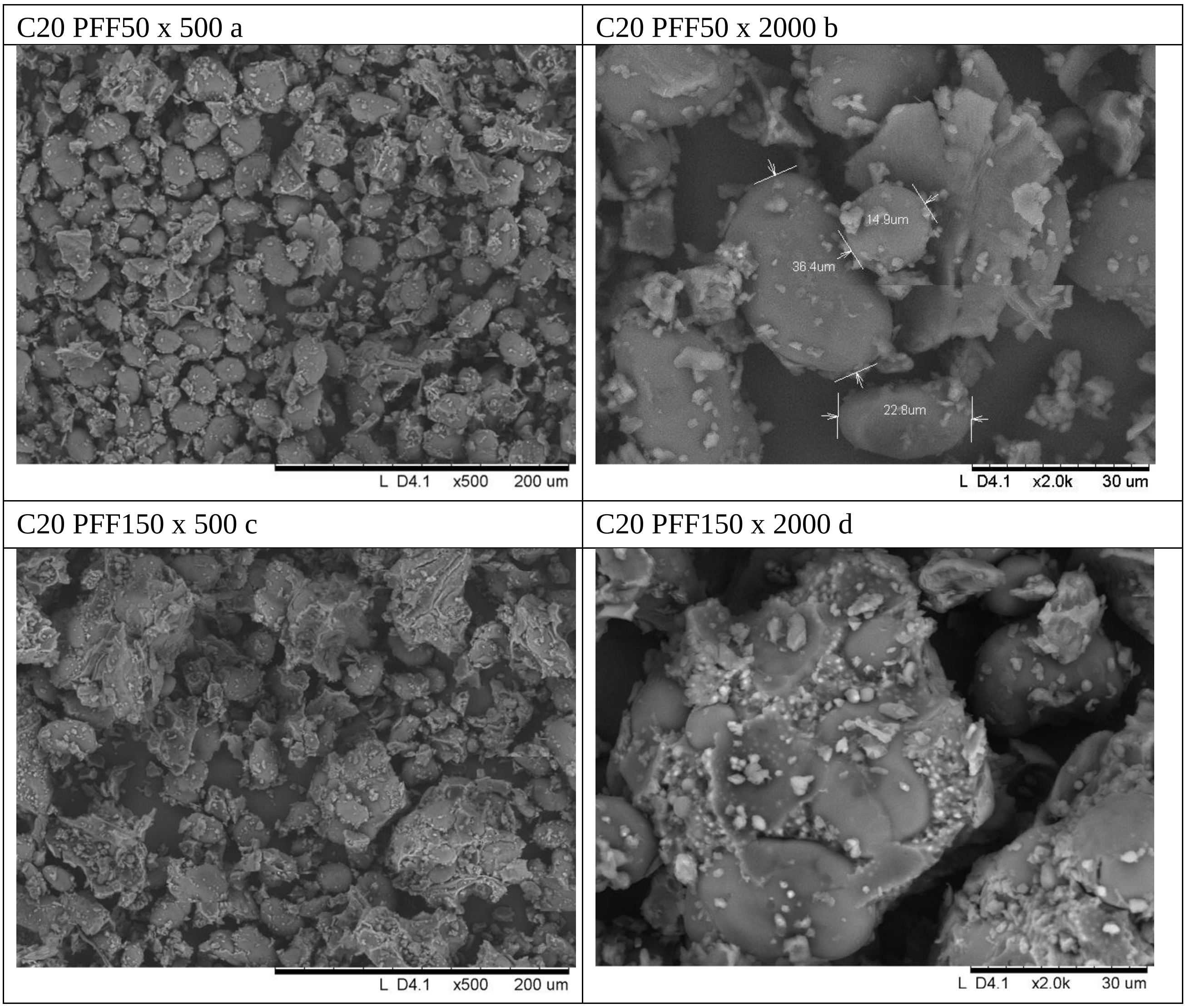


Figure 5. SEM-pictures of PFF50 and PFF150 of pea variety Clara harvested in year 2020, in magnifications x 500 (a and c) and x 2000 (b and d). The bar in the lower right corner represents 200 μm (a and c) and 30 μm (b and d).

4.4 Functional properties of the WPF

4.4.1 OAC & WAC

Table 8 shows the WAC and OAC of the WPF, with values expressed as means by variety and year. Based on variety, the WAC ranged from 2.29 (Ingrid and Clara) to 2.57 g/g flour (Rokka). Balder had a significantly higher WAC (2.39 g/g flour) compared to Clara and Ingrid. There was no significant difference detected in OAC between the varieties. Values of OAC ranged from 1.90 (Ingrid) to 1.93 g/g flour (Rokka). WAC and OAC of yellow peas have been reported in a range of 0.8-4.04

(Maninder et al. 2007; Agboola et al. 2010; Ferawati et al. 2019; Young et al. 2020) and 0.9-2.49 g/g flour (Maninder et al. 2007; Agboola et al. 2010; Ferawati et al. 2019). Values reported in this study lies within the reported range.

There were significant, although small, differences shown in WAC between the years. Where samples from 2018 showed the highest WAC (2.36 g/g flour) and 2020 showed the lowest WAC (2.29 g/g flour). Based on these results, differences in WAC of the analysed varieties appears to be affected by both genetic factors as well as possible environmental and storage factors (differences by year). Content of polar amino acid residues, which have an affinity for water, could be influencing the WAC of the different samples. Moreover, carbohydrates and their composition also presents as a possible component influencing the differences in WAC (Sreerama et al. 2012). Whereas, the main factors influencing OAC is interactions with nonpolar groups with the fatty acid carbon chain (Shevkani et al. 2021). Furthermore, WAC and OAC are important indicators for the behavior of samples in food product. OAC indicates the ability to retain lipids, hence, has large influence on the flavor retaining ability of food products. WAC is important for predicting the ability to form viscous solutions, important for products such as soups and gravy (Sreerama et al. 2012).

Table 8. Water absorption capacity (WAC) and oil absorption capacity (OAC) of whole pea flours, values expressed as means by variety as well as means by year of cultivation

	Variety				**Year**		
Parameter	Ingrid	Clara	Balder	Rokka[1]	2018	2019	2020
WAC (g/g flour)	2.29[b]	2.29[b]	2.39[a]	2.57	2.36[a]	2.33[b]	2.29[c]
OAC (g/g flour)	1.90[a]	1.91[a]	1.92[a]	1.93	1.89[a]	1.92[a]	1.92[a]

Values in the same row with different letters represent a significant difference, although, by variety only and year only.
[1]Data of Rokka was only available from 2019 and is not included in the means of the years

4.4.2 Pasting properties by RVA

Pasting properties of the WPF analysed by RVA is shown in Table 9 below, as well as illustrated in pasting curves in Figure 6 and 7. Means by variety show varying differences in the different pasting parameters, although non-significant. Most notable is the difference in final viscosity (FV) ranging from 56.46 rapid viscosity units (RVU) for Rokka to 83.19 RVU for Balder. Furthermore, FV for field peas have been reported in ranges of 578-4736 centipoise (cP) (Maninder et al. 2007; Chung et al. 2008; Singh et al. 2010; Santos et al. 2019; Young et al. 2020), indicating that the results of the pasting properties of pea flour, from this study, is comparatively low, although within the reported range since 1 RVU equals 12 cP.

Table 9. Pasting properties of the WF in RVU, values expressed as means based on variety as well as year of cultivation. Peak viscosity (PV), holding strength (HS), breakdown (B), final viscosity (FV), setback (S), peak time (PeakT) and pasting temperature (Ptemp)

Variety	**PV**	**HS**	**B**	**FV**	**S**	**PeakT**	**Ptemp**
Ingrid	53.22^{a}	51.03^{a}	2.21^{a}	76.00^{a}	24.97^{a}	5.88^{a}	78.35^{a}
Clara	50.57^{a}	48.44^{a}	2.13^{a}	66.64^{a}	18.19^{a}	5.59^{b}	78.08^{a}
Balder	55.04^{a}	53.31^{a}	1.74^{a}	83.19^{a}	29.89^{a}	5.98^{a}	77.49^{a}
Rokka[1]	41.92	40.50	1.42	56.46	15.96	5.83	79.05
Year							
2018	56.92^{a}	55.61^{a}	1.31^{a}	81.60^{a}	25.99^{a}	6.28^{a}	79.74^{a}
2019	55.54^{a}	53.01^{a}	2.53^{a}	77.96^{a}	24.94^{a}	5.79^{ab}	77.77^{ab}
2020	46.37^{a}	44.15^{a}	2.24^{a}	66.28^{a}	22.13^{a}	5.38^{b}	76.41^{b}

Values in the same column with different letters represent a significant difference, note that this applies by year only and variety only.

[1]Data of Rokka was only available from 2019 and is not included in the means by years

Means by year also show variations, where, for instance, whole flour from 2020 showed a lower PV and FV, although non-significant. Furthermore, the statistical analysis showed significant differences in peak time and pasting temperature between samples from 2018 and 2020. Where samples from 2020 showed a lower peak temperature and an earlier peak time. These observations are also illustrated in Figure 6, depicting a pasting curve of the whole flours of variety Clara from the three harvest years. Furthermore, apart from lower viscosity, the samples from 2020 also showed a lower WAC, indicating that differences in functional properties between the WPF cultivated in different years exist. This could be due to environmental factors and the effects of storage.

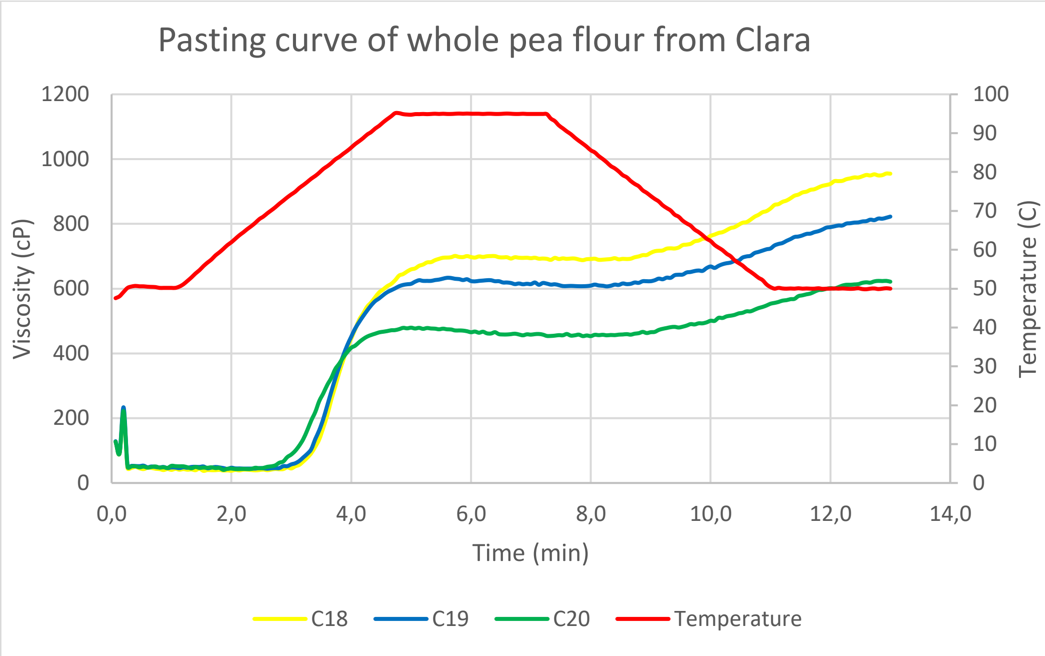


Figure 6. Pasting curves of three whole pea flours, variety Clara, from year 2018 (C18), 2019 (C19) and 2020 (C20). Curves are mean values of two replicates

Furthermore, when studying the pasting curves of the individual samples from each year which can be seen in Appendix 2 – pasting curves, it becomes apparent that the pasting curve of Ingrid WPF does not follow the same trend as the samples of Clara and Balder. While I20 is still the sample with the lowest viscosity, I18 is lower than I19. This indicates that all pea varieties are not affected by environmental or storage conditions in the same way.

Pasting curves of each variety from the year 2019 is shown in Figure 7. This curve shows that R19 has the lowest FV and PV, while I19 has the highest PV and B19 the highest FV. Furthermore, all pasting curves in Figure 7 follows the typical pasting curve of pea flours, representative of the starch properties of peas. The holding period leads to a low breakdown, indicating the slow granular swelling of pea starch. Moreover, the FV is higher than the PV for all samples, indicating the strong tendencies of pea starch to form strong gels during gelatinization and retrogradation (Martinez & Boukid 2021).

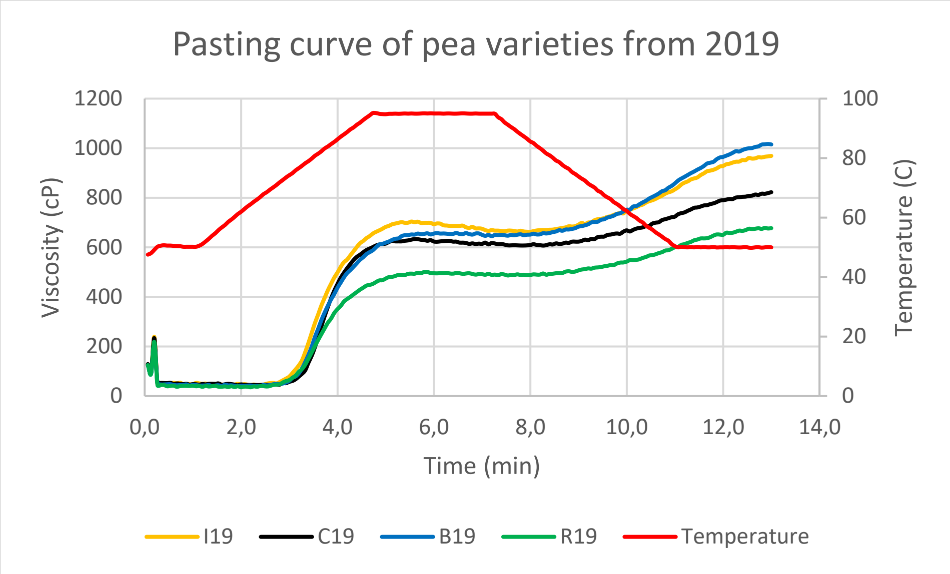


Figure 7. Pasting curves whole pea flours from four different varieties, cultivation year 2019, variety Clara (C19), Ingrid (I19), Balder (B19) and Rokka (R20). Curves are mean values of two replicates

4.5 Chemical properties of the WPF

4.5.1 Starch content

Table 10 shows the starch content presented as means by variety and means by year. There are no significant differences shown in starch content by variety. The starch content varies from 47.7% of dry matter (dm) for Ingrid to 50.0% of dm for Clara. When looking at the average of starch expressed as means by year, the range is smaller, 48.5-48.9% of dm, for 2020 and 2018, respectively. Further, no significant differences are shown between the years. Starch content of peas have been reported in ranges of 33.4-53.6% of dm (Nikolopoulou et al. 2007; Chung et al. 2008; Young et al. 2020). Results reported in this study are within these ranges. Further, in the study by Nikolopoulou et al. (2007) the effect of variety and year on the chemical composition of peas was examined. The authors studied seeds from three white-colored varieties that were grown in three locations for two cultivation years. They found that both location and variety had a significant effect on the proximate composition, however, they could find no effect of cultivation year on starch content.

There does not seem to be a correlation between starch content and pasting parameters. For instance, Clara showed a lower viscosity compared to Balder and Ingrid, while having a higher starch content. This could suggest that the starch content is less relevant for the viscosity parameters compared to the starch composition. Therefore, to study the amylose:amylopectin ratio of the WPF would be interesting, this could possibly help in explaining the differences in pasting properties. Further, more insight into the effect of chemical composition on functional properties could be developed if protein and fat composition of the pea samples were also available.

Table 10. Starch content in the whole pea flours based on variety as well as year of cultivation

	Variety				Year		
Parameter	Ingrid	Clara	Balder	Rokka[1]	2018	2019	2020
Starch (% dm)	47.7[a]	50.0[a]	48.6[a]	47.7	48.8[a]	48.9[a]	48.5[a]

Values in the same row with different letters represent a significant difference, although, by variety only and year only.
[1]Data of Rokka was only available from 2019 and is not included in the means of the years

4.5.2 Dietary fibre analysis

The main dietary fibre components in all the samples were, in descending order, glucose, arabinose, xylose and uronic acid residues. Only trace amounts of Klason lignin was shown, indicating that it is probably present in the hulls in small amounts. In Table 11 it can be seen that Clara had a significantly lower percentage of arabinose residues compared to Ingrid and Balder, otherwise there were no significant differences shown in the dietary fibre composition of the different pea varieties. Moreover, the total dietary fibre content of the varieties ranged from 12.7-13.2 % of dm.

The content of galactose residues was significantly lower in 2018 compared to 2020, 0.58 and 0.65 % of dm, respectively.

Table 11. Sugar residues, uronic acids, Klason lignin and total dietary fibre content presented in % of dm in the whole pea flours, values expressed as means by variety as well as means by year*

Variety	**rha**	**ara**	**xyl**	**man**	**gal**	**glc**	**UA**	**KL**	**TDF**
Ingrid	0.21[a]	3.40[a]	1.19[a]	0.31[a]	0.61[a]	5.85[a]	1.00[a]	0.18[a]	12.7[a]
Clara	0.21[a]	2.93[b]	1.23[a]	0.29[a]	0.63[a]	6.25[a]	1.01[a]	0.13[a]	12.7[a]
Balder	0.18[a]	3.46[a]	1.25[a]	0.28[a]	0.59[a]	6.20[a]	0.99[a]	0.22[a]	13.2[a]
Rokka[1]	0.19	3.15	1.27	0.30	0.63	6.03	0.97	0.16	12.5
Year									
2018	0.21[a]	3.25[a]	1.21[a]	0.29[a]	0.58[b]	6.09[a]	0.97[a]	0.16[a]	12.6[a]
2019	0.20[a]	3.22[a]	1.19[a]	0.29[a]	0.60[ab]	6.06[a]	1.00[a]	0.21[a]	12.6[a]
2020	0.19[a]	3.32[a]	1.27[a]	0.29[a]	0.65[a]	6.14[a]	1.03[a]	0.16[a]	12.9[a]

*) Rhamnose (rha), arabinose (ara), xylose (xyl), mannose (man), galactose (gal), glucose (glc), uronic acids (UA), Klason lignin (KL) and total dietary fibre (TDF).
Values in the same column with different letters represent a significant difference, although, by year only and by variety only.
[1]Data of Rokka was only available from 2019 and is not included in the means of the years

The relative cell wall composition is presented in Table 12. Relative rhamnose, mannose, uronic acids and xylose residues still showed no significant difference between varieties. However, the two major components, relative glucose residues, most likely corresponding to cellulose content, and arabinose residues, most likely corresponding to hemicellulose and pectic content, showed significant differences between the varieties. Clara had a lower relative arabinose residue content compared to Ingrid and Balder, while simultaneously showing a significantly higher relative glucose residue content compared to the other varieties. Indicating that cellulose and arabinose residues could be negatively correlated. Moreover, Clara also showed a significantly higher galactose residue content compared to Balder. Moreover, there were no differences shown in the relative cell wall composition by years compared to the dietary fibre content presented above in table 11.

Table 12. Relative cell wall composition of the whole pea flours, values expressed as means by variety as well as means by year, presented as a percentage of the total relative cell wall composition. Rrha (relative rhamnose), Rara (relative arabinose), Rxyl (relative xylose), Rman (relative mannose), Rgal (relative galactose), Rglc (relative glucose) and RUA (relative uronic acids)

Variety	**Rrha**	**Rara**	**Rxyl**	**Rman**	**Rgal**	**Rglc**	**RUA**
Ingrid	1.7^{a}	27.0^{a}	9.5^{a}	2.4^{a}	4.8^{ab}	46.6^{b}	8.0^{a}
Clara	1.7^{a}	23.4^{b}	9.8^{a}	2.3^{a}	5.0^{a}	49.8^{a}	8.1^{a}
Balder	1.4^{a}	26.7^{a}	9.6^{a}	2.1^{a}	4.6^{b}	47.9^{b}	7.6^{a}
Rokka[1]	1.5	25.1	10.1	2.4	5.0	48.1	7.7
Year							
2018	1.6^{a}	25.8^{a}	9.6^{a}	2.3^{a}	4.6^{b}	48.3^{a}	7.7^{a}
2019	1.6^{a}	25.6^{a}	9.5^{a}	2.3^{a}	4.8^{ab}	48.3^{a}	7.9^{a}
2020	1.5^{a}	25.7^{a}	9.8^{a}	2.2^{a}	5.1^{a}	47.7^{a}	8.0^{a}

Values in the same column with different letters represent a significant difference.

[1]Data of Rokka was only available from 2019 and is not included in the means of the years

4.6 PCA of WPF

The biplot originating from the PCA is shown in Figure 8 below. All variables analysed for the WPF (loadings) are shown in light blue together with the scores of the individual whole flour samples, coloured by variety. A total of 50.2% of the variance were attributed to the first two principal components, where 26.9% and 23.3% were attributed to PC1 and PC2, respectively.

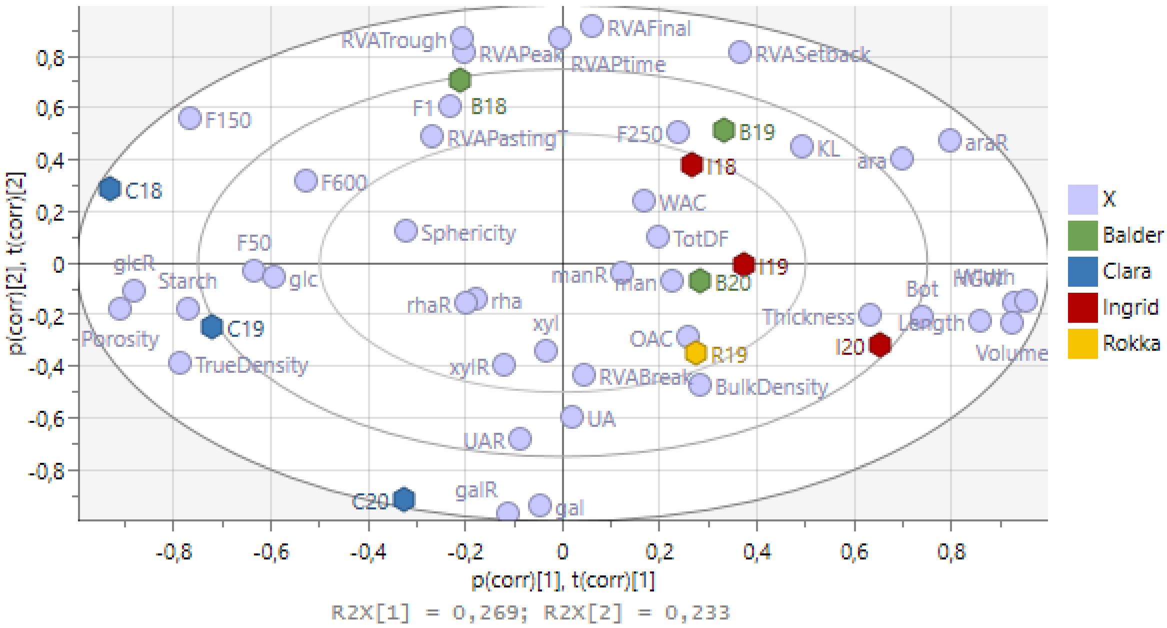


Figure 8. A biplot containing the loading, coloured in light blue, as well as the scores of the individual samples, coloured by variety and labelled with year (18 = 2018, 19 = 2019 and 20 = 2020). A total of 50.2% of the variation is attributed to the two first principal components shown in the biplot. Abbreviations of parameters analysed are explained in appendix 3

Results from the PCA show a difference between the different pea varieties. For instance, samples of Clara are leaning farther towards the left of the plot, demonstrating the higher values of starch content as well as relative glucose residues in the relative cell wall composition. Samples of Balder and Ingrid are closer together, although Balder samples are generally located more toward the upper part of the plot, where most of the pasting parameters are located. This indicates that Balder differentiates from Ingrid mainly by the higher values from the rapid visco analysis.

Further, the differentiation by cultivation year also can also be seen. While the samples are grouping by variety, they do so in a quite uniform matter. Where samples from cultivation year 2020 are located below samples from cultivation year 2019, which, in turn, is located below samples from cultivation year 2018. Values of average size, WAC, galactose residues in the dietary fibre content and pasting parameters were the main variables that differed when comparing means by year. Moreover, the PCA biplot appears to show these differences since galactose residues is in the bottom of the plot (values were lower for means of samples from 2018) and the pasting parameters are located at the top (values higher for means of samples from 2018).

Based on the PCA it seems that the differences are influenced by variety, genetic factors, and by year of cultivation, environmental conditions and storage conditions. It appears as if though Ingrid, Balder and Rokka are more similar, Clara stands out a bit more from the rest. Moreover, it appears as if the main factors influencing the differentiation are pasting parameters, average size, starch content, porosity and relative glucose, galactose and arabinose residues.

4.7 Functional properties of PFF

4.7.1 OAC & WAC

Figure 9 shows the WAC and OAC from PFF50 and PFF150 of I20 and C20 and their WPF counterparts. The WAC and OAC of the smallest fraction were lower than that of the whole flour for both I20 and C20. However, for the PFF150, there was a slight decrease in WAC compared to the whole flour, while the OAC was higher in the fractions. A similar initial increase in OAC with particle size reduction, followed by a decrease for the finest fractions has also been reported by Ahmed et al. (2016), while studying Indian and Turkish lentil flours. Further, a non-significant decrease in WAC has also been observed in Indian grass pea varieties in a study by (Bala et al. 2020).

Differences in the functional properties of different PFF compared to WPF could be explained by the differences in composition, shown in the SEM images. Cellular distribution of components such as fat, protein and starch, combined with further differentiation of these components by sieve fractionation, could change the functional properties.

As cell wall fragments, containing a large portion of the dietary fiber, where only visible in PFF250, it can be assumed that the smaller fractions contain less dietary fiber. Additionally, more independent starch granules were observed in the PFF50 compared to the WPF and PFF150, indicating a higher starch content.

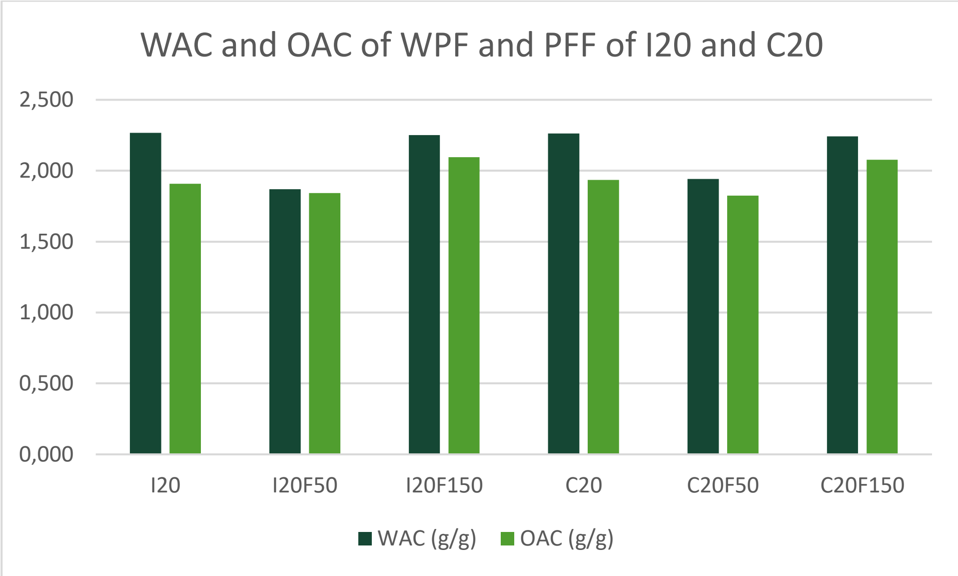


Figure 9. Water absorption capacity (WAC) and oil absorption capacity (OAC) of pea flour fractions (PFF) as well as the whole pea flour (WPF) of I20 and C20

4.7.2 Pasting properties by RVA

The pasting properties of PFF50 and PFF150 of samples I20 and C20 compared to the whole flours were also investigated. The results of the pasting properties are shown in table 13 as well as illustrated by pasting curve in figure 10. Comparing the pasting curves of PFF150 to their original WPF showed very similar results, as illustrated in figure 10. However, when comparing the smallest fractions to their WPF counterpart, there were clear differences. Where, for instance, the pasting temperature decreased, while the peak viscosity, holding strength, final viscosity and setback increased. Results showing a negative correlation between particle size and peak and final viscosity has been shown in hammer-milled yellow split peas by Kaiser et al. (2019). Where the authors discussed how the effect of decreased particle size leads to an increased exposure of starch to water as a possible explanation. Which could help in explaining the results of this study. Furthermore, as mentioned, the differences in composition shown in the SEM images, could also explain the different pasting properties of PFF50 compared to the original WPF. Based on these results it can be hypothesized that sieving, a relatively simple production step with low energy demand, is enough to change the functional properties of a pea flour. Perhaps leading to increased possibilities for uses of pea flour in the industry. Moreover, sieving could possibly be used in obtaining optimized fractions for further extraction of chemical components.

Table 13. Pasting properties of pea flour fractions as well as their whole flour (WF) counterpart. Peak viscosity (PV), holding strength (HS), breakdown (B), final viscosity (FV), setback (S), peak time (PeakT) and pasting temperature (Ptemp)

Sample	Flour fraction	PV	HS	B	FV	S	PeakT	Ptemp
I20	WF	48.25	46.63	1.67	69.38	22.75	5.70	77.20
I20	‹50 µm	66.42	64.92	1.50	100.38	35.46	5.60	75.50
I20	‹150 – 50 µm	49.13	46.71	2.42	69.92	23.21	5.70	76.33
C20	WF	40.08	37.67	2.42	51.79	14.13	5.13	76.58
C20	‹50 µm	61.46	58.08	3.38	85.46	27.38	5.10	75.90
C20	‹150 – 50 µm	40.13	36.88	3.25	50.83	13.96	4.63	76.63

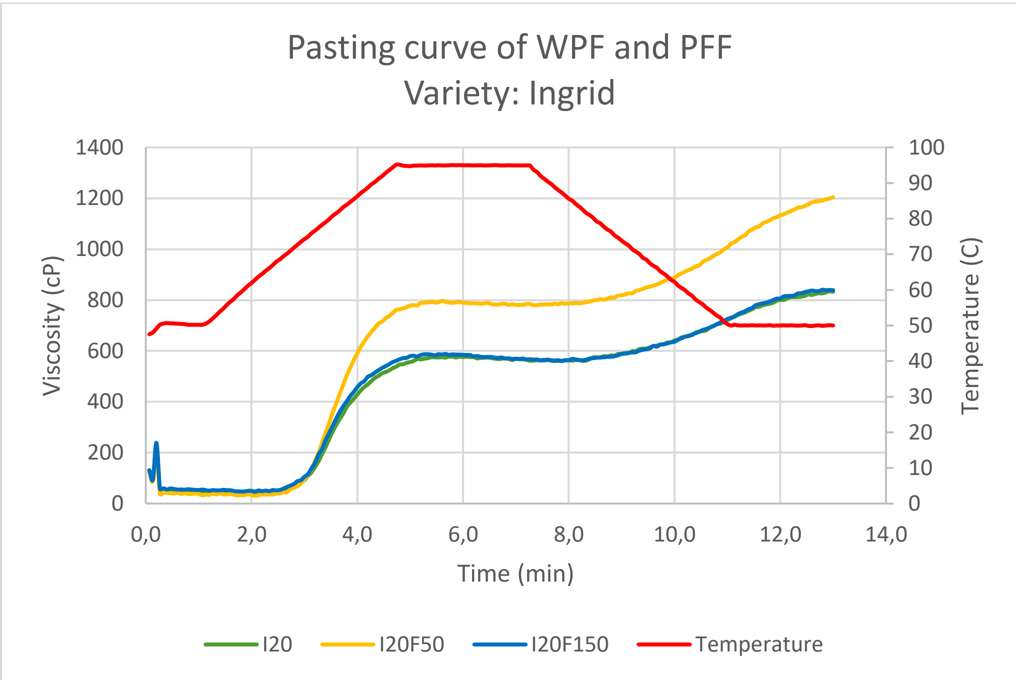


Figure 10. Pasting curve of the two flour fractions of I20 (I20F50 and I20F150) as well as the whole flour (I20)

4.8 Chemical properties of the PFF

4.8.1 Starch content

Compared to the WPF, the PFF50 had a higher starch content (Table 14), which is correlated by the SEM-images shown earlier, whereas PFF150 had a lower starch content compared to the WPF. These results further support the earlier discussed thesis that sieving is a relatively simple measure for changing the chemical as well as functional properties of a pea flour. However, to confirm this hypothesis, further studies need to be conducted, including more samples and preferably more compositional analyses.

Table 14. Starch content of pea flour fractions (PFF) as well as whole flour (WPF)

Sample	Flour fractions	Starch (% dm)
I20	WPF	47.24
I20	PFF50	53.99
I20	PFF150	45.13
C20	WPF	49.94
C20	PFF50	56.11
C20	PFF150	44.77

4.9 PCA of the PFF

Figure 11 shows a loading plot originating from a PCA of PFF50 and PFF150 of I20 and C20, which also included the respective WPF. A total of 88.3% of the variance were attributed to the two principal components. Where 69 and 19.3% of the variance were attributed to PC1 and PC2, respectively.

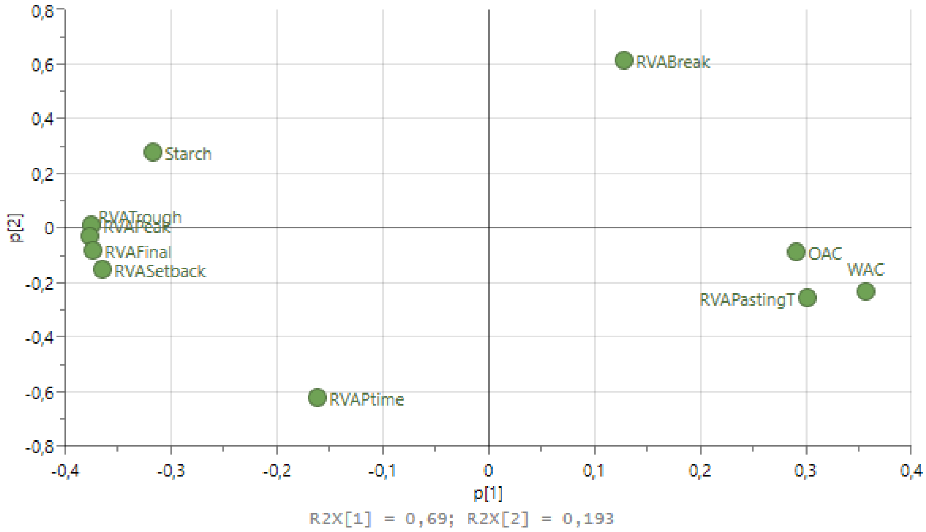


Figure 11. Loading plot of variables studied for the two flour fractions of I20 and C20. A total of 88.3% of the variation is attributed to PC1 and PC2 in the loading plot. Abbreviations of parameters analysed are explained in appendix 3

The scores are shown in Figure 12, coloured by variety, indicating a difference between the two varieties. The WPF as well as two PFF of Clara are located in the upper part of the score plot, while the Ingrid WPF and PFF are located in the lower part. Moreover, the PFF50 differs in a similar manner for both varieties and are located more to the left of the score plot indicating the higher starch content and pasting properties. Moreover, the PFF150 appear to have similar properties compared to their WPF counter parts, while being located slightly more to the right of the plot. This indicates a higher WAC and OAC of the PFF150 compared to the WPF. The PCA further confirms that the relatively simple process of sieving WPF is enough to alter the functional and chemical properties. Although, the major differences, in this case, was seen for the PFF50.

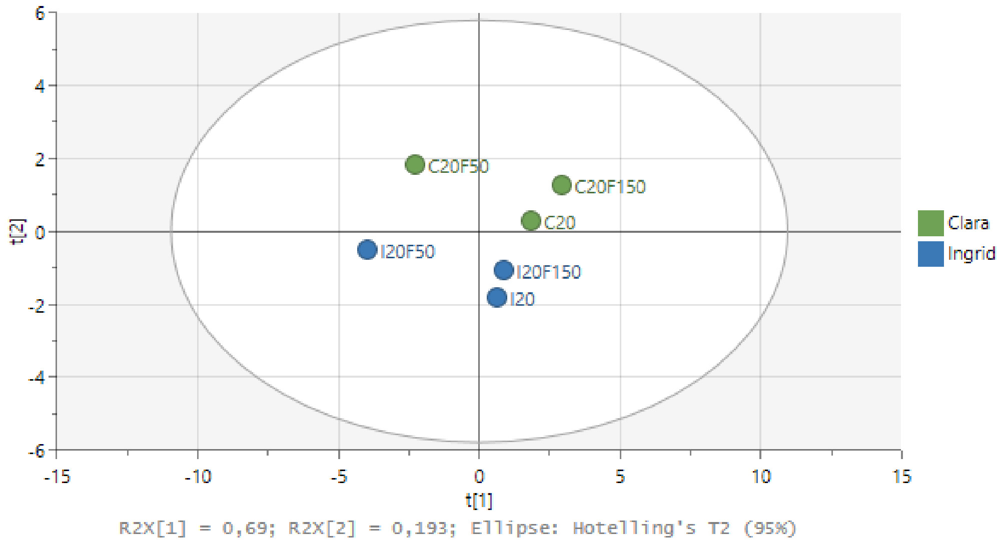


Figure 13. Score plot of flour fractions (F50 and F150) of Ingrid (blue) and Clara (green) as well as their whole flour counterparts (I20 and C20)

5. Conclusion

The purpose of this study was to provide information about the genetic and environmental effect on the physical, chemical and functional properties of peas. However, since one of the varieties was only available from one year, the ability to conduct detailed statistical calculations for all varieties became limited. Although, interesting conclusions could still be drawn from the rest of the results.

Several physical, chemical and functional properties do vary, between varieties as well as between years. Average size, for instance, was clearly impacted by the very dry conditions of 2018, while also being strongly tied to the genotype of the varieties. These variations will probably affect processing steps such as dehulling.

The PCA visualised the variations clearly by grouping of the samples by variety. Further, there was also a clear pattern in the way the years spread out. It appeared as if though Ingrid, Balder and Rokka were more similar, while Clara appeared to stand out from the rest, with a higher starch content and cellulosic content in the cell wall composition. The interesting variations by year, mainly WAC and pasting properties, could be starting points for further studies of how storage might affect the pea matrix, and, in turn, the functional properties of the WPF. Further, based on the PCA, it appeared as if the main factors influencing the differentiation are pasting parameters, average size, starch content, porosity and relative content of glucose, galactose and arabinose residues.

Based on the results of the flour fractions, there are positive indications showing that the relatively simple process of sieving could be enough to alter the chemical and functional properties of pea flour. Therefore, it could help in achieving optimized fractions for protein, starch or dietary fibre extraction.

The effect of average size and size distribution related to the dehulling process should be further studied to help implement optimal processing conditions of specific pea varieties. It is also recommended to continue research on the effect of storage related to the composition and functional properties of peas. A factor which was excluded from this research was the effect of site of cultivation, since all pea samples were grown in the same location. Further studies, also considering cultivation site, could help give a broader perspective on factors influencing physical, chemical and functional properties of peas.

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Popular science summary

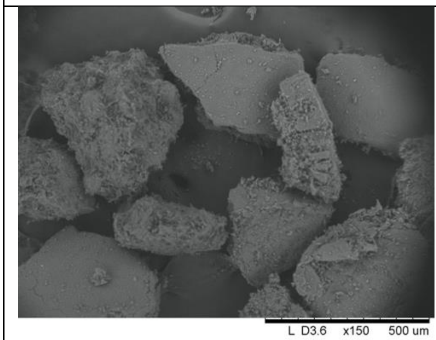
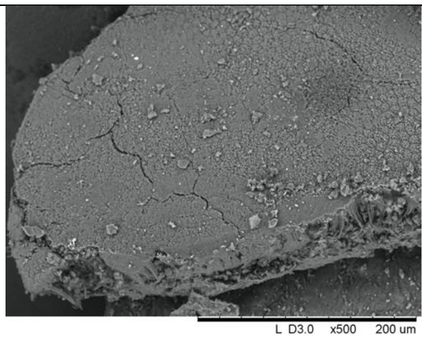
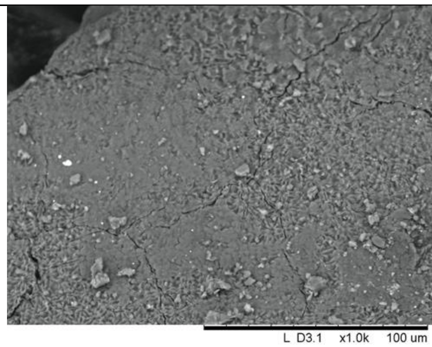
In Sweden, when you hear the words "yellow peas", most people generally think of pea soup Thursdays, which, eaten along with pork and mustard, is probably something they're extremely fond of or find impossible to eat. But, did you know that peas are actually used for so much more? For example, as food for animals, protein concentrates extracted and used in plant-based meat alternatives as well as dietary fibre components incorporated in breads.

When considering the multiple uses for pea components it becomes clear that peas are very valuable for the food industry. However, there are major variations in the nutritional content of peas. Also, the size and what you can produce from the peas varies. Depending on where they are grown, which variety of peas we are discussing and on the climate of the year the peas are grown, the content of the peas can vary greatly. Therefore, this project aimed at giving an overview of some of the factors influencing the value of peas as a food component. Four different peas grown in three different years were analysed.

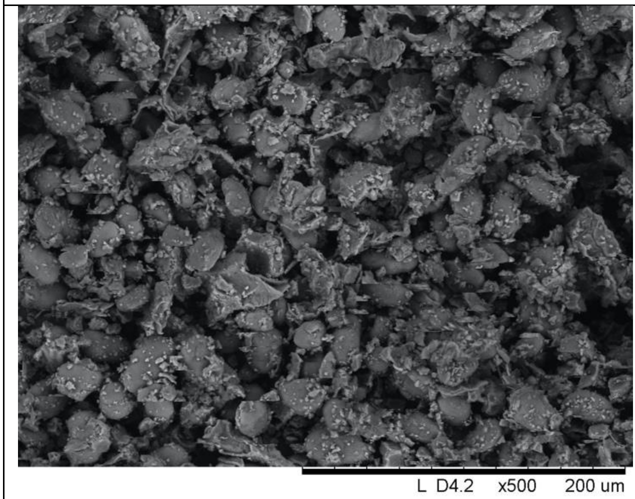
The results from the study showed variation in several factors such as average size of the seeds, which could be very important when deciding on how to process the peas in the future. There were variations based on the variety of pea as well as variations based on which year the pea was grown. For example, the peas that were grown in the very hot and dry year of 2018, were smaller compared to the years of 2019 and 2020. This could have implications for processing steps such as dehulling of the peas.

The take home message from this study is that variety and climatic conditions can affect the pea components in varying amounts. To produce pea components with the best properties and nutritional content, there is a need for knowledge of how the specific pea variety used behaves under the cultivation conditions.

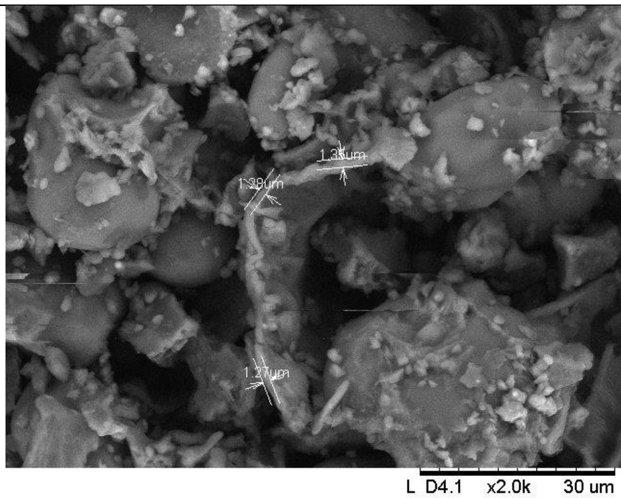
Appendix 1 – SEM-images

I20 PFF250 x150	I20 PFF250 x500	I20 PFF250 x1000
		

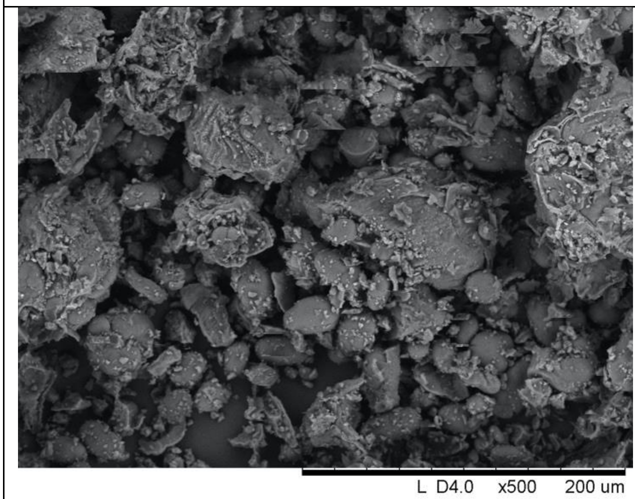
I20 PFF50 x500 a



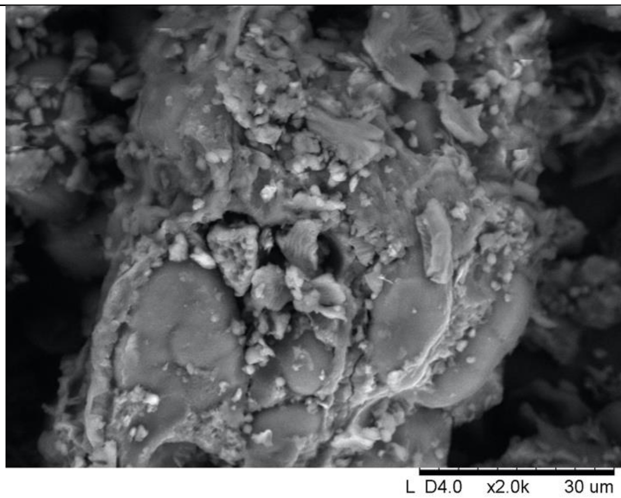
I20 PFF50 x2000 b



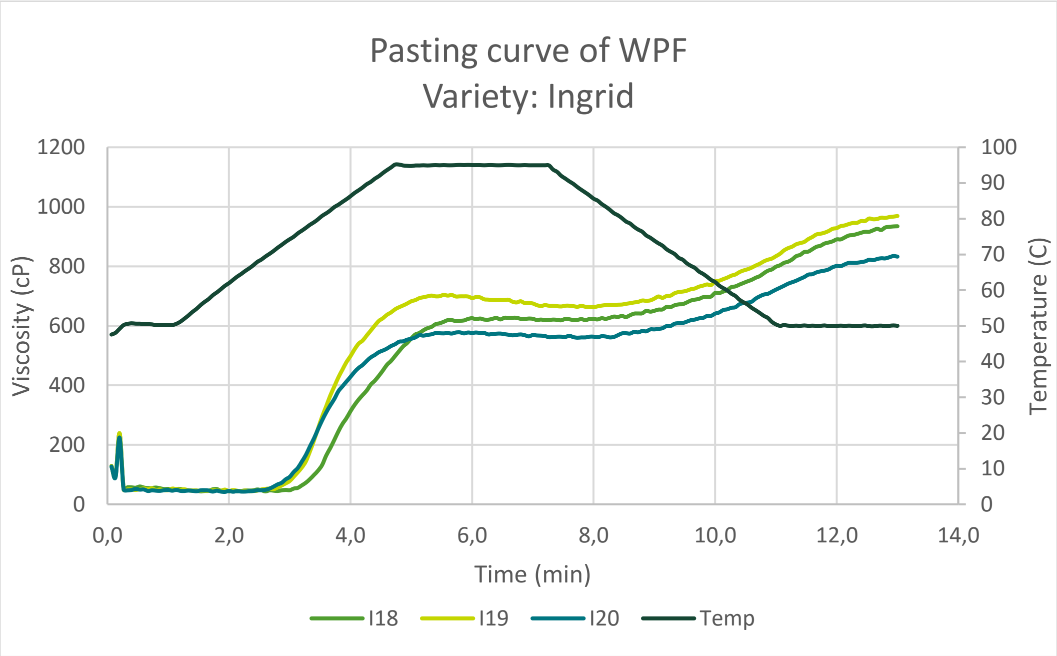
I20 PFF150 x500 c

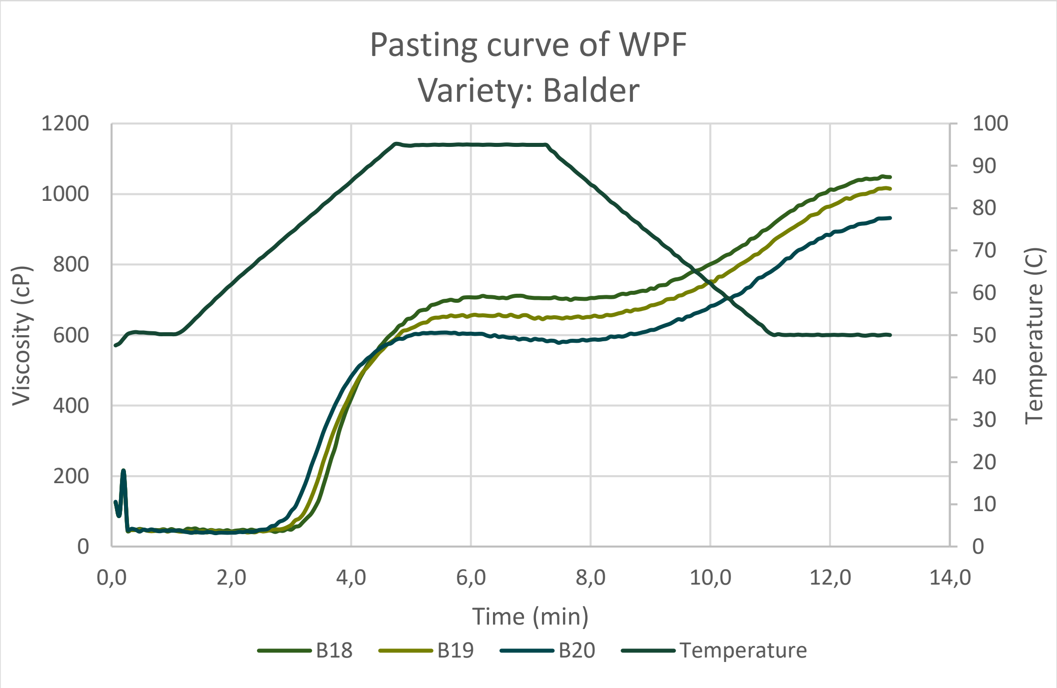


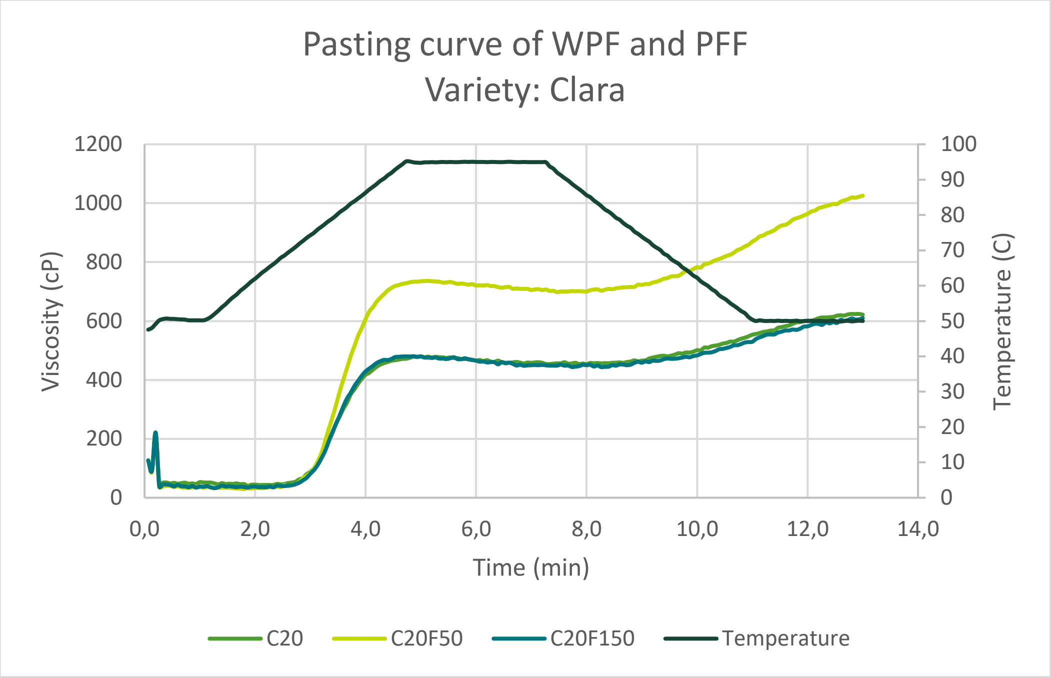
I20 PFF150 x2000 d



Appendix 2 – Pasting curves






Pasting curve of WPF and PFF
Variety: Clara
Viscosity (cP)
1200
1000
800
600
400
200
0
Temperature (C)
100
90
80
70
60
50
40
30
20
10
0
0,0
2,0
4,0
6,0
8,0
10,0
12,0
14,0
Time (min)
C20
C20F50
C20F150
Temperature

Appendix 3 – Parameters analysed

Parameter	Description	Unit	Analysis
ara	Arabinose residues	%	Dietary fibre
araR	Relative arabinose	%	Dietary fibre
Bot	Fraction <50μm	%	Particle size
BulkDensity	Bulk density of pea seeds	g/cm^3	Bulk density
F1	Fraction >1mm	%	Particle size
F150	Fraction <250μm	%	Particle size
F250	Fraction <600μm	%	Particle size
F50	Fraction <150μm	%	Particle size
F600	Fraction <1mm	%	Particle size
gal	Galactose residues	%	Dietary fibre
galR	Relative galactose	%	Dietary fibre
glc	Glucose residues	%	Dietary fibre
glcR	Relative glucose	%	Dietary fibre
HGW	100 grain weight	g	100 grain weight
KL	Klason Ligning	%	Dietary fibre
Length	Length of pea seed	mm	Average size
man	Mannose residues	%	Dietary fibre
manR	Relative mannose	%	Dietary fibre
OAC	Oil absorption capacity	g/g flour	OAC
Porosity	Porosity of pea seeds	%	Porosity
rha	Rhamnose residues	%	Dietary fibre
rhaR	Relative rhamnose	%	Dietary fibre
RVABreak	Breakdown of WPF	RVU	RVA
RVAFinal	Final viscosity of WPF	RVU	RVA
RVAPastingT	Pasting temperature of WPF	°C	RVA
RVAPeak	Peak Viscosity of WPF	RVU	RVA
RVAPtime	Pasting time of WPF	min	RVA
RVASetback	Setback of WPF	RVU	RVA
RVATrough	Holding Strength of WPF	RVU	RVA
Sphericity	Sphericity of pea seed		Average size
Starch	Starch content	%	Starch

Parameter	Description	Unit	Analysis
Thickness	Thickness of pea seed	mm	Average size
TotDF	Total dietary fibre content	%	Dietary fibre
TrueDensity	True density of pea seeds	g/cm^3	True density
UA	Uronic acids	%	Dietary fibre
UAR	Relative uronic acids	%	Dietary fibre
Volume	Volume of pea seed	µl	Average size
WAC	Water absorption capacity	g/g flour	WAC
Width	Width of pea seed	mm	Average size
xyl	Xylose residues	%	Dietary fibre
xylR	Relative xylose	%	Dietary fibre

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