The Influence of Starch Properties on Bread Quality

Exchange of Starch Phase Between Wheat Flours of High and Low Quality

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The Influence of Starch Properties on Bread Quality - Exchange of Starch Phase Between Wheat Flours of High and Low Quality

Inverkan av stärkelsens egenskaper på brödkvalitet – Utbyte av stärkelsefas mellan vetemjöl av hög och låg kvalitet

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Abstract

The quality of wheat bread depends on the molecular and structural composition of the utilized wheat flour, which is significantly impacted by the surrounding conditions during the cultivation of wheat grains. Thus, given the global warming, the quality of flours is altered. This thesis aimed to gather knowledge whether the quality of starch differ between two flours of low versus high quality, and whether any such differences impact the final bread quality. The examined flours were initially fractionated by kneading a wheat flour dough in excess water, with the obtained liquid starch phase being spray dried and the wet gluten phase freeze dried. The fractionation process was however not optimal, as the gluten phases still contained a large proportion starch. Analysis of the starch from quality wheat flour showed a slightly higher proportion of A-type granules, a significantly higher gelatinization temperature, and a significantly lower viscosity upon gelatinization.

Subsequently, to assess the impact of starch on final bread quality, six flours were analysed: two native flours of higher and lower quality, two reconstituted flours aimed to be identical with the native flours, one reconstituted flour made of gluten derived from high-quality flour and starch from low-quality flour, and the final flour reconstituted of gluten from low-quality flour and starch from high-quality flour. Breads made with the latter flour exhibited a significantly larger volume, darker crust, greater average crumb pore area, softer crumb texture, and less strong crust compared to the breads baked with the high-quality gluten and low-quality starch flour. These results could however not be predicted by viscoelastic measurements of doughs made of identical flours.

Regardless, the results indicates that the quality of starch indeed has a major impact on bread quality, and that flours of lower quality can be boosted by adding starch of higher quality. However, analysis of the reconstituted flours aimed to be identical with the native samples indicates that the starch separated from low-quality flour might be negatively affected by the fractionation process. Therefore, the results of this study may stem from quality differences obtained during the fractioning and reconstitution procedure. Still, the knowledge gathered is necessary for selection of wheat cultivars that can yield flours of high quality, regardless of global warming. Moreover, this offers a suggestion on how to improve the quality of already existing flours.

Keywords: Wheat flour, starch, bread quality, fractioning and reconstitution, rheology

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Abbreviations

Abbreviation	Description
AM	Amylose
AMP	Amylopectin
DSC	Differential Scanning Calorimetry
NNWF	Native normal wheat flour
NQWF	Native quality wheat flour
RNG+QSF	Reconstituted normal gluten and quality starch flour
RNWF	Reconstituted normal wheat flour
RQG+NSF	Reconstituted quality gluten and normal starch flour
RQWF	Reconstituted quality wheat flour
SAXS	Small-angle X-ray scattering
T_c	Gelatinization conclusion temperature
T_o	Gelatinization onset temperature
T_p	Gelatinization peak temperature
WAXS	Wide-angle X-ray scattering

1. Introduction

Wheat-based products are a staple food in many areas of the world, serving as the primary source of nourishment for about 2.8 billion people (Wei et al., 2023). The quality of wheat grains, largely determined by their molecular structure, is influenced by several factors, including genetic composition (Zhang et al., 2016), the geographical area for cultivation, environmental conditions (Rhazi et al., 2021), and atmospheric CO_2 levels (Wei et al., 2023).

Hence, as a result of global warming, the molecular composition of wheat grains has shifted (Wei et al., 2023). Research indicates that wheat grown under prolonged heat and drought conditions has enhanced protein contents and decreased starch concentrations (Shi et al., 2024). This shift presents a challenge for modern baking industries, which prioritize achieving consistent flour quality rather than optimal quality (Soba et al., 2024). The variations not only lead to unpredictable qualities of wheat yields, but also to food loss and economic damage in the food industry. This in turn creates a major obstacle in achieving the Sustainable Development Goal 12, Target 3, which aims to reduce food losses throughout both production and supply chains, including post-harvest losses (United Nations, n.d.).

Overall research indicates that a wheat protein called gluten is the primary factor influencing bread quality (Dizlek and Awika, 2023). However, other studies have shown that certain starch properties positively impact the quality of wheat flour doughs (Cao et al., 2019). However, to our knowledge, less research has focused on whether starch properties differ in flours of varying qualities, and whether these properties in such a case have an impact on final wheat bread quality. Considering the high consumption of wheat based products across the continent (Wei et al., 2023), and its changed molecular composition caused by global warming (Shi et al., 2024), it is of great interest to examine the impact of each specific component on bread quality. Above all, this knowledge is essential for breeding and selection of preeminent wheat cultivars as part of future food security strategies.

A commonly utilized approach for studying the properties of specific flour components is fractioning and reconstitution. In this method, the component aimed to be assessed is added to the flour in varying compositions. By analysing the quality of the resulting baked goods, the influence of the individual component can be determined. This approach is advantageous because it does not rely on any prior assumptions about which specific components in the flour determine its quality, nor whether these components are evenly distributed or concentrated in certain flour components (MacRitchie, 1985). Hence, the practice is based on a reverse engineering approach. This involves disassembling a product and analysing its

components in terms of physical properties, functionality, and structure, which provides valuable insights into the product's behaviour, and forms a basis for further development and improvement (Otto and Wood, 1998).

1.1 Aim

This thesis aims to broaden the knowledge about starch properties influence on wheat flour and bread quality through fractioning and reconstitution of wheat flours of higher quality and lower quality. By baking bread from the reconstituted flours and analysing the raw materials, doughs, and finished breads (Figure 1), this study aimed to determine whether gluten is the only factor influencing flour and bread quality, or if starch also plays a significant role.

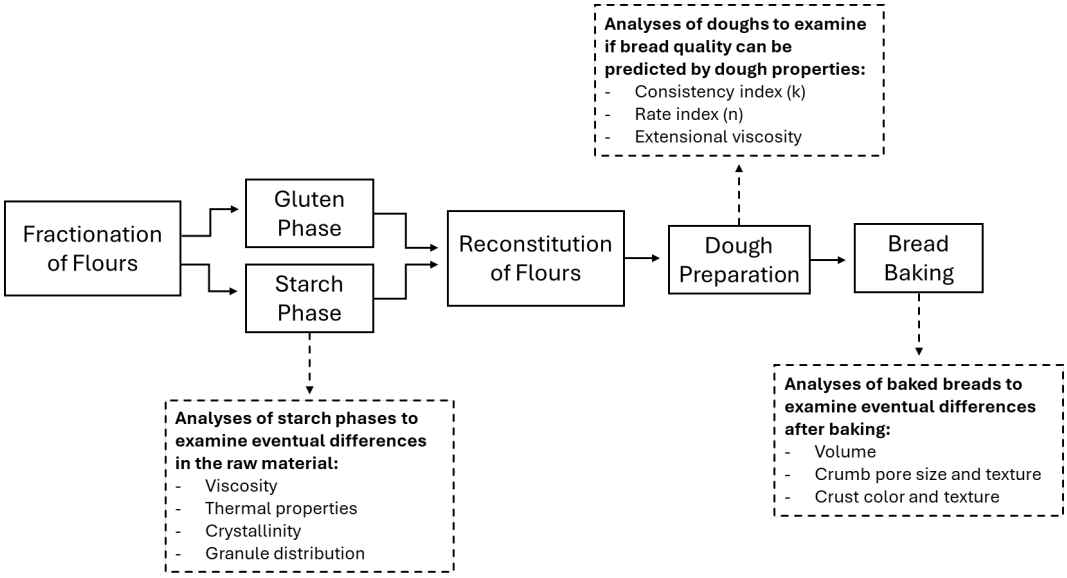

Figure 1. Schematic overview illustrating the various steps and analyses conducted in the present study.

2. Background

This chapter introduces the background of wheat flour and its constituents, with a primary focus on gluten and starch. Subsequently, methods commonly utilized to determine bread quality is presented.

2.1 Wheat Flour Composition

Wheat flour is a fine mixture of milled wheat grains, where the bran and germ has been removed (FAO and WHO, 2023). Wheat from *Triticum aestivum* is commonly utilized in flours made for bread baking (Arya et al., 2016). The wheat grains are composed of approximately 61% starch, 13% proteins, 12% moisture, and 2% lipids, among other smaller fractions of components. Up to 85% of the proteins within wheat are gluten (Golea et al., 2023), widely known having the major impact on bread quality.

2.1.1 Gluten

Gluten proteins are composed of two major components: Gliadin (60%) and glutenin (40%), both providing wheat doughs with its desirable properties for bread baking (Dizlek and Awika, 2023).

Gliadin
Gliadins are monomeric proteins responsible for the extensibility and plasticity of doughs (Dizlek and Awika, 2023). They are soluble within aqueous alcohols (Veraverbeke and Delcour, 2002), and are composed of three major components differing from each other in molecular weights and chemical composition. The smallest component, α-gliadins, has a molecular weight of approximately 31 kDa, whilst γ-gliadins weights about 35 kDa, and ω-gliadins, the biggest fraction, weights between 44 to 80 kDa (Dizlek and Awika, 2023).

Glutenin
Glutenin is a heterogenous group of polymeric proteins, insoluble in aqueous alcohols, bound together by disulfide bonds (Veraverbeke and Delcour, 2002). The protein is composed of two sub-groups differing from each other by their molecular weights. High molecular weight glutenin subunits (HMW-GS), 90-140 kDa, and low molecular weight glutenin subunits (LMW-GS), 30-75 kDa (Dizlek and Awika, 2023). While the HMW-GS acts as a linear backbone of the glutenin protein, LMW-GS are attached to them via intermolecular disulfide bonds, forming macro polymers (Yang et al., 2025).

The properties of each specific glutenin compound depend on the composition of HMW-GS and LMW-GS, whereas the former subunit contributes with elasticity of the dough, and the latter with strength. Flours with higher HMW-GS/LMW-GS ratio produce bread of better quality (Dizlek and Awika, 2023).

The Gluten Network

Once water is added to the wheat flour and mixing is initiated, a gluten network of gliadin and glutenin is generated, as illustrated in Figure 2. Gliadin binds to glutenin via hydrogen-, disulfide-, Van der Waals-, and ion bonds, resulting in the gluten network providing the dough with its characteristic elastic and plastic properties (Dizlek and Awika, 2023). Additionally, the polymerization of gluten proteins results from oxidation of free thiol groups to disulfide bonds, as well as through free thiol-disulfide exchange reactions (Ooms et al., 2018).

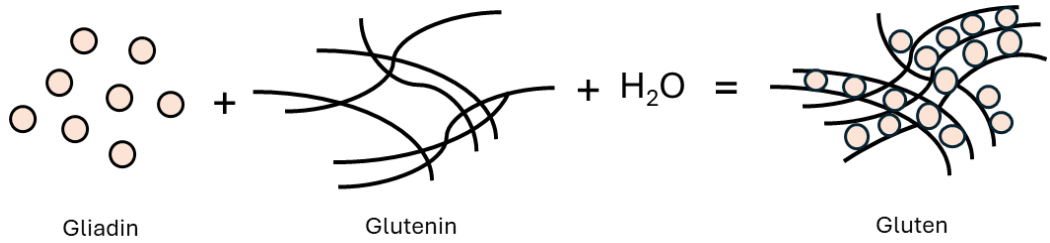

Figure 2. Illustration of gliadin, glutenin and water forming gluten upon mixing.

2.1.2 Starch

Starch is the major component of the wheat grain, accounting for approximately 60% of its dry weight (Rhazi et al., 2021), and up to 75% of the wheat flours weight (Cao et al., 2019). Amylose (AM) and amylopectin (AMP) are the two major sub-components of starch.

Amylose and Amylopectin

AM is a linear polysaccharide composed of D-glucopyranosyl units linked by α-(1,4)-bonds. A small proportion AM also have a few branches linked by α-(1,6)-bonds (Fig. 3A). AMP on the other hand is a highly branched molecule, also composed of glycosyl subunits linked by α-(1,4)-linkages (Fig. 3B) (Rhazi et al., 2021).

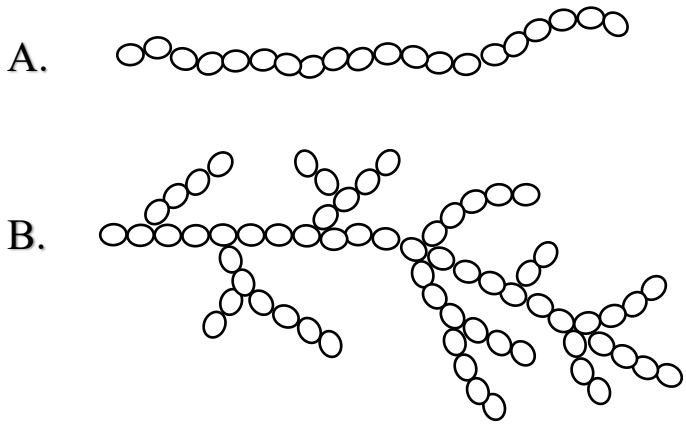

Figure 3. The linear amylose molecule (A) and the branched amylopectin molecule (B).

Approximately 18% to 35% of the starch in wheat are AM, whilst 65% to 82% are AMP (Rhazi et al., 2021), depending on wheat variety (Cao et al., 2019). Starch appears in the form of semicrystalline structures, with its degree of crystallinity primarily being impacted by the structure of AMP. An increased level of polymerization leads to increased crystallinity (Nivelle et al., 2019).

Starch Granules

The two sub-components of starch are organised in A- and B-type granules, distinguished by size and morphology, as seen in Figure 4. A-type granules are usually lenticular shaped with diameters >10 µm, whereas B-type starch granules are round with diameters <10 µm. Approximately 70% of the wheat weight are contributed to A-type granules, whilst B-type granules account for about 90% of the granule count (Zhang et al., 2016, Cao et al., 2019). However, the ratio varies depending on wheat genotype (Zhang et al., 2016). The molecular composition of the two granule types also differs, as A granules typically contain more AM than B granules (Shang et al., 2020).

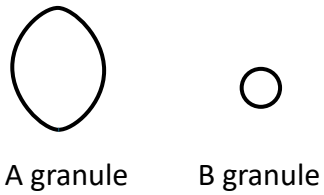

Figure 4. Illustration of the major morphological differences between A- and B granules.

Gelatinization of Starch

When starch is heated in excess water, the stability of its crystals is disrupted, which leads to gelatinization (Nivelle et al., 2019). This phenomenon occurs in three major steps. During the first step, the starch granules initiate absorption of a limited amount of water. However, at this point, the absorption is reversible and does not affect the granule crystallinity (Zhang et al., 2013).

As the temperature rises, the second, irreversible stage of gelatinization starts. At a certain temperature, the chemical bonds within starch break down. This causes the crystalline areas to become amorphous, resulting in a drastic expansion in granule volume (Zhang et al., 2013). The granules swelling power is impacted by the ratio of AM to AMP and the distribution of their chain lengths. More short chains, and less AM increase the swelling power (Cao et al., 2019). AMP has a greater absorption capacity than AM due to its branched structure (Li et al., 2020). As a result of the water absorption, starch can fill up the empty space within the gluten network as an inert filler (Li et al., 2021).

At the final gelatinization stage, at an even higher temperature, the starch granules disrupts and a high viscosity gel is produced (Zhang et al., 2013). As a result of the granule disruption, AM leach out to the environment. As the starch subsequently cools down, the leached AM creates a new semicrystalline network upon gelation. (Nivelle et al., 2019). Short AM chains leach more easily than long AM chains. Consequently, a higher content of the latter also improves granules swelling power (Li et al., 2020).

Studies show that flours with a dominating AMP ratio requires more energy (enthalpy) and higher temperatures to gelatinise (Nivelle et al., 2019). However, the gelatinization temperature is also impacted by the composition of granules. A-type granules require a higher temperature to initiate gelatinization, whilst the B-type granules need a greater temperature to complete the process (Zhang et al., 2016, Shang et al., 2020, Song et al., 2024). A-type granules higher onset temperature could be explained by their greater relative crystallinity (31.95%), in contrast to B-type granules crystallinity of 29.38% (Zhang et al., 2013). Finally, the viscosity of starch obtained upon gelatinization is also affected by the granules. A-type granules tend to generate thicker viscosities (Zhang et al., 2016, Song et al., 2024).

2.1.3 Experimental Examination of Starch Properties

Morphological Examination

Morphological properties of starch are commonly observed using a light microscope. For example, the distribution of A- and B granules within a sample can be analysed using a light microscope coupled with a digital image analysis. Upon this method, both number of the various granule types as well as their volume ratio can be determined. The granule count analysis typically shows a monomodal distribution, peaking at granules smaller than 5 µm in diameter (Wilson et al., 2006).

Viscosity Measurements

The changes of starch viscosity during gelatinization are usually measured by controlled stirring during a temperature ramp. Upon such analysis, four distinguished pasting values can be determined. First, peak viscosity (PV), implying the greatest viscosity of starch upon heating, which also gives information about the granules swelling power. Following the PV, the viscosity decreases. The extent of which the viscosity is reduced indicates the granules' resistance to rupture at high temperatures, called breakdown viscosity (BV). Final viscosity (FV), measured after the starch has cooled down, reflects the stability of the swollen granule structure, while setback viscosity (SV) gives a value of starch gelation capacity (Cao et al., 2019).

Thermal Properties

Starch thermal properties is often examined using differential scanning calorimetry (DSC) (Zhang et al., 2013). Upon DSC measurements, gelatinization onset temperature (T_o), gelatinization peak temperature (T_p) and gelatinization conclusion temperature (T_c) can be determined. These values reflect the quality of the starch crystalline structures. Moreover, a DSC measures the gelatinization enthalpy (ΔH), thus, the energy required to break the starch crystalline structures (Cao et al., 2019). Previously conducted DSC analysis of starch resulted in T_o from 55.1 to 58.0˚C, T_p between 60.5 and 62.5˚C, and T_c from 65.5 to 68.9˚C (Song et al., 2024, Eliasson and Karlsson, 1983).

Crystallinity Assessment

The crystallinity of starch can be examined using small-angle X-ray scattering (SAXS) and wide-angle X-ray scattering (WAXS) analyses. The former primarily investigate the starch's lamellar structure, thus, the alternating amorphous and crystalline layers, and how this arrangement changes during gelatinization. In contrast, the latter method focuses on the short-range crystalline structure, providing information about the specific type of crystal. Starch crystals can be

monoclinic, hexagonal, or a combination of both, and each type is identified by its characteristic diffraction pattern on an X-ray graph, with distinct peaks at specific angles (Xu et al., 2020).

2.1.4 High-Quality and Low-Quality Wheat Flour

Wheat flours are usually defined as either high-quality or low-quality, with their main differences outlined in Table 1. The most frequently noted difference between them is their protein concentration, whereas high-quality flour has a greater protein content. Low-quality flour on the other hand tends to have a higher total starch content. As a result, the flours have varying properties. For instance, due to high-quality flours enhanced protein concentration, it exhibits better water absorption capacity (60.7%) compared to low-quality flour, absorbing only 56% (Setya Budi Muhammad et al., 2024).

The composition and properties of starch also vary between the two flour types. For example, previous studies have shown that starch from high-quality flour contains more AM (Shang et al., 2020) and B-type granules, while flours of lower quality tends to have more A-type granules (Setya Budi Muhammad et al., 2024, Shang et al., 2020). Moreover, Setya Budi Muhammad et al. (2024) found that starch from high-quality flour exhibited a higher PV. However, Shang et al. (2020) found the opposite, with starch from high-quality flour showing a lower PV. Additionally, their gelatinization temperatures also differ, with low-quality flour starch having slightly higher T_o, T_p and T_c values (Setya Budi Muhammad et al., 2024). Still, low-quality starch has a lower relative crystallinity, accordingly to Shang et al. (2020).

Table 1. The major differences between high-quality and low-quality flour. ↑ represent a higher content or value, whilst ↓ represent a lower content or value

	High-quality flour	Low-quality flour
Protein content	↑	↓
Total starch content	↓	↑
Water absorption capacity	↑	↓
Amylose content	↑	↓
B granule content	↑	↓
A granule content	↓	↑
Gelatinization temperature	↓	↑
Relative crystallinity	↑	↓

2.2 Bread Quality

Bread quality is typically distinguished in terms of the loafs' volume and texture (MacRitchie, 2016), whereas a large volume and soft crumb texture usually is aimed. The texture of breadcrumbs is commonly analysed objectively using an instrument, such as a texturometer. The results obtained upon such analysis have been found consistent with sensory analyses of bread, making it an effective method to evaluate bread quality (Scheuer et al., 2015).

Previous research has demonstrated that the hardness of bread increases with higher AM content. The hardness is a result of a reduced number of crumb pores and a lower loaf volume. The authors attribute this phenomenon to two structural properties. First, they found that high AM starch granules (84% AM) had more irregular shapes than the control sample (32% AM). Thus, they become unevenly distributed within the gluten network, making it more fragile, which could restrict the dough expansion. Second, they saw that the swelling of granules in high AM wheat flour was limited, which leads to impaired gelatinization. Consequently, swollen granules are trapped within the gluten network, which also could restrict the dough development (Li et al., 2022). Additionally, previous studies have demonstrated a linear relationship between protein content and bread volume (MacRitchie, 2016, Graßberger et al., 2003), indicating that protein has a major impact on the capacity of dough expansion.

The colour of the breadcrust is also a factor influencing the perception of bread quality, as it indicates both aroma and texture (Ahrné et al., 2007). The characteristic crust results from quick water evaporation from the surface of the dough upon baking. Simultaneously as the water level decreases, the temperature rises, whereas Maillard reactions occur. These reactions are responsible for the crusts desirable colour and taste. However, they also lead to the formation of a toxic compound called acrylamide. The concentration of this compound can though be reduced by adding steam during baking (Ahrné et al., 2007). Upon bread baking using reconstituted flours, Graßberger et al. (2003) obtained a darker crust in wheat breads. The authors discuss the explanation being an enhanced level of Maillard-reaction occurring due to the increased level of free amino acids and reducing sugars generated during the procedure of fractionating the native flour.

2.2.1 Fractioning and Reconstitution

A method commonly utilized to examine the impact certain flour components have on bread quality is fractioning and reconstitution using two flours of different qualities (Sollars, 1973, Arya et al., 2016, MacRitchie, 2016). This method complies of two major steps. First, the component aimed to be analysed is separated

from both flours. Second, the separated fractions from the two flours are recombined. This means that one component from the high-quality flour, such as starch, is combined with another component from the low-quality flour, such as gluten. Consequently, this allows for determination of how these fractions influence baking performance (MacRitchie, 1985).

Previous studies have utilized this method to examine for example glutens influence on chapatti bread (Arya et al., 2016), the role of starch AM content and granule distribution on durum spaghetti quality (Soh et al., 2006), and starch impact on the quality loss of frozen wheat doughs (Tao et al., 2016). Moreover, in a study conducted by Soulaka and Morrison (1985), the influence of granule, AM, and lipid content on wheat breads was analysed. In summary, they found no impact of AM and lipid content on baking quality. However, they found that total starch gelatinization temperatures and granule distribution affected bread volume. Higher gelatinization temperatures and 25% B-granules (by weight) resulted in larger loaves. The authors suggest that the former property may be related to the bread's internal temperature during baking, which is time-dependent. Thus, if gelatinization is delayed due to a higher gelatinization temperature, the loaf has more time to expand.

2.2.2 Dough Properties Indicate Bread Quality

The bread quality outcome can often be predicted already by its dough properties (Tronsmo et al., 2003). Therefore, dough rheology analysis is necessary when aiming to improve bread quality. Two key properties commonly discussed are strain hardening and extensional viscosity.

Strain Hardening

Strain hardening is a phenomenon occurring upon dough proofing, meaning that the dough stiffness increases as the dough inflates. This property strengthens the thin bubbles produced upon proofing, allowing the dough to expend further. Consequently, wheat doughs with prominent strain hardening usually generate breads with greater volume and more desirable crumb structures. Strain hardening is usually presented by a strain hardening coefficient (k), and a strain hardening index (n), whereas higher values are associated with better bread quality (Tronsmo et al., 2003).

Extensional Viscosity

Dough extensional rheology properties can be analysed using hyperbolic contraction flow. Upon this method, the dough is pressed through a hyperbolic-shaped nozzle at a constant extension rate, whereas the force generated on the nozzle is measured. This provides a value of the extensional viscosity of the dough,

which together with the level of strain hardening highly impacts the capability of bubble growth, and thus, the quality of bread (Stading, 2011).

Both Gluten and Starch Impact Dough Properties

The strength of wheat dough is commonly determined by its dough development time and stability. These values are highly impacted by the quality of gluten proteins, and especially HMW-GS. However, in a study comparing three wheat varieties with identical HMW-GS content but varying starch compositions, they identified that starch components also had a great influence on wheat dough properties. The authors found that the wheat variety yielding the preeminent dough quality had significantly higher concentration of B-type granules and AM, greater granule swelling power, higher short-range ordered degree, lower relative crystallinity, and reduced starch gelatinization enthalpy. Based on these findings, the authors presume that these factors may also determine dough properties (Cao et al., 2019).

3. Materials and Methods

3.1 Materials

Native quality wheat flour (NQWF) (14.3% protein content) and native normal wheat flour (NNWF) (11.5% protein content) from Lantmännen Cerealia AB (Malmö, Sweden) was utilized as the baseline for this study. NQWF is a spring wheat flour, whilst NNWF is an autumn wheat flour. Additional detailed information about the two flours is available in Appendix 1.

3.2 Fractioning and Reconstitution of Wheat Flour

The starch and gluten in the two native flours was separated and subsequently recombined into four new flours accordingly to the methodology outlined below.

3.2.1 Fractioning of Flours

The fractionation of the native flours to gluten- and starch rich phases were performed accordingly to the recommended scheme of MacRitchie (1985). A dough composed of 930 g flour and 570 g water were kneaded for 45 s in a dough mixer (Electrolux BM 20 AS) at its lowest intensity. The starch phase was then washed out by mixing the dough with 1 875 g of water (15˚C) in the dough mixer at its lowest intensity for 1 min. The liquid starch phase obtained was strained and collected in buckets. The washing procedure was repeated six times per dough batch. Thus, a total of 11 250 g water was utilized. At the end, the remaining gluten phase was evenly separated in aluminium muffin liners. Both phases were frozen in -20˚C, and four replicates were conducted per native flour type.

The fractionation outcome was calculated based on the input weights of the native flours and water, the output weights of the gluten and starch fractions, the moisture content of the wet gluten phase, the protein content of the native flours, and an assumption that the concentration of starch within the native flour accounts for the remaining weight. This calculation was subsequently validated with an analysis outsourced to Eurofins Food and Feed Testing (Sweden).

3.2.2 Drying of Starch and Gluten Phases

Drying of Starch Phase

The frozen starch phase was thawed and subsequently stirred (OHS 200 Advance Overhead Stirrer, VELP SCIENTIFICA) until all starch chunks had dissolved. The liquid starch phase was thereafter dried to starch powder using a spray dryer (APT-5.0, APT SOL), as illustrated in Figure 5. The spray dryer's heater had a setpoint of 200˚C, the blower an output of 50 Hz, and the atomizer a setpoint of 600 Hz. The liquid starch phase was pumped into the spray dryer at a rate of 39 to 41 rpm. The rate was adjusted depending on the outlet temperature, which were aimed at 75 to 77˚C to ensure a mild treatment. The obtained starch powder was collected in buckets and frozen in -20˚C.

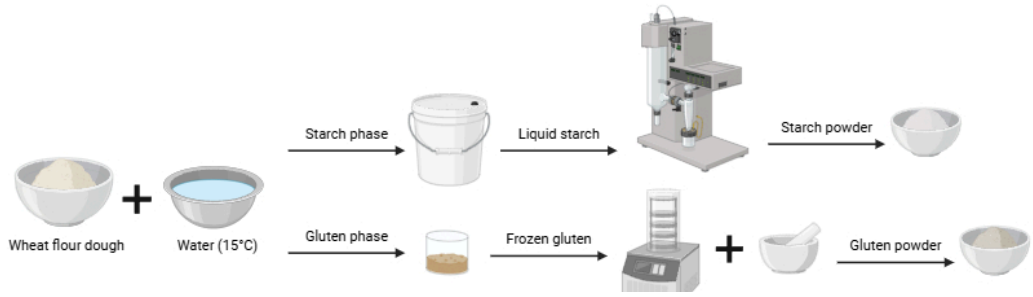

Figure 5. The utilized process of separating native wheat flours into starch rich powders and gluten rich powders.

Drying of Gluten Phase

The frozen gluten phase was dried for approximately 48 h in a Freeze-dryer (Alpha 1-2 LD plus, Christ) with the ice condenser set at -52˚C and vacuum at 0.31 mbar. Using a mortar, the freeze-dried gluten was grounded to big chucks to examine whether the whole sample was dried, and to prepare for subsequent grinding. A knife mill (Grindomix GM 200, Retsch) was utilized at 10 000 rpm for 20 s to grind the gluten chunks into a homogenous powder.

Measurement of Moisture Content

The moisture content of the native flours, the dried starch and gluten phases, and the wet gluten fractions was determined using a vacuum oven at 105˚C. Firstly, an empty aluminium cup was weighted. Subsequently, approximately 1 g sample was placed in the cup and the combined weight of the sample and cup was noted. Five replicates were prepared for each sample, which were placed in the vacuum oven overnight. The cups together with the dried sample was afterwards weighted once again, and the moisture content was calculated as

$$Moisture\ \% = \frac{Weight_{cup+sample} - Weight_{cup+dried\ sample}}{Weight_{sample} \cdot 100}.$$

3.2.3 Reconstitution of Flours

Four reconstituted flours were prepared by combining the gluten and starch powders. Two of them were designed to match the native flours and functioned as control samples, whilst the other two would exchange starch phase, as illustrated in Figure 6. The mixtures were aimed to have identical protein concentrations as their native precursors. Hence, 14.3% protein in NQWF, and 11.5% in NNWF. Therefore, the reconstituted quality wheat flour (RQWF) was made of 55% quality gluten and 45% quality starch. The reconstituted normal wheat flour (RNWF) on the other hand was made of 48% normal gluten and 52% normal starch.

The reconstituted quality gluten and normal starch flour (RQG+NSF) were made through mixture of 55% quality gluten and 45% normal starch. The normal gluten and quality starch flour (RNG+QSF) were made of 48% normal gluten and 52% quality starch.

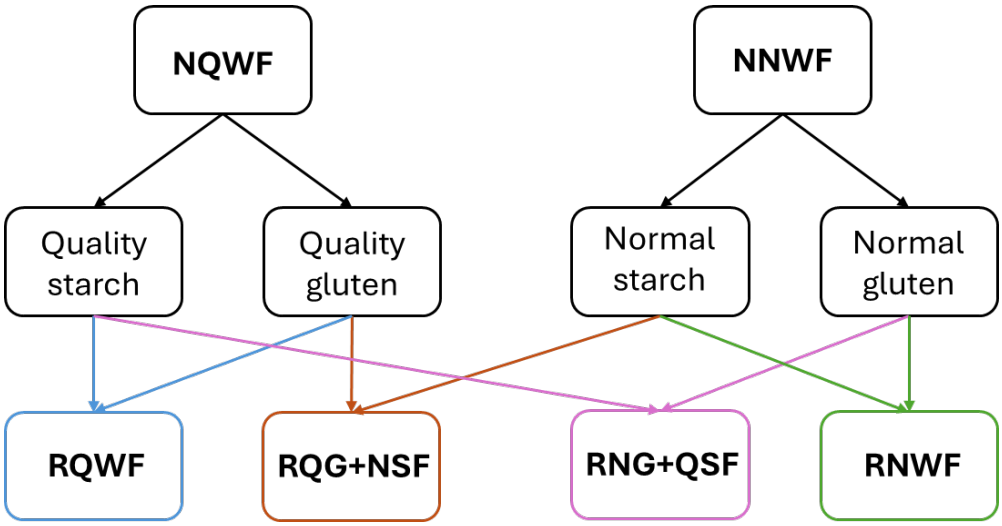

Figure 6. Illustration demonstrating the construction of the reconstituted flours.

3.3 Analysis of Raw Material

To examine whether the raw material utilized in this study had distinguished properties prior to baking, the starch, gluten, and native flours were analysed via light microscopy, DSC, and viscosity measurements.

3.3.1 Light Microscopy

Light microscopy (Olympus BX53F2) equipped with a CMOS colour camera (Olympus SC50) and the software Olympus CellSense Entry was utilized to examine the ratio of A- and B starch granules, and whether the gluten fraction still contained starch granules.

Ratio of A- and B Starch Granules

The distribution of A- and B granules in the starch samples were examined through data analysis of light microscope images. Initially, a slurry composed of 1.5 mg starch, three drops iodine (≈0.1 g), and 25 drops distilled water (≈0.9 g) was mixed with a plastic Pasteur pipette. 8 µg of the slurry was pipetted onto a glass slide with a Secure-Seal spacer (0.12 mm deep, ø=9 mm). The sample was subsequently covered with a cover glass (22×22 mm). 46 to 48 light microscope images covering the entire sample were captures at 10× magnification (Fig. 7A). This was replicated in triplets per starch sample.

To enable data analysis of the images, the sample edge was removed from the images where it was visible using the software ImageJ (Fig. 7B). Then, the granule distribution was analysed using the software Matlab (MathWorks). Initially, all images were converted to binary versions (Fig. 7C). Using the binarized images, the software counted the total number of A- and B granules and their total volume ratio. The granules were distinguished based on their diameter, whereas A granules were ≥10 µm, and B granules <10 µm. Finally, the distribution by count was normalized to enable a more accurate comparison between the two samples.

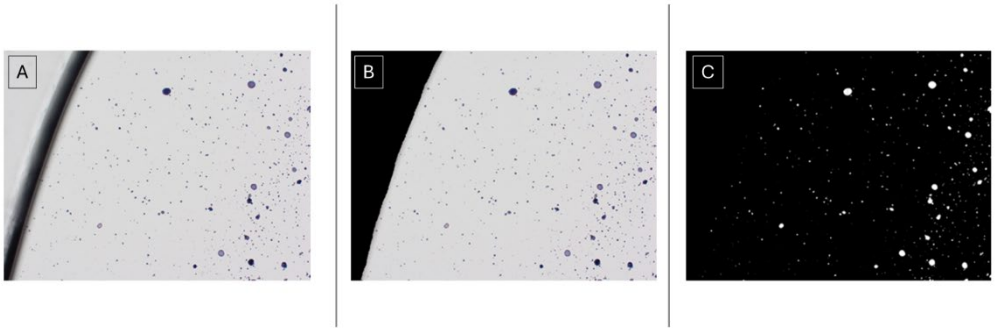

Figure 7. A) light microscope image of starch granules (purple stained dots) and the sample edge, B) sample edge is removed, C) binary measured version of the image.

Imaging of Gluten Fractions

Light microscope images of the gluten fractions were achieved through cryosection of gluten doughs. The doughs (60 mg) composed of 50% gluten powder and 50% deionized water was carefully kneaded with a small spoon. Subsequently, it was sectioned using a cryostat (Leica, CM3050 S) at -12˚C. Sections (8 µm) were obtained at three levels of the dough and each section was pressed onto a microscope slide. The samples were stained with iodine, covered with a cover glass and thereafter examined in the microscope. Representative images were captured at 20× magnification.

3.3.2 Thermal Properties of Starch

Differential Scanning Calorimetry (DSC) was utilized to study the thermal properties of starch, complying its gelatinization temperature and enthalpy. A SAXS/WAXS analysis was conducted by outsourced external partners to examine potential crystallinity differences between of the two samples.

Differential Scanning Calorimetry

An aluminium crucible (40 µL) was loaded with approximately 2 mg starch. About 5.3 µL deionized H_2O was pipetted to the sample, giving a starch to water ratio 1:3, as by Eliasson and Karlsson (1983). After careful stirring using a micro lab spatula, a cap was placed on the crucible, which were sealed together using a crucible sealing press (Mettler Toledo).

The sealed sample were placed in a DSC 1 STARe System (Mettler Toledo, Switzerland) calibrated with Indium. Moreover, a blank aluminium crucible was utilized as reference. The starch sample was heated from 20 to 120˚C with a heating rate of 4˚C per min. Gelatinization onset (T_o), peak (T_p), and conclusion (T_c) temperatures as well as melting enthalpies (ΔH) was determined using the Star^e Evaluation (Mettler) software. The crucible was weighted after the heating program to ensure no loss of sample. The analysis was replicated thrice per sample.

SAXS/WAXS Analysis

The SAXS/WAXS measurement was performed accordingly to the method detailed in Yulianingsih and Gohtani (2019). A SAXSpoint 2.0 (Anton Paar) with a Cu Kα radiation (λ=1.54 Å) and an Eiger R 1M Horizontal Detector was utilized. The distance from the sample to the detector was 561.9 mm for SAXS, and 109.1 mm for WAXS. The acquisition time was 10 min per frame.

3.3.3 Viscosity Measurements

The viscosity of the starch samples and the native flours upon heating was measured using a rheometer (ARES-G2, TA Instruments) equipped with a 34 mm in diameter

cup and a helical ribbon tool. For the starch samples, a slurry (45 g) made of 8% starch (dry basis) and 92% deionized water was loaded to the machine and a heating program starting at 30˚C was initiated. The temperature increased with 1.5˚C per min until the end temperature of 97˚C was reached. A soak time of 30 min was performed at 97˚C to ensure that all starch had been gelatinized. Afterwards, the temperature decreased with 1.5˚C per min until the end temperature of 30˚C was fulfilled. Peak-, trough- (lowest viscosity at maximum temperature), final-, breakdown- (peak minus trough), and setback (final minus trough) viscosities was collected in the software TRIOS (Ta Instruments). Three replicates were performed per starch sample.

Viscosity measurements were also performed on NQWF and NNWF in triplets. Identical heating programs were conducted on flour slurries made of 8% flour (dry basis) and 92% deionized water.

3.4 Dough Analysis

Dough rheological properties of the six samples were examined by their consistency index (k), rate index (n), and extensional viscosity. The doughs utilized for the measurements were assembled accordingly to Table 2. The amount of water added was based on ReoMixer analysis results. Moreover, 0.18 g salt, 0.18 g sugar and 0.18 g rapeseed oil were added to the mixture before the dough was kneaded using a ReoMixer (Reologen i Lund AB, Lund, Sweden).

Table 2. Dough formulations and blending conditions for rheological analysis

	NQWF	NNWF	RQWF	RNWF	RQG+NSF	RNG+QSF
Flour (g)	10	10	10	10	10	10
Water (g)	6.4	6	7	6.7	7	6.5
Blending	9.5 min	9 min	9 min	9 min	9 min	9 min

Determination of Consistency (k) and Flow Index (n)

The consistency- and rate index values were measured using a rheometer (HR 30, TA instruments) equipped with two parallel stainless-steel plates, whereas the upper plate had a diameter of 25 mm. A dough ball (1 g) covered with paraffin oil was compressed between the plates with a gap of 1.5 mm. The dough was left to rest until an applied force of 0.8 N was reached. Then, the gap was adjusted to achieve the set force threshold. After the initial force had been reached an oscillatory frequency sweep of 15-0.1 Hz with a strain of 0.1% was conducted. The 0.1% strain had previously been found to be within the linear viscoelastic region of the sample. Using the Cox Merz law, the oscillatory frequency sweep was converted to a flow sweep. In a flow sweep the dynamic viscosity in addition to

shear stress vs shear rate are shown. The power law function was fitted to the shear stress curve, resulting in consistency (k)- and rate (n) index values, which were required for subsequent extensional viscosity measurements. This was replicated twice per sample.

Extensional Viscosity Measurements

The doughs extensional viscosity was analysed through hyperbolic contraction flow measurements using a mechanical testing machine (Instron 68SC-05) equipped with a hollow compression fixture. A cylinder-shaped sample holder together with a feeding piston was filled with about 10 g of dough, and a contraction nozzle with an exit diameter of 3 mm was placed at the top, as illustrated in Figure 8. The sample holder was fitted into the testing machine so that only the nozzle was in contact with the measuring system. The k and n values previously calculated was inserted into the software method, and a compression was subsequently conducted at a rate of 1 mm/s. The dough was pressed out of the nozzle until it was in contact with the measuring system, whereas the measurement was stopped as soon as a plateau was reached. Two 10 g doughs were analysed per sample, allowing for approximately five measurements per dough.

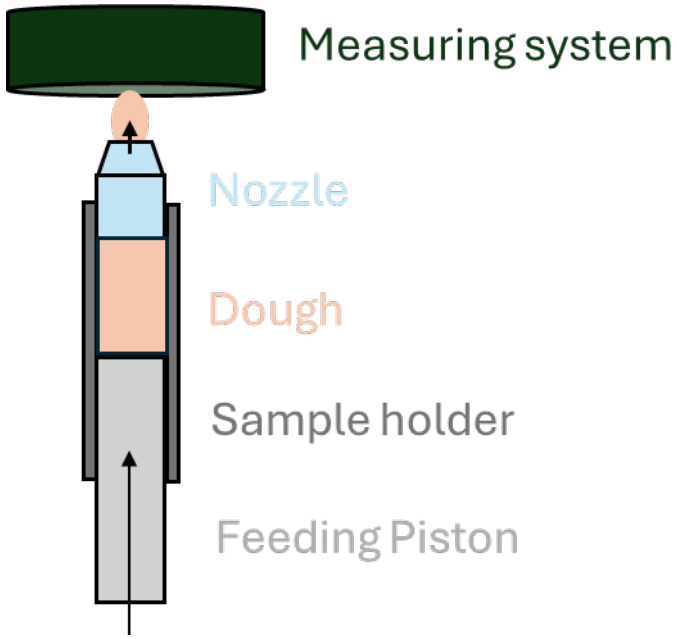

Figure 8. Diagram of the hyperbolic contraction flow apparatus for extensional viscosity measurements.

3.5 Bread Analysis

Six bread samples were made of the native and reconstituted flours. These were subsequently analysed through volume-, texture-, crust colour-, and crumb pore size measurements.

3.5.1 Baking

The recipe used for baking was derived from Lantmännen Cerealia AB, and the amount of flour and water used per bread sample (Table 3) stemmed from Reomixer analysis results with small adjustments based on manual dough tests. Moreover, based on the flour weight, 1.8% salt, 1.8% sugar, 1.8% rapeseed oil, 5% fresh yeast, and 50 ppm ascorbic acid were included in the recipes.

Table 3. The flour and water composition and blending conditions per bread sample

	NQWF	NNWF	RQWF	RNWF	RQG+NSF	RNG+QSF
Flour (g)	152.4	156.3	147	149.7	147	151.5
Water (g)	97.6	93.8	103	100.3	103	98.5
Blending	9.5 min	9 min	9 min	9 min	9 min	9 min

The doughs were initially kneaded 4 min at level one in a dough mixer (Ultra Power, KitchenAid, USA), followed by 4 or 4.5 min at a higher speed (level four). Subsequently, the dough was placed in a proofing chamber (Fermatic, Sveba-Dahlen AB, Fristad, Sweden) at 35˚C and 80% humidity for 30 min. To stretch out the gluten network, the doughs were formed to stiff balls by stretching its edges downwards, as illustrated in Figure 9A. The doughs were put in the proofing chamber for 5 min to release its stiffness before they were folded into bread loaves, as visualized in Figure 9B.

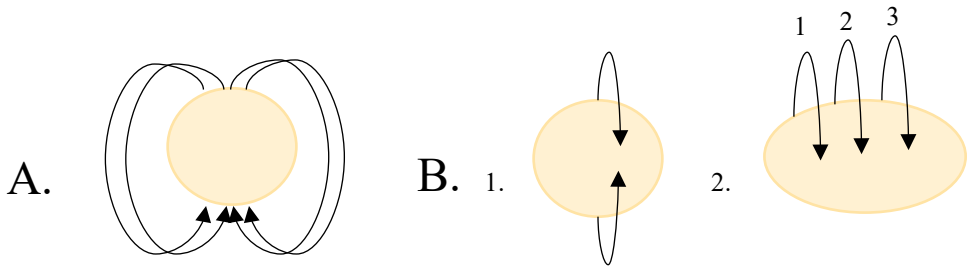

Figure 9. Illustration of the dough folding after 30 min proofing (A), and before the 60 min proofing (B).

Subsequently, the loaf was put in a baking pan (115×210×75 mm) sprayed with a releasing agent (Bakels Sprink). The combined pan and dough were placed in the proofing chamber for additionally 60 min before they were baked in a rotating oven (S8, Sveba-Dahlen AB, Fristad, Sweden) at 220˚C for 15 min, initiated with 10 s

of steam to improve the colour and texture of the crust. Four replicates were baked per flour sample. The replicates were baked on different days, with the order varying each time.

3.5.2 Volume Measurements

The volume of the bread loaves was measured two hours after baking, using the seed displacement method. The loaf was placed in a baking pan with a volume of 1.67 L. Subsequently, the empty area within the pan was filled with seeds (δ=0.7724 kg/L), whereas the weight of the seeds required to fill up the pan was measured. The bread volume was calculated as

$$Volume_{bread} = Volume_{baking\ pan} - \frac{Weight_{seeds}}{\delta_{seeds}}.$$

3.5.3 Texture Analysis

Texture measurements were conducted after 24 h of storage in closed plastic bags at room temperature.

Texture Analysis of Crumb

Crumb textures were examined through compression testing. A 10 mm thick bread slice from the midpart of the loaf were prepared using an electrical food slicer (Compact 1, Ritter, Germany). Cylinder shaped crumb samples (ø = 13 mm) were punched out of the slice and placed in a closed container to prevent them from drying. A mechanical testing apparatus (Instron 68SC-05) equipped with a flat upper plate and a stable lower plate was used for the measurements. The compression rate was 30 mm/min, and the compressive strain was 70%. Young's Modulus at 10-25% compression was measured and used to represent crumb firmness. The compression was replicated approximately 60 times per bread sample.

Texture Analysis of Crust

The crust was analysed via tensile testing using the mechanical testing apparatus supplied with an upper and a lower clamp positioned 50 mm from each other. Crust samples (80×10×4 mm) was prepared by slicing the loaf with the electrical food slicer, then the crust was carved out using a knife. The sample was locked between the clamps, and a tensile movement was initiated with a rate of 30 mm/min. Maximum tensile stress right before the crust ruptured was examined, which was replicated about 20 times per bread sample.

3.5.4 Crust Colour and Crumb Structure Analysis

Crust colour and crumb pore structure of each bread replicate were analysed through images captured with a DigiEye (VeriVide, Leicester, England).

Crust Colour

The crust colour analysis was conducted on images captured of the whole loaf. Twelve rectangular areas were examined per bread using the colour measurement function. Subsequently, the crusts level of lightness, yellowness and redness was analysed.

Crumb Pore Size

After the colour measurement, a 10 mm thick bread slice were prepared from the midpart of the loaf using the electrical food slicer. An image of the bread slice's crumb was captured, whereas its average crumb pore area was measured accordingly to the method used by Pietiäinen et al. (2024).

3.6 Statistical Analysis

Statistical analysis was conducted on all test parameters except from the A- and B granule ratio analysis. First, values exceeding the average testing value with three standard deviations was excluded as outliers. Secondly, a F-test was exhibited between all samples to determine eventual variance. Depending on the F-test outcome, statistical significance was examined using a homoscedastic or a heteroscedastic T-test with a 95% confidence level. The statistical analysis was conducted using the software Excel (Microsoft Office 365).

4. Results

4.1 Fractioning of Wheat Flours

4.1.1 Liquid Starch and Wet Gluten Constituents

The fractioning of NQWF yielded 46 220 g liquid starch phase and 3 890 g liquid gluten phase. However, the separation process was not optimal, as the wet gluten phase content was calculated to about 26% protein and 74% starch (+ other constituents). The analysis conducted by Eurofins confirmed this calculation, as their measurements gave 25.8 ± 7% raw protein, and 58.9% starch.

The fractioning of NNWF resulted in 46 540 g liquid starch phase and 3 420 g liquid gluten phase. The wet gluten phase constituents were calculated to approximately 24% protein and 76% starch (+ remaining constituents), and the Eurofins analysis results gave 25.1 ± 7% raw protein, and 59.2% starch.

4.1.2 Dry Starch and Gluten Powder Yields

Starch Powder Yield

Approximately 1 031 g (dry basis) quality starch powder from NQWF (moisture content 11.10%) was obtained from spray drying of the liquid starch phase. Given the initial usage of about 3 215 g (dry matter) NQWF with an assumed starch concentration of 73%, the process yield was 43.9%.

Considering NNWF, about 1 200 g (dry basis) normal starch powder (moisture content 10.45%) was collected. Given the total usage of 3 220 g (dry matter) NNWF with an assumed starch concentration of 76%, the process yield was 49%.

Gluten Powder Yield

The freeze drying of NQWF gluten yielded about 1 547 g powder (moisture content 1.9%), whilst the gluten from NNWF yielded about 1 280 g (moisture content 1.2%).

4.1.3 Composition of Reconstituted Flours

Given the composition of the gluten fractions presented in chapter 4.1.1, a complete exchange of starch phase between RQG+NSF and RNG+QSF was not achievable. Consequently, those samples had a mixture of normal and quality starch, as presented in Table 4. RQG+NSF contained 14.2% protein, 40.8% quality starch and 45% normal starch. The constituents of RNG+QSF on the other hand was

12.1% protein, 36% normal starch and 52% quality starch. Moreover, RNWF and RNG+QSF had a slightly higher protein content than their native protein precursor.

Table 4. The composition of gluten and starch in each flour sample

	Quality gluten (%)	Normal gluten (%)	Quality starch (%)	Normal starch (%)
NQWF	14.3		86.7	
RQWF	14.2		85.8	
RQG+NSF	14.2		40.8	45.0
NNWF		11.5		88.5
RNWF		12.1		87.9
RNG+QSF		12.1	52.0	36.0

4.2 Analysis of Raw Materials

4.2.1 Light microscopy

Distribution of A- and B Starch Granules

Data analysis of light microscope images shows only a small difference between quality and normal starch regarding ratio of A- and B granules. Diameter measurements of 136 688 quality starch granules (Fig. 10) and 189 568 normal starch granules (Fig. 11) reveal that the former consisted of 4.8% A-type granules, while the latter had 4.7%. However, quality starch had a greater proportion of B granules with smaller diameters (between 1 and 2 µm) than normal starch.

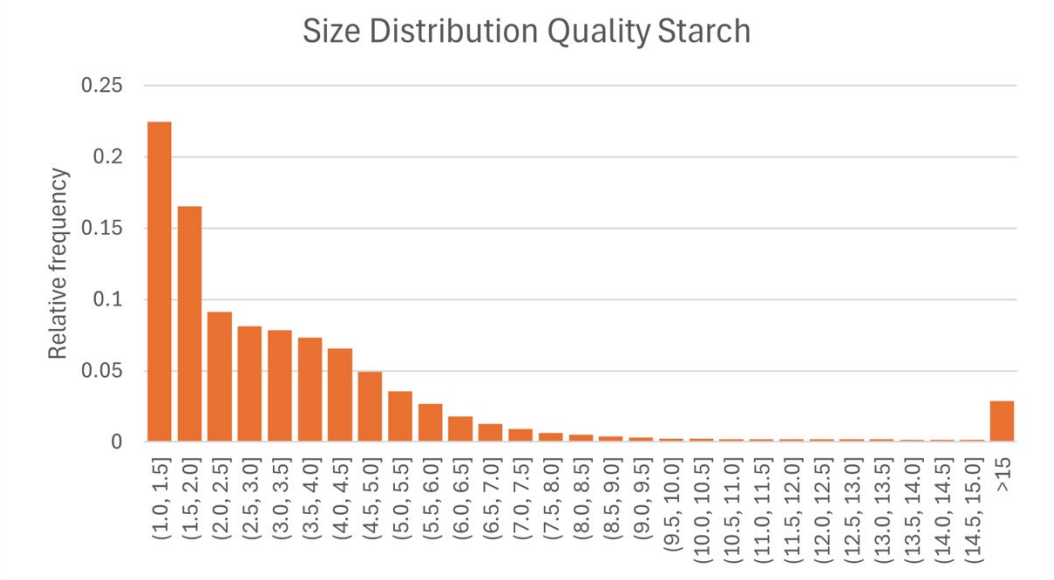

Figure 10. Size distribution of 136 688 quality starch granules, with relative frequency plotted against granule diameter.

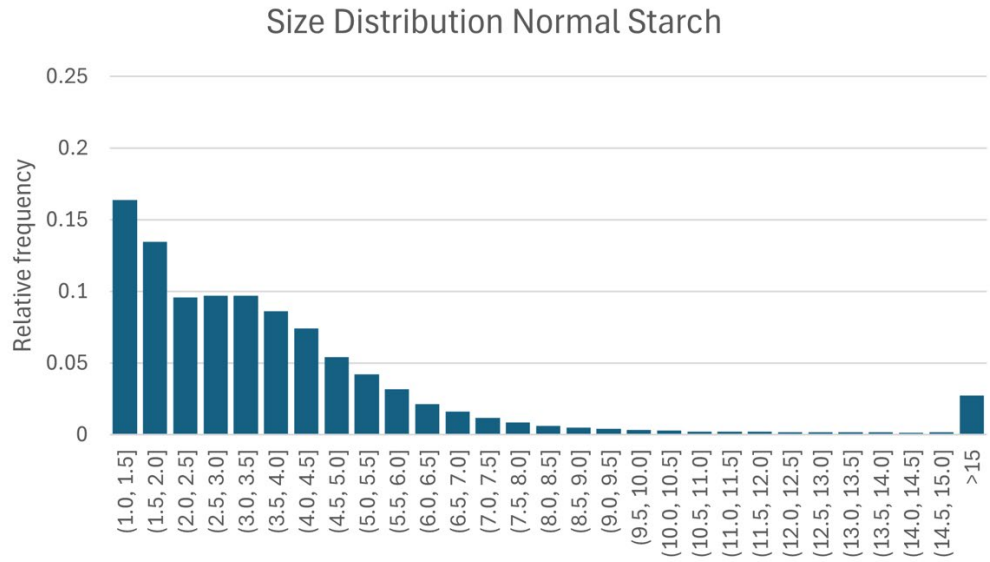

Figure 11. Size distribution of 189 568 normal starch granules, with relative frequency plotted against granule diameter.

A bigger distinction was however seen between the samples considering total volume measurements. The volume ratio of A granules to B granules in quality starch was 5.9, whilst the ratio in normal starch was 5.1.

Imaging of Gluten Fractions

Imaging of the two gluten fractions (Fig. 12) confirms that both samples still contained large amounts of starch, as presented in chapter 4.1.1. The starch is stained purple, whilst gluten is stained yellow.

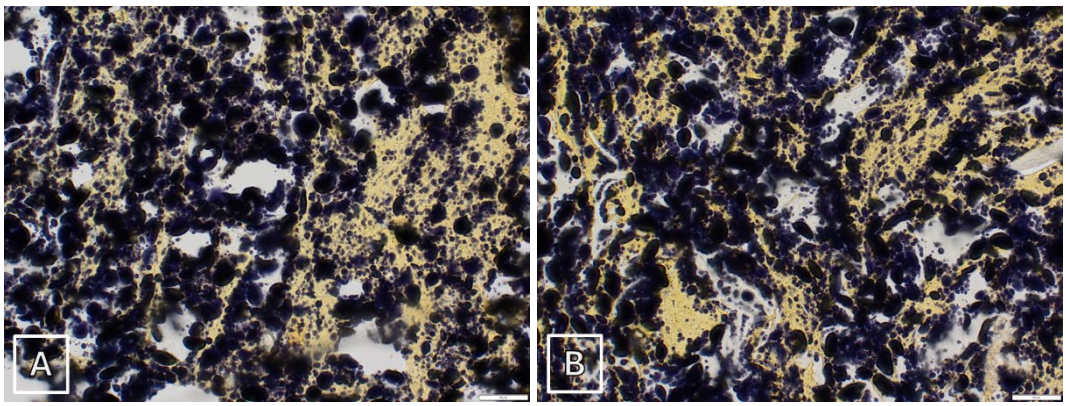

Figure 12. Light microscope images (×20) of a quality gluten dough (A) and a normal gluten dough (B). Yellow stained parts is gluten, whilst purple stained sections are starch.

4.2.2 Thermal Properties of Starch

The results from the DSC analysis show that quality starch requires a significantly higher temperature to gelatinise compared to normal starch, (Table 5 and Figure 13). Quality starch had a higher onset temperature (T_o), peak temperature (T_p) and conclusion temperature (T_c).

However, as visualized in Figure 14, the enthalpy (ΔH) required to melt the starch crystals did not differ significantly between the two starch samples.

Table 5. Average gelatinization parameters of normal and quality starch determined by DSC

Starch Sample	T_o (˚C)	T_p (˚C)	T_c (˚C)	ΔH (J/g)
Quality starch	55.3	61.5	66.6	1.98
Normal starch	54.2	60.1	65.4	2.15

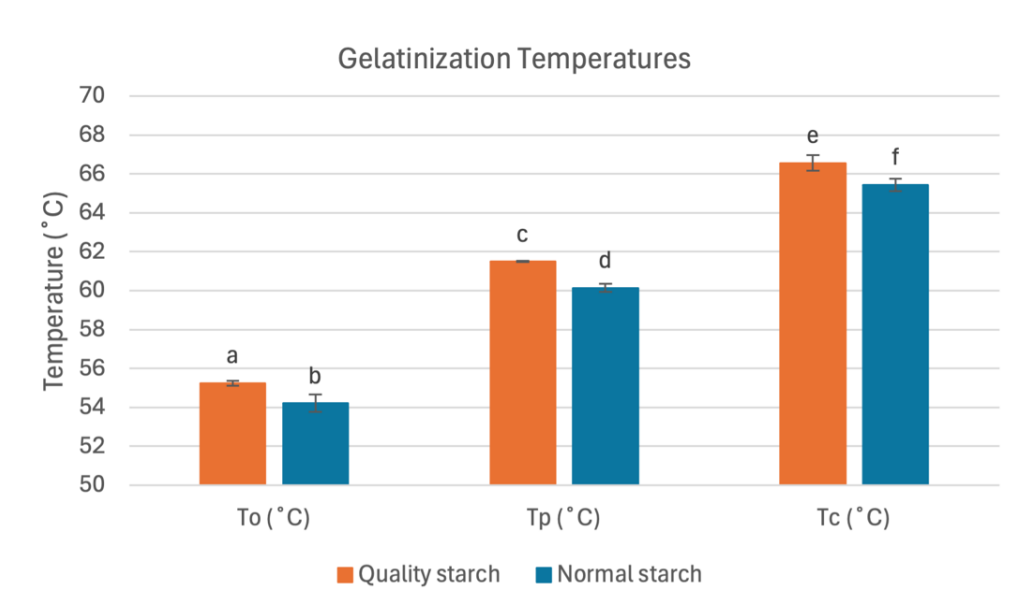

Figure 13. Gelatinization temperatures of quality and normal starch. T_o represents the temperature needed to initiate gelatinization, T_p the peak of gelatinization, and T_c the conclusion gelatinization. Error bars represent standard deviations.

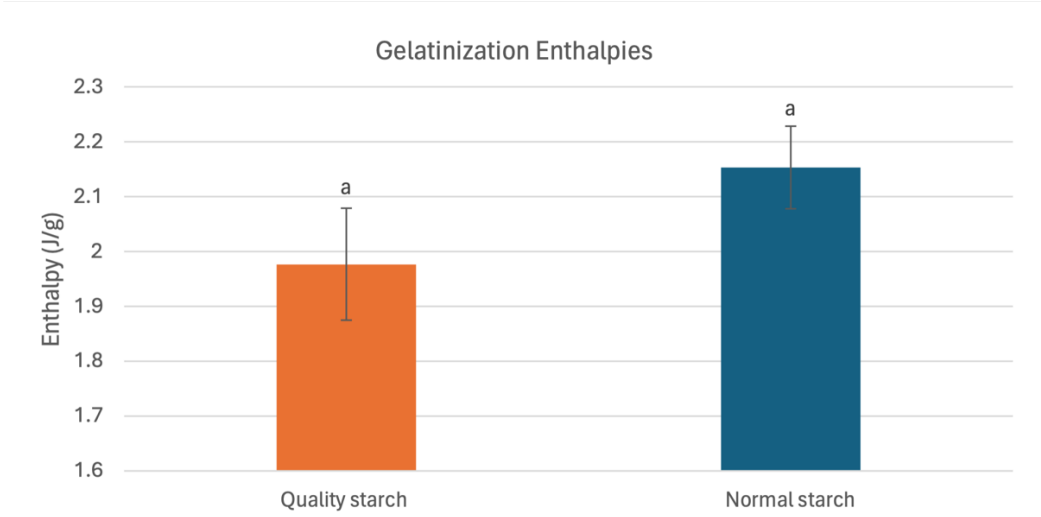

Figure 14. Enthalpy required to gelatinize quality starch (orange bar) and normal starch (blue bar). Error bars represent standard deviations.

Moreover, the SAXS/WAXS analysis found no difference in crystallinity profile between quality and normal starch, as seen in Figure 15 and 16.

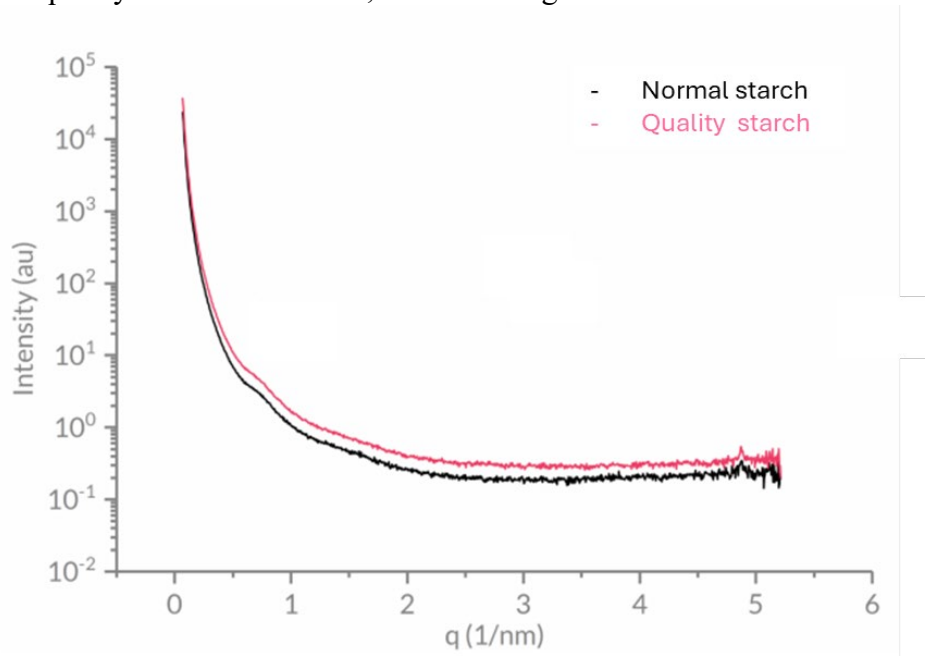

Figure 15. The crystal lamellar structure of quality and normal starch determined by SAXS analysis.

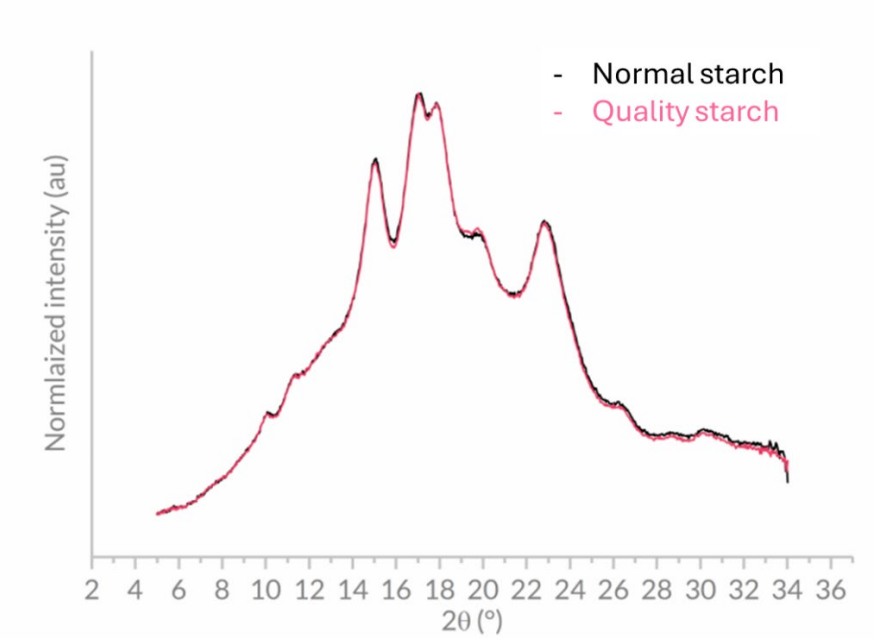

Figure 16. The structural crystallinity profile of quality and normal starch determined by WAXS analysis.

4.2.3 Viscosity Measurements

The viscosity of NQWF, NNWF and their respective starch phases was examined with a rheometer. Analysis of the two native flours upon heating indicate that all measured parameters, hence, start (SV)-, peak (PV)-, trough (TV)-, and final viscosity (FV) differed significantly between the two samples. NNWF had a higher viscosity than NQWF in all four cases. As a result, breakdown and setback viscosity also varied (Table 6, Fig. 17).

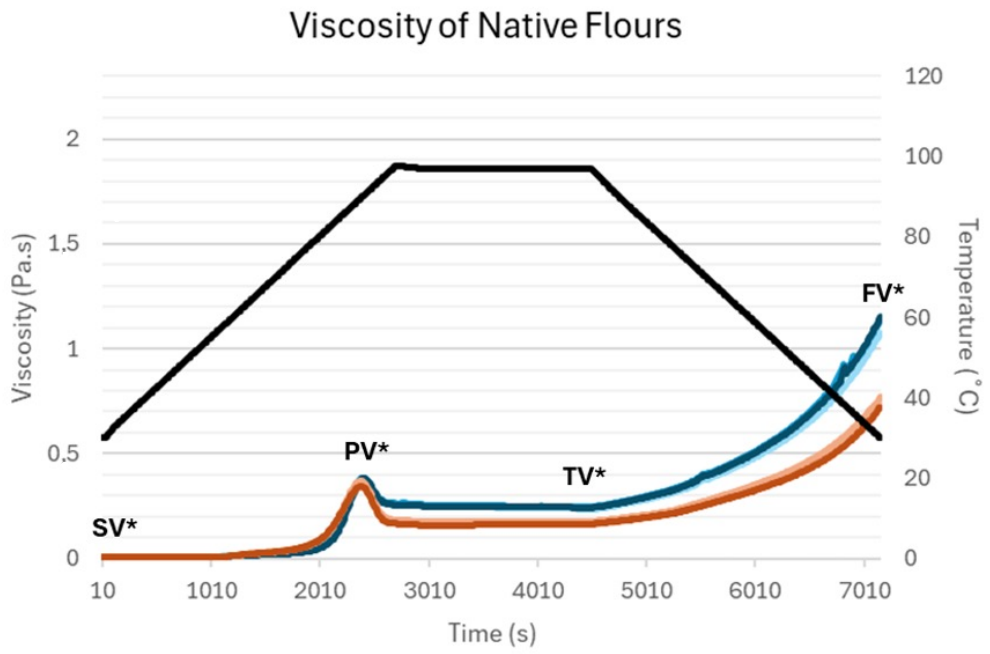

Figure 17. Viscosity of NNWF (blue graph) and NQWF (orange graph) as a function of temperature (black graph). The asterisks represent a significant difference between the two samples.

When the gluten had been removed from the native flours, the viscosity increased further in both samples. Moreover, upon comparison of the two starch samples, normal starch still showed significantly higher viscosity than quality starch at all temperatures, except from the SV (Fig. 18).

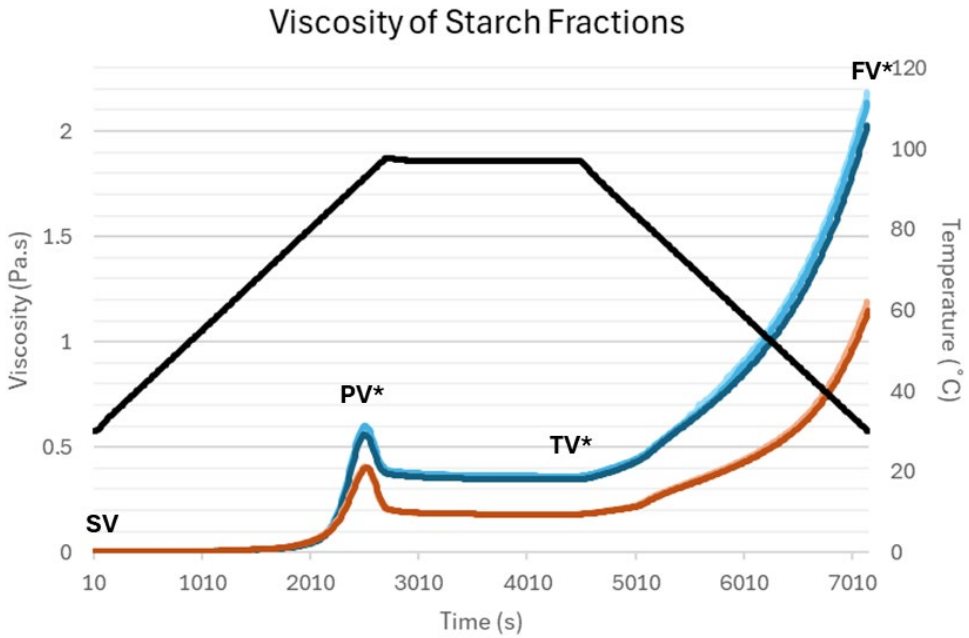

Figure 18. Viscosity of normal starch (blue graph) and quality starch (orange graph) as a function of temperature (black graph). The asterisks represent a significant difference between the two samples.

Table 6. Average viscosity values ± standard deviations of NQWF, NNWF and their respective starch phases at different temperatures

	NQWF	***NNWF***	***Quality starch***	***Normal starch***
Start viscosity (SV)	0.0093 ± 2.7×10^{-4}	0.0079 ± 8.0×10^{-4}	0.0067 ± 9.4×10^{-4}	0.005 ± 7.8×10^{-5}
Peak viscosity (PV)	0.35 ± 1.4×10^{-3}	0.38 ± 6.2×10^{-3}	0.4 ± 2.5×10^{-3}	0.59 ± 2.3×10^{-2}
Trough viscosity (TV)	0.16 ± 7.9×10^{-3}	0.24 ± 5.6×10^{-3}	0.18 ± 6.5×10^{-4}	0.36 ± 8.5×10^{-3}
Final viscosity (FV)	0.74 ± 2.4×10^{-2}	1.12 ± 4.2×10^{-2}	1.16 ± 2.7×10^{-2}	2.12 ± 7.9×10^{-2}
Breakdown viscosity	0.19	0.14	0.22	0.23
Setback viscosity	0.58	0.88	0.98	1.76

4.3 Dough Analysis

4.3.1 Dough Rheology

Extensional viscosity was utilized to examine the flour samples dough rheological properties. First, consistency- (k) and rate (n) index values was determined through

a frequency sweep, whereas the obtained values (Fig. 19 and 20) was utilized to enable subsequent dough rheology measurements. However, no significant difference was obtained between any of these values.

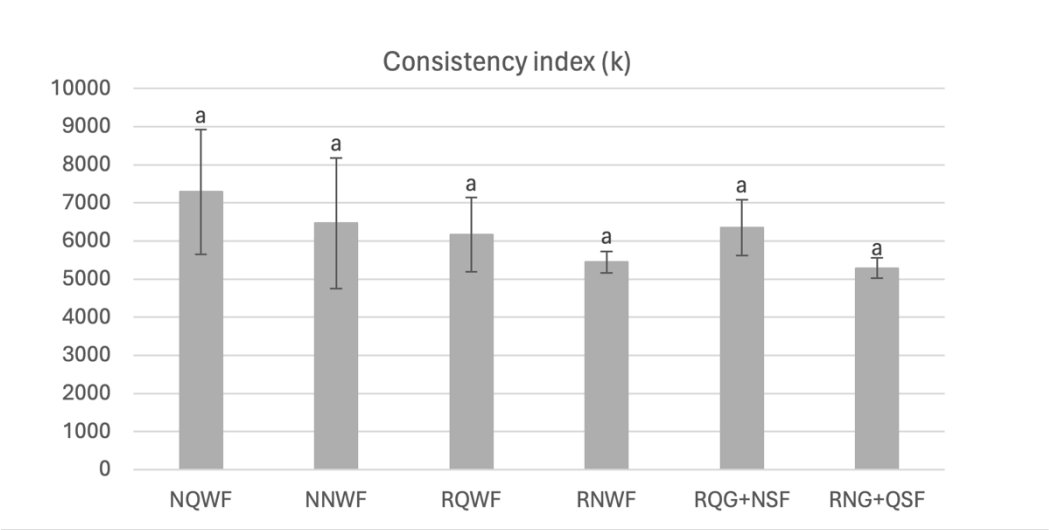

Figure 19. Consistency index (k) of the dough samples. Error bars represent standard deviation.

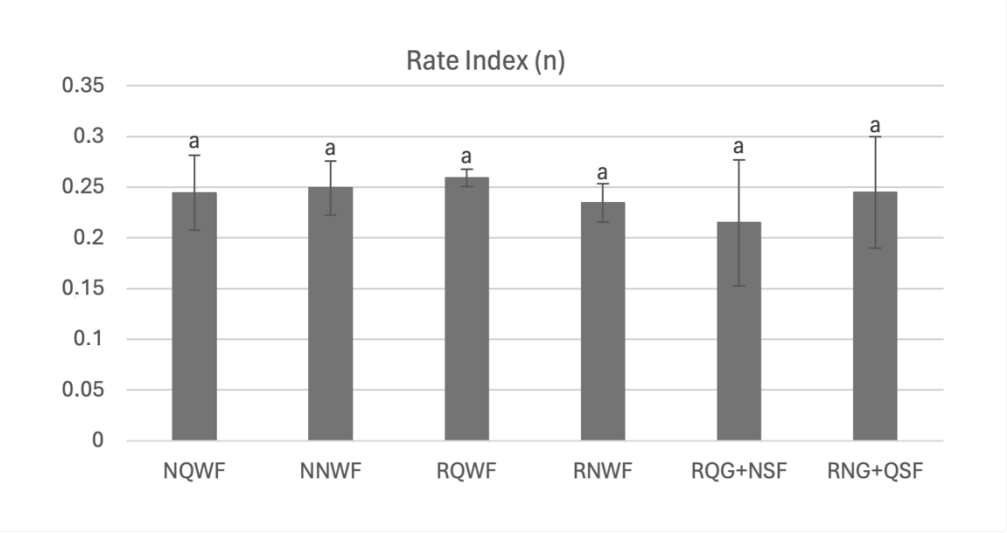

Figure 20. Rate index (n) of the dough samples. Error bars represent standard deviation.

The dough's viscoelastic properties were then analysed via hyperbolic contraction flow. This analysis revealed that the reconstituted flours yielded significantly lower extensional viscosity than their native precursors, as seen in Figure 21. Additionally, NNWF exhibited the highest extensional viscosity, while RQWF, RNWF, RQG+NSF and RNG+QSF showed the lowest values, with no significant difference between them.

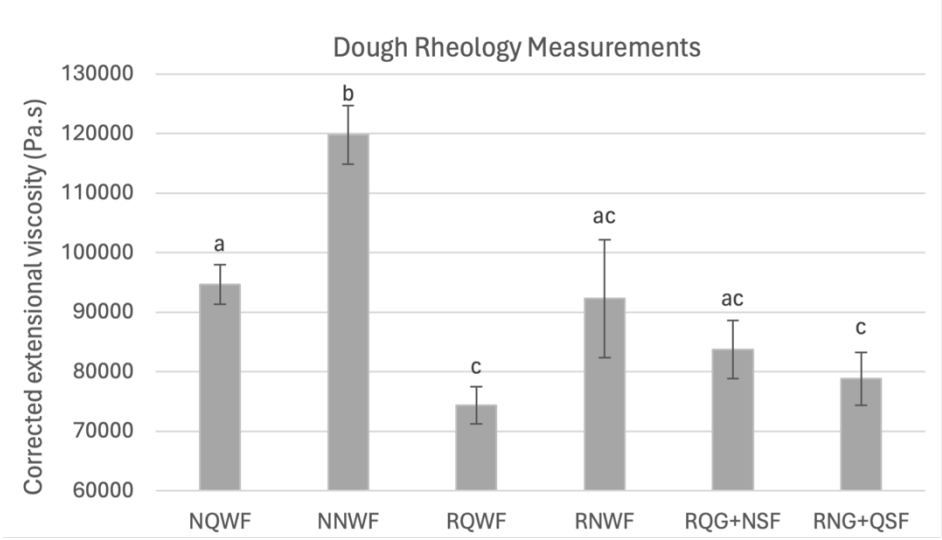

Figure 21. Extensional viscosity of the dough samples. Error bars represent standard error.

4.4 Bread Analysis

4.4.1 Loaf Volume

The volume of the six breads differed significantly between several of the samples (Fig. 22 and 23). Bread baked of NQWF and RQWF exhibited the greatest volumes of all samples, with no significant difference between them. The volume of the NNWF loafs on the other hand was significantly bigger than the RNWF breads. Moreover, when comparing RQG+NSF bread with RNG+QSF bread, the latter was significantly larger.

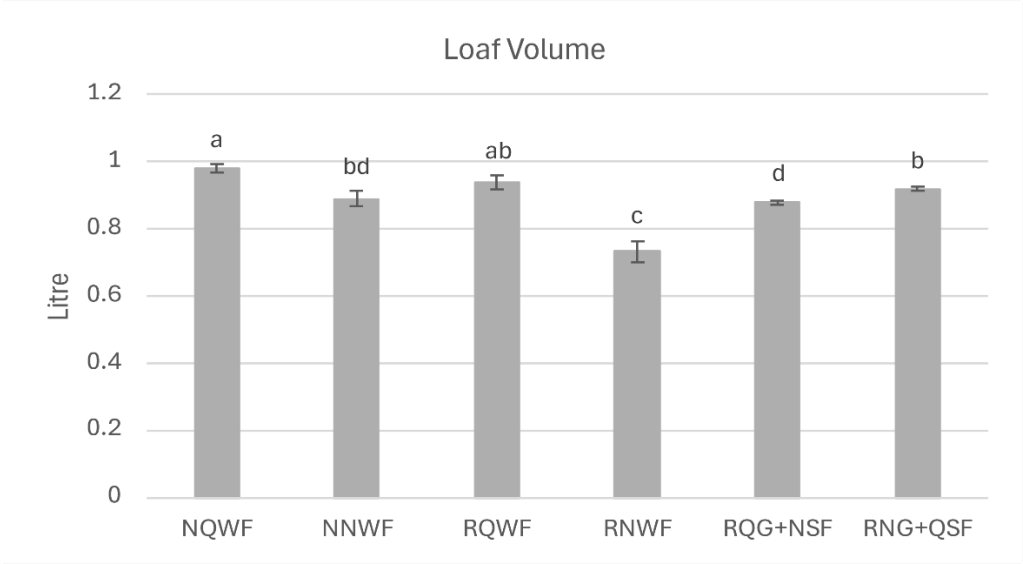

Figure 22. Average volume of the bread samples. Error bars represent standard error.

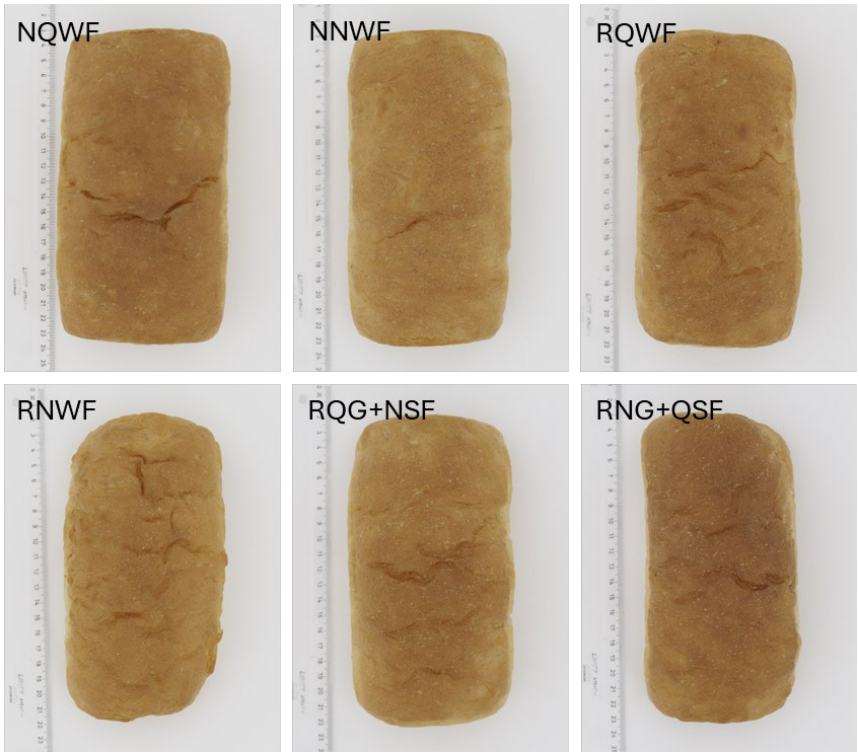

Figure 23. Representative images of bread samples demonstrating the average volume of each sample.

4.4.2 Crust Colour

Representative images showing the breadcrust's colours are presented in Figure 24.

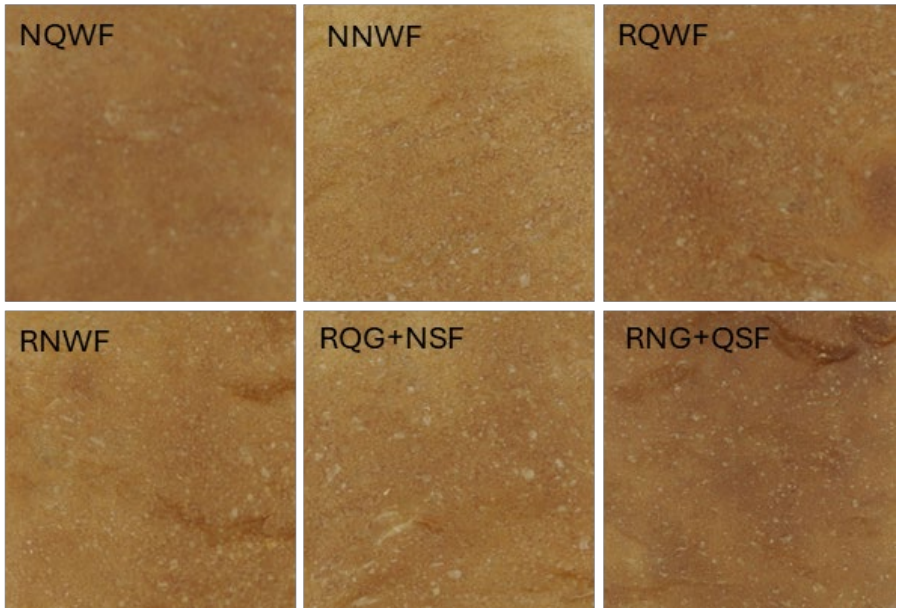

Figure 24. Representative images of the crust colour of each bread sample.

The colour analysis of these images measured the crusts level of lightness, yellowness, and redness, illustrated in Figure 25, 26 and 27. A higher bar implies a lighter, yellower or redder crust. Consequently, NNWF and RNWF was significantly lighter and had more yellow tones than the other samples. Moreover, bread made of RQG+NSF was both lighter and yellower than bread made of RNG+QSF. Additionally, the reconstituted flour breads were significantly darker than their native precursors in both cases.

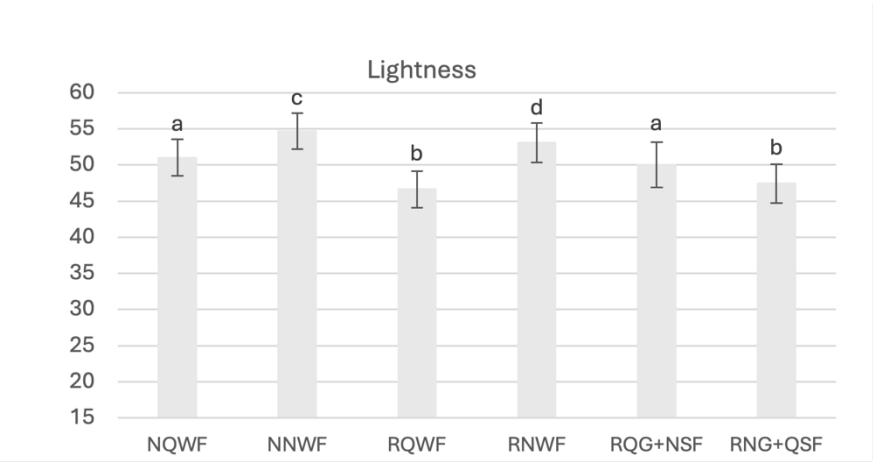

Figure 25. Average crust lightness of the loaf samples. The error bars represent standard deviation.

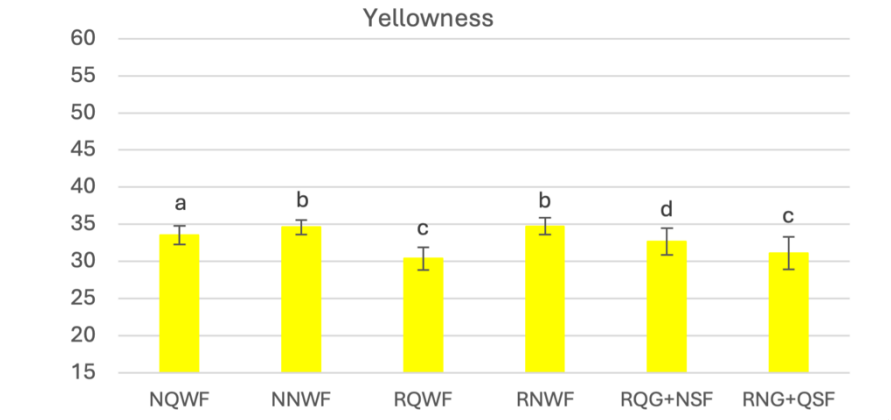

Figure 26. Average crust yellowness of the loaf samples. The error bars represent standard deviation.

The redness analysis shows that NQWF and RQWF had significantly redder crusts than NNWF and RNWF (Fig. 27). Moreover, the reconstituted samples were redder than the native samples, and no difference was detected between RQG+NSF and RNG+QSF.

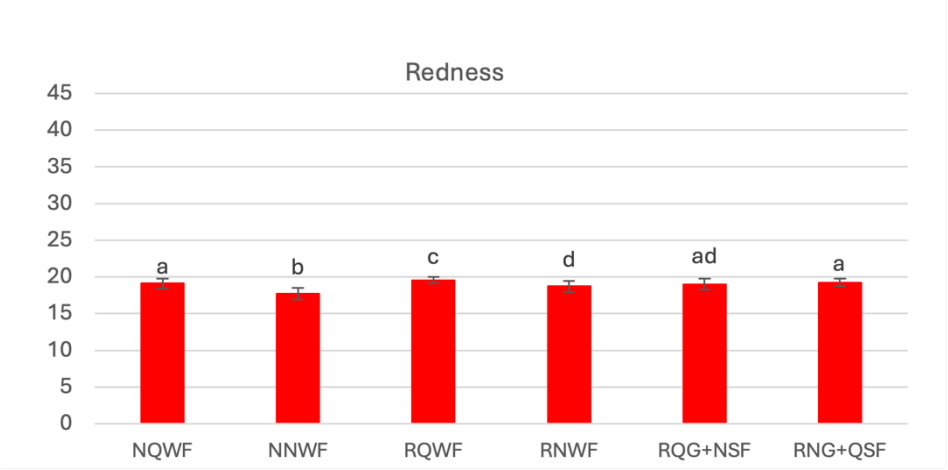

Figure 27. Average crust redness of the bread samples. The error bars represent standard deviation.

4.4.3 Crumb Pore Size

Representative images of the breadcrumbs are seen in Figure 28, and the binarized version of these images upon which the measurements were conducted are presented in Figure 29.

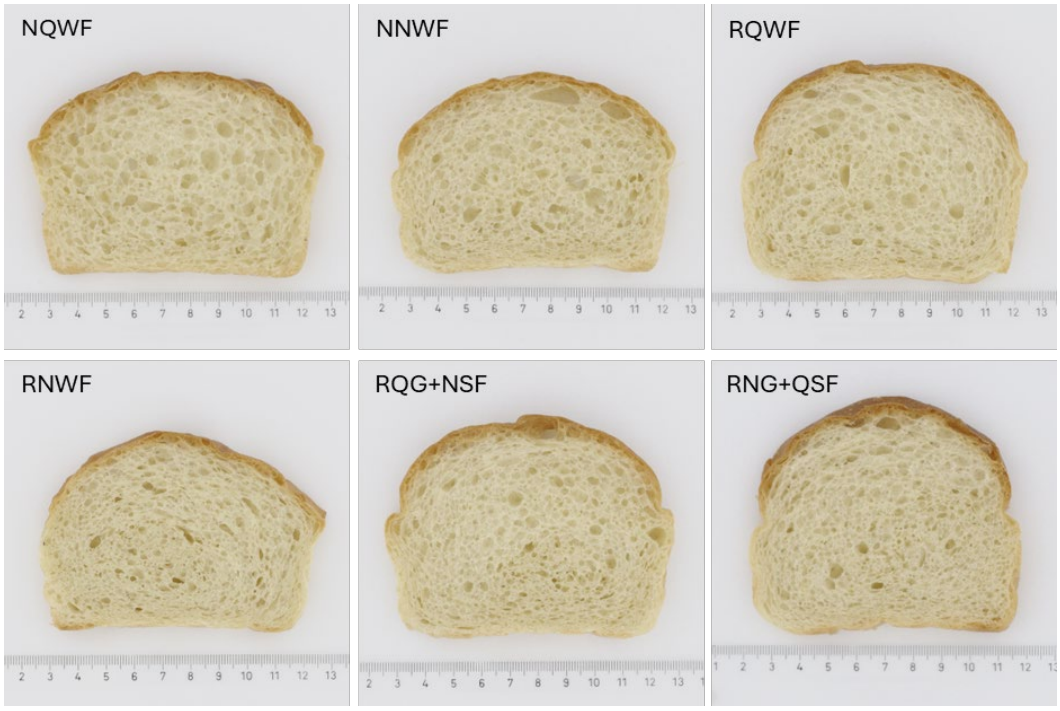

Figure 28. Visual appearance of the crumb of representative samples.

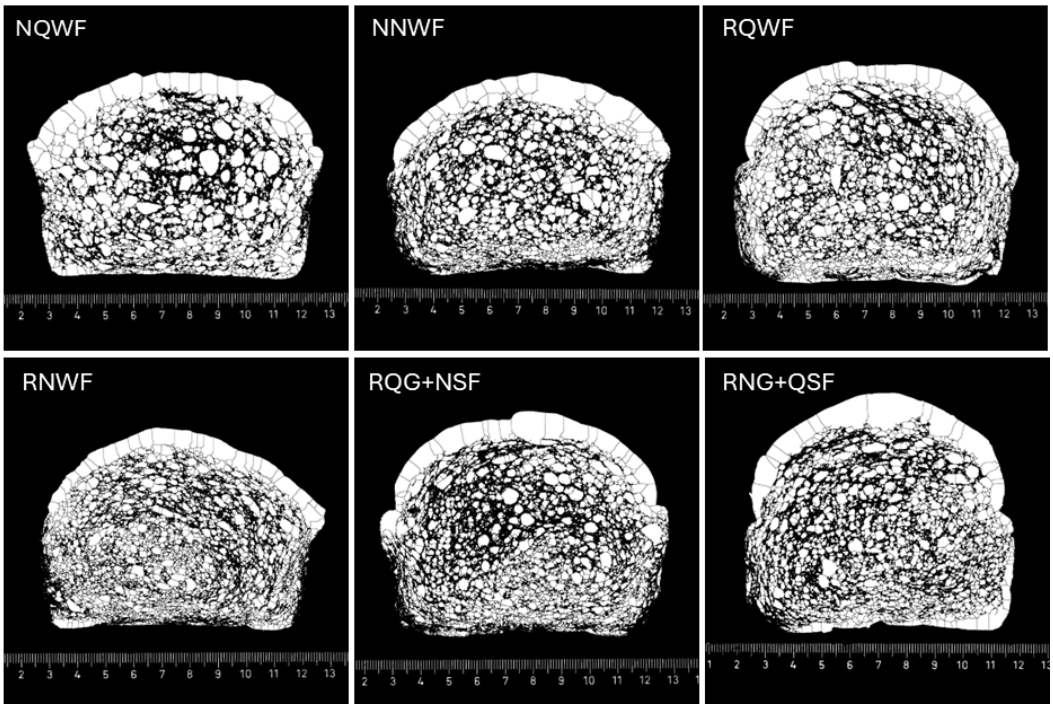

Figure 29. Binarized images of the crumbs demonstrating the pore structures.

A digital image analysis of the crumbs pore structures reveals that NQWF and RQWF had the significantly biggest average pore areas, as seen in Figure 30. There is a bigger distinction between NNWF and RNWF, as the former had the second largest average pore area, whilst the latter had the smallest average pore area. Moreover, RNG+QSF had larger pore areas than RQG+NSF.

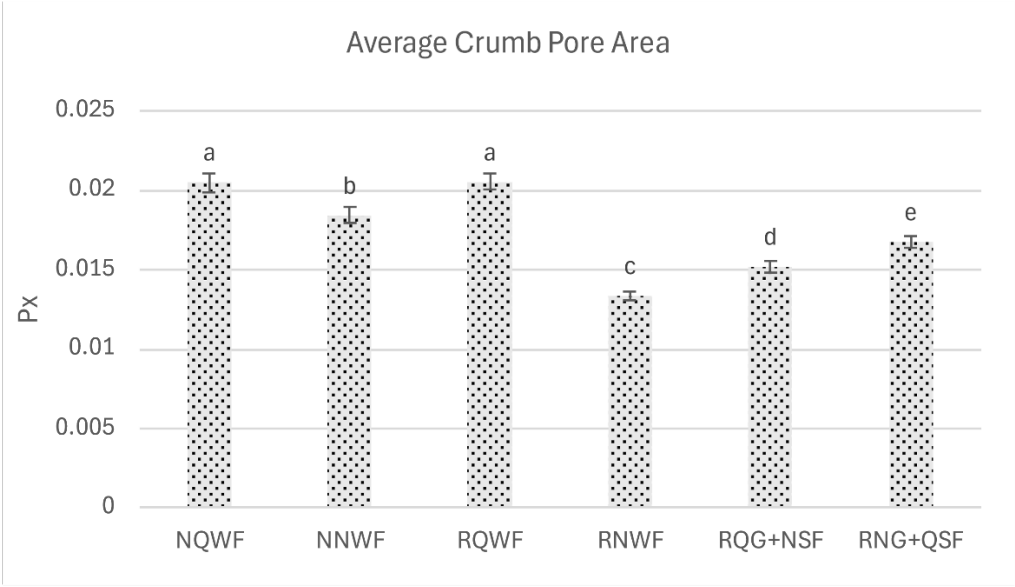

Figure 30. Average crumb pore area of each bread sample. The error bars represent standard error, and Px represent pixels.

4.4.4 Crumb and Crust Texture

Crumb Texture

To determine crumb texture, their Young's Modulus value upon compression was detected. A higher Young's Modulus implies a stiffer crumb. Breads made of NQWF, NNWF, RQWF and RNG+QSF showed the lowest and significant similar values, as seen in Figure 31. Moreover, the stiffness of RQG+NSF crumb did not differ significantly from NNWF. However, it was stiffer than the other three mentioned samples. Finally, the crumb from RNWF bread had a significantly higher Young's Modulus value than all other samples.

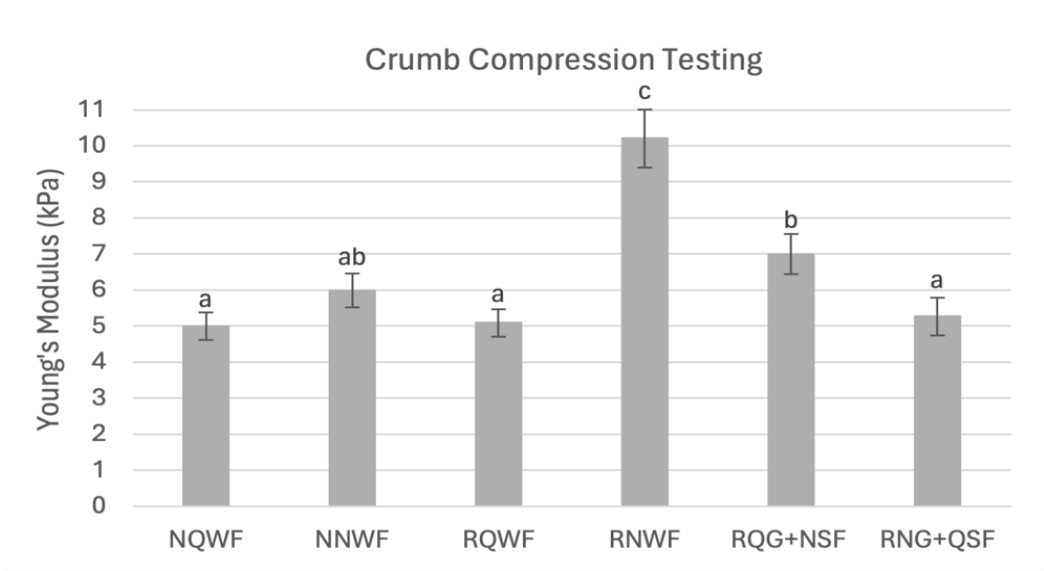

Figure 31. The breadcrumbs Young's Modulus value upon compression. The error bars represent standard error.

Crust Texture

The texture of the bread samples crust was analysed through tensile testing, whereas the maximum tensile stress was measured right before the crust ruptured. No significant difference was identified between the crusts from NQWF, RQWF, RNWF and RQG+NSF, as seen in Figure 32. However, the crusts from NNWF and RNG+QSF had, compared to the previous mentioned samples except from NQWF, significant lower maximum tensile stress right before rupture.

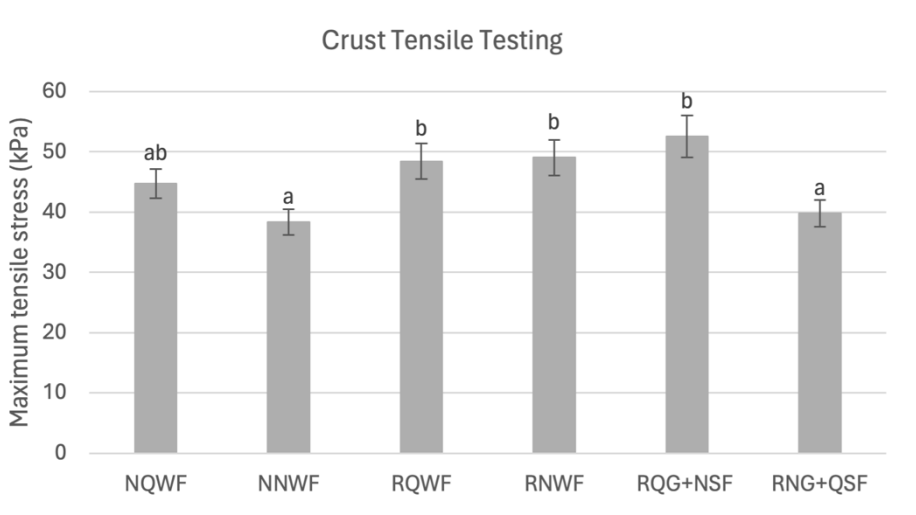

Figure 32. The crusts maximum tensile stress prior to rupture upon tensile testing. The error bars represent standard error.

5. Discussion

The aim of this study was to examine whether the quality of starch had an impact on the outcome of bread baking, or if only the quality of gluten played a major role. The results achieved upon analysis of the raw materials utilized, the doughs and the final baked breads indicate that the quality of starch indeed influences the quality of bread. In this chapter, the results will be discussed in relation to the literature and the aim of this study.

5.1 The Difference Between the Raw Starch Samples

Gelatinization Temperatures

As a result of heating in presence of water, starch gelatinises (Nivelle et al., 2019). The two starch samples analysed in this study showed significant distinguished gelatinization properties. For example, the DSC analysis showed that quality starch required approximately +1˚C to gelatinize compared to normal starch. However, the gelatinization enthalpy displayed no significant difference, and the SAXS/WAXS analysis revealed no difference in crystallinity profile. A higher gelatinization temperature is commonly attributed to a higher relative crystallinity (Zhang et al., 2013). However, given the SAXS/WAXS analysis results, this was not the case in this study.

Moreover, gelatinization onset temperatures are positively impacted by A-type granules (Zhang et al., 2016). In the present study, the quality starch sample had a slightly higher content of A granules than normal starch, as measured by both number and total volume ratio. However, the higher onset temperature of A-type granules may also origin from their greater level of crystallinity (Zhang et al., 2013), which, as already discussed, did not differ between the samples.

Starch gelatinization temperatures can also be impacted by its AMP and AM ratio, whereas the firmer increase the onset temperature. The concentration of AMP and AM was not analysed in this study. However, the primarily reason their ratio affects thermal properties is because it typically influences overall crystallinity (Nivelle et al., 2019). Thus, to summarize, exiting literature attributes many starch properties to differences in crystallinity. However, this study found no difference in crystallinity between the two starch samples. Hence, previously discussed results must stem from other factors.

Starch Viscosities

The viscosity obtained upon gelatinization differed between the two starch samples. Normal starch developed significantly higher viscosity than the quality sample, with the biggest distinction found at final viscosity. Accordingly to Zhang et al. (2016), A-type granules usually enhance the viscosity of starch. This theory does not align with our results, as the normal sample had a lower concentration of A granules than the quality sample.

However, starch viscosities are also influenced by AM. A high AM content limits granules swelling power, which negatively impacts starch gelatinization properties (Li et al., 2022). Granules swelling power is indicated by their PV (Cao et al., 2019), and this study found that normal starch had a higher PV than quality starch, indicating that the former granules have greater swelling power. Therefore, as Li et al. (2022) suggested, quality starch may have a higher AM content than normal starch, which could explain its overall lower viscosity. A greater AM concentration is also associated with improved dough quality (Cao et al., 2019), which is consistent with the dough measurements observed in this study. However, according to Soulaka and Morrison (1985), is the final bread quality not impacted by the AM concentration.

Interestingly, NNWF had a lower PV than its separated starch phase. Hence, the granules swelling power are degraded when combined with the remaining flour constituents. This is expected, since the protein in the flour also absorbs water (Setya Budi Muhammad et al., 2024). However, there was no such distinguished difference between NQWF and the separated quality starch's PV. This indicates a molecular difference between NQWF and NNWF that highly impacts their gelatinization capacities. Moreover, although there was only a small significant difference in PV between the two flours, a larger significant difference was observed in the FV, where NNWF exhibited a higher viscosity. Since the FV results from leached AM (Nivelle et al., 2019), it could be suggested that NNWF contains a higher AM content. However, this contradicts the earlier findings, where quality starch was discussed having a higher AM concentration. Thus, no conclusions can be drawn concerning eventual difference in AM content in the two samples.

Finally, breakdown and setback viscosities were measured. The former informs if the samples have a variating capacity to withstand granule rupture upon high temperatures (Cao et al., 2019). However, no distinguished difference was observed between quality and normal starch. The setback viscosity on the other hand, which gives an indication of starch gelation capacity upon cooling (Cao et al., 2019), was much higher for NNWF, and especially for normal starch. This enhanced viscosity might influence the stiffer breads in the samples containing normal starch.

5.2 Bread Quality is Improved by Quality Starch

A bread of good quality exhibits a soft texture. Among the samples examined in this study, bread baked of NQWF, NNWF, RQWF, and RNG+QSF exhibited the significantly softest breads. However, when the fractioned normal starch phase was added to the recipe, hence RNWF and RQG+NSF, the bread stiffness increased. Still, it slightly decreased when combined with quality gluten (RQG+NSF). The texture analysis was consistent with the crumb pore size and bread volume analysis, whereas softer breads had both larger average pore areas and total loaf volumes, which aligns with Li et al. (2022).

Properties Influencing Bread Volume

The deflated bread volume especially found in RNWG and RQG+NSF could result from an underdeveloped gluten-starch matrix. Li et al. (2022) concluded that starch with irregular shaped granules gave smaller breads. The authors suggest that this might hamper the distribution of starch within the gluten network, restricting its development. However, morphological examination of the starch granules under a light microscope revealed no distinct differences between the morphology of normal and quality starch granules. Moreover, the distribution of granule types may have a role in the gluten-starch matrix outcome. Both Setya Budi Muhammad et al. (2024) and Shang et al. (2020) found that starch from high-quality flours have more B-type granules. Perhaps small granules also more evenly distribute within the gluten network, improving the matrix. In this study, normal starch, which yielded smaller loaves, had the highest concentration of B-type granules, which contradicts this theory. However, based on the normalized granule distribution, the quality starch sample had a lower content of B granules in total but a higher proportion of small B granules (1-2 µm in diameter), which could be advantageous considering the distribution within the gluten matrix.

Bread volume can moreover be influenced by starch gelatinization temperatures and the exact proportion of B-type granules. Thus, higher gelatinization temperatures and 25% B-granules (by weight) yield larger breads (Soulaka and Morrison, 1985). In this study, quality starch exhibited a higher gelatinization temperature and gave larger loaves. The distribution of granules measured by weight was not assessed in this study, however, total volume was. The total volume of quality B granules was 14.5%, whilst normal B granules accounted for 16.4%. Hence, the latter had a content closer to the optimum. Nevertheless, normal starch resulted in smaller loaves. However, since both starch samples differed a lot from the optimum value, it might not have given any distinct effect on final bread volume. Moreover, the influence from the different gelatinization temperatures was likely more pronounced.

More Proteins Yields Darker Crusts

The bread samples having a higher protein content got significantly darker crusts. Moreover, the samples made of reconstituted flours, except from RQG+NSF, also yielded darker crusts, as already seen in Graßberger et al. (2003). However, it is questionable whether the differences observed in present study has an impact on consumer perception.

The Reliability of Crust Texture Measurements

The strength of the breadcrusts was measured via tensile testing. Although RQG+NSF had a significantly stronger crust than RNG+QSF, no other distinguished trend was observed. However, the results obtained in this analysis should be evaluated with careful consideration. There were difficulties in preparing the sample with the equipment to ensure accurate measurements. If the clamps were tightened too much, the crust tended to rupture near the clamping points. Hence, the equipment may have influenced the sample. Nevertheless, all measurements were included in the results, regardless of the rupture location. Conversely, if the clamps were not sufficiently tightened, they lost the grip on the sample. However, in such cases, the measurements were excluded from the results.

Impact on Starch of the Fractionation Process

The results obtained in this thesis indicate that normal starch exhibits a lower quality, which negatively impacts the bread outcome. However, upon comparison of the native and reconstituted breads, no significant difference was observed between NQWF and RQWF on volume and texture measurements. In contrast, comparison between the normal samples revealed significant differences, with the reconstituted sample showing lower quality in both aspects. Thus, the differences observed in this study may stem from that normal starch was harmed during the fractionation process, whilst quality starch was not.

MacRitchie (2016) emphasize that a vital criterion for drawing conclusions from research conducted using the fractioning and reconstitution method is that the functional properties of the examined components remain the same after the process. In this study, this concern was considered through analysis of RQWF and RNWF, which both functioned as control samples. Although analysis of them indicates that the functional properties of RNWF was altered, it is still unclear how it was harmed. However, since both samples underwent identical treatments, it can be concluded that the components of RNWF in such a case is more fragile.

5.2.1 Did Dough Properties Predict Bread Qualities?

Tronsmo et al. (2003) explains that doughs with high strain hardening coefficient-(k) and index (n) values are associated with good bread quality. This phenomenon

was somewhat consistent with the k values obtained in this study, but not with the n values. For instance, NQWF had a higher k value than RNWF, which was expected given the bread quality differences between them. However, RQG+NSF had a greater value than RNG+QSF. Nevertheless, the latter exhibited a better baking performance. Moreover, no significant difference was found between the k and n values. Thus, no conclusion can be drawn.

The quality of the final breads could neither be fully predicted by the extensional viscosity of its dough. For instance, although NQWF and NNWF doughs differed significantly in extensional viscosity, the baked breads showed no significant difference in texture and only a small, but still significant, difference in volume.

Interestingly, a clearer trend was observed when comparing the native and reconstituted flours. Hence, doughs made of the native flours exhibited significantly higher extensional viscosity than the reconstituted ones. Among the reconstituted samples, those containing normal starch displayed the highest values, even though the differences were not statistically significant. This indicates that the fractionated normal starch impaired the doughs extensional capacity, which may also be a reason for the deflated bread volumes.

However, due to time constraints, the dough rheology measurements were performed with less replicates than the conducted texture analyses. Hence, increasing the number of replicates could have led to a bigger and more plausible difference between the samples, aligning more closely with the final bread textures.

5.3 The Unsuccessful Fractioning Method

The aim of the wheat flours fractionation was to achieve a complete separation of starch and gluten. However, this was not fulfilled, as a large proportion of starch remained in the gluten phase. Wheat flour fractionation can be carried out either mechanically, as in the present study, or manually through traditional seitan-style kneading by hand. Due to time constraints, it was not possible to conduct a comparative test to determine the most efficient method. Nevertheless, the former method was selected as it was expected to provide the most equal treatment across all samples. However, complete separation was not achieved, which may have been improved by extending the kneading time above 1×6 min. This duration was initially chosen to minimize its impact on the gluten properties, as the gluten network begins to develop when kneaded in the presence of excess water (Dizlek and Awika, 2023). Thus, it is possible that the hand-kneading method could have produced a more desirable outcome, which should be considered in future research.

5.4 Sustainability Implications

Global warming is altering the quality of wheat grains (Wei et al., 2023). In the worst-case scenario, reduced grain quality can lead to unusable wheat yields and increased food losses, directly conflicting with the United Nations Sustainable Development Goal 12.3, which aims to reduce food loss. However, this present study has identified certain starch properties that contribute to breads of higher quality. Consequently, these properties should be considered both when selecting wheat varieties for cultivation and in breeding programs for future cultivars. The findings in this study clearly demonstrate that flour quality is influenced not only by protein content, but also by certain starch properties. Thus, focusing solely on protein aspects when aiming to improve wheat quality may hinder progress toward achievement of the United Nations goals.

5.5 Future Research

The disparities between the findings of this study compared to previous research highlight the complexity of the topic and the need for further investigation. Although all analyses conducted consistently show that the quality of starch has an impact on the bread outcome, this thesis could not determine why it has such a big influence.

Primarily, previous publications emphasize the impact of the starch AM and AMP ratio on bread quality. This property was not examined in this study, but analysing it would have provided a better molecular understanding of the samples and thereby a possible explanation why quality starch yielded better quality.

Moreover, there are indications that the normal starch was harmed during the fractionation process. Hence, to enable improvement of the processing method and thereby receiving more reliable results, the degree of damage should be examined. This could for example be conducted by analysing the level of damaged starch using a light microscope.

Finally, since the fractioning of gluten and starch in this study was unsuccessful, also the exchange of starch phase between the two flours fell short. However, by repeating the study with an optimized fractioning method, it could be determined whether the disparities observed in this study increase as the starch exchange rate rises, which in that case would confirm the results obtained in present measurements.

5.6 Conclusion

The present study clearly demonstrates that bread quality is influenced not only by gluten properties but also significantly by starch characteristics. Thus, bread made from a reconstituted flour having degraded gluten and preeminent starch properties yielded breads with larger volumes and softer textures compared to those made from a flour with preeminent gluten and degraded starch properties. Hence, this provides a suggestion on how the quality of already existing flours can be enhanced.

The high-quality starch exhibited a slightly higher A granule content, a significantly higher gelatinization temperature, and a significantly lower viscosity upon gelatinization. Hence, these properties may play a key role in improving bread quality and should be considered in the selection and breeding of future wheat cultivars that can continue to produce high-quality wheat despite the challenges caused by global warming.

Additionally, fractionating wheat flours with a kneading machine appears to require a longer kneading time than the 1×6 min used in this study. The results also suggest that starch from low-quality flour may require a gentler treatment, as it may have been damaged by the present processing method. Consideration of these aspects is necessary for improvement of future research on this topic.

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Popular Science Summary

The main ingredient in wheat bread is wheat flour. However, there is a wide variety of wheat flour types that can be used for different purposes, with the main difference being their composition. The primary component in wheat flour, making up about 75% of its weight, is a carbohydrate named starch. The second major component is a protein called gluten. However, even if the gluten is present in much smaller quantities, it is usually considered having the biggest impact on the baked bread quality. Consequently, flours of high quality typically contain a higher proportion gluten compared to flours of lower quality.

However, the exact composition of these components can vary based on the environmental conditions during the growth of wheat grains. An increased temperature, which is becoming more common due to global warming, have been shown to increase the gluten concentration, while reducing the concentration of starch. Nevertheless, there is a lack of research examining whether high-quality flour is better due to its starch also being of higher quality, not just its gluten. Understanding the flour components and its properties is crucial, as we may need to select specific wheat cultivars in the future that can produce flours of high quality, even in higher temperatures. Therefore, this study aimed to explore whether starch also plays a role in the outcome of bread baking.

This question was investigated by baking breads using different flours having various starch and gluten properties. Several quality measurements were then conducted on the baked breads, and the results from these revealed that breads made of a flour containing low-quality gluten and high-quality starch had greater volume, a softer texture, and a darker crust, compared to breads made of high-quality gluten and low-quality starch flour. These results indicates that not only gluten influences bread quality, also starch does.

The results obtained in this study is important for two main reasons. Firstly, it shows that the quality of already existing flours can be improved by adding starch with certain properties. Secondly, it reveals that starch properties also should be considered when deciding which wheat grains to breed and cultivate in the future, and not only their gluten properties.

Appendix 1

Table 7. Detailed information about the two native flours

	NQWF	NNWF
Protein content (%)	14.3	11.5
Ash content (%)	0.69	0.59
Water absorption (%)	64.2	62.4
Falling number (s)	343	369
Bread pores (Dallman)	6.5	6
Dough development time (min)	4.2	2.6
Dough stability (min)	6.2	6.6

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