Characterization of pea starch to increase its use in food industry

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Degree project/Independent project • 30 credits
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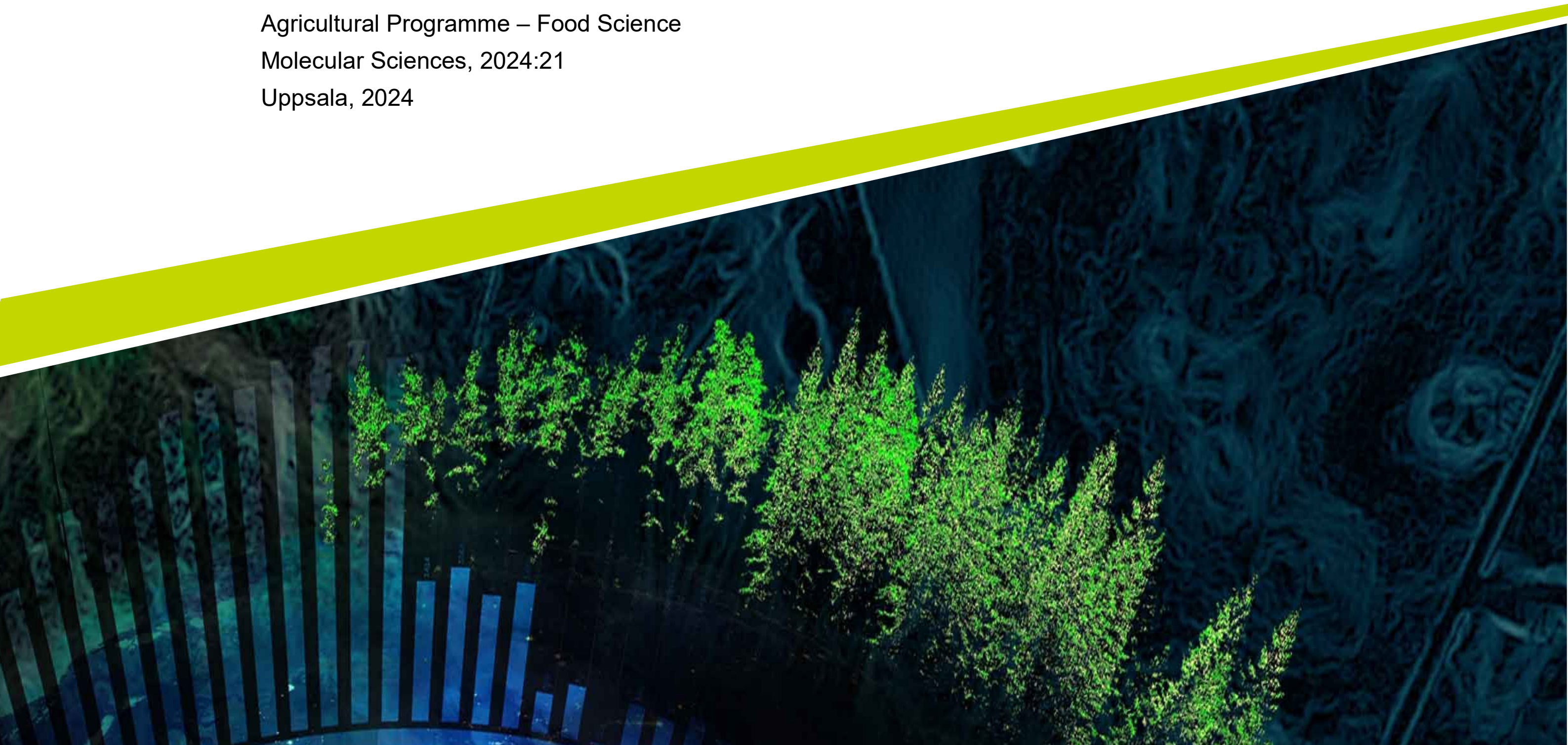
Molecular Sciences, 2024:21
Uppsala, 2024



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Credits: 30 credits
Level: Second cycle, A2E
Course title: Master thesis in Food Science
Course code: EX0877
Programme/education: Agricultural Programme – Food Science
Course coordinating dept: Department of Molecular Science
Place of publication: Uppsla
Year of publication: 2024
Copyright: All featured images are used with permission from the copyright owner.
Title of series: Molcular Sciences
Part number: 2024:21

Keywords: **Pea starch, wheat starch, chemical properties, physical properties**

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Abstract

There is a growing demand of natural food additives where pea starch which is extracted during extraction of pea protein could have a possible use. The aim of this study was to investigate chemical, molecular as well as physical characteristics of pea starches in comparison to wheat starch. This was done by investigating the amylose content, resistant starch content, chain length distribution and the starch gel microstructure by light microscopy. Water holding capacity, oil absorption capacity, gelatinization and retrogradation parameters as well as the pasting properties of the starch samples and syneresis of starch gel samples were also analyzed. The results showed distinct differences between the starch varieties (pea and wheat) in their chemical and molecular characteristics which could be linked to the differences in their physical attributes. In conclusion pea starch has a potential use in the food industry and could be beneficial for human health due to its high formation of resistant starch.

Keywords: pea starch, wheat starch, chemical properties, physical properties

Sammanfattning

Det finns en växande efterfrågan efter naturliga livsmedelstillsatser där ärtstärkelse, som produceras som en biprodukt vid extraktion av ärtprotein, har en potentiell användning. Syftet med studien var att undersöka de kemiska, molekylära och fysikaliska egenskaperna hos ärtstärkelse i jämförelse med vetestärkelse. Detta gjordes genom att analysera amyloshalt och kedjelängdsföredelning samt genom att studera av den gelatiniserade stärkelsen i mikroskop. Mängden resistent stärkelse, dess funktionella egenskaper samt dess syneres analyserades också. Resultatet visade på skillnader mellan de olika stärkelsevarianterna för de kemiska och molekylära egenskaperna. Dessa skillnader kunde sedan kopplas till resultatet från de fysikaliska undersökningarna. Sammanfattningsvis har ärtstärkelse en potentiell användning inom livsmedelsindustrin och kan vara fördelaktig för människors hälsa på grund av dess höga halt av resistent stärkelse.

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Abbreviations

ANOVA	Analysis of Variance
AMG	Amyloglucosidase
B	Breakdown
cP	Centipoise
DMSO	Dimethyl Sulfoxide
DP	Degree of Polymerization
DS	Digestible Starch
DSC	Differential Scanning Calorimetry
FV	Final Viscosity
GOPOD	Glucose Oxidase/Peroxidase
ΔH	Enthalpy
HPLC	High Performance Liquid Chromatography
HPSEC	High-performance Size Exclusion Chromatography
HS	Holding Strength
OAC	Oil Absorption Capacity
PAA	Peracetic α-amylase
PeakT	Peak Time
PES	Polyethersulfone
Ptemp	Pasting Temperature
PV	Pasting Viscosity
SDS	Slowly Digestible Starch
RDS	Rapidly Digestible Starch
RS	Resistant Starch
RVA	Rapid Visco Analyzer
RVU	Rapid Visco Units
S	Setback
T_o	Onset Temperature
T_p	Peak Temperature
T_e	Endset Temperature
WHC	Water Holding Capacity

1. Introduction

A growing interest of plant-based meat as an alternative has resulted in a growing demand of protein concentrates and isolates from pea (Martinez & Boukid 2021). In Europe an increase of legume cultivation are being considered suitable, especially in the Northernmost of Europe, where pea is probably the best suited legume for this growth (Carlson-Nilsson *et al.* 2021). Pea has been cultivated in the Nordic countries for a long time, possible as long as since the Bronze Age. However, the cultivation of legume is mainly located in the southern parts of the Nordic countries due to yield security. During the extraction of pea protein, fractions of pea starch, fibers and protein are being separated (Yang *et al.* 2021). Since the dry mass of pea contains a large portion of starch, pea starch is considered to be an abundant byproduct resulting from the fractionation of pea (Martinez & Boukid 2021).

Furthermore currently there are concerns about the usages of artificial food additives to food products (Lacerda *et al.* 2024). Therefore, the demand of natural food ingredients such as native starch has increased. Starch is often used in food products as a thickener agent, stabilizer agent, viscosity builder or as a gel former (Geerts *et al.* 2017). Pea starch is used as a thickening agent as well as a stabilizing agent and to bind water in the food industry (Shevkani *et al*. 2024). It can be used in gluten-free products as a texturizing agent as well as it can replace gelatin in confectionery products and work as an anticaking agent in cheese. Pea starches also have a nonfood application and can be used in paper, textile, cosmetics and animal feed industries.

Pea starch has been reported to consist of more slowly digestible starch then other beans and lentils (Ratnayake *et al.* 2002). Compared with rice the digestion rate of pea starches is very slow, which could be of important nutritional aspects and enable pea starches to be used as dietetic food. Pea is also low in allergens and have a low glycemic index (Manickavasagan & Thirunathan 2020).

The application of starch in food are determined mainly by physical and thermal properties which depends on the purity as well as molecular architecture of the starches (Shevkani *et al.* 2024). Therefore, the findings (physical and thermal properties along with chemical profile) in this study could be interesting for food industries to find usage of pea starch.

1.1 Aim

The aim of this study was to perform chemical and molecular characterization of pea and wheat starches, and try to find how these characteristics are linked to pasting and syneresis behavior.

1.2 Limitation of the study

The project has limitation, as only two different samples of pea starches (pea (a) and pea (b)) along with one sample of wheat starch were studied. Pea (a) as well as the wheat starch were commercial starches with unknown extraction methods. Pea (b) was provided from the project The Øresund Centre for Starch Biopolymer Functional Profiling (2005) lead by Andreas Blennow, University of Copenhagen. The amount of pea (b) starch was limited and therefore some investigations were not performed on the pea (b) sample.

2. Background

The background is divided into four different parts. In the first part, general information about pea as well as the Swedish pea production is described, while the second part is about starch. The third part, chemical characteristics of starch is described under different subheadings where firstly the amylose and amylopectin are explained and later the digestible starch as well as the resistant starch is discussed. Fourth part, physical characteristics of starch is discussed under subheadings where firstly the thermal properties of starches are described, then the pasting properties and syneresis phenomena are covered.

2.1 Pea

Pea (*Pisum sativum* L.) is cultivated globally predominantly within the zone of temperate climate and can be cultivated both for food and feed (Fogelfors 2023). Feed peas contain more tannins than food peas and is therefore not suitable for human consumption.

The pea seed is spherical and has an outer skin layer, called testa (Möller *et al.* 2021). The core of the pea is di-cotyledonous which has a storage function in the pea. The storage tissue in the cotyledons consist mainly of protein bodies, fibers and starch granules. Cotyledons has an outer layer and an inner layer (Otto et al. 1997). The inner layer has a looser structure and contains more starch than the outer layer. Pea starch granules has an irregular shape, it varies from round to elliptical (Ratnayake *et al.* 2001). Pea starch granules size varies from a length of 18-46 µm and a width of 13-32 µm (Chung *et al.* 2008). The dry matter of a pea seed consist of 59–70 % carbohydrate, 20-25 % protein, 3-7 % lipids and 3 % ash (Wu *et al.* 2023). The carbohydrate consists of 39-46 % starch and 23-31 % dietary fiber (4-8 % soluble fiber and 19-23 % insoluble fiber).

2.1.1 Swedish pea production

In year 2023, the total amount of arable land in Sweden was 2529800 hectares, out of these, legumes were cultivated on 55000 hectare (Jordbruksverket 2024). Yellow peas were cultivated on 27700 hectares of land year 2023 (Jordbruksverket 2024), and green peas were cultivated on 6200 hectares (Jordbruksverket 2023a). The number of cultivated hectares with yellow pea increased between 2022 and 2023 while the number of cultivated hectares with green peas decreased during the same period (Jordbruksverket 2023b).

Yellow peas are harvested after ripening and can be used as both food and feed (Jordbruksverket 2022). They can be used as a protein source for feed but also as a protein source in vegetarian meals. Green peas are harvested before the pea ripening, and after the harvesting the peas can be frozen as a preservation method (Fogelfors 2023).

2.2 Starch

Starch is an important storage carbohydrate in plants but also the largest source of carbohydrate in human food (Singh *et al.* 2010). The starch is present as granules in the endosperm in cereals and legumes as well as in tubers and unripe fruits (Raigond *et al.* 2015). These granules can be present in different shapes such as round, lenticular, oval and angular. Its size can vary between 1 to 100 µm. Pea starch is commonly shaped as an ellipsoid or spherical, and the granules diameter ranges from 25 to 38 µm (De Souza Gomes *et al.* 2018). Wheat starch consist of both A and B granules (Kumar & Khatkar 2017). A granules diameter ranges from 13 to 35 µm, and they can have the shape of a disk or lenticular and are commonly smooth. B granules diameter ranges from 2 to 6 µm and has a spherical or polygonal shape.

Pea starch granules contains an amorphous region and a crystalline region (Sajilata *et al.* 2006). Pea starch contains 20.4-25.5 % of crystalline structure (Ratnayake *et al.* 2001; Chung *et al.* 2008). The amorphous region contains the main part of the amylose, the crystalline region mainly consists of amylopectin (Sajilata *et al.* 2006). Starch can be divided into three different types; type A, type B and type C, where type C is found in pea starch (Ratnayake *et al.* 2001). Type C crystalline polymorph is a mixture and therefore contains both the crystalline polymorph A and B. Type A and B starch are based on parallel stranded double helices In type A the double helices are closely packed whereas in type B the double helices are loosely packed. For pea starch type A and B crystalline polymorph are in the same granule, where type B is in the center of each granule and surrounded by type A.

2.3 Chemical characteristics of starch

2.3.1 Composition

Starch contains two different types of molecules; amylose and amylopectin (Singh *et al.* 2010). Amylose is a linear polymer, slightly branched (Wang 2020). It consists of glucose units which are linked to 99 % with (1,4)-α-glycosidic linkages and 1 % with (1,6)-α-glycosidic linkages. Due to the linkage the amylose molecule can be relatively long (Wang 2020). The amylose chains can form single as well as double helices (Singh *et al.* 2010). Amylopectin is a branched polymer which consist of many short chain α-(1,4)-linked D-glucosyl units which forms a cluster (Wang 2020). The chains are linked together through the reducing end by α-(1,6)-linkages, which result in a highly branched structure. Amylopectin consists of about 95 % of α-(1,4) and 5 % of α-(1,6)-linkages. Pea starch is high in amylose (Martinez & Boukid 2021; Xie *et al.* 2024). Chung *et al.* (2008) found in their study that pea starch contains between 34.9-37.1% apparent amylose. Amylose can have a degree of polymerization (DP) up to 6000 compared with amylopectin which can have a DP of up to $2x10^6$ (Raigond *et al.* 2015). Ratnayake *et al.* (2001) reported in their study that the DP for amylose varied between 1300-1350 for pea starches depending on the pea variety. For debranched amylopectin the average chain length varied between a DP of 22.9 to 24.2 for the different pea variety, and the longest detected chain length had a DP of 71. Content of amylose together with the length and placement of the branches in amylopectin affect water absorption, gelatinization, pasting as well as the retrogradation (Wang *et al.* 2011).

2.3.2 Digestible starch

Based on the digestibility starch can be classified into three different types (Singh *et al.* 2010). These three starch types are rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). RDS and SDS is classified as digestible starch since they are digestible by body enzymes (Sajilata *et al.* 2006). RDS is measured as the amount of glucose that is released within 20 minutes (Sajilata *et al.* 2006). SDS is digested in the small intestine and the glucose is released between 20 to 120 minutes (Singh *et al.* 2010). Food which is rich in SDS is beneficial for the human health since it delays diabetes and cardiovascular diseases as well as the origin of metabolic syndrome (Raigond *et al.* 2015).

2.3.3 Resistant starch

Resistant starch is affected by the proportion of amylose and amylopectin, where amylose is digested slowly and amylopectin is digested rapidly after retrogradation (Raigond *et al.* 2015). RS is a linear molecule composed of α-1,4-D-glucan, which originates from retrograded amylose. Generally, the amount of RS formed due to cooking and the amount of amylose in the starch positively correlate.

Resistant starch is not hydrolyzed in the small intestine and therefore reaches the large intestine (Singh *et al.* 2010), and is indigestible for the body enzymes (Sajilata *et al.* 2006). In the large intestine the RS can be fermented by the colonic microflora (Wang *et al.* 2012). The fermentation of resistant starch is associated with health benefits, since the fermentation in gut results in short-chain fatty acids. These short-chain fatty acids can be absorbed in the colon and transported in the bloodstream, where it can lower the cholesterol levels, mostly for people suffering from hypercholesterolemia (Gutiérrez & Tovar 2021).

Resistant starch is subdivided into five different fractions; RS1 physically inaccessible starch, RS2 resistant granules, RS3 retrograded starch, RS4 chemically modified starch and RS5 amylose-lipid complex (Raigond et al. 2015).

RS3 retrograded starch, are mostly retrograded or recrystallized amylose which is formed during the cooling of the gelatinized starch in cooked foods that are stored at low or room temperature (Raigond *et al.* 2015). This type of resistant starch is very thermally stable and is formed in moist-heated foods. Therefore, most of the moist-heated foods contains RS3 (Sajilata *et al.* 2006). This type represents the most resistant starch fractions. Compared to granular starch, RS_3 has a higher water holding capacity (Raigond *et al.* 2015). During storage of starch gel or starch paste the amylose double helices aggregates by hydrogen bonded network formation and a B-type crystalline structure which is more thermostable is formed due to amylopectin reassociation. The crystallization rate of amylopectin is slower, and the structure is less stable than the crystalline structure of amylose. These crystalline structures are more resistant to the amylolytic enzymes.

2.4 Physical characteristics of starch

2.4.1 Thermal properties

Gelatinization is the process in which starch granules swell and undergo an irreversible phase transition (order to disorder transition) where the well-ordered structure is disturbed (Wang 2020). The process occurs when starch is heated in the presence of excess water. During heating, the granules absorb water (Liu *et al.* 2019). The water first enters the amorphous regions and expands the region which results in a disruption in the crystalline regions (Wang 2020). The starch granules swell, take up more water with heat and looses the crystalline order *(Ratnayake et al.* 2002). In the crystalline regions the double helices dissociate, and the amylose leach out. During higher temperatures the remaining crystallites melts. For pea starch during the gelatinization the crystals structure is firstly disrupted in the hilum area and then spread through the central part of the granule (Ratnayake *et al.* 2002). This results in the central part of the granule swelling.

To study gelatinization process in starches differential scanning calorimetry (DSC) is commonly used (Punia *et al.* 2020), which measure the transition temperatures (T_o, T_p, T_e) (Shevkani *et al.* 2024). The transition temperatures for pea starch varied from 56.7 – 60.5 °C for onset (T_o), 61.9 – 66.1 °C for peak (T_p) and 67.6 – 74.6 °C for end (T_e) temperature, the enthalpy, ΔH varied from 7.67 to 11.08 J g^{-1} (Shevkani *et al.* 2024).

In gelatinized starch, the disturbed amylose and amylopectin chains due to migration of moisture (Donmez *et al.* 2021) recrystallize into a new ordered structure during cooling, called retrogradation (Wang 2020). The retrogradation begins with recrystallization of amylose (Punia *et al.* 2020). This is a rapid process due to the linear structure of amylose followed by a slow recrystallization of amylopectin. Amylose forms double helical structures with 40 to 70 glucose units during the retrogradation while the recrystallization of amylopectin occurs with the reassociation of the outermost short branches (Ratnayake *et al.* 2002; Wang *et al.* 2015). This recrystallization of amylopectin can result in B-type crystalline polymorph (Wang *et al.* 2015).

Retrogradation as well as the gelatinization have been studied by DSC, for pea starch after storage for two weeks at 5 °C. For the retrogradation of amylopectin T_o was within 43.0 – 43.5 °C, T_p within 59.6 – 60.3 °C and ΔH in the range of 6.9 to 8.1 J g^{-1} (Chung *et al.* 2008). It is the retrogradation of amylose which influences the initial hardness of the gel as well as the stickiness and the digestibility (Wang 2020). The recrystallization of amylopectin contributes to the staling of cereal based products like bread and cakes.

2.4.2 Pasting properties

Pasting properties are the changes which occur in starch – water systems through the gelatinization under controlled temperature and shear forces (Wang & Ren 2020). The disruption of starch granules is defined as the pasting. Through the heating the viscosity increases to its maximum with increasing swelling of the starch granules, however further heating and shearing motion leads to decreases in the viscosity. Throughout the cooling the viscosity again increases, as an indication of starch retrogradation. Pasting properties are commonly studied in a Rapid Visco Analyser (RVA).

Chung *et al.* (2008) reported the pasting properties of pea starch; the pasting temperature was between 69.5 and 69.7 °C, the peak viscosity between 1129 and 1371 cP, the breakdown within the range of 93 to 172 cP, the setback between 576 to 706 cP and the final viscosity within the range 1554 to 1870 cP.

2.4.3 Syneresis of starch gel

Syneresis is a measurement of starch gel retrogradation (Byars & Singh 2016; Bhat & Riar 2017). The increase of syneresis during storage might be because of reassociation of leached amylose and chains of amylopectin which cause shrinkage of the gels and release of water from the gel (Bhat & Riar 2017). During storage of the starch gel at lower temperature reassociation/crystallinization of amylose begins within the first few hours. Reassociation of amylopectin begins later during storage in low temperature. For pea starch the high amylose content with long chains together with amylopectin's outer branch results in a large retrogradation and hence higher syneresis phenomena (Ratnayake *et al.* 2002).

3. Method and Material

3.1 Literature review

A literature review was implemented by using different databases such as Primo and Google Scholar. Search words such as for example: Pea, Starch, Amylose, Amylopectin, Retrogradation, Cooking properties, Resistant starch, Behavior, Rheological, Pasting, Seed, Anatomy, Water holding capacity, Oil Absorption Capacity, RVA were used in different combinations.

3.2 Material

Two different pea starches and a wheat starch were used. Pea starch (a) and the wheat starch were commercial starches, while pea starch (b) was provided by the project "The Øresund Centre for Starch Biopolymer Functional Profiling (2005)" lead by Andreas Blennow. The known chemical compositions, provided by the producer and the projects database is presented in table 1.

Table 1. Known chemical composition (% of sample) for respective starch

Variety of starch	Starch	Protein	Total ash
Pea (a)	> 90	<0.30	<0.10
Pea (b)		0.26	0.11
Wheat		<0.3	

3.3 Amylose/Amylopectin

Amylose and Amylopectin contents were measured by an enzymatic assay. Concavalin A was used for precipitation of amylopectin fractions according to the accompanying assay protocol for Amylose/Amylopectin Assay kit provided by Megazyme (Megazyme 2023a). The absorbance was read at 510 nm against reagent blank.

3.4 Resistant starch

Resistant starch was determined by using the Resistant Starch Assay Kit (rapid) from Megazyme (Megazyme 2023b). Resistant starch content method was adopted from McCleary *et al.* (2020). Starch samples cooked in the RVA as described in section 3.9 were stored overnight in the refrigerator before being analyzed. Gelatinized starch gel samples were incubated with amylase and amylo-glucosidase enzymes at a roller mixer in an oven for 4 h at 37°C. This step helped in solubilization and hydrolysis of starch to D-glucose. The pellet and solution obtained were used for resistant starch and digestible starch measurements by reading absorbance at 510 nm against the reagent blank.

3.5 Chain length distribution

Chain length distribution was studied using a high-performance size exclusion chromatography (HPSEC). The method was conducted as described by Jayarathna *et al.* (2024).

3.6 Water holding capacity (WHC)

WHC was measured according to the American Association of Cereal Chemists method 56-20 (AACC 1999) with minor modifications. One gram of starch and 20 ml of distilled water were added to a pre-weighed centrifuge tube and the suspension was shaken. The suspension was left in room temperature for 10 minutes, with intermittently inverting of the tube for three times. Then the suspension was centrifuged for 15 minutes at 1000×g and the excess water was removed. The WHC was calculated as the amount of absorbed water divided by the amount of dry starch sample, M represent the weigth in gram.

$$WHC = \frac{M\ (pellet) - M(dry)}{M\ (dry)} * 100$$

3.7 Oil absorption capacity (OAC)

OAC was measured according to the method described by Gustafsson (2022) with minor modifications. One gram of starch and 10 ml of rape seed oil (ICA, Solna, Sweden) were added to a pre-weighed centrifuge tube. To investigate the remaining oil in the tubes 10 ml of the rape seed oil were added to pre-weighted tubes. The tubes were then vortexed every five min for 30 minutes (in room temperature), and later centrifuged at 3000 g for 25 minutes. The excess oil was removed, and the tube was left in inverted position for 10 minutes. The amount of remaining oil was calculated from the weight differences between before and after centrifugation of the tubes with oil. The remaining oil in the centrifuge tube was also taken into account for the calculation. OAC was calculated as the amount of absorbed oil divided by amount of starch, M represent the weigth in gram.

$$OAC = \frac{M\,(pellet - remaining\ oil) - M(dry)}{M\,(dry)} * 100$$

3.8 Gelatinization and retrogradation parameters

Differential Scanning Calorimetry (DSC) (TA Instrument, New Castle, USA) was used to measure the gelatinization parameters as well as the retrogradation parameters. In a DSC pan 60 µl of distilled water and 20 mg of starch were added, then the pan was sealed and left to equilibrate for 2 hours at room temperature. Later DSC scanning was run from 20 °C to 130 °C with a heating rate at 5 °C/minutes. During the DSC scanning, the thermogram was recorded with an empty sealed DSC pan as a reference. Three transition temperature were determined, the onset (T_o), peak (T_p) and end set (T_e) as well as the gelatinization enthalpy (ΔH). Thereafter the pans were stored in a refrigerator for three days.

The retrogradation parameters were measured after three days of refrigerated storage. The pans were then rescanned in the DSC, with the scanning condition of; initial temperature 5 °C, proceding to temperature – 5 °C where it was kept for 5 minutes and followed by heating to 120 °C with a heating/cooling rate at 10 °C/minutes. The T_o, T_p and ΔH were determined.

3.9 Rapid Visco Analyzer (RVA)

Pasting properties of the starches were quantified by a rapid visco analyser (RVA) (Newport Scientific Works, Warriewood, Australia). Firstly the RVA was equilibrated for 30 minutes and then two grams of starch and 25 grams of distilled water were added to a canister. The canister was then placed in the RVA, and the measurement started according to the program 1STD1. For the first 10 seconds, the rotation speed was 960 rpm, later the speed was 160 rpm through the whole measurement. The first 60 seconds the temperature was 50 °C, followed by heating to 95 °C for 3 minutes and 42 seconds, held at 95 °C for 2 minutes and 30 seconds and cooled to 50 °C for 3 minutes and 48 seconds and lastly hold at 50 °C for 2 minutes. The total run time was 13 minutes and all relevant pasting parameters (peak viscosity, final viscosity, setback etc.) were evaluated from the software.

3.10 Light microscopy

Starch gel samples were studied with a light microscope using a Nikon Eclipse Ni-U microscope. Starch gel samples stored at room temperature and refrigerator were studied for understanding the gel microstructure. The starch samples were stained with diluted 1:2 iodine and then studied with a light microscope.

3.11 Syneresis study for gel swelling

3.11.1 Petri dishes

Eight grams of cooked starch gel sample from the RVA measurement, as described in section 3.9, were placed on petri dishes, where the samples were allowed to form set gels. These petri dishes were stored for five days with lid on, to measure the amount of water coming out at the surface of the gels and were monitored for five days. One set of petri dishes was stored at room temperature and another set in a refrigerator. Each day the petri dishes were decanted, and the surface water was removed, then the petri dishes were weighed again. The amount of leached water coming from the starch gel sample was measured.

3.11.2 Falcon Tubes

Around 5 ml of cooked starch gel sample obtained from the RVA pasting study, were placed in 15 ml falcon tubes. One group of tubes with lid on were stored at room temperature while the other group of tubes with lid on were stored in the refrigerator. Syneresis was monitored with storage time for the gel samples. The tubes were decanted, and the amount of surface water was weighed and later returned into the tubes again. Thereafter the tubes were centrifuged at 4000 rpm for 10 minutes, and after that the amount of released water was weighed and later returned into the tubes again. The gel was then cut into a cross with a spatula before being centrifuged at 4000 rpm for 10 minutes and then again, the amount of released water was weighed. This procedure was done for the second, third-, fifth- and fifteenth day of storage of the starch gel samples. The percentage of syneresis from both petri dishes and falcon tubes was calculated with the following equation:

$$\%\,Syneresis = \frac{Mass\ of\ water\ lost}{Mass\ of\ initital\ gel} \text{x} 100$$

3.12 Statistical analysis

Analysis of variance (ANOVA) was done to analyze the data statistically with the programme Minitab® 19. A general linear model was used to investigate the significant difference between samples, with Tukey pairwise comparisons with a significant level at 95 %.

4. Results & Discussion

4.1 Chemical characteristics of starch

4.1.1 Amylose content

Amylose content was investigated by complex formation between amylopectin and Concavalin A, using the amylose/amylopectin determination kit provided by Megazyme (Megazyme 2023a). Pea starches had a higher amylose content (34-35 % of the starch) than the wheat starch (27 % of the starch), as shown in table 2, but it was not statistically significant. The values are means of two duplicates. These duplicates are presented in Appendix 1, where also the standard deviation is presented. The standard deviation for pea (a) is the highest at 4.9, while wheat has the lowest standard deviation at 0.5.

Table 2. Amylose content for respective starch sample. The values are means of two duplicates

Starch sample	Amylose content (g amylose/100 g starch)
Pea (a)	35^{a}
Pea (b)	34^{a}
Wheat	27^{a}

Values with different letters varies significantly ($p< 0.05$).

Chung *et al.* (2008) found in their study the apparent amylose content for pea starch to be in the range of 34.9 – 37.1 %. Another study found pea starch to have an amylose content around 35 % and the wheat starch to be around 25 % (Conde-Petit *et al.* 1998). Lan *et al.* (2008) found that the apparent and total amylose content of wheat starch to be in the range of 23.2 – 23.3 %. The measured amylose values for pea starches are slightly lower than reported by Chung *et al.* (2008) and Conde-Petit *et al.* (1998). The measured amylose content for the wheat starch was slightly higher compared to the value presented by Conde-Petit *et al.* (1998) and higher compared to value presented by Lan *et al.* (2008) . The method of measuring the amylose content as well as difference in cultivars, environmental conditions and the seeds physiological condition will influence the amylose content in pea samples

(Ratnayake *et al.* 2002). These are reasons which can explain the difference between the measured amylose content of the starch samples in the study and the reported amylose content in the published scientific articles.

4.1.2 Resistant starch

Resistant starch was measured using the resistant starch kit provided by Megazyme, (Megazyme 2023b). Pea starches had a statistically higher amount of resistant starch, compared to wheat starch, table 3. For the pea starches there was no significant difference between the samples ($p<0.5$). For the digestible starch the statistical analysis did not show any significant difference between the starch varieties.

The higher amount of resistant starch in pea starch gel samples was expected since the amount of RS and the amount of amylose in the starch are correlated (Raigond *et al.* 2015). For these starch varieties in the study, pea had the highest amylose content and the highest RS content, while wheat had the lowest amylose content as well as the lowest RS content.

Table 3. The amount of resistant starch and digestible starch in the starch gel samples. The values are means of two duplicates

Starch sample	Resistant starch content (g resistant starch /100 g starch)	Digestible starch content (g digestible starch /100 g starch)
Pea (a)	9^a	74^a
Pea (b)	9^a	73^a
Wheat	6^b	78^a

Values with different letters varies significantly ($p< 0.05$) within the columns.

Resistant starch formation from cooked starch, stored in a refrigerator is affected by the degree of retrogradation (Sangokunle *et al.* 2020). RS type 3 is formed during cooling of gelatinized starch which is stored in refrigerator or at room temperature (Raigond *et al.* 2015). Most of the moist-heated food contains RS3 (Sajilata *et al.* 2006). Since the gel samples were gelatinized and then stored in a refrigerator overnight before being studied the measured RS in these starch varieties are most likely to be RS3. Vasanthan & Bhatty (1998) reported in their article that the retrograded resistant starch type 3 from the field pea starch to be 8.4 %.

Due to the formation of resistant starch, pea starch has a possible application as a food additive for example in bread or other baked products where the RS3 formed will be beneficial for health (Sajilata *et al.* 2006). The amount of RS3 in food is in general low, in baked foods, pasta and processed cereals the levels has been reported to be 3 % (Vasanthan & Bhatty 1998). The resistant starch will contribute to a better nutritional value and will promote health benefits (Sajilata *et al.* 2006).

4.1.3 Chain length distribution

HPSEC was preformed to investigate the chain length distribution of de-branched pea and wheat starch. In the HPSEC chromatogram, figure 1, the fractions eluting during the time interval 24.5 to 31.7 minutes are shown. The fractions eluting after 24.5 minutes are associated with chains coming from amylopectin. From the chromatogram, it can be seen that the chain distribution or de-branched wheat starch and the two pea starch amylopectin samples differs.

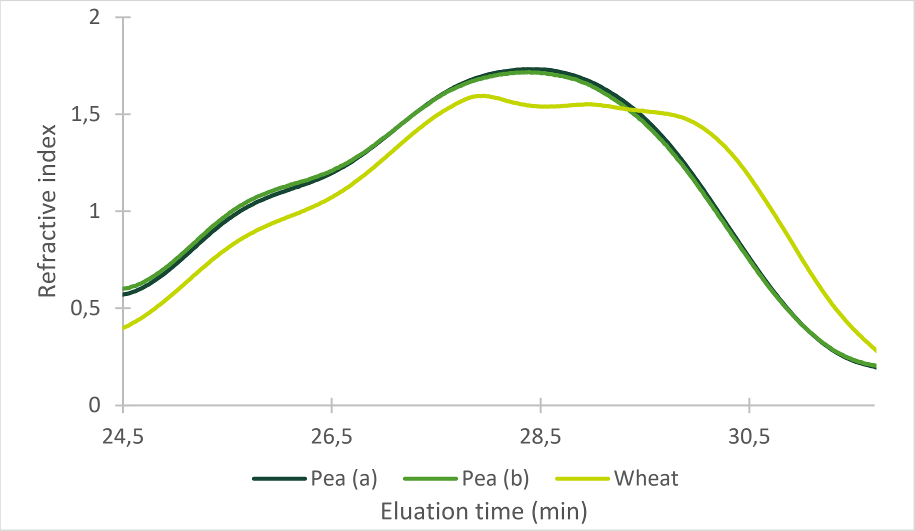


Figure 1. HPSEC chromatogram of chain length distribution of de-branched amylopectin. The chromatogram is based on the relative weight basis after the normalization for the area (24.5 – 31.7 minutes). Each value are means of two duplicates.

The difference in abundance of amylopectin chains units of the two pea starch varieties is presented in table 4. Between 24.5 and 26.5 minutes the longest amylopectin chains were eluted, whereas shorter amylopectin chains were eluted between 26.5 and 31.7 minutes (Jayarathna *et al.* 2024). The statistical analysis between the two pea starch varieties shows a significant difference for the normalized amylopectin fraction. Pea (a) has a significantly lower presented area of refractive index on relative weight basis in the interval of 24.5 to 26.5 minutes compared to pea (b). This indicates that there is difference in the chain length distribution in-between the two starch varieties and that pea (a) has a slightly lower amount of long amylopectin fractions than pea (b). Wheat starch has a lower area between 24.5 to 26.5 minutes than the pea starch (figure 1) which indicates that wheat starch has the lowest amount of long amylopectin fractions. Pea (a) has a slightly higher presented area in the time interval 26.5 to 31.7 minutes than pea (b) and therefore has a slightly higher amount of shorter amylopectin fractions than pea (b).

Table 4. Different categorization of amylopectins chain fraction from debranched whole starches based on the elution time. Values are means of two duplicates

Starch sample	24.5 – 26.5 min	26.5 – 31.7 min
Pea (a)	224^{b}	776^{a}
Pea (b)	230^{a}	770^{b}

Values with different letters varies significantly ($p< 0.05$) within the columns.

4.2 Physical characteristics

4.2.1 WHC and OAC

Water holding capacity is the amount of water that the starch can hold (Wani *et al.* 2016). Oil absorption capacity represents the ability of the starch powder to entrap oil (Sreerama *et al.* 2012). Water holding capacity and oil absorption capacity of the starch samples are presented in table 5. For the water holding capacity pea (b) and wheat starch had a significant difference while pea (a) did not have any significant difference to neither pea (b) nor with the wheat starch. Pea (b) had the highest water holding capacity while wheat starch had the lowest water holding capacity. For the oil absorption, all the starch variety differed significantly ($p<0.5$). Wheat starch had the highest oil absorption while pea (a) had the lowest oil absorption.

Table 5. Water holding capacity (WHC) and oil absorption capacity (OAC) for respective starch sample. Each value is means of two replicates except WHC for pea (a) which is a mean of four replicates

Starch sample	WHC (g water/100 g starch)	OAC (g oil/100 g starch)
Pea (a)	84^{ab}	45^{c}
Pea (b)	100^{a}	62^{b}
Wheat	70^{b}	84^{a}

Values with different letters varies significantly ($p< 0.05$) within the columns.

The measured water holding capacity for the pea starches were lower than the reported water holding capacity for pea starch by Geerts *et al.* (2017), which reported a WHC between 1-2 g water/g starch and the starch concentration varied between 52.5 and 96.2 g starch/ 100 g dry matter. Sangokunle *et al.* (2020) also presented a higher WHC, 1.65 g water/g starch for pea starch with unknown purity of the starch. Sangokunle *et al.* (2020) also presented in their study, the oil absorption capacity, which they found to be higher than the measured value in this study. In their study they investigated the OAC with 6 different types of oil, and found the OAC for pea starch to differ between the oil type from 0.86 g/g starch to 1.72 g/g starch. None of the oil-type were rape seed oil. Due to the high impurities in starch (high protein, ash, etc.) presented by Geerts *et al.* (2017) and the unknown starch purity from Sangokunle *et al.* (2020) work, it becomes extremely difficult to compare their results with the investigated WHC and OAC values of the starches in this study.

Pea (b) had granule width at 25.3 µm and a length at 33.3 µm (The Øresund Centre for Starch Biopolymer Functional Profiling 2005). Wheat starch contains both A-type granules as well as B-type granules, and the proportion between them range from 44 % to 65 % of A-type to 35 % to 56 % of B-type (Guo *et al.* 2023). This range is possibly due to differences in between the wheat varieties as well as in the determination methods. Studies have found the diameter of type A granules to range from 10 – 40 µm and the type B granules diameter to range from 2.3 – 7.1 µm. The size of the granules and pores in the granules affects the water holding capacity, smaller granules have a higher surface area per weight unit and therefore generally has a higher water holding capacity (Li *et al.* 2023). The analyzed wheat starch could consist of a large proportion of A-type granules and therefore has more large granules than the pea starches. This would then result in the pea starches getting a higher WHC. However, the purity of the starch sample (presence of protein, fibre, ash residues) also will hugely impact in the WHC and OAC.

Both oil absorption capacity and water holding capacity can be affected by the number of pores and pore size in the starch granule (Chen & Zhang 2012). Larger pores contribute to a higher absorption than smaller pores. A study conducted by Sujka & Jamroz (2010) presented the average pore radius for native wheat starch to be 3.47 µm. For native pea starches Sujka & Wiącek (2024) found the average pore diameter to be 14.01 nm. This pore size correlates to the results from the OAC where the wheat starch had a higher OAC than the pea starch. However, the pore size does not correlate with the result from the WHC where pea starch had the highest WHC. Therefore, the results of WHC and OAC in the present study are hard to interpret.

4.2.2 Thermal properties

Thermal properties, gelatinization and retrogradation were measured by using a DSC instrument. DSC is a thermoanalytical tool which identifies structural changes by measuring the energy flow (Schirmer *et al.* 2015). The gelatinization enthalpy is the integrated area below the thermal transition peak and represents the thermal energy needed for melting the clustered double helices of amylopectin chains.

Gelatinization

Onset temperature varied from 53.12 to 55.74 °C for all the starches, table 6. Pea (b) had the highest T_o, T_p and T_e, while wheat starch had the lowest measured temperature values. Interestingly the enthalpy for pea (b) was the lowest while the enthalpy for pea (a) was the highest. The onset temperature and endset temperature for pea (a) and pea (b) showed non-significant difference, whereas the pea starch varieties showed a significant difference compared to the wheat starch. For the peak temperature all starch samples were significantly different from each other and for the enthalpy pea (a) and pea (b) had a significant difference. Compared to gelatinization parameters presented by Shevkani *et al.* (2024) the measured T_o for the pea starches is slightly lower than the reported value, while T_p for the pea starches is within the reported value. T_e for the measured pea starches is slightly higher than the value reported by Shevanki *et al.* (2024). The enthalpy for pea (b) is slightly higher than the reported value while pea (a) is significantly higher than the reported value.

Table 6. Gelatinization parameters[1] for respective starch sample. The values are means of two replicates

Starch sample	ΔH (J/g)	ΔH (J/amylopectin)	T_o (°C)	T_p (°C)	T_e (°C)	T_e-T_o (°C)
Pea (a)	14.85^a	22.85^a	55^a	63^b	76^a	21^a
Pea (b)	11.67^b	17.68^b	56^a	65^a	78^a	22^a
Wheat	12.82^{ab}	17.56^b	53^b	60^c	71^b	17^b

1) T_o – onset temperature, T_p – peak temperature, T_e – endset temperature and ΔH – gelatinization enthalpy. Values with different letters varies significantly ($p< 0.05$) within the columns.

Pea (b) has the highest fraction of long amylopectin chains (figure 1) while pea (a) has a slightly lower fraction of amylopectin chains, which correlates to the gelatinization temperature, table 6. A study conducted by Gomand *et al.* (2010) found that starches with higher amount of short amylopectin chains had lower gelatinization T_o, T_p and T_e compared with starch with higher amount of long amylopectin chains which had higher gelatinization temperatures. The crystallinity is affected by the amylopectin's structural elements, shorter chains of amylopectin result in lower crystallinities. Longer chains with amylopectin results in increased crystallinities, which is suggested to result in more stable crystals and delayed gelatinization.

The gelatinization enthalpy indicates the loss of the molecular order inside the granule (Jayarathna *et al.* 2024). Although the degree of crystallinity and crystalline melting is associated with the crystalline structure of the starch granule. However, the melting enthalpy cannot be used alone to determine the crystallinity, as several factors affect the melting such as plasticization, swelling in water, competition for the melting and dissolving in water. Those other factors could affect the enthalpy and be the reason why pea (b) has a significantly lower enthalpy (J/amylopectin) than pea (a). Although pea (b) has a higher proportion of long chain amylopectin then pea (a), the enthalpy value indicates that the pea (a) has more crystalline structure and the corresponding heat required to melt those crystalline forms are therefore higher for pea (a).

T_o, T_p and T_e values are all affected by starch's building blocks composition (Zhao *et al.* 2023). Larger building blocks has a positive correlation with the gelatinization temperatures while smaller building blocks has a negative correlation with the gelatinization temperature. It has been suggested that building blocks of amylopectin with more short chains and a dense structure, result in formation of more prefect crystals which contributes to higher peak temperature. This is very likely to affecting the measured gelatinization parameters.

Retrogradation

Onset temperature varied from 50.9 to 53.0 °C where pea (b) had the lowest and pea (a) had the highest onset temperature (table 7). Peak temperature varied between 63.2 to 66.2 °C, wheat had the lowest and pea (a) the highest peak temperature. Enthalpy varied between 0.61 J/g for wheat starch to 2.81 J/g for pea (b). The statistical analysis showed non-significant difference between the starch varieties for the T_o and T_p. For the enthalpy the statistical analysis showed a significant difference between pea (b) and wheat. The endset temperature for the retrogradation was not measured as it was hard to define the end point correctly.

Table 7. Retrogradation parameters for each starch sample. Each value are means of two duplicates

Starch sample	ΔH (J/g)	ΔH (J/amylopectin)	T_o (°C)	T_p (°C)
Pea (a)	2.24^{ab}	3.44^{ab}	53^{a}	66^{a}
Pea (b)	2.81^{a}	4.27^{a}	51^{a}	66^{a}
Wheat	0.61^{b}	0.83^{b}	51^{a}	63^{a}

T_o – onset retrogradation temperature, T_p – peak retrogradation temperature and ΔH – retrogradation enthalpy. Values with different letters varies significantly ($p< 0.05$) within the columns.

For the retrogradation DSC measured the change of enthalpy and the transition temperature for melting of recrystallized amylopectin (Wang *et al.* 2015). For melting retrograded pea (b) starch, a significantly higher energy was needed than for melting the retrograded wheat starch. Pea (a) also had a higher enthalpy than the wheat starch. This indicates that the pea starches have retrograded more with refrigerated storage and therefore have more recrystallized amylopectin than the wheat starch. Interestingly pea (b) had the lowest onset temperature.

Retrogradation rate as well as the extent of the retrogradation is affected by the water content, starch source and the condition of storage (Wang *et al.* 2015). Therefore, it is hard to compare different published retrogradation data for pea and wheat starches with the measured retrogradation data.

Similar to gelatinization, retrogradation is also affected by the building blocks (Källman *et al.* 2015). Larger building blocks has showed to have a negative association with T_p and to have a positive association to the enthalpy. A lot of large building blocks results in earlier retrogradation of amylopectin and formation of crystals which melts at lower temperature. The building blocks structure of the pea starches and the wheat starches are not measured but is very likely to affect the measured retrogradation parameters. Therefore, for further studies it would be interesting to study the building blocks for the starches and study how they affect pea and wheat starches gelatinization parameters as well as retrogradation parameters.

4.2.3 Pasting properties

Pasting properties for the starch varieties were measured by using a RVA instrument. Pea (a) had the highest peak viscosity, holding strength, final viscosity, and setback as well as the lowest peak time and pasting temperature (Table 8 and figure 2). Pea (b) had the highest peak time and the lowest breakdown. Wheat starch had the highest pasting temperature and breakdown but the lowest peak viscosity, holding strength, final viscosity, setback, and peak time. For the statistical analysis, ANOVA showed significant differences between all the starch variety for the peak viscosity, holding strength, breakdown, final viscosity, and the peak time. The setback and the pasting temperature showed significant differences between the pea starches and the wheat starch. Furthermore the peak viscosity and the final viscosity for both pea starches was within the range as Chung *et al.* (2008) presented in their study. While both pea starches have a little lower setback and pea (b) had a lower breakdown compared to data reported by Chung *et al.* (2008). Both the pea starches had a lower pasting parameters compared with the values reported by Maninder *et al.* (2007), however pea (a)´s holding strength was slightly higher than the reported value.

These differences to the literature as well as the difference between the measured pasting properties for pea (a) and pea (b) could be due to internal and external factors (Schirmer *et al.* 2015). The internal factors include botanical source which affects the amorphous and the crystalline structure as well as the size granule, granule morphology and size distribution. The external factors include the area of cultivation as well as the climate. The gelatinization parameters are also affected by the water contents and process parameters such as temperature, time and mechanical stress.

Table 8. Pasting properties of respective starch sample in cP, the values are means of three replicates

Starch sample	PV (cP)	HS (cP)	B (cP)	FV (cP)	S (cP)	PeakT (min)	Ptemp (°C)
Pea (a)	1291^{a}	1176^{a}	115^{b}	1655^{a}	479^{a}	5^{c}	73^{b}
Pea (b)	1066^{b}	1046^{b}	19^{c}	1493^{b}	446^{a}	7^{a}	73^{b}
Wheat	839^{c}	658^{c}	182^{a}	969^{c}	312^{b}	6^{b}	91^{a}

Peak viscosity (PV), holding strength (HS), breakdown (B), final viscosity (FV), setback (S), peak time (PeakT) and pasting temperature (Ptemp). Values with different letters varies significantly ($p < 0.05$) within the columns.

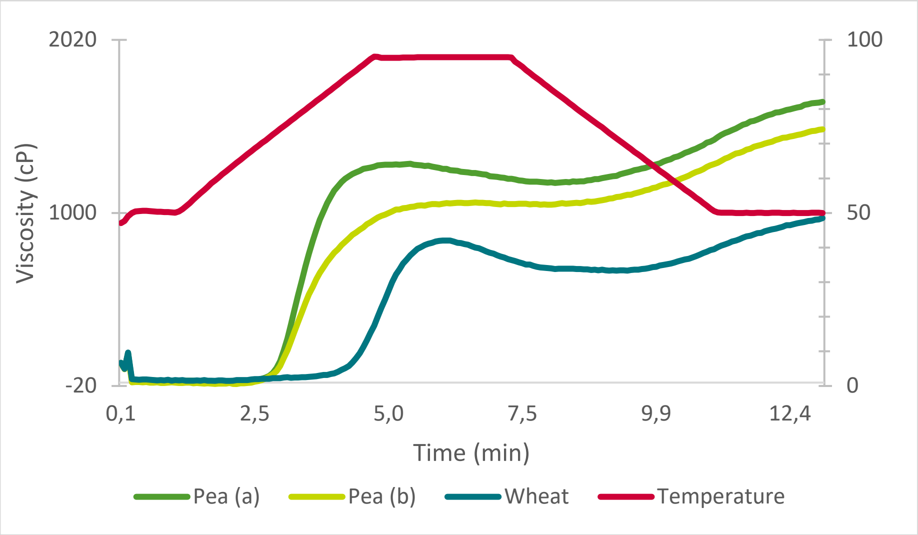


Figure 2. Pasting curves for each starch sample. The curves are means of three replicates

Peak viscosity is related to the swelling of the granules and water holding capacity of the starches (Joshi *et al.* 2013). Pea starches had a higher peak viscosity as well as a lower pasting temperature than the wheat starch, which means that pea starches start to gelatinize earlier. Pea starches start swelling at lower temperatures and have lower pasting temperatures, however the DSC data for gelatinization does not follow the same trend. The granule integrity and the amount of swelling is related to the viscosity of cooked paste (Joshi *et al*. 2013). For the wheat starch the higher thermal stability could therefore indicate a lower swelling capacity as well as higher granule integrity in comparison to the pea starches. Wheat starch gels also had more well-organized intact granules as evident from the light microscopy images and therefore the swelling is less compared to pea starch (Figure 3).

The setback value was measured to be higher for the pea starches compared to the value measured for the wheat starch. Setback viscosity is suggested to reflect the retrogradation of amylose in the starches (Wang *et al.* 2015). Commonly starch with a higher setback has a higher trend for retrogradation. Pea starches (a and b) showed a higher amylose amount (table 2) which could result in the higher setback.

The final viscosity also follows the association with measured amylose content (Table 2). Pea (a) which has the highest amylose content had the highest final viscosity. This is probably because of the fact that during the cooling phase of the RVA measurement the viscosity is depending on the composition of the starch (Joshi *et al.* 2013). The viscosity of the cold paste is affected by the amount of amylose which has leached out from the granules during the heating. These amylose molecules tend to reassociate during cooling and leading to a higher final viscosity.

Both pasting properties and gel formation properties are two important functional properties of starch to understand processing functionality for starch in the food industry (Joshi *et al.* 2013). Therefore, it is important to understand these properties for finding an application for pea starch in the food industry.

4.2.4 Microscopy of starch dispersion

The microscopy study of starch dispersion was conducted by studying the gelatinized starch gel obtained form RVA in light microscopy, by iodine staining method. In presence of iodine amylose is stained blue while amylopectin is stained brown/violet (Conde-Petit *et al.* 1998). In the light microscopy, figure 3, it is seen that all gels have some intact granules and that the two pea starches are following roughly the same pattern. For the pea starches difference between the freshly made gel and the gel stored at room temperature is just in the extent of how much the iodine has stained. Freshly made samples showed more homogeneous distribution of the amylose leakage phenomena and dark blue color throughout the image reflects that. While the samples which were kept in the cold storage, there was an increase in the amount of water pockets in between the gelatinized pea starch solution and damaged granules. The micrographs for pea starch also shows swollen blue stained granules. This could be due to that the amylose is still left in the intergranular space (Conde-Petit *et al.* 1998), and therefore never leaked out of the granule.

The wheat starch gels show distinctly amylose which is stained blue and amylopectin which is stained purple. This indicates that amylose has leaked out of the granule. For the wheat starch the increase of water present between the granules in the cold storage does not occur in the same extent as for the pea starches (Figure 3). It can also be seen in figure 3 that the granules for wheat starch and pea starch have different shapes and both partially maintained granule integrity. Light microscopy made it possible to see the differences between the cooked pea starch and wheat starch microstructure as well as how they behave differently under cold condition.

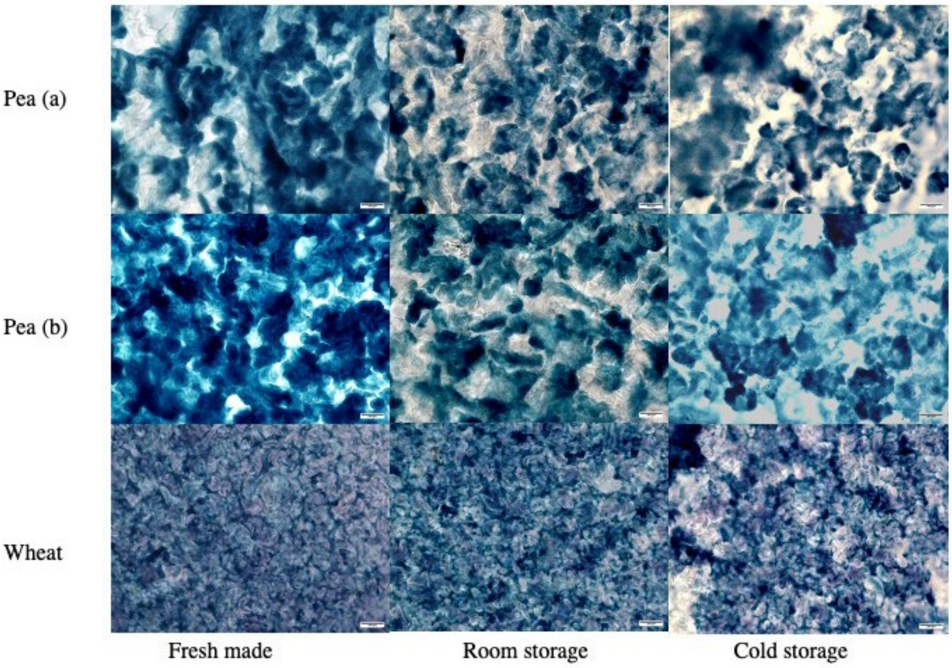


Figure 3. Starch gels studied with light microscope after different storage time and storage temperature. The scale bar at each picture shows 500 µm. Fresh starch represents gels which was studied on the same day when the gels were made, cold storage represent gels stored in a refrigerator for five days before being analysed and room storage represent gels stored at room temperature for five days before being analysed

4.2.5 Gel storage stability

The syneresis study was conducted by allowing the starch gel samples placed on petri dishes and in falcon tubes to stand during different storage conditions. All starch gels placed in petri dishes showed a reduction in weight with storage time, figure 4. Pea starches stored in a refrigerator had the highest reduction in weight when comparing the initial weight and the weight on day 5. The weight loss for the pea starches was highest between day 1 and day 2, figure 4. Wheat starch stored in a refrigerator had the lowest weight reduction (day 1 to day 5).

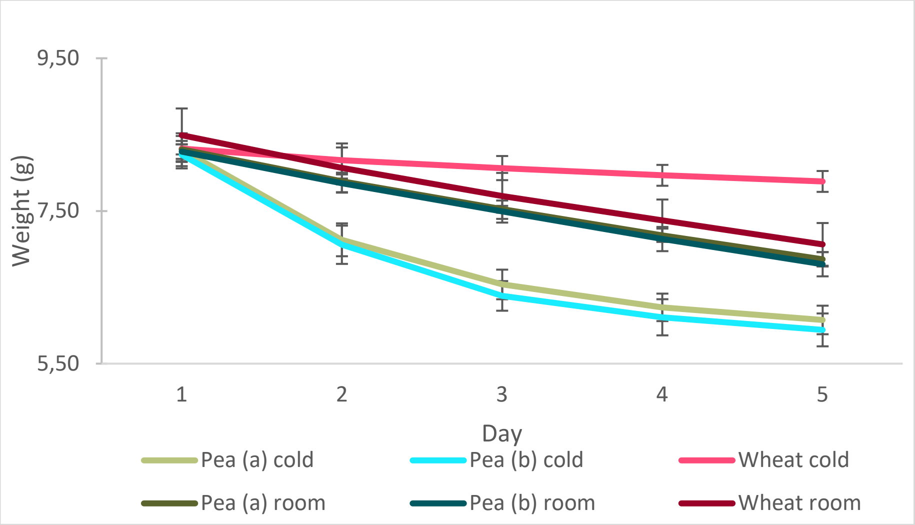


Figure 4. Weight of each starch gels stored at room temperature and refrigerator for five days in petridish. The curves are mean values of two duplicates

Since all the different starch varieties had reduction in gel weight, evaporation of water might have affected the result. Since the lid on the petri dishes was not taped and not completely airtight, minute amount of water evaporation could have happened.

Hence, the experiment was redesigned, and falcon tubes were used to study the syneresis. As can be seen in figure 5, pea starches stored in a refrigerator had a higher water loss than wheat starch. This can also be seen in the microscopic images, figure 2, where the pea starches showed a higher number of water pockets in between the granules than the wheat starch, after being stored in a refrigerator for five days. For the syneresis, pea starches (a and b) started the water loss phenomena from day 2 compared to wheat starch which first showed a loss of water on day 16 during refrigerated storage study. When comparing the different treatment of the gel it can be seen that for the pea starch the water loss was highest after the second centrifugation when the gel had been cut. For the gels stored at room temperature none of them showed water loss in initial days, as shown in figure 6. This was also reflected in the microstructure of the gels, between the freshly made starch gels and starch gels stored at room temperature for five days, figure 3. On day 5, wheat starch showed a little loss of water after being cut and centrifuged. On day 16, the pea starch showed water loss after the first centrifugation as well as after the second centrifugation when the gel was cut open.

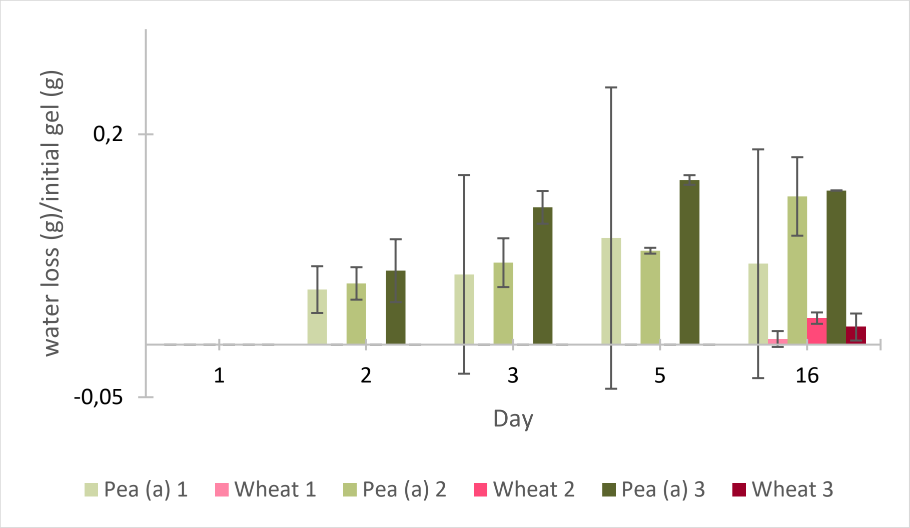


Figure 5. Water loss phenomena of starch gel samples during storage in cold temperature. Starch 1 represents the treatment with tilted tubes, starch 2 represents the starch after being tilted and then centrifuged and tilted once again. Starch 3 represents the starch that had been tilted, centrifuged, tilted and then cut, centrifuged and lastly tilted. Each value is a mean value of two replicates

The standard deviation for the water loss in pea starch samples was highest after the first treatment where the tubes were just tilted. This could be due to the fact that in some of the tubes the water was accumulated in the bottom below the gels, as shown in appendix 2. Therefore, no water was actually leaving some of the tubes although water was released from the gel.

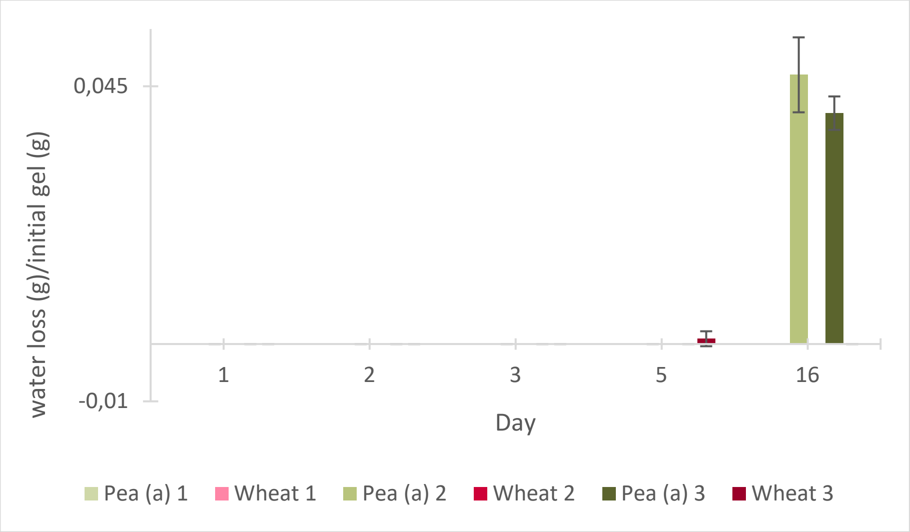


Figure 6. Water loss phenomena of starch gel samples during storage at room temperature. Starch 1 represent the treatment with tilted tubes, starch 2 represent the starch after being tilted and then centrifuged and tilted once again. Starch 3 represent the starch that had been tilted, centrifuged, tilted and then cut, centrifuged and lastly tilted. Each value is a mean value of two replicates

As syneresis phenomena is associated with retrogradation (Byars & Singh 2016; Bhat & Riar 2017). Pea (a) had a higher degree of syneresis than the wheat starch. This also is linked to the measured value of retrogradation enthalpy (Table 7), where pea (a) had much higher retrogradation than wheat starch under refrigerated storage. During the retrogradation the gel shrinks, and water is released, which is syneresis.

Temperature of storage affects the retrogradation of starch, both the amount as well as the rate of retrogradation (Chang *et al.* 2021). During storage at -18 °C the crystals only grow but during storage at 4 °C and 25 °C the crystals both grow and formation of new crystals occur (Aguirre *et al.* 2011). At 4 °C, overall rate of crystal growth is highest, which is linked to the highest retrogradation rate. As seen when comparing the gels stored at room temperature and the gels stored in refrigerator, more retrogradation occurs in the refrigerator storage. As the storage temperature is closer to 4 °C and therefore closer to the highest retrogradation rate due to higher chance of nucleation and crystal formation. However, in starch gels stored at room temperature, some retrogradation also occurs but the rate is much slower compared to refrigerated samples. Therefore, syneresis phenomena is almost absent for both the starch samples (pea and wheat) at room temperature.

4.3 Findings

The results from this project found pea starch to have a higher amylose content as well as higher amount of long chain amylopectin fractions than wheat starch. Also, the microscopic images of the starches indicated difference between pea and wheat starch gels under different storage conditions. These findings could explain the difference in the physical characteristics like syneresis of the starch gel samples.

Pea starches have higher gelatinization transition temperature than wheat starch, and one factor behind this could be the higher amount of long fractionated amylopectin in pea starch. Pea starch therefore needs a higher temperature to melt than the wheat starch. On the other hand, the pasting properties indicates that pea starch starts swelling at a lower temperature and has a higher viscosity then the wheat starch. This is because pea starch granules are less well organized than wheat starch and therefore swells easier.

The retrogradation parameters show that the pea starch retrogrades more than the wheat starch which could be due to amylopectin's building blocks. The retrogradation affects the syneresis since the syneresis is a measure for how the gel retrograde. Therefor the pea starch had a higher syneresis. During storage in cold temperature the syneresis phenomena in starch gels was higher than when they were kept in the room temperature. The perfect temperature for retrogradation (crystal formation) is around 4 °C and the retrogradation was therefore much higher in cold storage. This was also revealed in the microstructure where pockets with water between granules were found in the pea starch gel stored in a refrigerator for five days. The retrogradation also affects the formation of resistant starch, the pea starches had a much higher amount of resistant starch than the wheat starch.

5. Conclusion

In this study the aim was to provide information about the chemical and molecular characteristics for pea and wheat starches and to link them to the physical characteristics. The findings from the study found that there are some minor differences between the two pea starches while the difference between the two pea starches and the wheat starch was larger. The chemical profile and molecular structure of the starches affects the physical and thermal attributes of the starch samples.

Pea starch had a higher degree of retrogradation which affected both the syneresis of the gel as well as the amount of resistant starch formed with storage. Pea starch is high in resistant starch, which is desirable for the food industry due to its health benefits.

Further studies including more molecular characterization such as studying the building blocks of the pea starch will be important for a more profound understanding of the pea starch molecular organization. Exploring new product development possibilities such as studying how pea starch would affect the quality of cereal products like bread and how its retrogradation rate would affect the product quality will be very useful for food industry.

References

AACC (1999). *Hydration Capacity of Pregelatinized Cereal Products (AACC Method 56-20).* American Association of Cereal Chemists

Aguirre, J.F., Osella, C.A., Carrara, C.R., Sánchez, H.D. & Buera, M. del P. (2011). Effect of storage temperature on starch retrogradation of bread staling. *Starch - Stärke*, 63 (9), 587–593. https://doi.org/10.1002/star.201100023

Andreas Blennow. (2005) *The Øresund Centre for Starch Biopolymer Functional Profiling*

Bhat, F.M. & Riar, C.S. (2017). Studies on effect of temperature and time on textural and rheological properties of starch isolated from traditional rice cultivars of Kashmir (India). *Journal of Texture Studies*, 48 (2), 151–159. https://doi.org/10.1111/jtxs.12226

Byars, J.A. & Singh, M. (2016). Rheological and textural properties of pulse starch gels. *Starch - Stärke*, 68 (7–8), 778–784. https://doi.org/10.1002/star.201500271

Carlson-Nilsson, U., Aloisi, K., Vågen, I.M., Rajala, A., Mølmann, J.B., Rasmussen, S.K., Niemi, M., Wojciechowska, E. & Leino, M.W. (2021). Trait Expression and Environmental Responses of Pea (Pisum sativum L.) Genetic Resources Targeting Cultivation in the Arctic. *Frontiers in Plant Science*, 12. https://doi.org/10.3389/fpls.2021.688067

Chang, Q., Zheng, B., Zhang, Y. & Zeng, H. (2021). A comprehensive review of the factors influencing the formation of retrograded starch. *International Journal of Biological Macromolecules*, 186, 163–173. https://doi.org/10.1016/j.ijbiomac.2021.07.050

Chen, G. & Zhang, B. (2012). Hydrolysis of granular corn starch with controlled pore size. *Journal of Cereal Science*, 56 (2), 316–320. https://doi.org/10.1016/j.jcs.2012.01.011

Chung, H.-J., Liu, Q., Donner, E., Hoover, R., Warkentin, T.D. & Vandenberg, B. (2008). Composition, Molecular Structure, Properties, and In Vitro Digestibility of Starches from Newly Released Canadian Pulse Cultivars. *Cereal Chemistry*, 85 (4), 471–479. https://doi.org/10.1094/CCHEM-85-4-0471

Comparative study of the functional, thermal and pasting properties of flours from different field pea (Pisum sativum L.) and pigeon pea (Cajanus cajan L.) cultivars (2007). *Food Chemistry*, 104 (1), 259–267. https://doi.org/10.1016/j.foodchem.2006.11.037

Conde-Petit, B., Nuessli, J., Handschin, S. & Escher, F. (1998). Comparative Characterisation of Aqueous Starch Dispersions by Light Microscopy, Rheometry and Iodine Binding Behaviour. *Starch - Stärke*, 50 (5), 184–192. https://doi.org/10.1002/(SICI)1521-379X(199805)50:5<184::AID-

STAR184>3.0.CO;2-7

De Souza Gomes, D., Do Prado Cordoba, L., Rosa, L.S., Spier, M.R., Schnitzler, E. & Waszczynskyj, N. (2018). Thermal, pasting properties and morphological characterization of pea starch (Pisum sativum L.), rice starch (Oryza sativa) and arracacha starch (Arracacia xanthorrhiza) blends, established by simplex-centroid design. *Thermochimica Acta*, 662, 90–99. https://doi.org/10.1016/j.tca.2018.02.011

Donmez, D., Pinho, L., Patel, B., Desam, P. & Campanella, O.H. (2021). Characterization of starch–water interactions and their effects on two key functional properties: starch gelatinization and retrogradation. *Current Opinion in Food Science*, 39, 103–109. https://doi.org/10.1016/j.cofs.2020.12.018

Fogelfors, H. (ed.) (2023). *Vår mat.* 2nd edn. Studentlitteratur. 301–311, 391

Geerts, M.E.J., Strijbos, M., Van Der Padt, A. & Van Der Goot, A.J. (2017). Understanding functional properties of mildly refined starch fractions of yellow pea. *Journal of Cereal Science*, 75, 116–123. https://doi.org/10.1016/j.jcs.2017.03.025

Gomand, S.V., Lamberts, L., Derde, L.J., Goesaert, H., Vandeputte, G.E., Goderis, B., Visser, R.G.F. & Delcour, J.A. (2010). Structural properties and gelatinisation characteristics of potato and cassava starches and mutants thereof. *Food Hydrocolloids*, 24 (4), 307–317. https://doi.org/10.1016/j.foodhyd.2009.10.008

Guo, L., Chen, H., Zhang, Y., Yan, S., Chen, X. & Gao, X. (2023). Starch granules and their size distribution in wheat: Biosynthesis, physicochemical properties and their effect on flour-based food systems. *Computational and Structural Biotechnology Journal*, 21, 4172–4186. https://doi.org/10.1016/j.csbj.2023.08.019

Gustafsson (2022). *Screening of pea varieties of Sweden - for physical, chemical & functional properties. (*Molecular Sciences 2022:51). Swedish University of Agricultural Sciences. Department of Molecular Sciences/Agricultural Programme – Food Science. https://stud.epsilon.slu.se/18203/3/gustafsson-s-20220831.pdf

Gutiérrez, T.J. & Tovar, J. (2021). Update of the concept of type 5 resistant starch (RS5): Self-assembled starch V-type complexes. *Trends in Food Science & Technology*, 109, 711–724. https://doi.org/10.1016/j.tifs.2021.01.078

Jayarathna, S., Hofvander, P., Péter-Szabó, Z., Andersson, M. & Andersson, R. (2024). *GBSS* mutations in an *SBE* mutated background restore the potato starch granule morphology and produce ordered granules despite differences to native molecular structure. *Carbohydrate Polymers*, 331, 121860. https://doi.org/10.1016/j.carbpol.2024.121860

Jordbruketisiffror (2022). Gula ärtor till ärtsoppa och foder medan odlingen av de numera oftast frysta konservärterna ökar. *Jordbruket i siffror.* https://jordbruketisiffror.wordpress.com/2022/07/11/gula-artor-till-artsoppa-och-foder/ [2024-02-01]

Jordbruksverket (2023a). *Åkermarkens användning och antal företag med åkermark efter län och gröda. År 1981-2023.* https://statistik.sjv.se/PXWeb/pxweb/sv/Jordbruksverkets%20statistikdatabas/Jordbruksverkets%20statistikdatabas__Arealer__1%20Riket%20l%c3

%a4n%20kommun/JO0104B1.px/ [2024-02-28]

Jordbruksverket (2023b). *Jordbruksmarkens användning 2023. Preliminär statistik.* https://jordbruksverket.se/om-jordbruksverket/jordbruksverkets-officiella-statistik/jordbruksverkets-statistikrapporter/statistik/2023-05-24-jordbruksmarkens-anvandning-2023.-preliminar-statistik#h-Baljvaxter [2024-02-28]

Jordbruksverket (2024). *Jordbruksmarkens användning 2023. Slutlig statistik.* https://jordbruksverket.se/om-jordbruksverket/jordbruksverkets-officiella-statistik/jordbruksverkets-statistikrapporter/statistik/2024-02-07-jordbruksmarkens-anvandning-2023.-slutlig-statistik [2024-02-28]

Joshi, M., Aldred, P., McKnight, S., Panozzo, J.F., Kasapis, S., Adhikari, R. & Adhikari, B. (2013). Physicochemical and functional characteristics of lentil starch. *Carbohydrate Polymers*, 92 (2), 1484–1496. https://doi.org/10.1016/j.carbpol.2012.10.035

Källman, A., Vamadevan, V., Bertoft, E., Koch, K., Seetharaman, K., Åman, P. & Andersson, R. (2015). Thermal properties of barley starch and its relation to starch characteristics. *International Journal of Biological Macromolecules*, 81, 692–700. https://doi.org/10.1016/j.ijbiomac.2015.08.068

Kumar, R. & Khatkar, B.S. (2017). Thermal, pasting and morphological properties of starch granules of wheat (Triticum aestivum L.) varieties. *Journal of Food Science and Technology*, 54 (8), 2403–2410. https://doi.org/10.1007/s13197-017-2681-x

Lacerda, L.D., da Silveira, N.P., Bondam, A.F. & Hoffmann, J.F. (n.d.). Starch Gelatinization Behavior: The Impact of Granular Structure. *Starch - Stärke*, n/a (n/a), 2300143. https://doi.org/10.1002/star.202300143

Lan, H., Hoover, R., Jayakody, L., Liu, Q., Donner, E., Baga, M., Asare, E.K., Hucl, P. & Chibbar, R.N. (2008). Impact of annealing on the molecular structure and physicochemical properties of normal, waxy and high amylose bread wheat starches. *Food Chemistry*, 111 (3), 663–675. https://doi.org/10.1016/j.foodchem.2008.04.055

Li, M., Daygon, V.D., Solah, V. & Dhital, S. (2023). Starch granule size: Does it matter? *Critical Reviews in Food Science and Nutrition*, 63 (19), 3683–3703. https://doi.org/10.1080/10408398.2021.1992607

Liu, Y., Yu, J., Copeland, L., Wang, S. & Wang, S. (2019). Gelatinization behavior of starch: Reflecting beyond the endotherm measured by differential scanning calorimetry. *Food Chemistry*, 284, 53–59. https://doi.org/10.1016/j.foodchem.2019.01.095

Manickavasagan, A. & Thirunathan, P. (eds) (2020). *Pulses: Processing and Product Development*. Springer International Publishing. 245-261. https://doi.org/10.1007/978-3-030-41376-7

Martinez, M.M. & Boukid, F. (2021). Future-Proofing Dietary Pea Starch. *ACS Food Science & Technology*, 1 (8), 1371–1372. https://doi.org/10.1021/acsfoodscitech.1c00219

McCleary, B.V., McLoughlin, C., Charmier, L.M.J. & McGeough, P. (2020). Measurement of available carbohydrates, digestible, and resistant starch in food ingredients and products. *Cereal Chemistry*, 97 (1), 114–137. https://doi.org/10.1002/cche.10208

Megazyme (2023a). *Amylose/Amylopectin assay protocol.* Megazyme. https://www.megazyme.com/documents/Assay_Protocol/K-AMYL_DATA.pdf [2024-05-19]

Megazyme (2023b). *Resistant starch (Rapid) assay protocol.* Megazyme. https://www.megazyme.com/documents/Assay_Protocol/K-RAPRS_DATA.pdf [2024-05-19]

Möller, A.C., Van Der Padt, A. & Van Der Goot, A.J. (2021). Abrasive milling: A method to pre-fractionate testa and embryonic axis from yellow pea. *LWT*, 151, 112087. https://doi.org/10.1016/j.lwt.2021.112087

Otto, T., Baik, B.-K. & Czuchajowska, Z. (1997). Microstructure of Seeds, Flours, and Starches of Legumes. *Cereal Chemistry*, 74 (4), 445–451. https://doi.org/10.1094/CCHEM.1997.74.4.445

Punia, S., Sandhu, K.S., Dhull, S.B., Siroha, A.K., Purewal, S.S., Kaur, M. & Kidwai, Mohd.K. (2020). Oat starch: Physico-chemical, morphological, rheological characteristics and its applications - A review. *International Journal of Biological Macromolecules*, 154, 493–498. https://doi.org/10.1016/j.ijbiomac.2020.03.083

Raigond, P., Ezekiel, R. & Raigond, B. (2015). Resistant starch in food: a review. *Journal of the Science of Food and Agriculture*, 95 (10), 1968–1978. https://doi.org/10.1002/jsfa.6966

Ratnayake, W.S., Hoover, R., Shahidi, F., Perera, C. & Jane, J. (2001). Composition, molecular structure, and physicochemical properties of starches from four field pea (Pisum sativum L.) cultivars. *Food Chemistry*, 74 (2), 189–202. https://doi.org/10.1016/S0308-8146(01)00124-8

Ratnayake, W.S., Hoover, R. & Warkentin, T. (2002). Pea Starch: Composition, Structure and Properties — A Review. *Starch - Stärke*, 54 (6), 217–234. https://doi.org/10.1002/1521-379X(200206)54:6<217::AID-STAR217>3.0.CO;2-R

Sajilata, M. g., Singhal, R.S. & Kulkarni, P.R. (2006). Resistant Starch–A Review. *Comprehensive Reviews in Food Science and Food Safety*, 5 (1), 1–17. https://doi.org/10.1111/j.1541-4337.2006.tb00076.x

Sangokunle, O.O., Sathe, S.K. & Singh, P. (2020). Purified Starches from 18 Pulses Have Markedly Different Morphology, Oil Absorption and Water Absorption Capacities, Swelling Power, and Turbidity. *Starch - Stärke*, 72 (11–12), 2000022. https://doi.org/10.1002/star.202000022

Schirmer, M., Jekle, M. & Becker, T. (2015). Starch gelatinization and its complexity for analysis. *Starch - Stärke*, 67 (1–2), 30–41. https://doi.org/10.1002/star.201400071

Shevkani, K., Singh, N., Kaur, N., Isono, N. & Noda, T. (2024). Characterization of Starch Properties in Diverse Pea Accessions: Structural, Morphological, Thermal, Pasting, and Retrogradation Analysis. *Starch - Stärke*, 2300174. https://doi.org/10.1002/star.202300174

Singh, J., Dartois, A. & Kaur, L. (2010). Starch digestibility in food matrix: a review. *Trends in Food Science & Technology*, 21 (4), 168–180. https://doi.org/10.1016/j.tifs.2009.12.001

Sreerama, Y.N., Sashikala, V.B., Pratape, V.M. & Singh, V. (2012). Nutrients and antinutrients in cowpea and horse gram flours in comparison to chickpea flour: Evaluation of their flour functionality. *Food Chemistry*, 131 (2), 462–

468. https://doi.org/10.1016/j.foodchem.2011.09.008

Sujka, M. & Jamroz, J. (2010). Characteristics of pores in native and hydrolyzed starch granules. *Starch - Stärke*, 62 (5), 229–235. https://doi.org/10.1002/star.200900226

Sujka, M. & Wiącek, A.E. (2024). Physicochemical Characteristics of Porous Starch Obtained by Combined Physical and Enzymatic Methods, Part 1: Structure, Adsorption, and Functional Properties. *International Journal of Molecular Sciences*, 25 (3), 1662. https://doi.org/10.3390/ijms25031662

Vasanthan, T. & Bhatty, R.S. (1998). Enhancement of Resistant Starch (RS3) in Amylomaize, Barley, Field Pea and Lentil Starches. *Starch - Stärke*, 50 (7), 286–291. https://doi.org/10.1002/(SICI)1521-379X(199807)50:7<286::AID-STAR286>3.0.CO;2-O

Wang, N., Maximiuk, L. & Toews, R. (2012). Pea starch noodles: Effect of processing variables on characteristics and optimisation of twin-screw extrusion process. *Food Chemistry*, 133 (3), 742–753. https://doi.org/10.1016/j.foodchem.2012.01.087

Wang, N., Shi, N., Fei, H., Liu, Y., Zhang, Y., Li, Z., Ruan, C. & Zhang, D. (2022). Physicochemical, structural, and digestive properties of pea starch obtained via ultrasonic-assisted alkali extraction. *Ultrasonics Sonochemistry*, 89, 106136. https://doi.org/10.1016/j.ultsonch.2022.106136

Wang, S. (ed.) (2020). *Starch Structure, Functionality and Application in Foods*. Springer Singapore. 9-21, 77-131. https://doi.org/10.1007/978-981-15-0622-2

Wang, S., Li, C., Copeland, L., Niu, Q. & Wang, S. (2015). Starch Retrogradation: A Comprehensive Review. *Comprehensive Reviews in Food Science and Food Safety*, 14 (5), 568–585. https://doi.org/10.1111/1541-4337.12143

Wang, S. & Ren, F. (2020). Rheological, Pasting, and Textural Properties of Starch. In: Wang, S. (ed.) *Starch Structure, Functionality and Application in Foods*. Springer. 121–129. https://doi.org/10.1007/978-981-15-0622-2_7

Wang, S., Sharp, P. & Copeland, L. (2011). Structural and functional properties of starches from field peas. *Food Chemistry*, 126 (4), 1546–1552. https://doi.org/10.1016/j.foodchem.2010.11.154

Wani, I.A., Sogi, D.S., Hamdani, A.M., Gani, A., Bhat, N.A. & Shah, A. (2016). Isolation, composition, and physicochemical properties of starch from legumes: A review. *Starch - Stärke*, 68 (9–10), 834–845. https://doi.org/10.1002/star.201600007

Wu, D.-T., Li, W.-X., Wan, J.-J., Hu, Y.-C., Gan, R.-Y. & Zou, L. (2023). A Comprehensive Review of Pea (Pisum sativum L.): Chemical Composition, Processing, Health Benefits, and Food Applications. *Foods*, 12 (13), 2527. https://doi.org/10.3390/foods12132527

Xie, S., Li, Z., Duan, Q., Huang, W., Huang, W., Deng, Y., Chen, P. & Xie, F. (2024). Reducing oil absorption in pea starch through two-step annealing with varying temperatures. *Food Hydrocolloids*, 150, 109701. https://doi.org/10.1016/j.foodhyd.2023.109701

Yang, J., Zamani, S., Liang, L. & Chen, L. (2021). Extraction methods significantly impact pea protein composition, structure and gelling properties. *Food Hydrocolloids*, 117, 106678. https://doi.org/10.1016/j.foodhyd.2021.106678

Zhao, X., Hofvander, P., Andersson, M. & Andersson, R. (2023). Internal structure and thermal properties of potato starches varying widely in amylose content. *Food Hydrocolloids*, 135, 108148. https://doi.org/10.1016/j.foodhyd.2022.108148

Popular science summary

When Swedes hear yellow pea most of the swedes will think of the Swedish pea soup, which is a traditional dish in Sweden. However, this pea soup has divided the country into two camps: those who love it and those who hate it. For those who dislike it, this may be welcoming news. There are other uses for the Swedish yellow pea.

Peas are made up of both protein and starch. The protein can be used as an alternative protein in vegetarian meals, but the use of the starch is not that well-known. This study intended to investigate the chemical, microstructural and physical characteristics of the pea starch, for a better understanding for potential use of this starch in the food industry.

The results from the study showed that when looking at the chemical and physical characteristics of this pea starch, it becomes clear that it has a very valuable function for the food industry. Cooked pea starch that´s allowed to stand for a while develops a lot of resistant starch. This resistant starch is well-known for its health benefits. By comparing the pea starch and the wheat starch it was found that the pea starch has a greater health benefit since the pea starches develops more resistant starch. Therefore, a good application of pea starch can be to being used as a food additive in cooked or baked foods in future. One possible use for the pea starch will be to add it to bread or other baked goods and make the bread healthier.

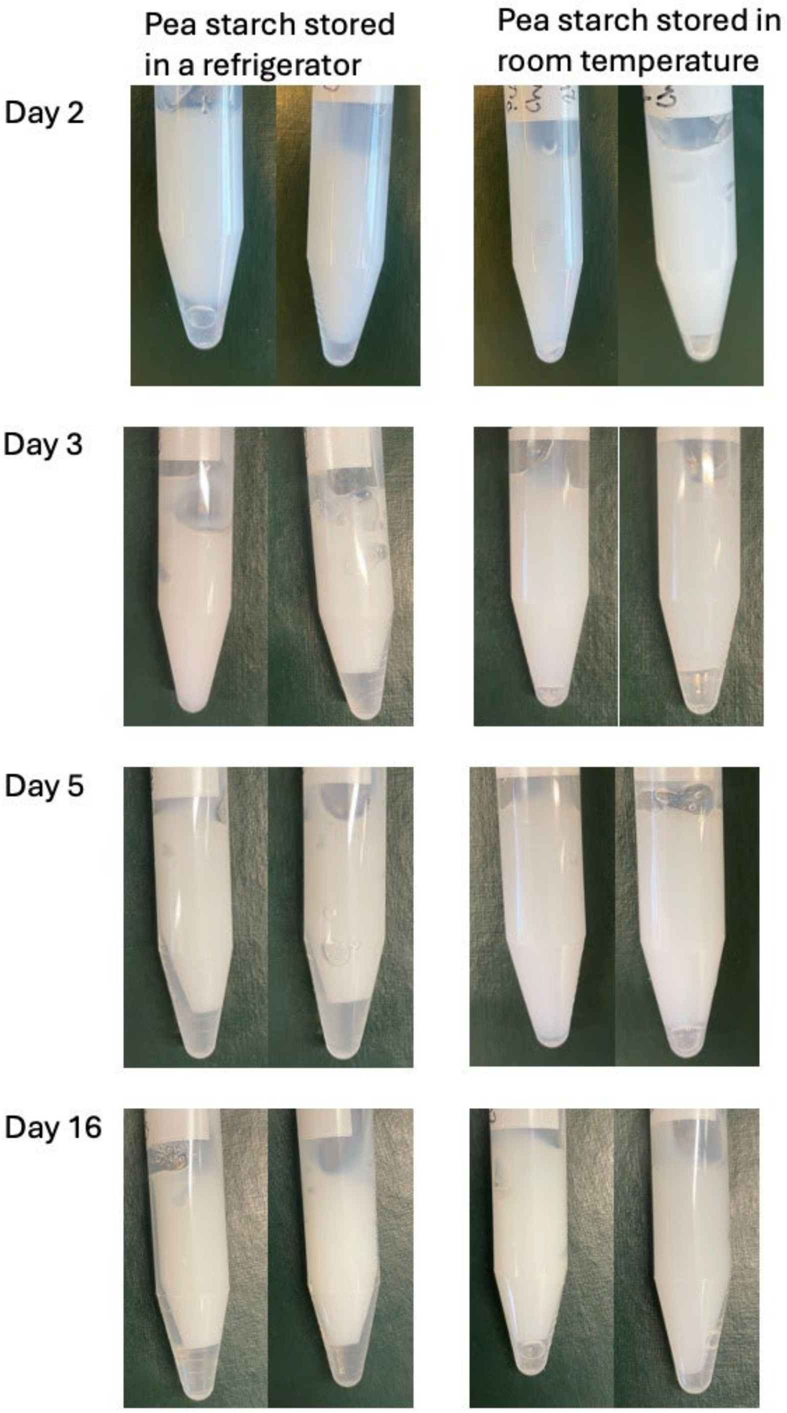
Acknowledgements

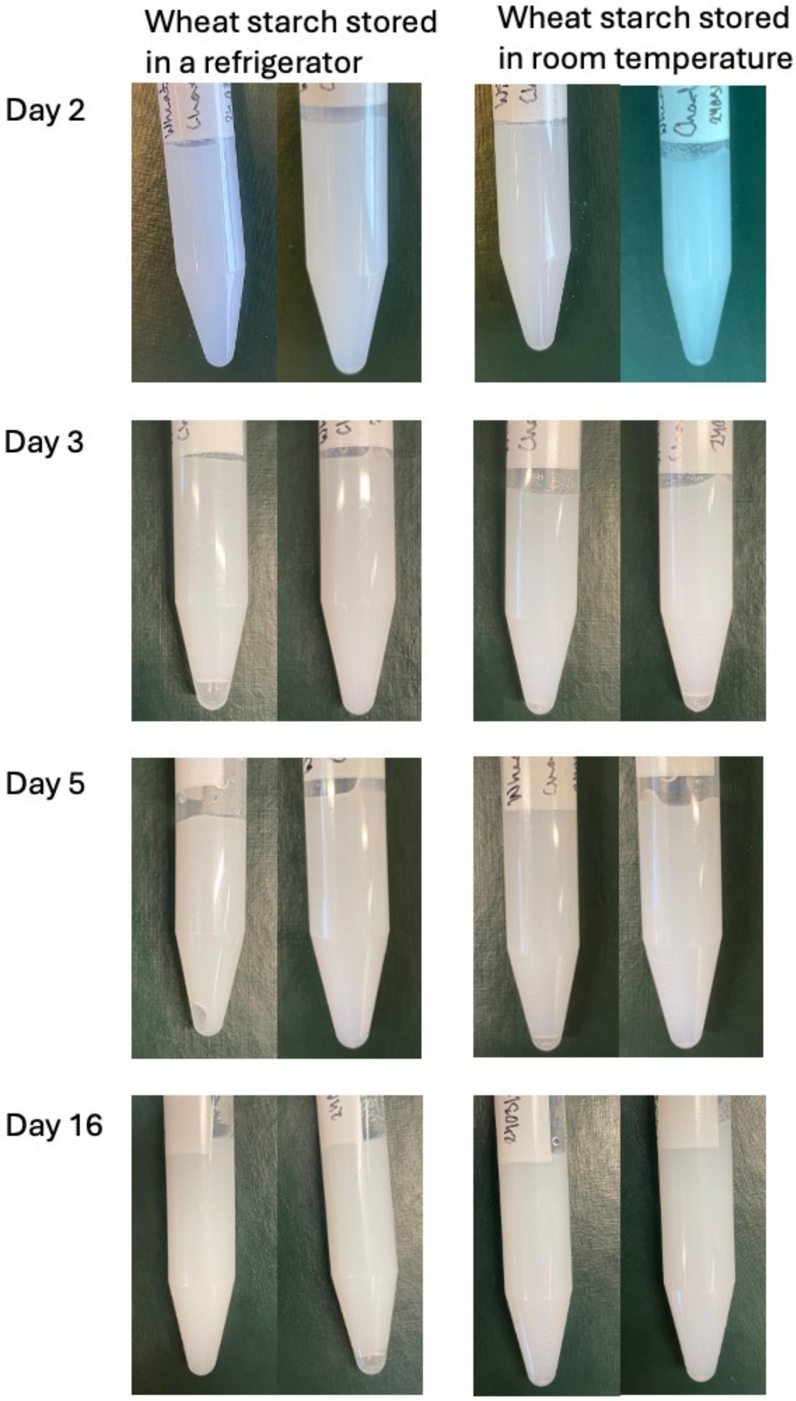
I would like to thank my two supervisors, Santanu Basu and Roger Andersson, for their invaluable support and guidance throughout the whole master thesis. Without any hesitation they have assist me when needed. I would also like to extend my thanks to Shishanthi Jayarathna for all her help and guidance throughout the whole thesis as well as for her interest for the thesis. Furthermore, I would like to thank Sunera Zulficar Nurmomade for her assistance in learning the laboratory techniques regarding the resistant starch as well as Jing Lu for helping me with the Light Microscope.

Appendix 1 – Amylose content

Starch variety	Amylose content %
Pea (a) 1	38.2
Pea (a) 2	31.4
Average Pea (a)	34.8
Diff %	19.8
Standard deviation Pea (a)	4.9
Pea (b) 1	34.5
Pea (b) 2	32.6
Average Pea (b)	33.6
Diff %	5.8
Standard deviation Pea (b)	1.4
Wheat 1	26.7
Wheat 2	27.4
Average Wheat	27.0
Diff %	2.7
Standard deviation Wheat	0.5

Appendix 2 – Falcon tubes




Wheat starch stored in a refrigerator
Wheat starch stored in room temperature
Day 2
Day 3
Day 5
Day 16

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