



Effect of hydrothermal treatment and drying temperature on the pasting properties of whole grain cereal flours

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Degree project/Independent project • 15 credits
Swedish University of Agricultural Sciences, SLU
Department of Molecular Sciences
Agriculture Programme – Food Sciences
Molecular Sciences, 2026:05
Uppsala, 2026



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Credits: 15 hp
Level: G2E
Course title: Bachelor Thesis in Food Science, G2E
Course code: EX0876
Programme/education: Agriculture programme - Food
Course coordinating dept: Department of Molecular Sciences
Place of publication: Uppsala
Year of publication: 2026
Title of series: Molecular Sciences
Part number: 2026:05
Keywords: hydrothermal treatment, starch annealing, rheology, wheat, rye, barley, whole grain, phytate degradation.

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Abstract

Whole cereal grains are increasingly promoted as sustainable and healthy food ingredients, and hydrothermal treatment can be used to improve mineral bioavailability through phytate degradation. Such treatment may however also influence starch functionality. The aim of the study was to investigate how hydrothermal treatment and different drying temperatures affect the rheological properties of whole grain wheat, rye, and barley flour. Rheological measurements, microscopy, and particle size distribution analyses were performed on untreated and hydrothermally treated cereal samples dried at different temperatures, which were supplied by the company Good Grains. The barley samples generally exhibited rheological behaviour most consistent with starch annealing, including increased pasting onset together with reduced peak, final and breakdown viscosity, while the wheat and rye samples instead showed more mixed responses. Differences in particle size distribution and possible partial pre-gelatinization may also have contributed to the observed rheological behaviour. Overall, the results suggest that hydrothermal treatment may influence starch functionality in whole grain cereal flours in ways partly consistent with starch annealing, particularly in barley, which may have potential applications in food products where improved thermal and shear stability are desirable.

Keywords: hydrothermal treatment, starch annealing, rheology, wheat, rye, barley, whole grain, phytate degradation.

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Abbreviations

Abbreviation	Description
BD	Breakdown Viscosity
GS	Gelatinisation Slope
HT	Hydrothermally treated whole grain flour dried at 50 °C
HTF	Hydrothermally treated whole grain flour dried at 70 °C
HTF85	Hydrothermally treated whole grain flour dried at 85 °C
OB	Untreated whole grain flour
PO	Pasting onset
PV	Peak viscosity
FV	Final viscosity
RG	Retrogradation
RVA	Rapid Visco Analyser

1. Introduction

Especially in high-income countries there is a growing shift toward a more plant-based diet. This shift is primarily driven by environmental and health concerns, with an emphasis on the benefits of foods such as cereal grains and legumes. The production systems for cereal grains and legumes are generally more sustainable compared to animal-based food production, leading to lower greenhouse gas emissions, reduced resource depletion, and less biodiversity loss overall. Whole cereal grains and legumes are also associated with positive health outcomes, such as lowering the risk of non-communicable diseases (Poutanen et al., 2022; Rööös et al., 2020).

A nutritional challenge arising from this dietary shift is micronutrient deficiency, particularly iron deficiency, partly due to anti-nutritional compounds such as phytate, which reduce mineral bioavailability in grains and legumes (Leonard et al., 2023). Even though the prevalence of iron deficiency is lower in the Nordic countries compared to the global average, there are key risk groups with higher iron requirements, such as young children, adolescent girls, women of childbearing age, and pregnant women (Domellöf and Sjöberg, 2024), which may face an increased risk when shifting towards a more plant-based diet (Rööös et al., 2020). This challenge is also part of a broader global issue. The World Health Organization has reported that around 2 billion people suffer from micronutrient deficiencies worldwide, with vitamin A, iodine, iron, and zinc being among the most common deficiencies (World Health Organization, 2021).

One way to address this nutritional challenge is to increase mineral bioavailability in cereal grains through hydrothermal treatment, in which whole grains are exposed to excess water and moderate heat (50-60 °C) for an extended duration (12-24 hours). These conditions are favourable for endogenous phytase enzymes, which degrade phytate and thereby increase the bioavailability of minerals such as iron and zinc. With optimal conditions it is possible to achieve a near complete reduction of phytate levels in this way (Fredlund et al., 2006).

While hydrothermal treatment can improve mineral bioavailability and be a solution to the challenges of micronutrient deficiency, it may also affect the functional properties of cereal grains. Starch is the major structural component of cereal grains, and changes in its properties could for example influence texture, processing behaviour, and thermal stability. Understanding these possible effects is therefore important when evaluating the types of food products that hydrothermally treated cereal grains could be suitable for.

Starch is composed of two glucose polymers, amylose and amylopectin. These polymers are organized into amorphous and semi-crystalline regions that together form starch granules. The arrangement and interactions of these polymers within the granule strongly influence starch functionality. When starch is exposed to heat and excess water its structure begins to change. The initial structural changes occur once the glass transition temperature is exceeded, resulting in a transition from a rigid and glassy state to a more flexible and rubbery one, with increased molecular mobility (Jayakody and Hoover, 2008). As the temperature is increased further and reaches the gelatinization temperature, the granular structure starts to be irreversibly disrupted. Granules absorb water and swell, lose crystalline order, and leach amylose, eventually leading to granule rupture and loss of structural integrity (Li, 2022).

If starch is held above the glass transition temperature but below the gelatinization temperature in the presence of excess water, its properties can be altered through a process called annealing. Annealing induces molecular rearrangement within the starch granule, leading to increased structural order and crystalline perfection. Annealed starch generally exhibits increased thermal stability, resulting in higher gelatinization temperatures and a narrower gelatinization temperature range. Annealing is generally also associated with reduced granule swelling and decreased amylose leaching, which may result in a lower peak viscosity and less retrogradation. This physical modification is mainly performed on isolated starches in order to improve its functional properties for food production (Jayakody and Hoover, 2008), due to native starches having several limitations that restrict their applications. They do for instance become unstable during heating, shear, and acidic conditions, and also exhibit undesirable retrogradation, syneresis, and viscosity loss during processing and storage (Tharanathan, 2005).

While the effects of hydrothermal treatment on mineral bioavailability in cereal grains have been investigated, less attention has been given to changes in starch functionality. Since the conditions used during hydrothermal treatment overlap with those used during starch annealing, it was hypothesised that hydrothermally treated whole grains may exhibit annealing-like changes in starch functionality, such as increased pasting onset and reduced viscosity development. Annealing has been shown to occur in situ, producing structural changes comparable to those observed in isolated starch (Tester et al., 1997). In addition, the subsequent drying temperature may further influence starch structure and functionality, particularly at higher temperatures where partial gelatinisation may occur.

Rheological measurements were performed to investigate the effects of hydrothermal treatment on the functional properties of whole grain cereal flours.

Rheology is the study of the deformation and flow behaviour of materials, and rheological measurements can be used to analyse changes in starch functionality and their implications for food processing during mixing, heating, and cooling (Otegbayo et al., 2024). The present study was conducted in collaboration with the company Good Grains, which produces hydrothermally treated cereal grains. Whole grain samples of wheat (Dala lantvete), rye, and barley supplied by the company were analysed.

In addition to rheological measurements, microscopy and sieving analyses were performed to further characterise the grain samples. Brightfield microscopy, polarized light microscopy, and iodine staining were used to provide qualitative information regarding starch gelatinization, morphology, and the presence of damaged starch in the different samples. Sieving analysis was also performed to determine particle size distribution. The degree of gelatinization, starch accessibility, and particle size distribution were used to support the interpretation of the rheological results.

The aim of this study was to investigate how hydrothermal treatment and subsequent drying temperature affect the pasting behaviour of whole grain wheat, rye, and barley flours. The study specifically evaluated changes in operationally defined rheological parameters derived from viscosity–time curves, including pasting onset, gelatinisation slope, peak viscosity, breakdown viscosity, final viscosity, and viscosity increase during cooling, and assessed whether the observed rheological responses were consistent with annealing-like changes in starch functionality. Microscopy and particle size distribution analysis were used to support the interpretation of the rheological results.

2. Method

2.1 Materials

Milled whole grain samples of wheat (Dala lantvete), rye, and barley were supplied by the company Good Grains. The grains originated from different farmers in the Sörmland region. Rye and wheat were harvested in 2025, while the barley was harvested in 2024. For rye and wheat, three treatment groups were analysed: untreated grains (OB), hydrothermally treated grains dried at 50 °C (HT), and hydrothermally treated grains dried at 70 °C (HTF). Barley samples included an additional treatment group consisting of hydrothermally treated grain dried at 85 °C (HTF85). All samples were milled prior to analysis using a KoMo Duo mill. The moisture content of each sample was determined by oven drying and calculated from the difference in mass before and after drying.

2.2 Rheometer sample preparation

Slurries were prepared by mixing flour with water to obtain a total mass of 28 g, corresponding to 16% dry matter and 84% water. All slurries were prepared manually in glass containers by mixing the flour and water with a spoon. A pre-treatment step was performed on the wheat samples to ensure full hydration and to enable earlier onset of viscosity development during rheological measurements. The slurry was held at 50 °C for 30 min under continuous stirring (Fisherbrand magnetic hotplate stirrer, Fisher Scientific, Waltham, MA, USA) at 200 rpm. This shifted the onset of viscosity development to earlier times without altering the overall curve profile, thereby allowing more informative data to be collected within the measurement window. For rye and barley samples, slurries were prepared by direct mixing without additional pre-treatment. Some degree of thickening was observed in OB rye and in HTF85 barley during pre-treatment, and it was therefore excluded from all rye and barley samples.

2.3 Rheological measurements

Rheological measurements were carried out using a TA Instruments Discovery HR-3 hybrid rheometer (TA Instruments, USA) equipped with a DIN concentric cylinder geometry and Peltier steel temperature control. A vaned stainless-steel Smart-Swap rotor (bob diameter 28 mm, bob length 42 mm) was used.

Measurements were conducted using TA Instruments Trios software (version 5.0.0.44608).

Apparent viscosity measurements were performed to characterise starch pasting and viscosity development during heating and cooling under continuous shear conditions. Samples were analysed using the following protocol:

1. Flow step at 50 °C for 10 s at 20.94 rad/s
2. Flow step at 50 °C for 30 s at 100 rad/s
3. Flow step at 50 °C for 5 s at 20.94 rad/s
4. Temperature ramp from 50 °C to 95 °C at 7 °C/min at 20.94 rad/s
5. Isothermal hold at 95 °C for 1800 s at 20.94 rad/s
6. Cooling ramp from 95 °C to 25 °C at 4 °C/min at 20.94 rad/s, followed by a 300 s hold at 25 °C

Measurements were initiated after equilibration to the target temperature. Data were collected at sampling intervals between 3 and 10 s depending on the measurement step. Apparent viscosity was recorded continuously as a function of time and temperature.

To reduce evaporation during measurements, a plastic cover consisting of two semicircular parts surrounding the rheometer spindle was placed over the geometry during each run.

For each grain type and treatment condition, three biological replicate measurements were performed, resulting in a total of 30 rheological runs. Each replicate consisted of an independently prepared slurry.

Raw rheological data were exported from the Trios software as text files and processed in Microsoft Excel. The initial homogenisation and equilibration stages conducted at 50 °C (stages 1-3) prior to the heating ramp were excluded from subsequent analysis, as these stages were used to standardise sample conditions before thermal treatment.

To reduce measurement noise while preserving overall curve morphology, viscosity data were smoothed using a five-point moving average. All subsequent parameter calculations were based on the smoothed viscosity curves. Mean curves from the three biological replicates were used for graphical presentation, while parameter extraction and statistical analyses were performed on individual replicate curves. Data are presented as mean values \pm standard deviation.

Since the rheological measurements were performed using a custom protocol rather than a standardized RVA method, the rheological parameters used in the

present study were operationally defined based on characteristic features of the viscosity curves (Figure 1). Parameter definitions were developed to allow consistent comparison across grain types and treatment conditions.

Pasting onset was defined using a combined viscosity- and slope-based threshold approach. A stable baseline region was first established from the initial low-viscosity portion of the smoothed curve prior to starch pasting. The mean baseline viscosity and mean baseline slope, together with their corresponding standard deviations, were calculated within this region. Thresholds for both viscosity and slope were subsequently defined as the respective baseline means plus five standard deviations. Pasting onset was identified as the first point at which both the calculated viscosity and slope exceeded their respective thresholds for five consecutive measurements. This approach was selected to improve robustness across samples exhibiting different pasting behaviours, including both gradual and sharp viscosity increases.

Gelatinisation slope was defined as the slope between the pasting onset point and the first local maximum identified in the smoothed viscosity curve following pasting onset. The first local peak was used as an intermediate reference point for the calculation of gelatinisation slope. To account for potential delayed peak formation and minor fluctuations in the viscosity profile, true peak viscosity was subsequently defined as the maximum viscosity observed within a defined interval surrounding the first local peak.

Breakdown viscosity was defined as the difference between true peak viscosity and the minimum viscosity observed after peak development and prior to the increase in viscosity during cooling. Final viscosity was determined from a conservatively defined interval within the cooling phase that covered the majority of the cooling region while excluding the endpoint regions of the measurement. Within this interval, the highest viscosity value was identified, after which final viscosity was calculated as the mean of the ten preceding viscosity measurements. This approach was applied to minimise the influence of end-of-measurement fluctuations and improve consistency across samples. Retrogradation was finally defined as the difference between final viscosity and minimum viscosity.

One-way analysis of variance (ANOVA) was performed using the Data Analysis ToolPak in Microsoft Excel. When significant differences were observed ($p < 0.05$), pairwise comparisons were conducted using Tukey's honestly significant difference (HSD) test calculated manually in Excel.

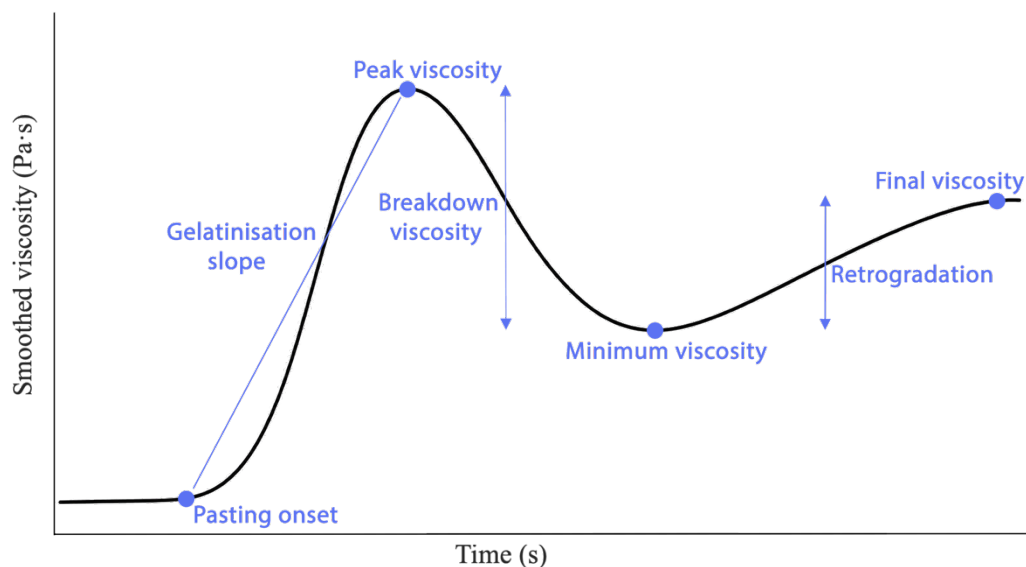


Figure 1. An illustration of the operationally defined rheological parameters derived from the viscosity–time curves used in this study: pasting onset, gelatinisation slope, peak viscosity, breakdown viscosity, final viscosity, and retrogradation. The minimum viscosity after peak development is shown as a reference point for calculation of breakdown viscosity and retrogradation. The figure is illustrative and does not represent measured data from a specific sample.

2.4 Microscopy analysis

Microscopy was performed in order to qualitatively evaluate starch granule morphology, degree of gelatinization, and the presence of damaged starch in the grain samples. Brightfield microscopy, polarized light microscopy, and iodine staining microscopy were carried out using a light microscope (Nikon Eclipse Ni-U, Nikon Corporation, Tokyo, Japan). Images were captured using a Nikon Digital Sight DS-Fi2 camera.

For all microscopy analyses, slurries were prepared with a 1:10 flour-to-water ratio. The slurry was mixed and directly pipetted onto a microscope slide before being covered with a coverslip. For brightfield microscopy and polarized light microscopy, the unstained slurry was analysed directly. Brightfield and polarized images were obtained at 20 \times magnification. Polarized light microscopy was performed using the polarized light function of the same microscope.

For iodine staining, 1 mL of slurry was mixed with 20 μ L of Lugol’s solution (0.05 g/L iodine). The stained slurry was then pipetted onto a microscope slide and covered with a coverslip. Images were captured immediately after staining in order to visualize accessible starch regions before further diffusion of the stain. Since iodine primarily stains accessible starch, the staining was used as a

qualitative indicator of starch damage. Iodine-stained samples were imaged at 4×, 10×, and 20× magnification.

Microscopy observations were evaluated qualitatively. No quantitative image analysis or scoring system was applied. The microscopy results were used to support interpretation of rheological behaviour and differences in starch functionality between treatments.

2.5 Particle size distribution analysis

Particle size distribution of the milled grain samples was determined by dry sieve analysis using a Retsch AS 200 Control sieve shaker (Retsch GmbH, Germany). Stainless steel analytical sieves with mesh openings of 1000, 600, 425, 250, 150, 75, and 50 μm were stacked in descending order, with a collection pan positioned below the 50 μm sieve to collect the remaining fine material.

90 g of flour was loaded directly into the sieve stack for each analysis. One representative measurement was performed for each sample. Sieving was carried out under dry conditions at an amplitude of 1.10 mm with an interval time of 10 s and a total sieving time of 6 min. After sieving, the mass retained on each sieve fraction was determined gravimetrically. The total recovered sample mass after sieving was slightly lower than the initial 90 g, due to minor material losses during handling. All sieve fractions were therefore normalized to the total recovered sample mass and expressed as the percentage retained in each particle size fraction. Particle size distribution curves were subsequently generated in Microsoft Excel. The obtained particle size distributions were used as supporting data for the interpretation of rheological behaviour.

3. Results

3.1 Effect of hydrothermal treatment on wheat pasting properties

Several pasting properties of whole wheat flour differed significantly between treatment groups (Table 1; Figure 2). Pasting onset was significantly higher in the HTF sample compared to the OB and HT samples, while no significant difference was observed between OB and HT. The HT sample exhibited the highest gelatinisation slope, indicating a more rapid viscosity development during gelatinisation compared to both OB and HTF.

HT and HTF samples exhibited significantly higher peak viscosity values compared to the OB sample, while no significant difference was observed between HT and HTF. In the case of breakdown viscosity, HT showed the highest value, differing significantly from OB, whereas HTF displayed intermediate behaviour and did not differ significantly from either HT or OB.

Final viscosity increased significantly with treatment intensity. The HTF sample exhibited the highest value, followed by HT and OB. Retrogradation did not differ significantly between treatments.

Table 1. Effect of thermal treatments on the rheological pasting properties of whole wheat flour determined using the custom protocol.

Sample	PO (s)	GS (Pa·s/s)	PV (Pa·s)	BD (Pa·s)	FV (Pa·s)	RG (Pa·s)
OB	581.40 ± 22.15 ^b	0.00066 ± 0.00003 ^c	0.48 ± 0.02 ^b	0.12 ± 0.00 ^b	0.57 ± 0.05 ^c	0.21 ± 0.07
HT	572.40 ± 4.61 ^b	0.00121 ± 0.00003 ^a	0.67 ± 0.03 ^a	0.25 ± 0.02 ^a	0.73 ± 0.02 ^b	0.31 ± 0.03
HTF	631.38 ± 14.80 ^a	0.00103 ± 0.00003 ^b	0.71 ± 0.04 ^a	0.13 ± 0.08 ^{ab}	0.85 ± 0.05 ^a	0.27 ± 0.10

Note: Values are presented as mean ± standard deviation ($n = 3$). PO = pasting onset; GS = gelatinisation slope; PV = peak viscosity; BD = breakdown viscosity; FV = final viscosity; RG = retrogradation. OB = untreated whole grain flour; HT = hydrothermally treated whole grain flour dried at 50 °C; HTF = hydrothermally treated whole grain flour dried at 70 °C. Different superscript letters within a column indicate significant differences ($p < 0.05$) according to Tukey's honestly significant difference (HSD) test. No significant differences were observed for RG.

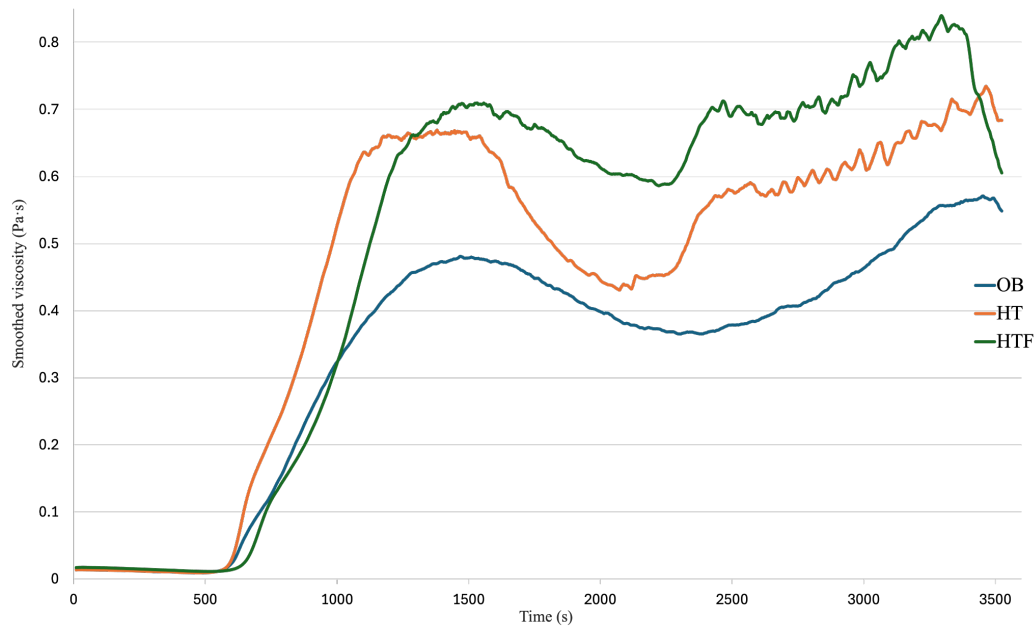


Figure 2. Smoothed viscosity curves of whole wheat flour samples during rheological analysis using the custom protocol. OB = untreated whole grain flour; HT = hydrothermally treated whole grain flour dried at 50 °C; HTF = hydrothermally treated whole grain flour dried at 70 °C. Curves represent the mean smoothed viscosity profiles of three biological replicates.

3.2 Effect of hydrothermal treatment on rye pasting properties

Several pasting properties of whole rye flour differed significantly between treatment groups (Table 2; Figure 3). Pasting onset was significantly higher in the HT sample compared to both OB and HTF samples, while no significant difference was observed between OB and HTF. The HTF sample exhibited the lowest gelatinisation slope, indicating a less rapid viscosity development during gelatinisation compared to OB and HT samples.

Peak viscosity increased with treatment intensity. The HTF sample exhibited the highest value, followed by HT and OB, with all groups differing significantly from each other. Breakdown viscosity showed the opposite trend, where HTF exhibited the lowest value and differed significantly from both OB and HT, while no significant difference was observed between OB and HT.

Final viscosity was significantly higher in HTF compared to both OB and HT, while no significant difference was observed between OB and HT. Retrogradation did not differ significantly between treatments, although OB showed higher values compared to HT and HTF.

Table 2. Effect of thermal treatments on the rheological pasting properties of whole rye flour determined using the custom protocol.

Sample	PO (s)	GS (Pa·s/s)	PV (Pa·s)	BD (Pa·s)	FV (Pa·s)	RG (Pa·s)
OB	439.40 ±	0.00260 ±	0.69 ±	0.41 ±	0.66 ±	0.38 ±
	1.74 ^b	0.00017 ^a	0.02 ^c	0.04 ^a	0.01 ^a	0.02
HT	479.38 ±	0.00269 ±	0.82 ±	0.31 ±	0.74 ±	0.23 ±
	6.89 ^a	0.00024 ^a	0.01 ^b	0.07 ^a	0.03 ^a	0.10
HTF	434.42 ±	0.00152 ±	0.87 ±	0.15 ±	0.97 ±	0.25 ±
	13.52 ^b	0.00003 ^b	0.01 ^a	0.03 ^b	0.08 ^b	0.05

Note: Values are presented as mean ± standard deviation ($n = 3$). PO = pasting onset; GS = gelatinisation slope; PV = peak viscosity; BD = breakdown viscosity; FV = final viscosity; RG = retrogradation. OB = untreated whole grain flour; HT = hydrothermally treated whole grain flour dried at 50 °C; HTF = hydrothermally treated whole grain flour dried at 70 °C. Different superscript letters within a column indicate significant differences ($p < 0.05$) according to Tukey's honestly significant difference (HSD) test. No significant differences were observed for RG.

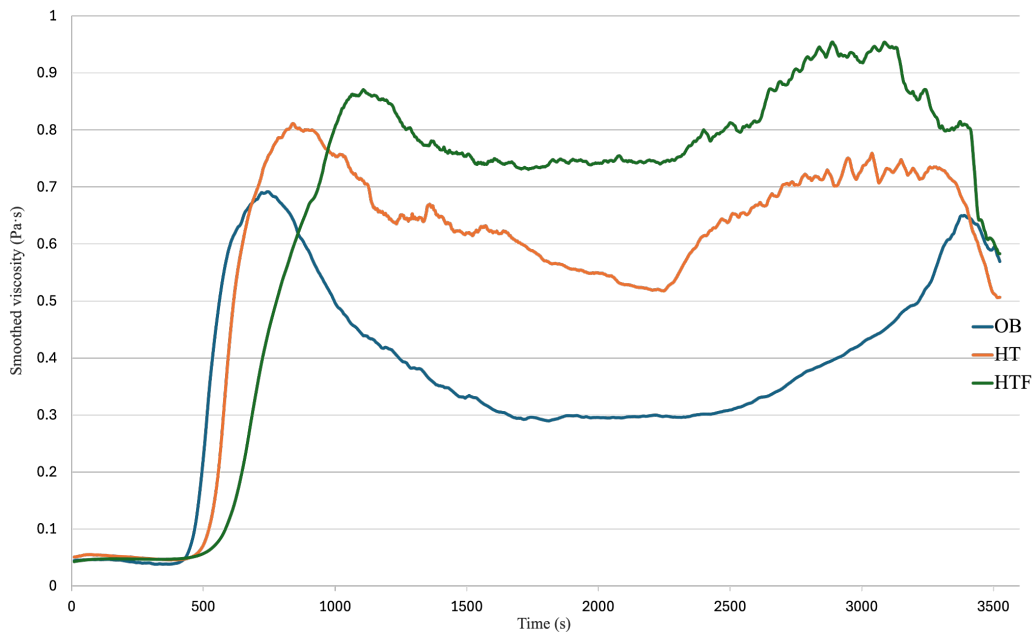


Figure 3. Smoothed viscosity curves of whole rye flour samples during rheological analysis using the custom protocol. OB = untreated whole grain flour; HT = hydrothermally treated whole grain flour dried at 50 °C; HTF = hydrothermally treated whole grain flour dried at 70 °C. Curves represent the mean smoothed viscosity profiles of three biological replicates.

3.3 Effect of hydrothermal treatment on barley pasting properties

Several pasting properties of whole barley flour differed significantly between treatment groups (Table 3; Figure 4). Pasting onset was significantly higher in both HT and HTF compared to OB. Gelatinisation slope decreased with increasing treatment intensity. OB and HT exhibited similar values, whereas HTF and HTF85 showed significantly lower values.

Peak viscosity differed significantly between treatments. OB exhibited the highest value, while HT showed the lowest. HTF displayed intermediate behaviour and did not differ significantly from either OB or HTF85. Breakdown viscosity generally decreased with treatment intensity, where OB exhibited the highest value and HTF85 the lowest.

No significant differences were observed for final viscosity or retrogradation, although HTF and HTF85 showed higher final viscosity values, and HTF85 had the lowest retrogradation value.

Table 3. Effect of thermal treatments on the rheological pasting properties of whole barley flour determined using the custom protocol.

Sample	PO (s)	GS (Pa·s/s)	PV (Pa·s)	BD (Pa·s)	FV (Pa·s)	RG (Pa·s)
OB	438.40 ± 7.97 ^a	0.00258 ± 0.00008 ^a	1.02 ± 0.05 ^a	0.47 ± 0.05 ^a	0.75 ± 0.08	0.19 ± 0.08
HT	481.43 ± 19.99 ^b	0.00240 ± 0.00021 ^a	0.84 ± 0.04 ^b	0.39 ± 0.05 ^{ab}	0.66 ± 0.14	0.21 ± 0.12
HTF	480.38 ± 10.80 ^b	0.00154 ± 0.00007 ^b	0.96 ± 0.04 ^{ac}	0.29 ± 0.08 ^{bc}	0.85 ± 0.05	0.18 ± 0.03
HTF85	405.41 ± 8.98 ^{ab}	0.00120 ± 0.00002 ^c	0.89 ± 0.03 ^c	0.18 ± 0.01 ^c	0.82 ± 0.07	0.11 ± 0.04

Note: Values are presented as mean ± standard deviation ($n = 3$). PO = pasting onset; GS = gelatinisation slope; PV = peak viscosity; BD = breakdown viscosity; FV = final viscosity; RG = retrogradation. OB = untreated whole grain flour; HT = hydrothermally treated whole grain flour dried at 50 °C; HTF = hydrothermally treated whole grain flour dried at 70 °C; HTF85 = hydrothermally treated whole grain flour dried at 85 °C. Different superscript letters within a column indicate significant differences ($p < 0.05$) according to Tukey's honestly significant difference (HSD) test. No significant differences were observed for FV or RG.

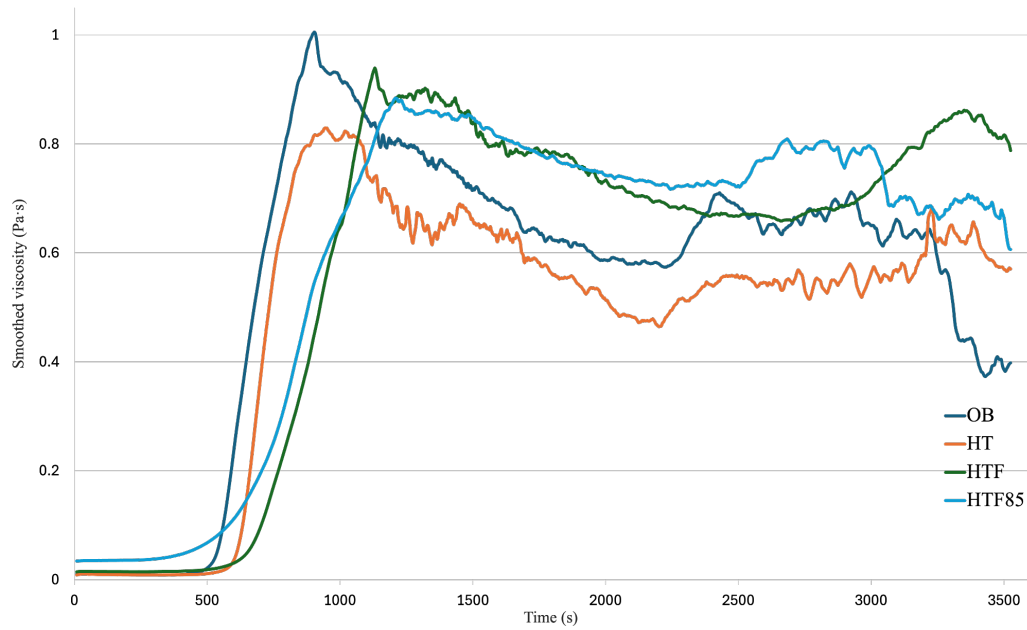


Figure 4. Smoothed viscosity curves of whole barley flour samples during rheological analysis using the custom protocol. OB = untreated whole grain flour; HT = hydrothermally treated whole grain flour dried at 50 °C; HTF = hydrothermally treated whole grain flour dried at 70 °C; HTF85 = hydrothermally treated flour dried at 85 °C. Curves represent the mean smoothed viscosity profiles of three biological replicates.

3.4 Particle size distribution

Particle size distribution differed substantially between treatment groups across all cereal grains, as can be seen in Figure 5. OB samples generally exhibited broader particle size distributions with higher proportions of larger particle fractions, whereas hydrothermally treated samples showed distributions shifted towards smaller particle sizes. This effect was most clear in the wheat and rye samples. For barley, the HT treatment had the highest fraction of smaller particles, while HTF and HTF85 showed a more intermediate distribution between HT and OB.

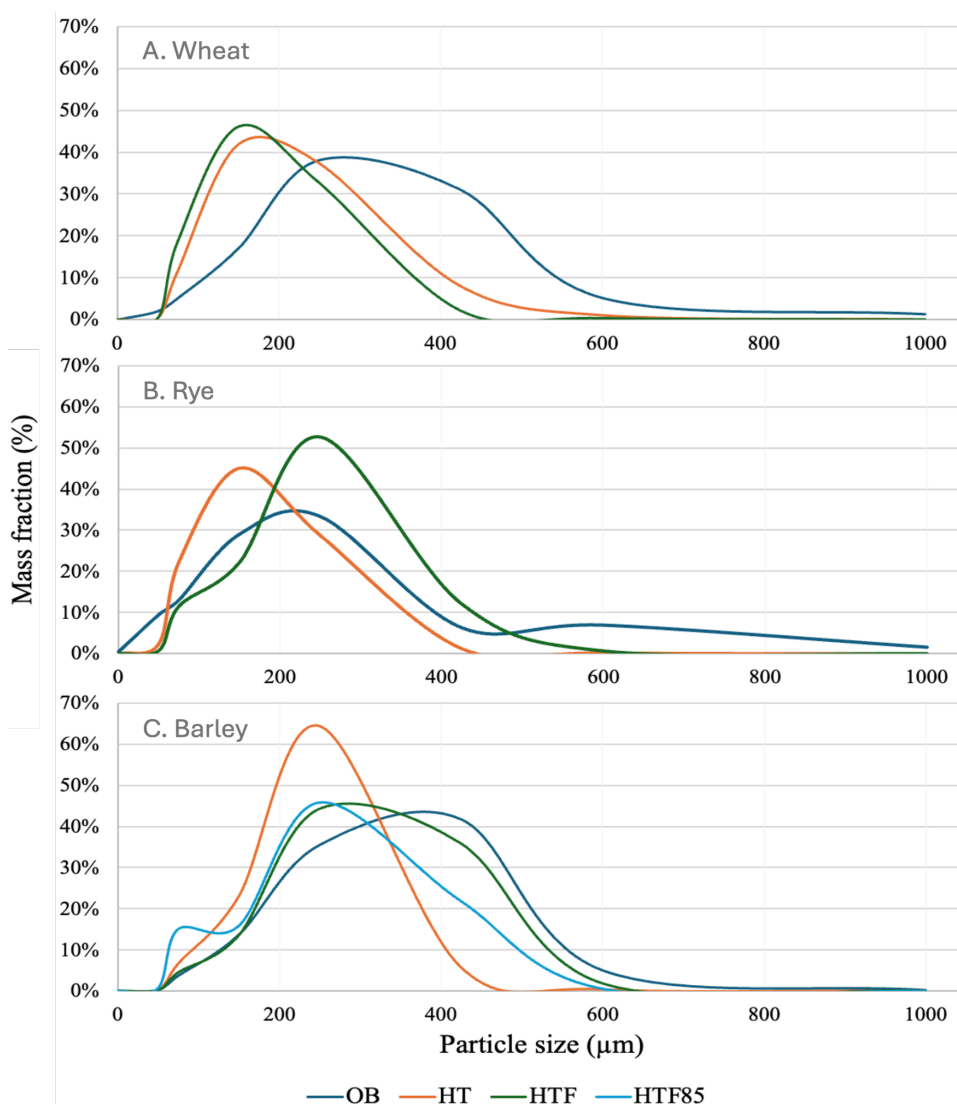


Figure 5. Particle size distribution curves for whole grain wheat (A), rye (B), and barley (C) flour samples. Curves represent the mass fraction (%) of particles across particle size classes for untreated whole grain flour (OB), hydrothermally treated whole grain flour dried at 50 °C (HT), hydrothermally treated whole grain flour dried at 70 °C (HTF), and, in the case of barley, hydrothermally treated whole grain flour dried at 85 °C (HTF85).

3.5 Microscopy

3.5.1 Brightfield microscopy

Brightfield microscopy showed limited differences in granule morphology between OB and HT, as can be seen in Figure 6-8. The starch granules in the OB samples generally appeared more uniform and had more clearly defined granule outlines, while in the HT samples some granules displayed less distinct outlines together with surface indentations and slightly irregular shape. These changes were more pronounced in the HTF and HTF85 samples, which had a greater proportion of granules with irregular shape and less clearly defined outline.

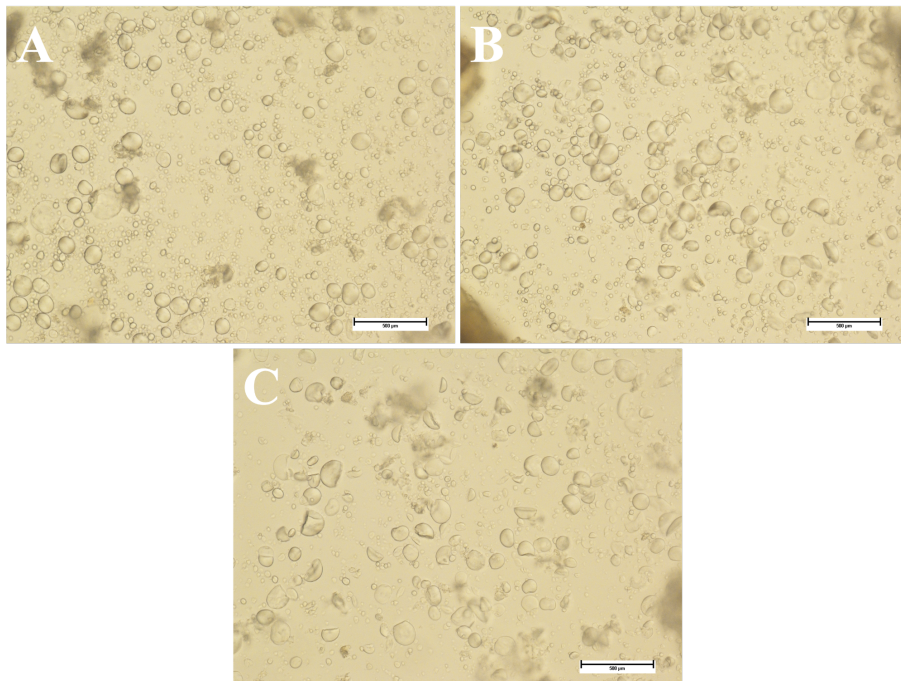


Figure 6. Brightfield microscopy images (20× magnification) of whole grain wheat flour samples: (A) untreated whole grain flour (OB), (B) hydrothermally treated whole grain flour dried at 50 °C (HT), and (C) hydrothermally treated whole grain flour dried at 70 °C (HTF). Scale bars = 500 µm.

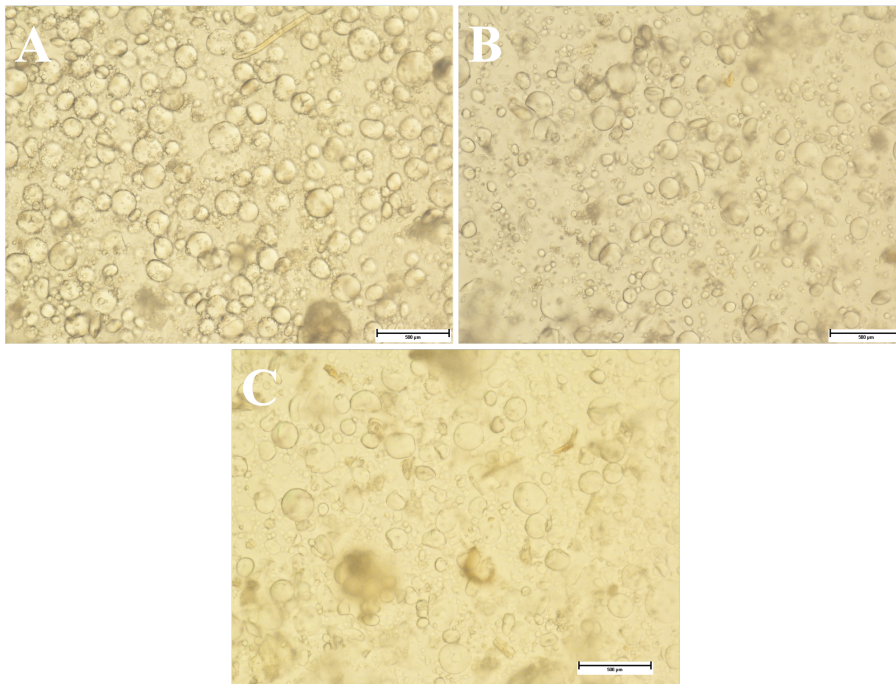


Figure 7. Brightfield microscopy images (20× magnification) of whole grain rye flour samples: (A) untreated whole grain flour (OB), (B) hydrothermally treated whole grain flour dried at 50 °C (HT), and (C) hydrothermally treated whole grain flour dried at 70 °C (HTF). Scale bars = 500 µm.

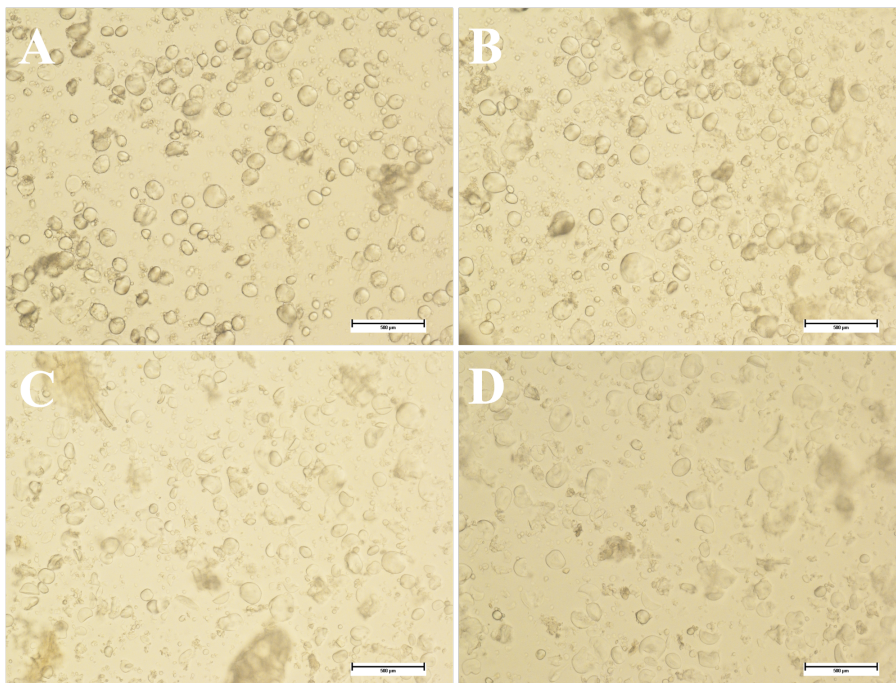


Figure 8. Brightfield microscopy images (20× magnification) of whole grain barley flour samples: (A) untreated whole grain flour (OB), (B) hydrothermally treated whole grain flour dried at 50 °C (HT), (C) hydrothermally treated whole grain flour dried at 70 °C (HTF), and (D) hydrothermally treated whole grain flour dried at 85 °C (HTF85). Scale bars = 500 µm.

3.5.2 Polarized light microscopy

Polarized light microscopy revealed differences in starch birefringence between OB and hydrothermally treated whole grain flour samples, as can be seen in Figures 9-11. The OB and HT samples generally exhibited similar birefringence, where numerous starch granules showed distinct Maltese crosses, which is characteristic of preserved crystalline structure. In the HTF samples there instead was a clear reduction in both the amount and intensity of visible Maltese crosses, which indicates partial pre-gelatinisation. These changes were even more clear in the barley HTF85 sample.

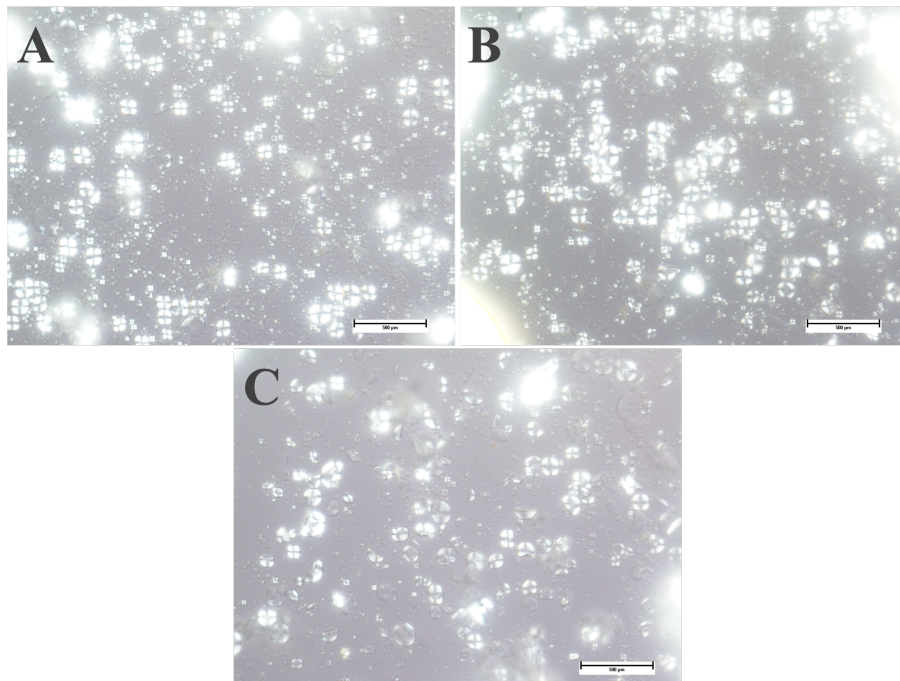


Figure 9. Polarized light microscopy images (20× magnification) of whole grain wheat flour samples: (A) untreated whole grain flour (OB), (B) hydrothermally treated whole grain flour dried at 50 °C (HT), and (C) hydrothermally treated whole grain flour dried at 70 °C (HTF). Scale bars = 500 µm.

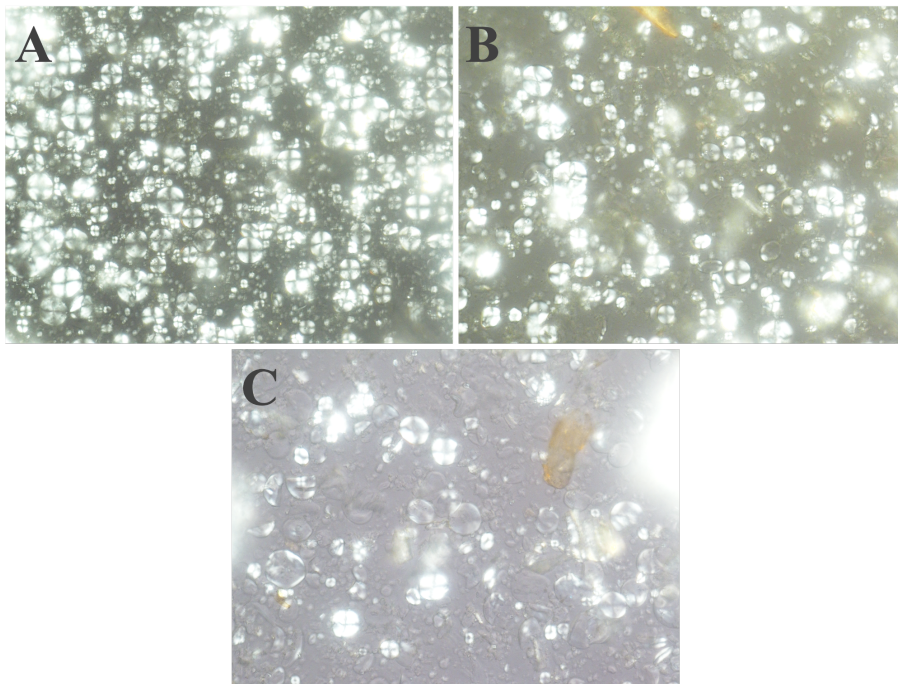


Figure 10. Polarized light microscopy images (20× magnification) of whole grain rye flour samples: (A) untreated whole grain flour (OB), (B) hydrothermally treated whole grain flour dried at 50 °C (HT), and (C) hydrothermally treated whole grain flour dried at 70 °C (HTF). Scale bars = 500 μm.

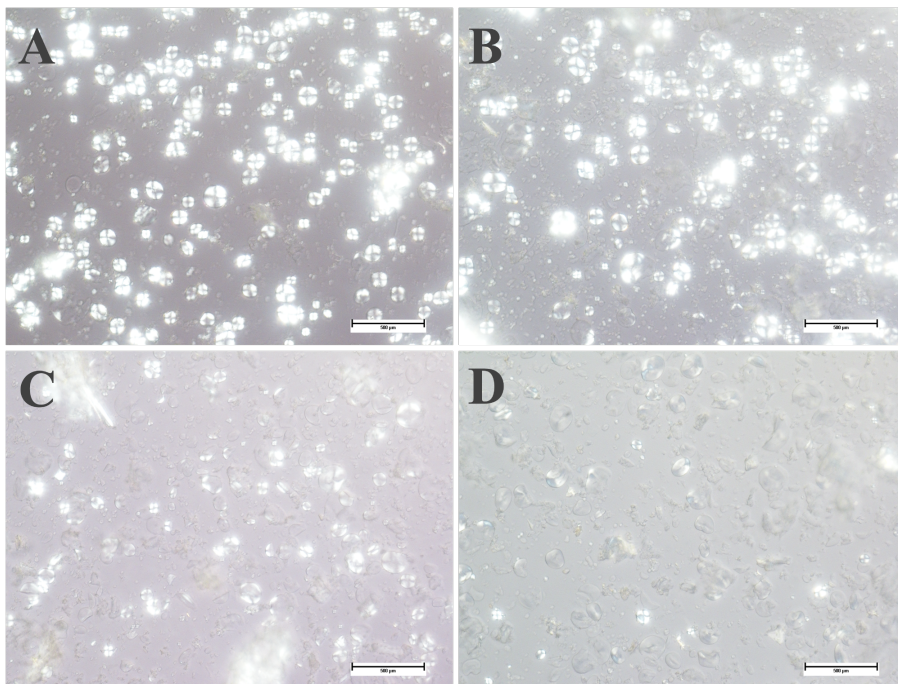


Figure 11. Polarized light microscopy images (20× magnification) of whole grain barley flour samples: (A) untreated whole grain flour (OB), (B) hydrothermally treated whole grain flour dried at 50 °C (HT), (C) hydrothermally treated whole grain flour dried at 70 °C (HTF), and (D) hydrothermally treated whole grain flour dried at 85 °C (HTF85). Scale bars = 500 μm.

3.5.3 Iodine staining microscopy

Iodine staining microscopy revealed differences in the amount of damaged starch between OB and the hydrothermally treated whole grain flour samples, as can be seen in Figures 12-14. The OB samples exhibited fewer stained regions compared to HT, HTF, and HTF85. Only minor visual differences were observed between the HT, HTF, and HTF85 treatments. Since imaging was performed immediately after staining, the iodine-stained regions likely represent accessible starch, such as damaged or structurally disrupted starch granules.

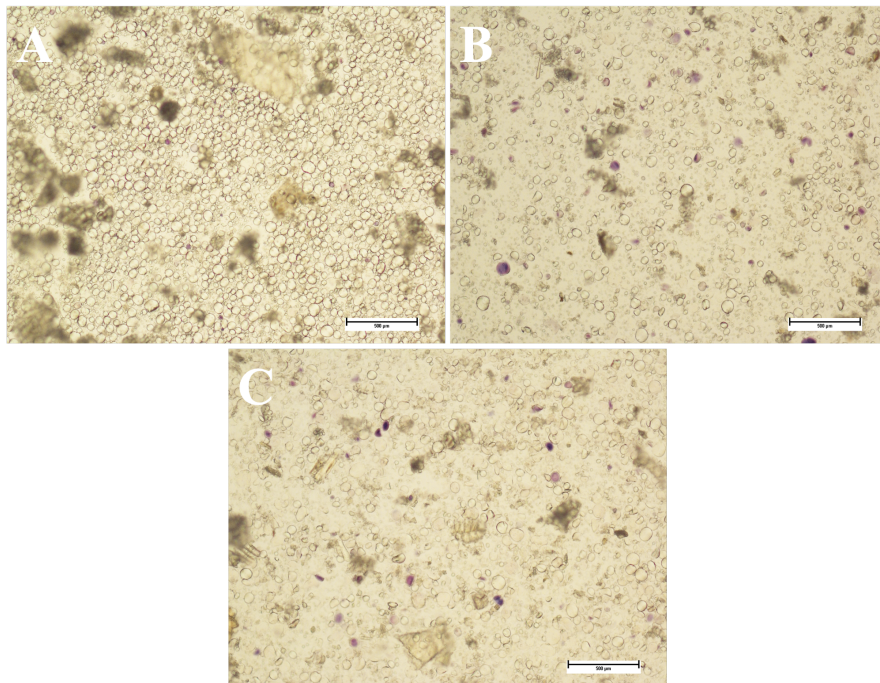


Figure 12. Iodine-stained microscopy images (10× magnification) of whole grain wheat flour samples: (A) untreated whole grain flour (OB), (B) hydrothermally treated whole grain flour dried at 50 °C (HT), and (C) hydrothermally treated whole grain flour dried at 70 °C (HTF). Scale bars = 500 μm.

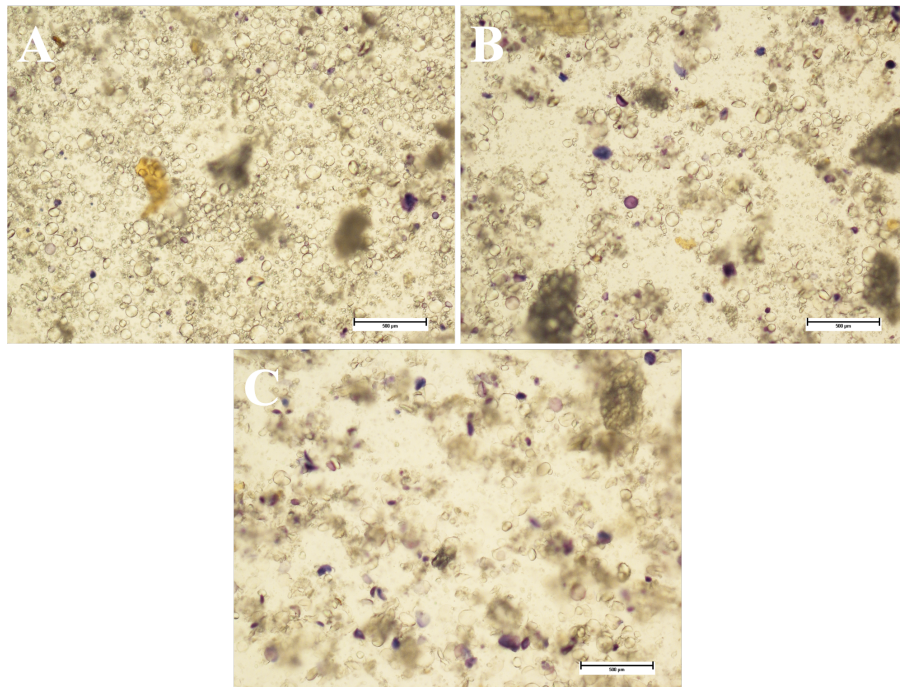


Figure 13. Iodine-stained microscopy images (10× magnification) of whole grain rye flour samples: (A) untreated whole grain flour (OB), (B) hydrothermally treated whole grain flour dried at 50 °C (HT), and (C) hydrothermally treated whole grain flour dried at 70 °C (HTF). Scale bars = 500 µm.

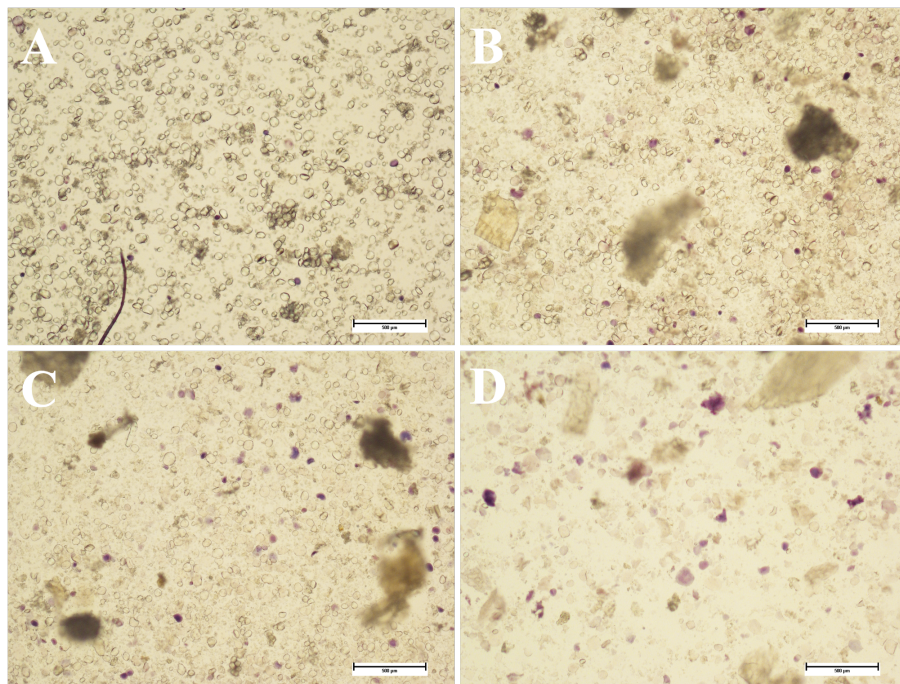


Figure 14. Iodine-stained microscopy images (10× magnification) of whole grain barley flour samples: (A) untreated whole grain flour (OB), (B) hydrothermally treated whole grain flour dried at 50 °C (HT), (C) hydrothermally treated whole grain flour dried at 70 °C (HTF), and (D) hydrothermally treated whole grain flour dried at 85 °C (HTF85). Scale bars = 500 µm.

4. Discussion

4.1 General considerations for interpreting the rheological results

Before discussing the rheological behaviour of the individual cereal flours, it is important to consider the differences observed between the different samples during microscopy and sieving. The particle size distribution analysis showed that the hydrothermally treated samples generally contained a higher fraction of smaller particles compared to the untreated samples. Smaller particle size increases the available surface area and improves water accessibility, and is generally expected to increase viscosity development (Guan et al., 2020). The higher fraction of smaller particles in the hydrothermally treated samples is therefore an important factor to keep in mind when interpreting the rheological results.

Polarized light microscopy showed a loss of birefringence in the hydrothermally treated samples dried at higher temperatures, which indicates that these samples were partly pre-gelatinized. Partial pre-gelatinization is generally expected to reduce viscosity development, since some of the starch granules' structure has already been disrupted (Pramana et al., 2024). The rheological results does however seem to suggest that partial pre-gelatinization was not the dominant factor in most samples. The HTF samples generally showed higher viscosity development compared to OB and HT samples in wheat and rye, despite showing some loss of birefringence. This indicates that the degree of partial pre-gelatinization was not sufficient to drive down the viscosity development overall. An exception was the barley HTF85 sample, where partial pre-gelatinization had a clearer effect. It had a considerably higher initial viscosity before pasting onset, and a slightly lower viscosity development, which is consistent with the fact that it showed the lowest birefringence during polarized light microscopy

The iodine staining microscopy showed that the hydrothermally treated samples appeared to contain more damaged starch than the untreated samples. A greater amount of damaged starch is generally expected to reduce viscosity development, as damaged granules may have a reduced ability to swell and maintain structure during heating (Guan et al., 2020). However, since viscosity development was generally higher in the hydrothermally treated wheat and rye samples, and the pasting onset was higher in both HT and HTF in the barley samples compared to OB, the presence of damaged or accessible starch does not appear to have been

the main factor controlling the rheological behaviour. It is possible that the increased amount of damaged starch was partly a consequence of the higher fraction of smaller particles in the hydrothermally treated flours. The positive effect in viscosity development of smaller particle size may therefore have outweighed the reducing effect from the damaged starch.

Another possible factor to consider in relation to the rheological results is protein denaturation during drying. The higher drying temperatures used for HTF and HTF85 may have affected the protein in the grains, which in turn could have influenced their viscosity development. Protein denaturation was however not measured in the present study, which makes the influence of protein denaturation on the viscosity development difficult to judge.

In addition to these sample-specific factors, the rheological results should also be interpreted in relation to what is typically observed for annealed starch. Annealed starches generally exhibit an increased pasting onset, where greater thermal energy is required to disrupt the more stable crystalline structure. Annealing is also generally associated with lower peak viscosity, lower breakdown viscosity, and reduced final viscosity and retrogradation, due to less swelling of the granules and higher stability during heating and cooling (Ariyantoro, Katsuno and Nishizu, 2018). Most evidence for these effects comes from studies focusing on isolated starches rather than whole grain flour systems. Direct comparisons should therefore be made with some caution, as starch granules in whole grain flour are embedded in their native cereal matrix.

Previous studies have nevertheless reported significant correlations between whole grain wheat and barley flours and their respective isolated starches in terms of pasting properties (Srichuwong et al., 2017; Fan et al., 2019), which suggests that overall changes in whole grain flour behaviour may still provide useful indications of changes in starch functionality. There have also been limited studies focusing directly on flour systems (Purnomo et al., 2024), in which the annealed flour exhibited similar changes in rheological behaviour compared to isolated starch.

4.2 Wheat

The wheat samples showed the least clear evidence of annealing-like behaviour among the cereals investigated. The significantly higher pasting onset observed for HTF compared with OB and HT could indicate increased thermal stability,

which is consistent with what is typically reported for annealed starch (Su et al., 2020; Vamadevan et al., 2013). Both HT and HTF also exhibited higher gelatinisation slope values than OB, indicating that viscosity development occurred more rapidly once gelatinisation had initiated. This could possibly be related to a narrower gelatinisation temperature range resulting from increased crystalline homogeneity, which has previously been observed in annealed starches (Fonseca et al., 2014). However, this interpretation should be made with caution, since the gelatinisation slope measured in the present study is not directly equivalent to a gelatinisation temperature range. The higher pasting onset in HTF should also be interpreted cautiously. If this increase was mainly caused by annealing during the hydrothermal treatment, a similar increase might have been expected in HT, since both samples underwent the same hydrothermal treatment and differed mainly in drying temperature. The fact that pasting onset increased only in HTF suggests that the higher drying temperature may have contributed to this effect, possibly through changes in the flour matrix such as protein denaturation. However, since protein denaturation was not measured in the present study, its contribution cannot be determined.

The peak and final viscosity values did not follow a clear annealing-like behaviour. Both HT and HTF exhibited significantly higher peak- and final viscosity compared to OB. This is opposite to the reduction in peak and final viscosity often associated with annealing (Ariyantoro, Katsuno and Nishizu, 2018). Even though an increased peak viscosity has been reported in some cases of annealed wheat starch (Fonseca et al., 2014), the simultaneous increase in final viscosity observed here suggests that the higher viscosity development was more likely due to the higher fraction of smaller particles in the hydrothermally treated samples.

Breakdown viscosity differed between HT and HTF. HT showed a substantially higher breakdown viscosity compared with OB, which is not typical for annealed starch, where breakdown viscosity is generally reduced (Ariyantoro, Katsuno and Nishizu, 2018; Liu et al., 2024). HTF did not show the same increase in breakdown viscosity as HT, and was instead similar to OB. This suggests that the higher drying temperature may have limited breakdown during heating. One possible explanation is partial pre-gelatinisation. If some granule disruption had already occurred before the rheological measurement, less additional breakdown may have occurred during heating.

Overall, the wheat results suggest that hydrothermal treatment and drying temperature affected the functional properties of the flour, but the response cannot be clearly attributed to starch annealing. The increased peak and final viscosity, together with the differing pasting onset and breakdown viscosity values between

HT and HTF, suggest that particle size distribution and drying temperature were the major contributing factors.

4.3 Rye

The rye samples showed a more complex response than wheat, with some responses indicating possible annealing-like effects from the hydrothermal treatment, although the interpretation was complicated by similar factors as for wheat.

Both HT and HTF exhibited higher peak viscosity than OB, and HTF also showed significantly higher final viscosity than both OB and HT. Final viscosity was only numerically higher in HT compared to OB. This increase in viscosity development is not typical of annealed starch (Ariyantoro, Katsuno and Nishizu, 2018), and was likely partly due to the higher fraction of smaller particles in the hydrothermally treated rye samples.

Some responses nevertheless suggest a possible annealing-like effect, especially in the HT sample. HT had a significantly higher pasting onset compared to OB, which may indicate increased thermal stability consistent with annealed starches (Vamadevan et al., 2013; Liu et al., 2024; Schierbaum and Kettlitz, 1994). In addition, breakdown viscosity and retrogradation were numerically lower in HT compared to OB. This is notable, even though these differences were not statistically significant, since the HT sample had a clearly higher fraction of smaller particles, which would generally be expected to increase viscosity development. The combination of increased pasting onset and numerically lower breakdown viscosity and retrogradation may therefore suggest an annealing-like effect from the hydrothermal treatment.

The HTF sample had the lowest breakdown viscosity and a lower retrogradation value compared to OB, which could indicate improved stability during heating and cooling. However, HTF also had the lowest pasting onset value. The considerably lower pasting onset suggests that other factors, such as partial pre-gelatinisation caused by the higher drying temperature, influenced the rheological behaviour.

Due to the lack of research on the relationship between whole grain rye flour and isolated rye starch pasting behaviour there are some limitations compared to wheat and barley. Possible annealing effects in rye flour should therefore be interpreted with caution. The rye results did however show that the hydrothermal

treatment altered the rheological properties of the flour, and some of the variables, particularly in HT, may indicate some annealing-like effect. The increased peak and final viscosity values in the hydrothermally treated samples also suggest that particle size distribution were important and may have masked a clearer annealing response.

4.4 Barley

The barley samples showed the clearest indications of annealing-like behaviour among the cereals investigated. Both HT and HTF exhibited significantly higher pasting onset values compared to OB, which may indicate increased thermal stability and is consistent with what is commonly reported for annealed starches, including barley starch (Liu et al., 2024; Vamadevan et al., 2013). This response differed from wheat and rye, where increased pasting onset was either only observed in one treatment or was more difficult to interpret due to other conflicting variables.

The peak and final viscosity values also supported a more annealing-like interpretation in barley compared to wheat and rye. HT exhibited significantly lower peak viscosity and numerically lower final viscosity compared to OB, despite having the highest fraction of smaller particles. Since smaller particle size would generally be expected to increase water accessibility and viscosity development, the reduced peak viscosity in HT suggests that hydrothermal treatment may have restricted granule swelling and increased starch stability. HTF had a particle size distribution more similar to OB and did not differ significantly from OB in either peak or final viscosity. Therefore, the viscosity development in HTF was less clearly reduced compared to HT, but it also did not increase in the same way as observed for HT and HTF in the wheat and rye samples.

Breakdown viscosity also decreased in both HT and HTF compared to OB. Reduced breakdown viscosity is often associated with annealing, since increased granule stability can improve resistance to disruption during heating and shear (Ariyantoro, Katsuno and Nishizu, 2018; Liu et al., 2024). This further supports the interpretation that hydrothermal treatment had a stabilising effect on the barley samples. Unlike wheat and rye, where increased peak and final viscosity complicated the interpretation of possible annealing effects, barley generally followed the pattern more commonly reported for annealed starch, with increased pasting onset and generally reduced viscosity development.

HTF85 differed somewhat from the other hydrothermally treated barley samples. Since it was dried at 85 °C, partial pre-gelatinisation likely had a greater influence on the rheological behaviour of this sample. HTF85 exhibited the lowest breakdown viscosity value, a low peak viscosity value, the lowest gelatinisation slope, and a final viscosity value similar to OB. It also had a lower pasting onset than HT and HTF. Partial pre-gelatinisation of barley starch has previously been reported to reduce pasting onset, peak viscosity, breakdown viscosity, and final viscosity (Boyd, Storsley and Ames, 2017; Hickman, Janaswamy and Yao, 2008). The reduced birefringence observed in polarized light microscopy also supports the interpretation that HTF85 had undergone a greater degree of starch disruption before the rheological measurement.

Overall, the barley results suggest that the hydrothermal treatment produced a clearer annealing-like effect compared to wheat and rye. The increased pasting onset in HT and HTF, together with reduced breakdown viscosity and the lower peak- and final viscosity in HT, indicates improved thermal and shear stability. The HT sample showed the clearest annealing-like behaviour, especially since it had the highest fraction of smaller particles. HTF85, however, should be interpreted separately, since the higher drying temperature likely caused significant partial pre-gelatinisation, which was probably the dominant factor determining the rheological behaviour, rather than any possible annealing-like effect.

4.5 Conclusion

The hydrothermal treatment used by Good Grains is primarily intended to promote phytate degradation rather than to optimise starch annealing. Since effective starch annealing requires temperatures close to, but below, the gelatinization temperature of the starch, and since gelatinization temperatures differ between different cereal grains, the treatment conditions may not have been equally favourable for annealing across all samples. This may partly explain the mixed rheological responses observed. It could therefore be interesting for Good Grains to optimise their hydrothermal treatment for both phytate degradation and starch annealing. This could result in clearer annealing-like effects.

However, under the conditions used in the present study, the barley samples did exhibit behaviour generally consistent with annealing. These rheological changes could potentially be useful in food applications where improved thermal and shear stability, as well as reduced swelling and viscosity, are desirable.

Even though barley may not be the most suitable cereal for soft breads due to its lack of gluten, the hydrothermally treated barley flour could be useful in products where firmness, crispness, and structural stability are desirable, such as Scandinavian-style hard breads or crackers, where reduced dough viscosity, firmer gel structures, and more controlled retrogradation could contribute positively to texture and handling during processing (Yao, Sui & Janaswamy, 2023).

Due to the improved thermal stability and more controlled retrogradation associated with annealed starches, the hydrothermally treated barley flour could also be useful in frozen food applications. Since frozen ready-to-eat products often undergo quality deterioration during frozen storage and reheating, the improved freeze-thaw stability associated with annealed starch systems could contribute positively to texture stability in products such as frozen bakery products, pies, or similar convenience foods (Yao, Sui & Janaswamy, 2023).

Since annealing has been associated with reduced starch swelling and solubility, as well as improved shear stability and toughness, the treated barley flour may contribute positively to texture and processing stability in pasta products (Yao, Sui & Janaswamy, 2023). It could be interesting to investigate composite flour systems where hydrothermally treated barley flour is combined with wheat flour in order to balance an improved nutritional profile with sufficient gluten network formation.

Additional experimental replicates in this study could have helped strengthen the statistical reliability of the observed trends, particularly for rye and barley in terms of breakdown viscosity and retrogradation behaviour when comparing the hydrothermally treated grains with OB. It could furthermore be interesting to repeat the experiments using more standardized milling conditions and tighter particle size control in order to reduce variability and more clearly isolate the effects of the hydrothermal treatment itself.

Further studies would benefit from separating the starch from the whole grain flour prior to rheological analysis in order to more clearly determine to what extent the observed changes were caused by starch annealing rather than by whole grain matrix effects. It could also be interesting to further investigate how hydrothermal treatment and different drying temperatures affect the proteins and fibre fractions of the cereals, since these components may also influence the practical applicability of hydrothermally treated grains.

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