Evaluating the impact of vital gluten on the protein molecular size distribution, loaf volume and dough rheology of vital gluten wheat flour blends

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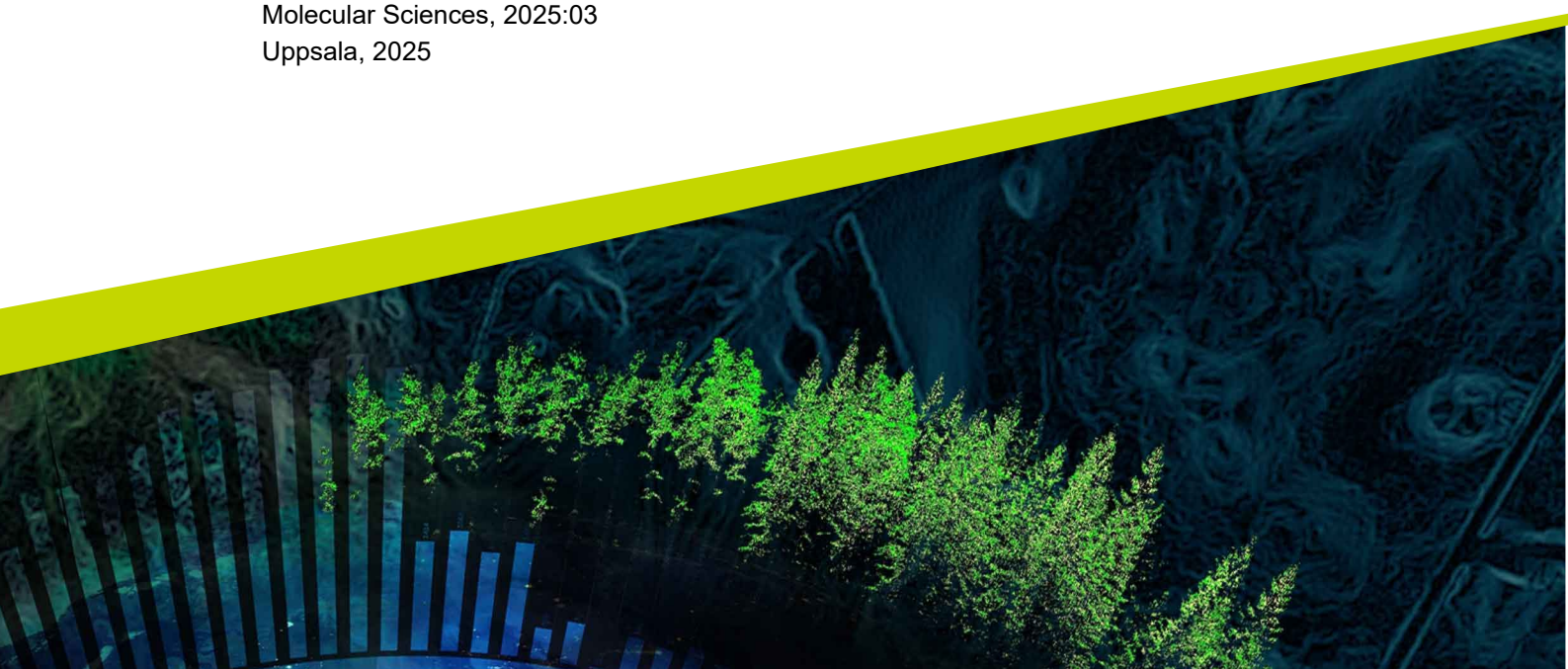

Evaluating the impact of vital gluten on the protein molecular size distribution, loaf volume and dough rheology of vital gluten wheat flour blends

Påverkan av vitalgluten på den molekylära storleksdistributionen av proteiner, brödvolymen samt degreologin hos blandningar av vetemjöl och vitalgluten

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Abstract

The breadmaking quality of wheat is generally attributed to its gluten proteins, which form the viscoelastic, gas-retaining network of wheat flour dough. The composition of gluten proteins, in particular protein molecular size distribution, is known to influence the rheological properties and baking quality of wheat flour. Vital gluten (VG) is a gluten protein concentrate that is added to wheat flour to improve dough and bread characteristics. However, VG is currently sold with limited quality specifications, including information regarding its protein composition. The aim of this project was to (1) investigate how the gluten protein molecular size distribution, loaf volume and dough rheological properties are affected in VG wheat flour blends by the addition of different VGs to wheat flour, and (2) compare chromatographic and rheological methods for describing VG quality in an industrial setting. Twelve wheat flour blends with 4% (w/w) of commercial VG were produced and evaluated in terms of protein molecular size distribution, loaf volume, and rheological properties using SE-HPLC, baking tests and empirical rheological methods, respectively. In the baking tests, both tin pan loaves and free-form loaves were produced. The VG wheat flour blends yielded varying loaf volumes, which could be attributed to differences in protein molecular size distribution and rheological properties. Free-form loaf volume was significantly correlated to the percentage of unextractable polymeric protein (%UPP) and dough strength, whereas tin pan loaf volume was significantly correlated with dough extensibility. While the alveograph test yielded significant correlations to free-form loaf volume, extensograph parameters were significantly correlated to the volume of both loaf types. In conclusion, the extensograph test may be suitable for VG quality control within the cereal and baking industries.

Keywords: vital gluten quality, protein molecular size distribution, SE-HPLC, loaf volume, dough rheology

Sammanfattning

Vetets brödbakningskvalitet tillskrivs generellt dess glutenproteiner, som bildar det viskoelastiska, gashållande nätverket hos vetemjölsdeg. Det är känt att sammansättningen av glutenproteiner, i synnerhet deras molekylära storleksfördelning, påverkar vetemjölets reologiska egenskaper och bakningskvalitet. Vitalgluten (VG) är ett glutenproteinkoncentrat som tillsätts vetemjöl för att förbättra degens och brödets egenskaper. För närvarande säljs dock VG med begränsade specifikationer av dess kvalitet, inklusive information om dess proteinsammansättning. Syftet med detta projekt var att undersöka hur den molekylära storleksfördelningen av glutenproteiner, brödvolymen och degreologin hos blandningar av VG och vetemjöl påverkas vid tillsats av olika VG till vetemjölet, samt att jämföra kromatografiska och reologiska metoder för att beskriva VG-kvalitet i ett industriellt sammanhang. Tolv blandningar av VG-mjölblandningar med 4% tillsatt VG producerades och utvärderades i termer av molekylär storleksfördelning av proteiner, brödvolym och reologiska egenskaper genom SE-HPLC, baktester, respektive empiriska reologiska metoder. I baktesterna producerades både formbröd och friformsbröd. De olika VG-mjölblandningarna gav varierande brödvolymer, vilket kunde kopplas till skillnader i proteinernas molekylära storleksfördelningen samt skillnader i reologiska egenskaper. Friformsbrödvolym korrelerade signifikant med andelen icke-extraherbart polymeriskt protein (%UPP) och degstyrka, medan formbrödsvolym korrelerade signifikant med degens tänjbarhet. Även om alveograf-parametrar var signifikant korrelerade med friformsbrödvolym, så var extensograf-parametrar signifikant korrelerade med både friformsbrödvolym och formbrödsvolym. Sammanfattningsvis skulle extensograftestet kunna vara en lämplig metod för kvalitetskontroll av VG inom spannmåls- och bageriindustrierna.

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Abbreviations

Abbreviation	Description
BU	Brabender units
db	Dry basis
EU	Extensograph units
FU	Farinograph units
GS	Glutenin subunit
HMW	High molecular weight
LMW	Low molecular weight
SV	Specific volume
VG	Vital gluten
wb	Wet basis

1. Introduction

Wheat (*Triticum aestivum*) is undoubtedly the most important cereal for breadmaking (Goesaert et al. 2005). Its unique breadmaking qualities are generally attributed to the physicochemical properties of its major storage proteins, gliadins and glutenins (Veraverbeke & Delcour 2002). When wheat flour is mixed with water, these proteins form a cohesive viscoelastic network, *gluten*, which is largely responsible for the rheological characteristics and gas-holding capacity of the dough, and ultimately contributes to the loaf volume and crumb structure of the resulting bread (Delcour et al. 2012). The breadmaking potential of wheat flour is influenced by both gluten protein content and composition (Veraverbeke & Delcour 2002), which in turn depends on a combination of genetic and environmental factors (Lan et al. 2023). To adjust for fluctuations in flour quality and achieve a stable end-product quality over time, industrial mills and bakeries produce wheat flour blends of uniform quality through the use of additives, such as ascorbic acid and enzymes, as well as vital gluten (VG) (Stauffer 1990).

Vital gluten is a gluten protein concentrate produced from sifted wheat flour. Upon hydration, VG rapidly regains its characteristic viscoelastic properties, which is a prerequisite for its application in baking (Day et al. 2006). In breadmaking, VG is generally added to wheat flour to improve dough and bread characteristics, such as dough strength, mixing tolerance and handling properties, as well as bread volume and texture (Ortolan & Steel 2017). Thus, it is no surprise that fortification of wheat flour with VG has become standard practice for bread manufacturers since the latter part of the 20th century (Day et al. 2006).

The quality of VG is known to vary depending on several factors, including wheat cultivar of origin and processing conditions (Wadhawan 1988). Industrial mills and bakeries typically evaluate VG quality through practical baking tests (Ortolan & Steel 2017), in which the loaf volume and crumb structure of bread made from VG fortified wheat flour is compared to that from non-fortified wheat flour (Wadhawan 1988). While the baking test is generally considered a reliable method for evaluating baking quality, it has the disadvantage of being resource-intensive, requiring experienced personnel, time and large amounts of sample material (Bouachra et al. 2017; Rakita et al. 2018). In addition, the choice of test protocol and wheat flour could influence the results obtained (Wadhawan 1988). Empirical rheological tests, such as the farinograph, extensograph and alveograph test, are standard flour quality evaluation tools within the modern cereal industry and have potential to be used in VG quality control (Ortolan & Steel 2017). Other approaches to evaluating VG quality have been explored in previous research, including intrinsic fluorescence (Wadhawan 1988; Guerrieri & Cerletti 1996), swelling index

(Hu & Shang 2007), gluten index (Ortolan et al. 2018), gluten aggregation behaviour (Melnyk et al. 2012; Schopf & Scherf 2020), microscale extension (Scherf et al. 2016; Schopf & Scherf 2020), and water absorption capacity (Schopf & Scherf 2021). Furthermore, the effects of VG addition to wheat flour on dough rheology and bread quality has been the subject of extensive research (Codina et al. 2008; Marchetti et al. 2012; Giannou & Tzia 2016; Rahman et al. 2022; Iqbal et al. 2023). Yet, commercially produced VG is currently sold with limited specifications of quality, in particular such pertaining to protein composition and functionality (Ortolan et al. 2018).

It is well established that the gluten protein composition – in particular, molecular size distribution – in wheat flour significantly affects dough and bread properties (Gupta et al. 1993; Johansson et al. 2002; Zhang et al. 2008). However, how adding VG to wheat flour influences the overall molecular size distribution of gluten proteins, and how this relates to variations in dough and bread properties, is not well understood.

1.1 Aims

The main aims of this project were to (1) investigate how the gluten protein molecular size distribution, loaf volume and dough rheological properties are affected in VG wheat flour blends by the addition of different VGs to wheat flour, and (2) compare chromatographic and rheological methods for describing VG quality in an industrial setting.

2. Background

2.1 Gluten proteins – an overview

Gluten proteins are the wheat proteins capable of forming gluten, the cohesive viscoelastic protein network that develops when wheat flour is mixed with water through application of mechanical energy (Delcour et al. 2012). The quantity and composition of gluten proteins is one of the main determinants of wheat flour baking performance (Veraverbeke & Delcour 2002; Goesaert et al. 2005). Some non-gluten proteins, such as endogenous enzymes, may also play a role in determining breadmaking quality, however their importance remains largely unknown (Veraverbeke & Delcour 2002).

Gluten proteins are found exclusively in the starchy endosperm of the wheat grain (Wieser et al. 2023). In contrast to most other wheat proteins, gluten proteins are largely insoluble in water and dilute salt solutions (Veraverbeke & Delcour 2002). Gluten proteins are divided into two subgroups based on their solubility in aqueous alcohol: gliadins, which are alcohol-soluble, and glutenins, which are alcohol-insoluble (Hu et al. 2023).

Gliadins are generally *monomeric* gluten proteins and exhibit a high degree of polymorphism (Goesaert et al. 2005). The term *monomeric* refers to the proteins' quaternary structure, meaning that they consist of single polypeptide chains (Veraverbeke & Delcour 2002). Gliadins typically have molecular weights around 30-80 kDa (Goesaert et al. 2005). Oligomeric gliadins, referred to as high-molecular-weight (HMW) gliadins, are also present in wheat, accounting for about 15% of total gluten proteins. These oligomers are larger than their monomeric counterparts, with molecular weights between 70-700 kDa (Wieser et al. 2023).

Glutenins are *polymeric* gluten proteins, each consisting of two or more polypeptide chains, called glutenin subunits (GSs), which are associated through interchain disulphide bonds (Delcour et al. 2012). They are very large proteins, with molecular weights ranging from 80 kDa to several million (Veraverbeke & Delcour 2002). The glutenins at the top end of this range are likely among the largest naturally occurring proteins known to man (Wieser et al. 2023). Glutenins generally exhibit poor solubility in most conventional buffers, and a majority cannot be fully solubilised without alteration to their native structure (Goesaert et al. 2005).

Glutenins can be degraded into their constituent GSs using a disulphide-reducing agent, such as dithiothreitol, which disrupts the interchain disulphide bonds (Veraverbeke & Delcour 2002; Goesaert et al. 2005). GSs are classified according to their molecular weight, as determined by sodium dodecyl sulphate-poly-

acrylamide gel electrophoresis (SDS-PAGE) (Wieser et al. 2023). High-molecular-weight glutenin subunits (HMW-GS) have molecular weights of 70-90 kDa, while low-molecular-weight glutenin subunits (LMW-GS) have molecular weights of 35-45 kDa (Delcour et al. 2012). Around 20 types of HMW-GS and over 40 types of LMW-GS have been identified to date (Hu et al. 2023), however only 3-6 HMW-GS and 7-16 LMW-GS are usually present in a single wheat genotype (Johansson et al. 2013).

2.2 Gluten proteins in breadmaking

A typical breadmaking process begins by kneading appropriate amounts of flour, water, yeast and salt into a viscoelastic dough, which is then fermented and baked (Scanlon & Zghal 2001; Goesaert et al. 2005). This process entails several biochemical and physical transformations of the various flour constituents (Goesaert et al. 2005). The following discussion will mainly focus on those of the gluten proteins.

Mixing evenly disperses the ingredients, which promotes the hydration of flour components, including the gluten proteins. With continued input of mechanical energy, hydrated gluten proteins are transformed into a continuous cohesive viscoelastic network, i.e. gluten, which encapsulates the other flour components and yields a pliable dough (Goesaert et al. 2005; Delcour et al. 2012). The properties of the dough are essentially the properties of the hydrated gluten proteins, although modified by the presence of other flour components as well as other dough ingredients (Singh & MacRitchie 2001; Cauvain 2015). Dough development is correlated to an increase in the dough's resistance to mixing, however overmixing ultimately leads to breakdown of the gluten structure, with a concomitant decrease in dough resistance. At the point of maximum resistance, the dough is considered optimally developed and will produce the maximum volume (Cauvain 2015). Mixing also incorporates small bubbles of air into the dough, which are stabilised within the gluten network. During fermentation, accumulation of carbon dioxide within these bubbles stretches the network, resulting in dough expansion and the formation of an open cellular dough structure (Ortolan & Steel 2017; Cauvain 2020). As the dough temperature increases during baking, yeast production of carbon dioxide increases and the gas volume expands, resulting in a phenomenon known as oven spring (Cauvain 2015). Gluten plays a major role in the gas retention capacity and expandability of the dough during fermentation and at the start of baking (Goesaert et al. 2005). Once the dough reaches a certain temperature, the gluten proteins coagulate and undergo extensive cross-linking, setting the gluten structure (Ortolan & Steel 2017). Thus, the gluten network contributes to both the volume and initial crumb structure of the baked bread (Delcour et al. 2012).

Glutenin and gliadin have contrasting roles in the gluten network. Due to their large molecular size, entanglement of glutenin chains provides cohesiveness to the dough system, yielding dough strength and elasticity. Meanwhile, gliadins have a plasticizing effect on the dough system by interfering with glutenin-glutenin interaction, thus contributing to dough viscosity and extensibility (Veraverbeke & Delcour 2002; Goesaert et al. 2005; Delcour et al. 2012). In addition, gliadins exhibit surface-active qualities, thereby contributing to the stability of gas cells within the gluten network (Hu et al. 2023).

Besides efficient gas retention, a ”good” dough for making high-quality bread is defined by an appropriate balance of dough elasticity to extensibility (Cauvain 2015). Elastic strength is required to prevent gas bubble coalescence and thus maintain the cellular dough structure, however some degree of extensibility is needed to allow for proper air incorporation during mixing and enable the dough to stretch in response to gas bubble expansion (Singh & MacRitchie 2001; Belton 2012). In effect, the ratio of monomeric gliadin to polymeric glutenin can be considered a key factor for quality breadmaking (Veraverbeke & Delcour 2002). However, the quality of the glutenin fraction is also important, as glutenin composition and molecular weight distribution are known to influence dough properties and baking quality (Wieser 2007). Particularly, a high proportion of SDS-unextractable polymeric protein, commonly measured as the percentage of unextractable polymeric protein in total polymeric protein (%UPP), is considered to be a major contributor to gluten and dough strength, as well as overall breadmaking quality (Gupta et al. 1993; Johansson et al. 2002; Zhang et al. 2008).

Because of the implications of dough rheology on bread quality, rheological tests have long been used within the cereal industry for quality control purposes and to evaluate wheat flour processing performance (Dobraszczyk & Morgenstern 2003). Dough is a viscoelastic material, meaning that it exhibits both elastic and viscous (or flow) characteristics when subjected to mechanical force (Belton 2012), and thus several rheological tests can be of use depending on the desired dough properties. Recording mixers, such as the farinograph, are used to monitor dough development and changes in dough resistance during mixing (Finnie & Atwell 2016). Other instruments, such as the alveograph and extensograph, are used to analyse the mechanical properties of dough after it has been mixed, shaped and rested under controlled conditions. The information obtained from these tests can give some insight into the flour’s suitability for specific applications and help predict its processing behaviour (de Beer 2023). One of the main quality traits of interest industrially is dough strength, or elasticity, which can be captured by several rheological parameters derived from these tests (Selga et al. 2024).

2.3 Vital gluten – production, composition, application

Vital gluten is produced through wheat flour fractionation and drying (Ortolan & Steel 2017). Wet-milling of whole wheat kernels is uncommon in industrial added production, due to the relatively high risk of gluten devitalisation and entanglement of gluten proteins with bran components. Therefore, sifted wheat flour is most often used as starting material (Van Der Borght et al. 2005). Various flour fractionation techniques have been developed; however most industrial processes follow the same principle. First, wheat flour is mixed with water, leading to hydration and agglomeration of gluten proteins. Second, starch granules are separated from the wet gluten by various means, often based on differences in size or density (Van Der Borght et al. 2005). Two main processing methods dominate commercially, namely the Martin process and the batter process, which have been described in detail elsewhere (Van Der Borght et al. 2005; Day et al. 2006). As the obtained wet gluten is sensitive to heat denaturation, the drying rate and temperature must be carefully controlled (Day et al. 2006). According to Marchetti et al (2012), loss of gluten functionality will typically occur at temperatures above 55-60°C.

Although a protein concentrate, non-protein components are also present in VG. On average, commercial VGs contain around 5-10% moisture, 73-82% protein, 3-20% carbohydrates, 5-8% total lipids and 0.5-1.5% ash (Marchetti et al. 2012). The carbohydrate content mainly consists of starch and smaller amounts of non-starch polysaccharides (Van Der Borght et al. 2005). More extensive washing can reduce the starch and fiber content, however their removal becomes increasingly difficult as the protein content rises (Day et al. 2006). Due to the hydrophobic nature of gluten proteins, they will frequently bind flour lipids during the washing process. Thus, the lipid content is chiefly determined by the lipid content of the flour source, and will not be altered by further washing (Day et al. 2006).

Vital gluten is mainly used in the milling and baking industry as a flour improver. The amount of VG to be added to the flour depends on the native flour quality, the desired end-product, as well as the quality of the VG itself (Ortolan & Steel 2017). In contrast to most additives, VG can be added to flour without legal restriction, as it is itself originally a flour component (Marchetti et al. 2012). Nonetheless, 2-10% VG on a flour weight basis is most common in bakery applications (Ortolan & Steel 2017).

3. Materials and methods

3.1 Materials

Twelve samples of commercial VG were obtained from a total of five manufacturing companies (A-E) (Table 1). Most VGs were known to be produced in 2023 or 2024. Four organic VGs were included in the sample set. Sample DB23 was produced from wheat grain of another geographical origin than the other VGs from company D.

Table 1. Description of commercial vital glutens.

Sample ID	Company	Agricultural system	Production year
ASX	A	Conventional	Unknown
BSX	B	Conventional	Unknown
CSX	C	Conventional	Unknown
DB23	D	Conventional	2023
DS23	D	Conventional	2023
DE23	D	Organic	2023
DN23	D	Organic	2023
DS24	D	Conventional	2024
DE24	D	Organic	2024
DN24	D	Organic	2024
EL24	E	Conventional	2024
EH24	E	Conventional	2024

To mimic the practical use of VG in an industrial bakery, analyses were performed on blends of wheat flour and VG. A concentration level of 4 g of VG per 100 g of flour blend was chosen to resemble the levels commonly applied in commercial bakery operations, however slightly elevated to better discern differences between the VG samples. An all-purpose winter wheat flour was obtained from a commercial mill in Malmö, Sweden, to be used as base flour for the flour blends. The winter wheat grain was cultivated in southern Sweden under 2023-2024 and harvested in 2024, and the flour was produced without typical flour additives such as ascorbic acid and malt.

Vital gluten flour blends for baking tests and rheological analyses were produced by mixing dry VG and dry wheat flour in a commercial baking mixer set at medium speed for 10 min. After mixing, the blends were transferred to transparent plastic bags, which were sealed and stored at room temperature until further use. Control (i.e., winter wheat flour without added VG) was stored under the same conditions.

A smaller batch of each VG wheat flour blend was produced for determination of protein molecular size distribution (Section 3.5.1).

3.2 Chemical composition using NIT

Moisture content and protein content of the VG wheat flour blends and the control, respectively, were determined using near-infrared transmittance spectroscopy (NIT; Infratec, Foss Analytical) according to the manufacturer's instructions for wheat flour testing.

3.3 Empirical rheology

Several empirical rheological tests were conducted according to standard analytical methods for evaluating wheat flour quality, including the farinograph test (ICC 115/1), the extensograph test (ICC 114/1), and the alveograph test (ICC 121/1). Farinograph and alveograph analyses were performed in duplicates. Extensograph analyses were majoritively performed in duplicate, however due to time restriction, some samples were analysed as singular replicates. Extensograph parameters were measured after 45-, 90- and 135-minutes resting. The required sample amount for each test replicate was calculated based on the sample moisture content, as determined by NIT.

In addition, wet gluten content, dry gluten content, gluten index and water binding capacity (ICC 155/1) was determined in triplicates using the Perten Glutomatic 2000 System (PerkinElmer). To enable comparison between samples, both wet and dry gluten contents were expressed on a 14% moisture basis. Calculations of the glutomatic parameters are described in Table 2.

Table 2. Calculation of glutomatic parameters.

Abbreviation	Description	Calculation
CWG	Corrected wet gluten content (%)	$\frac{Total\ weight\ of\ wet\ gluten\ (g) * 860}{100 - Sample\ moisture\ (\%)}$
CDG	Corrected dry gluten content (%)	$\frac{Total\ weight\ of\ dry\ gluten\ (g) * 860}{100 - Sample\ moisture\ (\%)}$
GI	Gluten index	$\frac{Weight\ of\ wet\ gluten\ on\ sieve\ (g) * 100}{Total\ weight\ of\ wet\ gluten\ (g)}$
WBC	Water-binding capacity (%)	$\mathrm{CWG} - \mathrm{CDG}$

Glutopeak (Brabender) results had been obtained previously on the twelve commercial VGs. These analyses were performed outside of this project using the pure commercial VGs as sample material and in the form of singular replicates.

Thus, the glutopeak results are not directly comparable with the other experimental data collected in this project, but was included to gain insight into the aggregation behaviour of the VG contained within each respective VG wheat flour blend.

3.4 Baking test

Baking tests were conducted by a professional baker. Each test batch used 3 kg of flour (either VG wheat flour blend or control), with water adjusted to farinograph absorption, and included 3.4% yeast, 1.2% salt, 50 ppm ascorbic acid, and 5 ppm amylase (to achieve a falling number of 280 s). Doughs were mixed to optimal development or until reaching 30°C, rested for 10 minutes, divided, and mechanically shaped into loaves. Loaves of 500 g were placed in tin pans for making tin pan bread, and loaves of 600 g were placed on a baking sheet for making free-form bread. Proofing was carried out at 38°C and 78% RH; tin pan loaves were allowed to ferment until 1 cm below the rim of the pan, while free-form loaves were fermented for 40 ± 5 minutes. Tin pan loaves were baked for 5 min at 250°C, then another 20 min at 220°C, and lastly 5 min at 200°C, whereas free-form loaves were baked for 5 min at 250°C and then another 25 min at 200°C. After baking and cooling, the loaves were weighed using a manual scale and bread dimensions were recorded using a Volscan Profiler (Stable Micro Systems). Tin pan bread results and free-form bread results were reported separately.

3.5 Molecular size distribution of gluten proteins by SE-HPLC

3.5.1 Sample preparation

A small batch of each VG wheat flour blend was prepared by adding 240 mg (± 0.5 mg) of winter wheat flour and 10 mg (± 0.5 mg) of VG into 2 ml Eppendorf tubes, followed by vortexing until thoroughly mixed. This resulted in a total VG concentration of 4.0 % (± 0.2 %) on a weight basis. Tube openings were covered with perforated parafilm, after which the samples were freeze-dried at -100°C for three days to remove all moisture.

3.5.2 Protein extraction

Proteins were extracted using a two-step protocol originally described by Gupta et al. (1993), with modifications according to Lan et al. (2023). Briefly, 16.5 mg (±0.05 mg) of freeze-dried sample and 1.4 mL of SDS-phosphate buffer (0.5% SDS, 0.05 M NaH_2POH_4, pH 6.9) was added to 1.5 mL Eppendorf tubes. The tubes were vortexed for 10 s, shaken at 2 000 rpm for 5 min, and then centrifuged at 9 600 x g for 30 min, after which the supernatants were decanted into 2 ml HPLC vials with screwcaps. Filled vials were placed in a 55°C water bath for 2 min to inactivate

endogenous proteases (Larroque et al. 2000), and then placed on ice for 2 min to reach a temperature suitable for HPLC injection. For the second extraction, the pellets were resuspended in 1.4 mL of SDS-phosphate buffer and sonicated for 45 s at an amplitude of 5 microns using a Soniprep 150 (MSE). The purpose of sonication is to increase the extractability of natively unextractable polymers, allowing for their quantification. Following this treatment, the tubes were centrifuged again and their supernatants were collected and heat-treated, as previously described. This two-step extraction procedure was repeated thrice for each of the twelve VG wheat flour blends, as well as for the control.

3.5.3 SE-HPLC procedure

All samples of extractable and unextractable proteins were separated and quantified by SE-HPLC (Waters) using a BioSep SEC-4000 Phenomenex column, as described by Lan et al. (2023). The eluant solution consisted of 50% water and acetonitrile (v/v), with 0.5% added triflouretic acid (TFA). Each sample injection (20 µl) was separated for 30 min at a flow rate of 0.2 ml/min. Upon elution, proteins were detected using a UV diode array detector set at 210 nm. Chromatograms were recorded and processed through the Waters Empower software, after which the data was exported to Microsoft Excel for further processing. Gluten protein fractions of the first extraction were termed SDS-extractable (e), and those of the second extractions SDS-unextractable (u).

As seen in Figure 1, the chromatogram peak areas were divided by retention time into four sections, each section representing a distinct gluten protein fraction: 8-10.05 min for larger polymeric proteins (LPPs), 10.05-14.3 min for smaller polymeric proteins (SPPs), 14.3-20.3 min for larger monomeric proteins (LMPs), and 20.3-23 min for smaller monomeric proteins (SMPs).

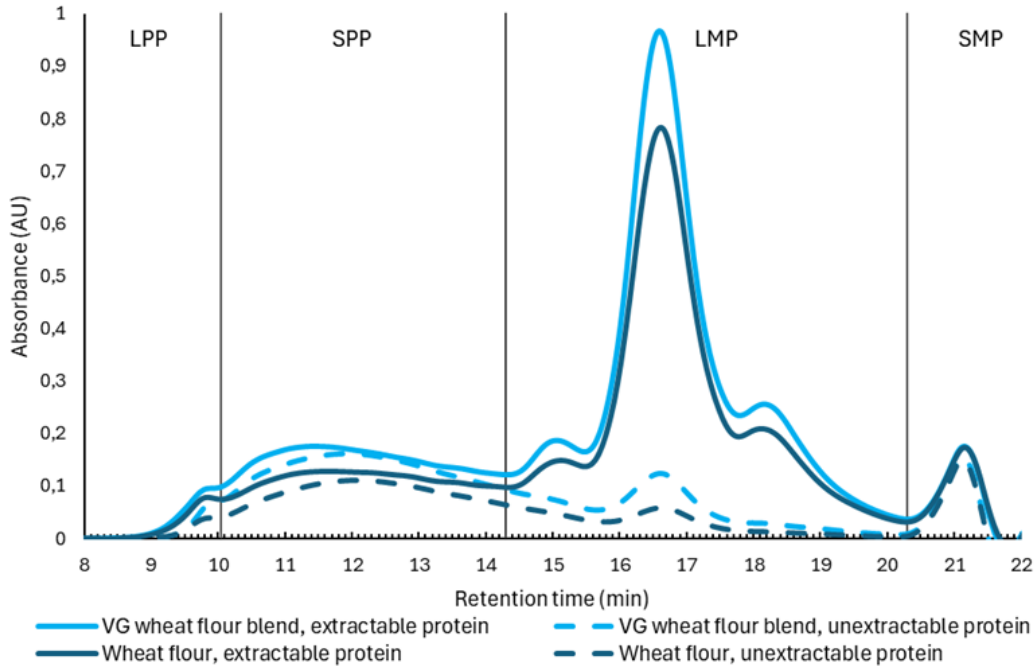

Figure 1. SE-HPLC chromatogram of extractable proteins (solid) and unextractable proteins (dashed) from a vital gluten wheat flour blend (light) and wheat flour (dark). VG: vital gluten; LPP: larger polymeric proteins; SPP: smaller polymeric proteins; LMP: larger monomeric proteins; SMP: smaller monomeric proteins.

The areas of the chromatogram sections were used to calculate five gluten protein parameters, as seen in Table 2. The parameters were total SDS-extractable protein (TOTE), total unextractable protein (TOTU), percentage of SDS-unextractable polymeric protein in total polymeric protein (%UPP), percentage of large unextractable monomeric protein in total large monomeric protein (%LUMP), and ratio of monomeric to polymeric protein (Mon/Pol).

Table 3. Calculation of gluten protein parameters describing protein molecular size distribution as determined by SE-HPLC.

Abbreviation	Description	Calculation
TOTE	Total SDS-extractable protein	$eLPP + eSPP + eLMP + eSMP$
TOTU	Total SDS-unextractable protein	$uLPP + uSPP + uLMP + uSMP$
%UPP	Percentage of unextractable polymeric protein in total polymeric protein	$\frac{(uLPP + uSPP) * 100}{eLPP + eSPP + uLPP + uSPP}$
%LUMP	Percentage of large unextractable monomeric protein in total large monomeric protein	$\frac{(uLMP) * 100}{eLMP + uLMP}$

Mon/Pol	Ratio of monomeric protein to polymeric protein	$\frac{eLMP + eSMP + uLMP + uSMP}{eLPP + eSPP + uLPP + uSPP}$

U: unextractable, referring to protein fractions from the first extraction; e: extractable, referring to protein fractions from second extraction. Names of chromatogram sections can be seen in Figure 1.

3.6 Statistical analysis

The data was subjected to statistical analysis using Microsoft Excel, SIMCA18 (Sartorius Stedim Data Analytics AB 2024), and RStudio (Posit team 2025). Descriptive statistical analysis (averages, standard deviations, etc.) was executed in Microsoft Excel, principal component analysis (PCA) analysis was performed in SIMCA 18 and Pearson's correlation analysis was conducted in RStudio.

4. Results and discussion

4.1 Loaf volume of VG wheat flour blends

Baking test results are summarised in Figure 2. While VG wheat flour blends were similar in total protein content, ranging from 13.9-14.2%, differences in loaf volume were observed. The VG wheat flour blends produced a higher average loaf volume than the control in both tin pan bread and free-form bread (Figure 2A), which is in line with the assumption that addition of VG to wheat flour contributes to a higher loaf volume. However, the average loaf volume obtained varied between VG wheat flour blends, indicating that the constituent commercial VGs were of varying quality. Of the VG wheat flour blends, ASX and BS2X produced the highest average tin pan loaf volume and DB23 had the lowest average tin pan loaf volume. Meanwhile, DB23 yielded the highest average free-form loaf volume.

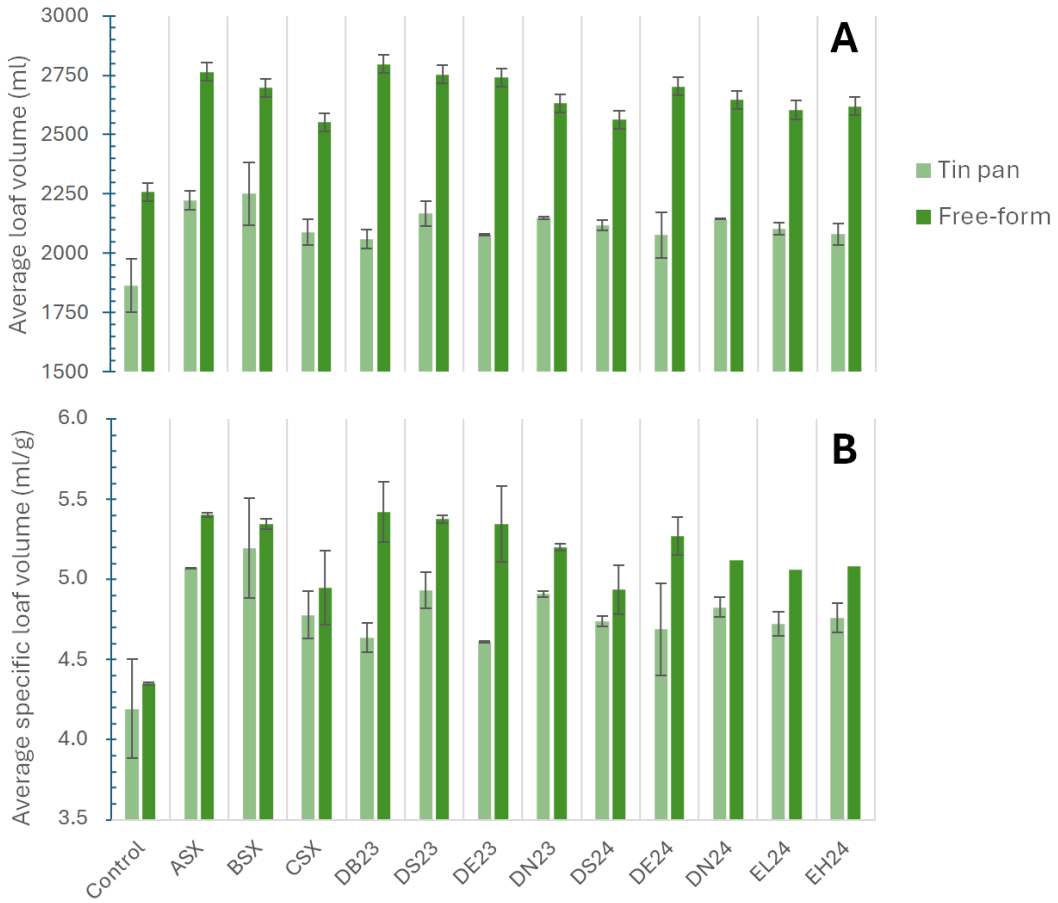

Figure 2. Average loaf volume (A) and specific volume (B) of vital gluten wheat flour blends and control. Length of error bars correspond to the standard deviation of duplicate measurements. Labels refer to sample names (Table 1).

Specific volume (SV) is the loaf volume divided by the loaf weight. The average specific loaf volume of tin pan loaves varied between 4.6-5.2 ml/g, while that of free-form loaves varied between 4.9-5.4 ml/g (Figure 2B). Differences in specific volumes between VG wheat flour blends followed a similar pattern as for loaf volume, with ASX and BSX yielding the highest average for tin pan loaves, and DB23 yielding the highest average for free-form loaves but the lowest for tin pan loaves. Average specific volume was generally higher in free-form loaves compared to tin pan loaves. This is likely due to differences in moisture evaporation during baking, which would influence the final bread weight.

Although the baking test is generally considered a reliable method for evaluating baking quality, the results obtained in this study showed some inconsistencies between duplicate measurements (Figure 2). This variation is likely the result of the manual steps involved in the breadmaking process.

4.2 Impact of gluten protein molecular size distribution on loaf volume of VG wheat flour blends

Figure 3 shows the average TOTE and TOTU of the VG wheat flour blends and the control. As extractability of polymers generally decreases with increasing molecular weight (Gupta et al. 1993), these parameters correspond to the content of smaller and larger gluten proteins in the samples, respectively. Both TOTE and TOTU were notably lower in the control than in the VG wheat flour blends. Meanwhile, only minor variations in TOTE and TOTU were observed between VG wheat flour blends (Figure 3A). Nonetheless, a positive relationship could be observed between TOTU and free-form loaf volume of the VG wheat flour blends, whereas TOTE demonstrated a negative association to free-form loaf volume (Figure 3B). Thus, TOTU and TOTE may be inversely related. There was no notable relationship between TOTU, TOTE and tin pan loaf volume.

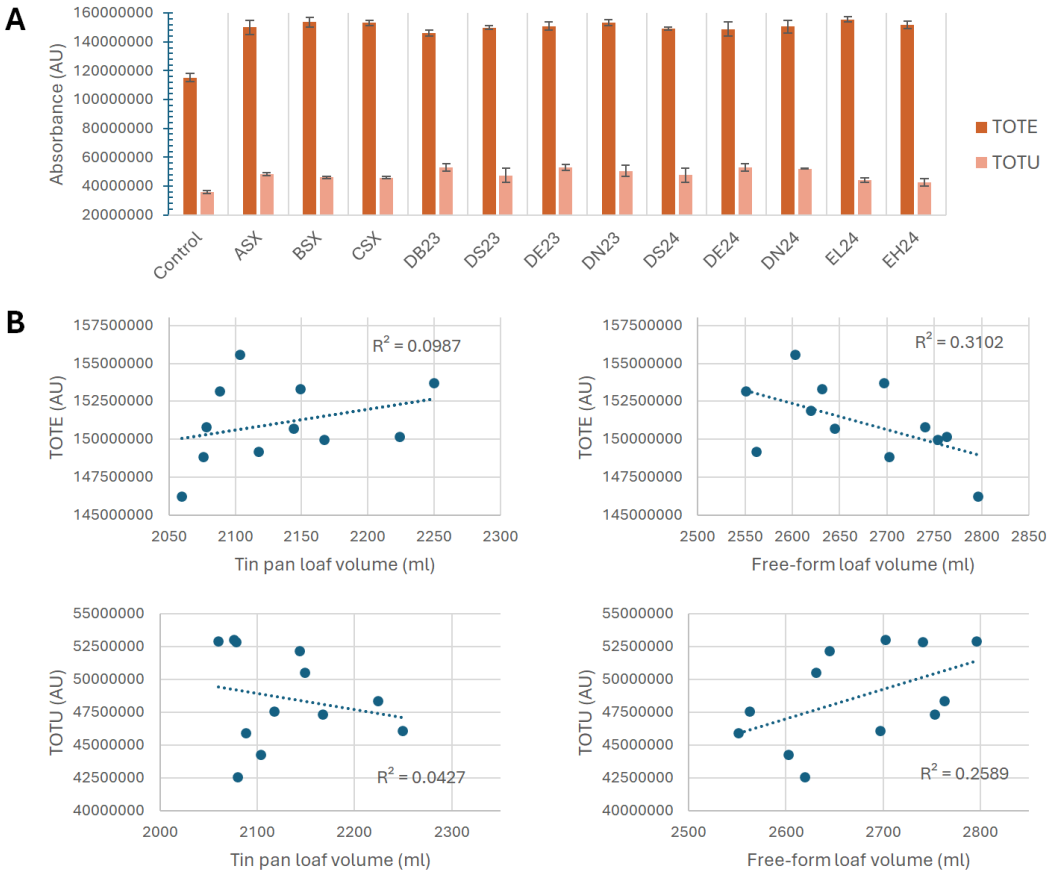

Figure 3. TOTE and TOTU of vital gluten flour blends and control (A), and the relationship between these gluten protein parameters and the loaf volume of vital gluten wheat flour blends (excluding the control) (B). In A, length of error bars correspond to the standard deviation of triplicate measurements. Labels refer to sample names (Table 1).

The average monomeric/polymeric ratio of VG wheat flour blends and control are shown in Figure 4. The VG wheat flour blends exhibited a lower average monomeric/polymeric ratio than the control (Figure 4), while yielding higher average loaf volumes (Figure 2). No major differences in monomeric/polymeric ratio could be observed between VG wheat flour blends (Figure 4), and there was no apparent relationship between monomeric/polymeric ratio and loaf volume (not shown). According to previous research, a higher monomeric/polymeric ratio would yield a more extensible and less elastic dough (Tronsmo et al. 2002), which can be explained by the contrasting behaviour of hydrated gliadins (viscous) and glutenins (elastic) (Southan & MacRitchie 1999).

Figure 4. Monomeric/polymeric ratio of vital gluten wheat flour blends and control. Length of error bars correspond to the standard deviation of triplicate measurements. Labels refer to sample names (Table 1).

Figure 5 shows the average %UPP and %LUMP values for the VG wheat flour blends and the control. Only one VG wheat flour blend produced a higher %UPP than the control (45.1%), namely DB23 (46.5%). The lowest %UPP (40.1%) was observed in samples EL24 and EH24. %UPP appeared positively related to loaf volume (Figure 5B), however this was only observed in FF loaves. Meanwhile, a negative relationship could be observed between %LUMP and loaf volume, but again only in free-form loaves.

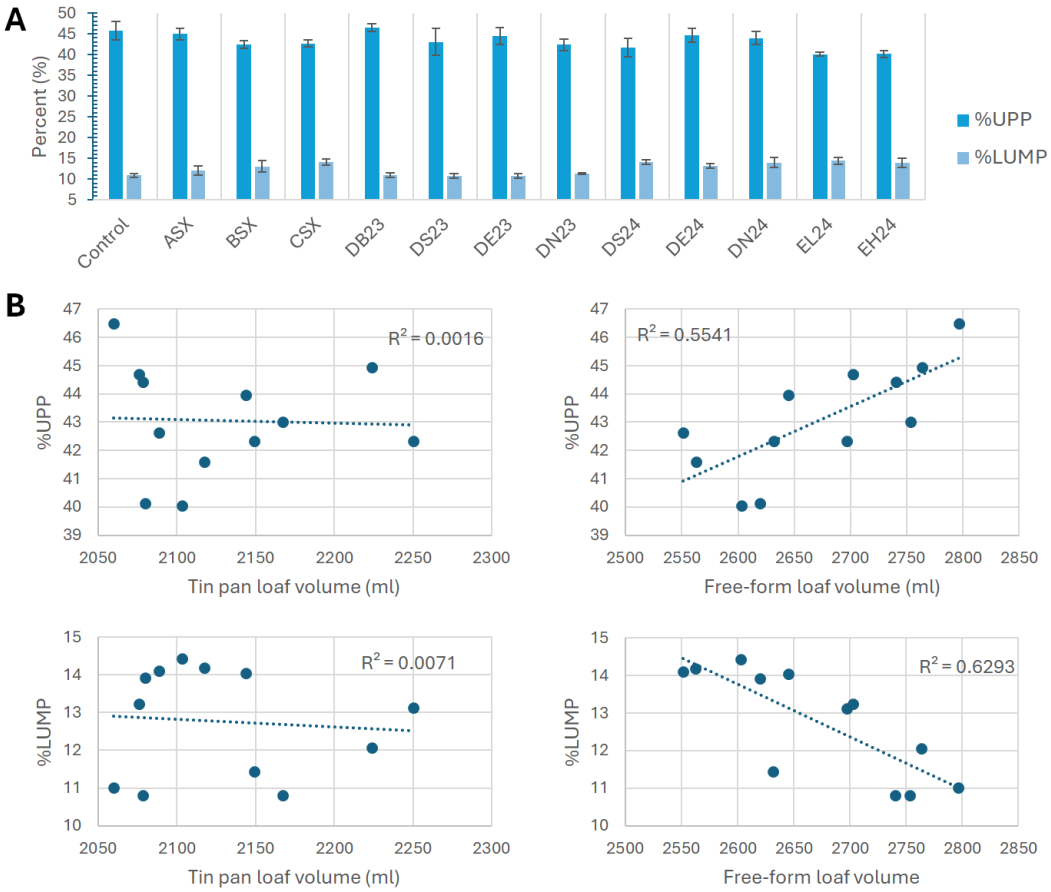

Figure 5. %UPP and %LUMP of vital gluten flour blends and control (A), and the relationship between these gluten protein parameters and loaf volume in vital gluten wheat flour blends (excluding the control). Length of error bars correspond to the standard deviation of triplicate measurements. Labels refer to sample names (Table 1).

Figure 6 shows a Pearson's correlation matrix of loaf volumes and gluten protein parameters, including only VG wheat flour blend results. As surmised in Figure 3B, TOTU was positively correlated to free-form loaf volume (0.51), while TOTE was negatively associated with free-form loaf volume (-0.56), however the results were not significant ($p > 0.05$). %UPP produced significant correlations to all other gluten protein parameters, except for Mon/Pol. In addition, as deduced from Figure 5B, %UPP was significantly positively correlated to free-form loaf volume (0.74**). A positive relationship between %UPP and loaf volume has been established in previous research on wheat flour (Johansson et al. 2002; Castellari et al. 2023). Meanwhile, %LUMP was significantly negatively correlated to free-form loaf volume as well as %UPP, which suggests that these parameters have an inverse relationship, and furthermore that they may have opposite effects on free-form loaf volume. No significant correlations were observed between tin pan loaf volume and gluten protein parameters, suggesting that protein molecular size distribution may be of a greater importance for free-form loaf volume. Mon/Pol did not produce significant correlations to neither baking test parameters nor gluten protein

parameters, perhaps due to a lack of variation in monomeric/polymeric ratio among the VG wheat flour blends.

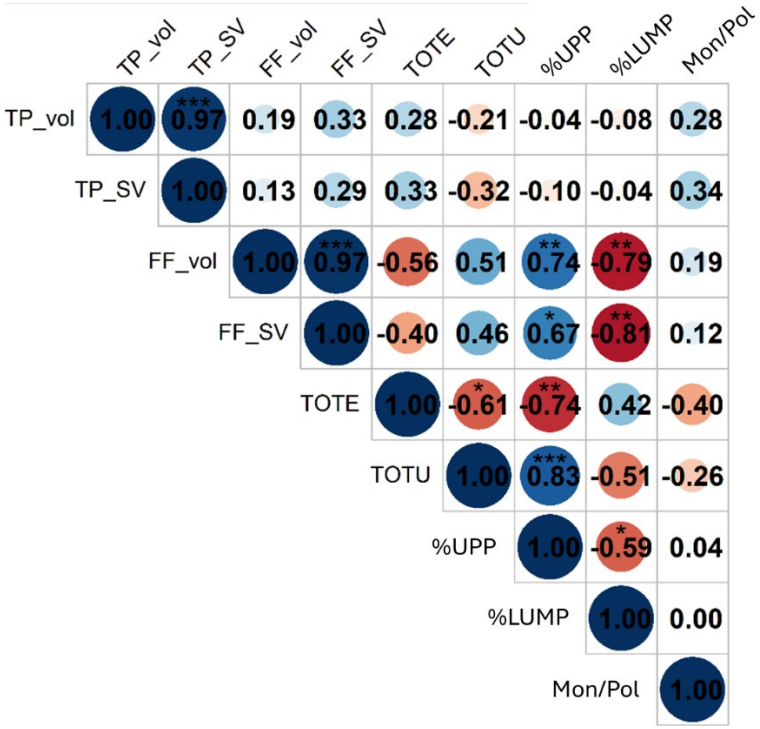

Figure 6. Pearson's correlation matrix of baking test parameters against protein molecular size distribution parameters (Table 3) from VG wheat flour blend results. Negative correlation coefficients (red) indicate a negative relationship between parameters, and vice versa (blue). Absence of correlation yields a coefficient of zero. Symbols (, **, ***) indicate significance at 95%, 99% and 99.9%, respectively. TP: tin pan bread; FF: free-form bread; vol: loaf volume; SV: loaf specific volume.*

Relative standard deviations were generally high (>10%) between SE-HPLC replicates for extractable and unextractable SMP, as well as unextractable LPP (data not shown). Other curve areas were more stable across replicates. For unextractable LPP, which is used to calculate %UPP, this is likely related to the low extractability of glutenins in their native form, in addition to slight variations in sonication time during the second protein extraction step. Furthermore, SE-HPLC analyses use much smaller sample sizes (16.5 mg) than rheological analyses (10-300 g for analysis methods evaluated here). Heterogeneity in the sample material would likely have a greater impact on results if a smaller sample size is used, and thus, this may partly explain the high standard deviations observed between SE-HPLC replicates.

4.3 Protein composition and loaf volume in relation to rheological behaviour of VG wheat flour blends

The data set was subjected to PCA analysis as a means of exploring relationships between gluten protein parameters, loaf volume and rheological parameters. In total, 44 variables were included in the PCA model. Gluten protein parameters have been described previously (Table 3). An overview of the other variables, including their abbreviations (in alphabetical order), units and analysis, is given in Table 4. The control was excluded from the PCA observation set.

Table 4. Overview of variables included in the PCA analysis.

Abbreviation	Description	Unit	Analysis
BEM	Maximum torque	BU	Glutopeak
CDG	Corrected dry gluten content	%	Glutomatic
CWG	Corrected wet gluten content	%	Glutomatic
DDT	Dough development time	min	Farinograph
DS	Degree of softening (10 min after start)	FU	Farinograph
DS(ICC)	Degree of softening (ICC / 12 min after start)	FU	Farinograph
E	Area under the curve	cm^2	Extensograph
Ext	Extensibility	mm	Extensograph
GI	Gluten index	%	Glutomatic
Ie	Elasticity index	unitless	Alveograph
L	Extensibility (curve length)	mm	Alveograph
P	Tenacity (curve height)	mm	Alveograph
P/L	Curve configuration	unitless	Alveograph
PMT	Peak maximum time	min	Glutopeak
R	Resistance to extension	EU	Extensograph
Rmax	Maximum resistance	EU	Extensograph
RN	Ratio number	unitless	Extensograph
S	Stability	min	Farinograph
SV	Loaf specific volume	ml/g	Baking test

Vol	Loaf volume	ml	Baking test
W	Deformation energy	10^{-4}J	Alveograph
WAM	Water absorption (14% moisture basis)	%	Farinograph
WBC	Water binding capacity	%	Glutomatic

Note: Extensograph parameters were assigned a number (45, 90 or 135) corresponding to the minutes after resting. Gluten protein parameters are described in Table 3. Two values (1 and 2) for BEM and PMT were recorded (4.3.1). BU: Brabender units; FU: farinograph units; EU: extensograph units; db = dry basis; wb = wet basis.

Figure 7 shows the first two principal components (PC), which together accounted for 63.8% of the total variance in the data set. In the loadings plot (Figure 7A), variation along PC 1 was dominated by extensograph parameters, possibly due to the strong internal correlations between parameters from this analysis (data not shown). Loaf volumes yielded high loading values on PC 2, however FF volumes were found on the opposite side of the PC1 as TP volumes.

Free-form loaf volume was closely associated to %UPP (Figure 7A), which was expected based on previously presented results. Furthermore, both free-form loaf volume and %UPP was located in proximity to several rheological parameters describing the gluten network strength and elasticity, such as gluten index (GI), elastic index (Ie), maximum resistance (RMax) and deformation energy (W). This would suggest that a high %UPP would yield a stronger gluten network, which in turn would produce more voluminous free-form bread, perhaps by improving gas retention. Meanwhile, %LUMP was located diagonally opposite free-form loaf volume and %UPP, indicating a negative association to these parameters, which is in line with previously presented results. In effect, a high %LUMP may negatively impact free-form loaf volume by contributing to a weaker dough. Tin pan loaf volume, on the other hand, was most closely associated with alveograph L and extensograph Ext, which both describe dough extensibility. In addition, tin pan volume appeared negatively associated with alveograph configuration ratio (P/L), which describes the balance between dough tenacity (P) and extensibility (L). This indicates that a weaker, more extensible dough, as indicated by a high Ext and low P/L, would be associated with a higher tin pan loaf volume, possibly by facilitating dough expansion.

Furthermore, TOTU appeared positively associated with maximum torque (BEM), which describes gluten network strength, as well as resistance to extension (R) and RMax, which describe the force required for dough extension. TOTE was positioned diagonally opposite TOTU, was expected based on previously presented results. Thus, it appears that a higher TOTU is associated with a stronger dough, while a higher TOTE is associated with a weaker dough. In contrast, Mon/Pol was

found relatively near origo, indicating that it was not a substantial source of variation between VG wheat flour blends, which is in line with results presented previously.

In the scores plot (Figure 7B), inclusion of selected PCA parameters resulted in the separation of samples along PC 1 and 2. Samples from company D (yellow) resembled a cluster near origo, suggesting that they performed similarly across analyses and did not contribute much to the overall variation in the data set. However, DB23 could be considered as an outlier to this central cluster, which is likely related to its comparatively high %UPP and high free-form loaf volume (Figure 5B). In contrast, EL24 and EH24 demonstrated the lowest %UPP of the VG wheat flour blends and among the lowest free-form loaf volumes, which would explain their position diagonally opposite DB23 in the scores plot.

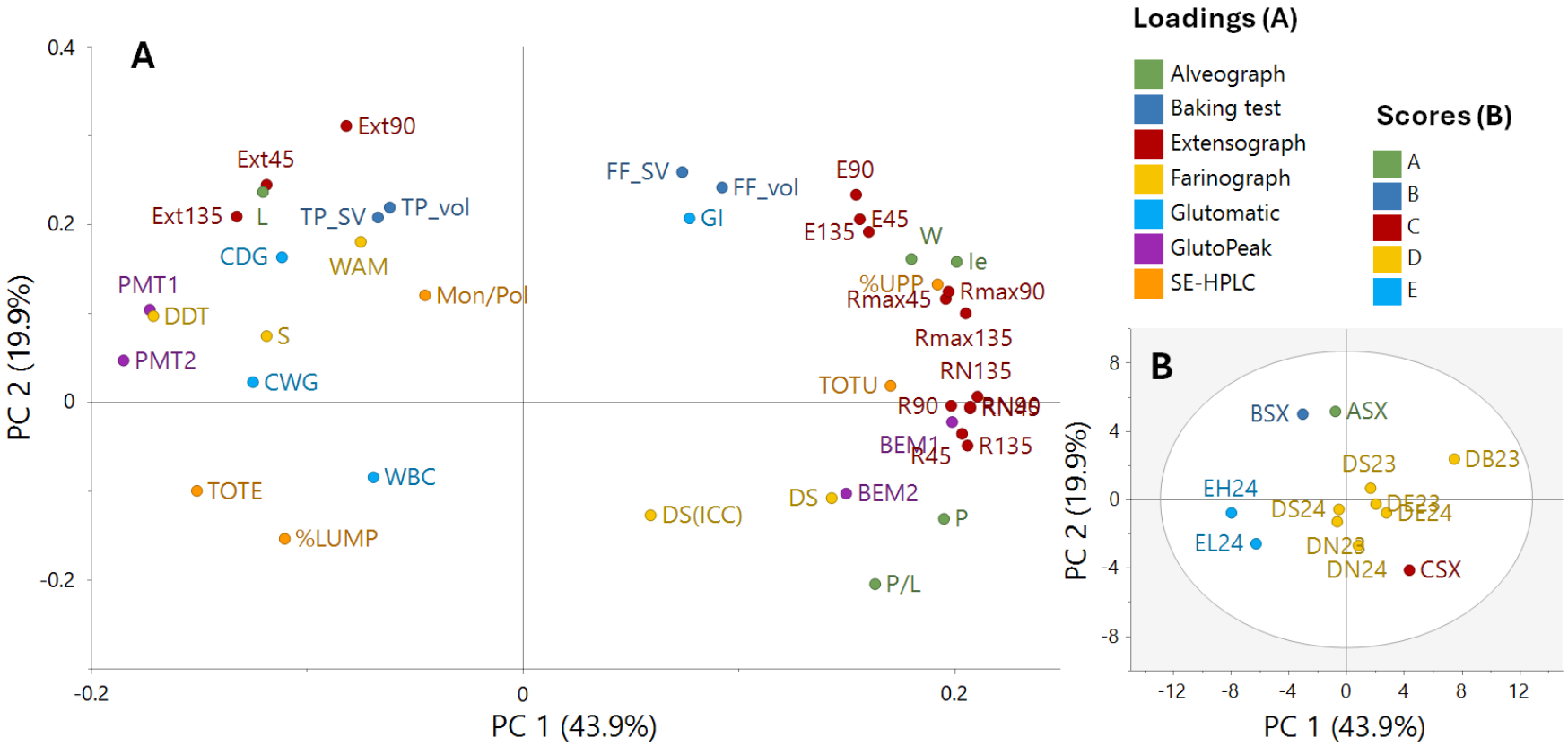

Figure 7. PCA loadings (A) and scores (B) of vital gluten flour blends. Labels in the loadings plot refer to variable names (Tables 3 and 4) are coloured according to method of origin, while labels in the score plot refer to sample names (Table 1) and are coloured according to manufacturing company. For variables (A), spatial proximity is indicative of positive association, while variables on opposite sides of origo are likely negatively associated. The distance from origo represents the strength of the association. For samples (B), spatial proximity is indicative of similar performance across analyses, and the distance from origo relates to their contribution to the overall variation in the data set. TP: tin pan bread; FF: free-form bread.

Pearson's correlation analysis results are presented in the form of a heatmap matrix (Figure 8). As surmised in the PCA loadings plot (Figure 7A), %UPP was significantly positively associated with parameters describing dough strength, including RMax ($p < 0.01$) and W ($p < 0.001$), which is in line with previous studies performed on wheat flour. Several publications (Gupta et al. 1993; Bangur et al. 1997; Zhang et al. 2008) have reported a positive association between %UPP and RMax, and Selga (2023) reported that %UPP was positively associated with W. Thus, there is reason to believe that the positive relationship between free-form loaf volume and %UPP in this study is linked to an increase in dough strength. This theory is also supported by the significant positive correlation between free-form loaf volume and W ($p < 0.01$). Moreover, Selga (2023) found a positive correlation between %UPP and P, which describes the dough's resistance to inflation. While the same positive correlation could also be observed in the current study, the results were not significant.

In contrast to %UPP, %LUMP demonstrated a significant negative relationship with W ($p < 0.05$), which supports the hypothesis that a higher %LUMP is associated with a reduction in dough strength. However, this finding contradicts Kuktaite et al. (2004), who found no relationship between %LUMP and dough strength in wheat flour. Meanwhile, Lama et al. (2022) reported a negative relationship between %LUMP in wheat flour and dough extensibility, which would suggest that the negative association between %LUMP and free-form loaf volume in the current study would be due to a reduction in dough extensibility. However, there were no significant correlations between %LUMP and parameters describing dough extensibility, such as L.

Furthermore, %UPP was significantly negatively correlated with farinograph dough development time (DDT) and stability (S). Dough development time refers to the mixing time required to reach default consistency, and stability refers to the time the dough is able to maintain this consistency before breakdown occurs. The results of the current study contradict previous studies on wheat flour, where %UPP was reported to positively correlate to dough development time (Gupta et al. 1993; Singh & Singh 2013) and stability (Zhang et al. 2008; Singh & Singh 2013). %UPP corresponds to the quantity of unextractable glutenins, and not necessarily polymer quality. Thus, the contrasting trend observed in the current results may be related to differences in GS composition, which are known to affect gluten strength and overall breadmaking quality (Sissons et al. 2005; Anjum et al. 2007). Moreover, the discrepancy between the results of previous studies and those of the current study could be related to differences in sample preparation and extraction methods.

TOTU and TOTE did not produce significant correlations with either free-form loaf volume or tin pan loaf volume. However, TOTU was significantly correlated to

alveograph parameters P, W and Ie, whereas TOTE was significantly negatively correlated to the same parameters ($p < 0.05$). A positive relationship between TOTU and W, as well as TOTE and L, was also reported by Selga (2023). Furthermore, in the current study, TOTU was significantly positively correlated to BEM and negatively correlated to peak maximum time (PMT), where a high BEM and a short PMT is indicative of a strong gluten network. Hence, it is reasonable to suspect that TOTU and TOTE have an indirect effect on loaf volume by influencing dough rheology.

Free-form loaf volume was positively correlated with extensograph energy (E), however only energy at 135 min (E135) yielded a significant correlation ($p < 0.05$). E corresponds to the total energy required to stretch the dough sample until rupture. Additionally, free-form loaf volume was significantly positively correlated to alveograph elasticity index (Ie; $p < 0.05$), which relates to the elastic properties of the dough. For optimal end-product quality, Ie needs to fall within a certain range, which is dependent on the type of end product (Finnie & Atwell 2016). Nonetheless, a positive correlation between Ie and loaf volume has been established in previous research (Duyvejonck et al. 2012). Notably, E and Ie were also significantly positively correlated with %UPP, reaffirming the role of %UPP in dough strength.

While farinograph water absorption has previously been observed to relate to VG quality (Wadhawan & Bushuk 1989), WAM did not produce significant correlations to neither tin pan loaf volume nor free-form loaf volume. However, this could be related to the lack of variation in this data set, as the VG wheat flour blends did not differ substantially in this regard, ranging from 59.0-59.5% on a 14% moisture basis.

Tin pan loaf volume was significantly positively correlated to extensograph extensibility at 45 min (Ext45; $p < 0.01$) and 90 min (Ext90; $p < 0.05$), but with no significance at 135 min (Ext135; $p > 0.05$). However, in the context of modern, short-time bread production, the first stretch (at 45 min) is likely the most important (Cauvain 2015). While tin pan loaf volume appeared positively correlated to alveograph L and negatively correlated to alveograph P/L and P in the PCA loadings plot (Figure 7A), correlations were not statistically significant (Figure 8).

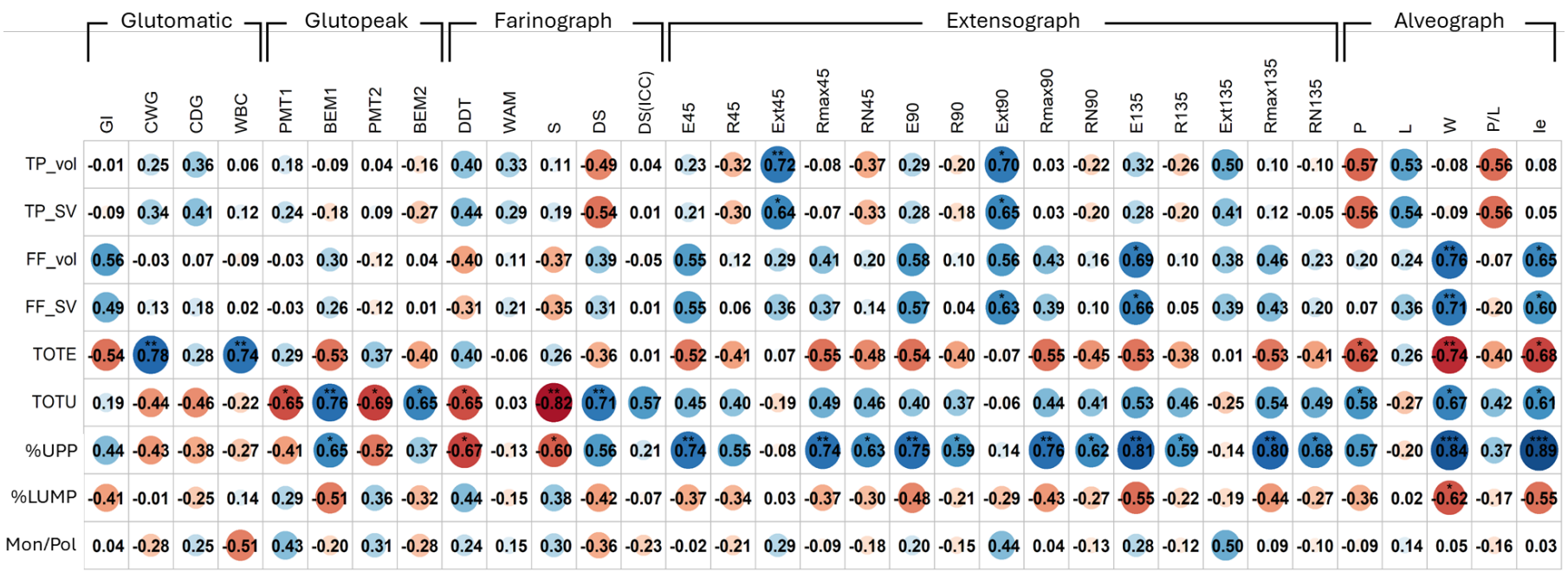

Figure 8. Pearson correlation matrix of baking test parameters and protein parameters against rheological parameters. A negative correlation coefficient (red) indicates a negative relationship between parameters, and vice versa (blue). Total absence of correlation between parameters produces a correlation coefficient of zero. Symbols (, **, ***) indicate significance at 95%, 99%, and 99.9%, respectively.*

Figure 9 shows the Glutopeak time-torque curves performed on the pure VG samples. Gluten aggregation is defined by a peak in the torque curve, after which point continuous mixing results in gluten network degradation with a subsequent reduction in torque. In general, strong glutens exhibit high peak torques (BEM) and short peak times (PMT), while weak glutens are characterised by low BEM and long PMT. This inverse relationship between BEM and PMT was visible in the PCA loadings plot (Figure 7A), as they were found on opposite sides of origo. Additionally, PMT was positively associated with DDT, which was unsurprising as gluten network development is an integral part of dough development (Cauvain 2015; Hu et al. 2023).

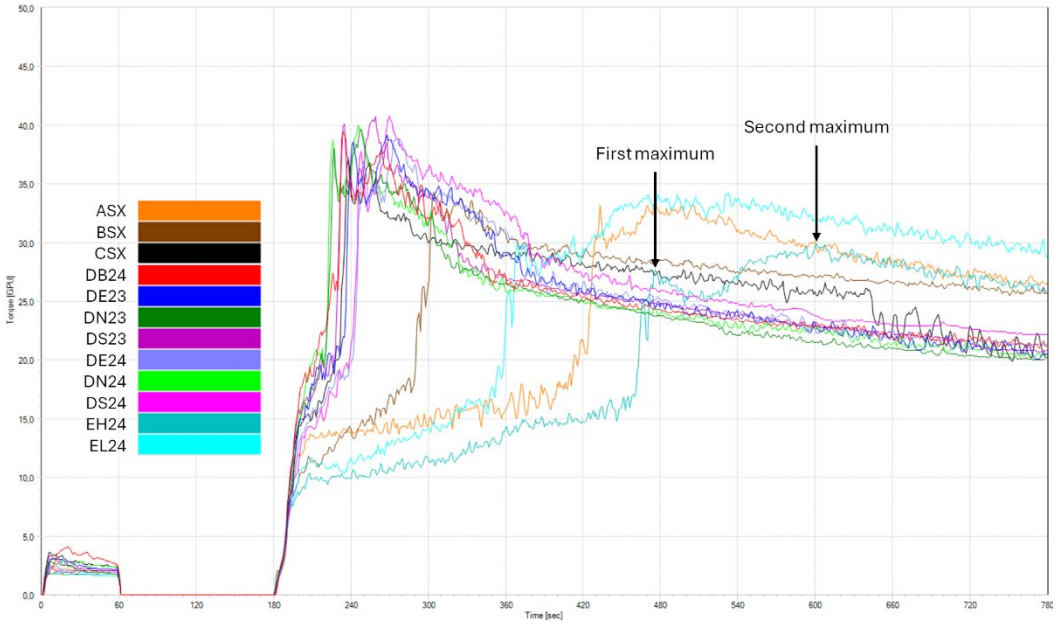

Figure 9. Glutopeak time-torque curves of pure vital gluten samples.

Compared to the other VGs, ASX, BSX, EL24 and EH24 demonstrated lower BEMs and longer PMTs. Long PMTs are attributed to delayed gluten network development (Güçbilmez et al. 2019) and have been linked to a high relative gliadin content (Melnyk et al. 2012). In contrast, short PMTs are associated with a high relative glutenin content (Melnyk et al. 2012). Thus, the glutopeak results of this study indicate that the glutens of these four VG samples were weaker and had a higher gliadin-to-glutenin ratio. However, as previously described, the VG wheat flour blends did not differ substantially in monomeric/polymeric ratio, and Mon/Pol did not yield significant correlations to other parameters. Meanwhile, in the scores plot, PMT appeared more closely associated with dough extensibility parameters and negatively associated with dough strength parameters, which suggests that the glutens of ASX, BSX, EL24 and EH24 were indeed of lesser strength, but instead more extensible. Nonetheless, BEM and PMT did not significantly correlate to free-

form loaf volume nor tin pan loaf volume (Figure 8). Additionally, inclusion or exclusion of BEM and PMT parameters from the PCA model did not substantially alter the spatial distribution of other variables (Figure 7A) nor observations (Figure 7B), suggesting that this source of variation was perhaps already captured by the combination of other analyses. As Glutopeak results stem from singular replicates, the results must be interpreted with caution.

Interestingly, ASX, EL24 and EH24 produced noticeable double peaks (see arrows in Figure 9). Therefore, two values for BEM and PMT were recorded for these samples. Güçbilmez et al. (2019) suggested that the presence of double peaks was indicative of gluten strength, as a second peak would entail that some gluten structures remain stable and continue to aggregate despite prolonged mixing. In addition, only flour from strong wheat cultivars demonstrated double peaks with high BEMs, while flour from weak wheat cultivars produced singular, late and low peaks. Meanwhile, in the current study, both BEMs of double-peak VGs were lower than the singular BEMs of the other VGs and their PMTs were longer, which would contradict this hypothesis. In the current study, it is possible that the double-peak phenomenon is related to differences in hydration rate within the VG samples, as gluten protein hydration precedes gluten network formation, and these differences could potentially be due to varying particle sizes from milling or the presence pre-existing gluten aggregates originating from VG production.

4.4 Comparing rheological methods for evaluating VG quality

As previously mentioned, baking tests are resource-intensive, and the observed inconsistency of baking test results seen in this project does not contribute to their appeal as an industrial quality control method. Finding other means of evaluating VG quality would be of interest for both manufacturers to reduce labour and materials costs, and for consumers as commercial VGs are currently sold with limited information of their quality.

According to Gupta et al. (1993), SE-HPLC is a simple, high-throughput and reliable method to analyse protein composition and molecular size distribution in wheat flour. As seen in this project, the analysis can also be performed on VG wheat flour blends. Compared to the baking test and rheological analyses, an advantage of SE-HPLC is the small sample size required (16.5 mg). Protein extraction and separation is time-consuming, however certain steps in the extraction procedure could potentially be automated. Nonetheless, SE-HPLC analysis requires access to advanced laboratory equipment and training, which may not be feasible in an industrial context. For routine industrial quality control, there is a need for resource-efficient analyses that are rapid and simple to perform (i.e., not requiring trained

personnel). If is not possible to invest in the equipment and laboratory skill required, this type of analysis may perhaps be purchased from an external laboratory, depending on the cost and availability of this service. However, while %UPP and %LUMP were significantly correlated to loaf volume and dough rheological properties of the VG wheat flour blends, this was only true for free-form breads, which limits their application as indicators of VG quality.

Of the five rheological analyses evaluated in this project, only extensograph and alveograph parameters produced significant correlations to loaf volume (Figure 8). Both the extensograph and alveograph constitute standard laboratory equipment in the cereal industry, however the available equipment may vary between cereal laboratories. A drawback of these analyses is that they still require a relatively large amount of sample material, although it is less than that needed for the baking test protocol used in this study. Another drawback is the time required per replicate: 45 min for the alveograph test, and at least 135 min for the extensograph test, if using the standard protocol. There is a shorter test protocol for the extensograph test, where the dough sample is instead stretched after 30, 60 and 90 minutes, respectively, however this protocol was not evaluated in this study. In contrast, the glutopeak instrument yields rapid results (in 10 min) using a comparatively small sample amount (2-10 g depending on the test protocol). While glutopeak analysis of the pure VGs demonstrated notable differences in BEM and PMT between the commercial VGs, as well as the presence of double peaks for some samples, results did not produce significant correlations to loaf volume in this study. Alveograph parameters significantly correlated to free-form loaf volume, whereas extensograph parameters significantly correlated to both free-form loaf volume and tin-pan loaf volume. Thus, if both loaf types are considered, the extensograph test would appear to be a promising method for VG quality evaluation within the cereal and baking industries.

5. Conclusions and recommendations for future research

Addition of VG to the wheat flour yielded a higher free-form loaf volume and a higher tin pan loaf volume compared to not adding VG. There were differences in loaf volume between VG wheat flour blends, which appeared to be related to differences in protein molecular size distribution and rheological properties. Free-form loaf volume, %UPP and dough strength were closely related, while tin pan loaf volume was instead associated with dough extensibility. If both free-form loaves and tin pan loaves are considered, the extensograph test may be a suitable for describing VG quality in an industrial setting. Future studies should investigate how to optimise the extensograph test for industrial VG quality evaluation, for example by shortening the time between stretches and potentially reducing the number of stretches, as well as define relevant VG quality specifications related to the extensograph parameters.

The results of this study, apart from glutopeak results, are unavoidably connected to the choice of wheat flour as base for the VG wheat flour blends. Therefore, future analyses using various flour types are needed to validate results. In addition, only one concentration level of VG was evaluated in this study, and thus the influence of VG concentration on the rheological behaviour and loaf volume of VG wheat flour blends warrants future research.

Since VG quality appears to be related to protein composition, it may be of interest for VG manufacturers to find means of improving the protein composition of their product. While it can be assumed that the protein composition of VG is related to the protein composition of its wheat flour source, there may be differences there between. Furthermore, potential effects of varying processing conditions, such as the choice of wheat flour fractionation method and drying temperature, on VG quality were not considered within the scope of this study. Thus, further research is required to examine the impact of the wheat flour raw material on VG protein composition, as well as investigating the impact of varying processing conditions on VG quality.

6. Acknowledgements

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References

Anjum, F.M., Khan, M.R., Din, A., Saeed, M., Pasha, I. & Arshad, M.U. (2007). Wheat Gluten: High Molecular Weight Glutenin Subunits—Structure, Genetics, and Relation to Dough Elasticity. *Journal of Food Science*, 72 (3), R56–R63. https://doi.org/10.1111/j.1750-3841.2007.00292.x

Bangur, R., Batey, I.L., McKenzie, E. & MacRitchie, F. (1997). Dependence of Extensograph Parameters on Wheat Protein Composition Measured by SE-HPLC. *Journal of Cereal Science*, 25 (3), 237–241. https://doi.org/10.1006/jcrs.1996.0098

de Beer, T. (2023). Chapter 24 - Quality assessment of wheat flour, dough, and bread. In: Shewry, P.R., Koksel, H., & Taylor, J.R.N. (eds) *ICC Handbook of 21st Century Cereal Science and Technology*. Academic Press. 225–233. https://doi.org/10.1016/B978-0-323-95295-8.00002-2

Belton, P.S. (2012). Chapter 12 - The molecular basis of dough rheology. In: Cauvain, S.P. (ed.) *Breadmaking (Third Edition)*. Woodhead Publishing. 373–390. https://doi.org/10.1016/B978-0-08-102519-2.00012-8

Bouachra, S., Begemann, J., Aarab, L. & Hüsken, A. (2017). Prediction of bread wheat baking quality using an optimized GlutoPeak®-Test method. *Journal of Cereal Science*, 76, 8–16. https://doi.org/10.1016/j.jcs.2017.05.006

Castellari, M.P., Simsek, S., Ohm, J.-B., Perry, R., Poffenbarger, H.J., Phillips, T.D., Jacobsen, K.L. & Van Sanford, D.A. (2023). Genetic Variation and Heritability of Sensory and Artisan Bread Traits in a Set of SRW Wheat Breeding Lines. *Foods*, 12 (13), 2617. https://doi.org/10.3390/foods12132617

Cauvain, S. (2015). *Technology of Breadmaking*. Springer International Publishing. https://doi.org/10.1007/978-3-319-14687-4

Cauvain, S.P. (2020). Chapter 1 - Introduction and overview to breadmaking. In: Cauvain, S.P. (ed.) *Breadmaking (Third Edition)*. 3. ed. Woodhead Publishing. 1–30. https://doi.org/10.1016/B978-0-08-102519-2.00001-3

Codina, G.G., Bordei, D. & Paslaru, V. (2008). The effects of different doses of gluten on Rheological behaviour of dough and bread quality. *ROMANIAN BIOTECHNOLOGICAL LETTERS*, 13 (6), 37–42

Day, L., Augustin, M.A., Batey, I.L. & Wrigley, C.W. (2006). Wheat-gluten uses and industry needs. *Trends in Food Science & Technology*, 17 (2), 82–90. https://doi.org/10.1016/j.tifs.2005.10.003

Delcour, J.A., Joye, I.J., Pareyt, B., Wilderjans, E., Brijs, K. & Lagrain, B. (2012). Wheat Gluten Functionality as a Quality Determinant in Cereal-Based Food Products. *Annual Review of Food Science and Technology*, 3 (Volume 3, 2012), 469–492. https://doi.org/10.1146/annurev-food-022811-101303

Dobraszczyk, B.J. & Morgenstern, M.P. (2003). Rheology and the breadmaking process. *Journal of Cereal Science*, 38 (3), 229–245. https://doi.org/10.1016/S0733-5210(03)00059-6

Duyvejonck, A.E., Lagrain, B., Dornez, E., Delcour, J.A. & Courtin, C.M. (2012). Suitability of solvent retention capacity tests to assess the cookie and bread making quality of European wheat flours. *LWT - Food Science and Technology*, 47 (1), 56–63. https://doi.org/10.1016/j.lwt.2012.01.002

Finnie, S. & Atwell, W.A. (eds) (2016). Chapter 5 - Wheat and Flour Testing. In: *Wheat Flour (Second Edition)*. AACC International Press. 57–77. https://doi.org/10.1016/B978-1-891127-90-8.50005-X

Giannou, V. & Tzia, C. (2016). Addition of Vital Wheat Gluten to Enhance the Quality Characteristics of Frozen Dough Products. *Foods*, 5 (1), 6. https://doi.org/10.3390/foods5010006
Goesaert, H., Brijs, K., Veraverbeke, W.S., Courtin, C.M., Gebruers, K. & Delcour, J.A. (2005). Wheat flour constituents: how they impact bread quality, and how to impact their functionality. *Trends in Food Science & Technology*, 16 (1), 12–30. https://doi.org/10.1016/j.tifs.2004.02.011
Güçbilmez, Ç.M., Şahin, M., Göçmen Akçacık, A., Aydoğan, S., Demir, B., Hamzaoğlu, S., Gür, S. & Yakışır, E. (2019). Evaluation of GlutoPeak test for prediction of bread wheat flour quality, rheological properties and baking performance. *Journal of Cereal Science*, 90, 102827. https://doi.org/10.1016/j.jcs.2019.102827
Guerrieri, N. & Cerletti, P. (1996). Effect of High-Temperature Short-Time Treatment of Wheat Flour on Gluten Vitality and Structure. *Cereal Chemistry*, 73 (3), 375–378
Gupta, R.B., Khan, K. & Macritchie, F. (1993). Biochemical Basis of Flour Properties in Bread Wheats. I. Effects of Variation in the Quantity and Size Distribution of Polymeric Protein. *Journal of Cereal Science*, 18 (1), 23–41. https://doi.org/10.1006/jcrs.1993.1031
Hu, X., Cheng, L., Hong, Y., Li, Z., Li, C. & Gu, Z. (2023). An extensive review: How starch and gluten impact dough machinability and resultant bread qualities. *Critical Reviews in Food Science and Nutrition*, 63 (13), 1930–1941. https://doi.org/10.1080/10408398.2021.1969535
Hu, X. & Shang, Y. (2007). A new testing method for vital gluten swelling index. *Journal of the Science of Food and Agriculture*, 87 (9), 1778–1782. https://doi.org/10.1002/jsfa.2925
Iqbal, S., Arif, S., Khurshid, S., Iqbal, H.M., Akbar, Q.-U.-A., Ali, T.M. & Mohiuddin, S. (2023). A combined use of different functional additives for improvement of wheat flour quality for bread making. *Journal of the Science of Food and Agriculture*, 103 (7), 3261–3271. https://doi.org/10.1002/jsfa.12508
Johansson, E., Malik, A.H., Hussain, A., Rasheed, F., Newson, W.R., Plivelic, T., Hedenqvist, M.S., Gällstedt, M. & Kuktaite, R. (2013). Wheat Gluten Polymer Structures: The Impact of Genotype, Environment, and Processing on Their Functionality in Various Applications. *Cereal Chemistry*, 90 (4), 367–376. https://doi.org/10.1094/CCHEM-08-12-0105-FI
Johansson, E., Nilsson, H., Mazhar, H., Skerritt, J., MacRitchie, F. & Svensson, G. (2002). Seasonal effects on storage proteins and gluten strength in four Swedish wheat cultivars. *Journal of the Science of Food and Agriculture*, 82 (11), 1305–1311. https://doi.org/10.1002/jsfa.1185
Lan, Y., Kuktaite, R., Chawade, A. & Johansson, E. (2023). Diverse wheat lines to mitigate the effect of drought on end-use quality. *Frontiers in Food Science and Technology*, 3, 1163412. https://doi.org/10.3389/frfst.2023.1163412
Larroque, O.R., Gianibelli, M.C., Sanchez, M.G. & MacRitchie, F. (2000). Procedure for Obtaining Stable Protein Extracts of Cereal Flour and Whole Meal for Size-Exclusion HPLC Analysis. *Cereal Chemistry*, 77 (4), 448–450. https://doi.org/10.1094/CCHEM.2000.77.4.448
Marchetti, L., Cardós, M., Campaña, L. & Ferrero, C. (2012). Effect of glutens of different quality on dough characteristics and breadmaking performance. *LWT - Food Science and Technology*, 46 (1), 224–231. https://doi.org/10.1016/j.lwt.2011.10.002
Melnyk, J.P., Dreisoerner, J., Marcone, M.F. & Seetharaman, K. (2012). Using the Gluten Peak Tester as a tool to measure physical properties of gluten.

Journal of Cereal Science, 56 (3), 561–567. https://doi.org/10.1016/j.jcs.2012.07.015
Ortolan, F. & Steel, C.J. (2017). Protein Characteristics that Affect the Quality of Vital Wheat Gluten to be Used in Baking: A Review. *Comprehensive Reviews in Food Science and Food Safety*, 16 (3), 369–381. https://doi.org/10.1111/1541-4337.12259
Ortolan, F., Urbano, K. & Steel, C.J. (2018). Simple tests as tools for vital wheat gluten evaluation. *British Food Journal*, 120 (7), 1590–1599. https://doi.org/10.1108/BFJ-06-2017-0356
Posit team (2025). *RStudio: Integrated Development Environment for R* (2024.12.1.563). R, Posit Software, PBC. http://www.posit.co/
Rahman, M.M., Ohm, J. & Simsek, S. (2022). Clean-label breadmaking: Size exclusion HPLC analysis of proteins in dough supplemented with additives vs hard red spring wheat flour. *Journal of Cereal Science*, 104, 103426. https://doi.org/10.1016/j.jcs.2022.103426
Rakita, S., Dokić, L., Dapčević Hadnađev, T., Hadnađev, M. & Torbica, A. (2018). Predicting rheological behavior and baking quality of wheat flour using a GlutoPeak test. *Journal of Texture Studies*, 49 (3), 339–347. https://doi.org/10.1111/jtxs.12308
Sartorius Stedim Data Analytics AB (2024). *SIMCA Multivariate Data Analytics Solution* (18.0.1). Sartorius Stedim Data Analytics AB.
Scanlon, M.G. & Zghal, M.C. (2001). Bread properties and crumb structure. *Food Research International*, 34 (10), 841–864. https://doi.org/10.1016/S0963-9969(01)00109-0
Scherf, K.A., Umseher, L., Kieffer, R. & Koehler, P. (2016). Optimization of a micro-scale extension test for rehydrated vital wheat gluten. *Journal of Cereal Science*, 68, 140–147. https://doi.org/10.1016/j.jcs.2016.01.008
Schopf, M. & Scherf, K.A. (2020). Predicting vital wheat gluten quality using the gluten aggregation test and the microscale extension test. *Current Research in Food Science*, 3, 322–328. https://doi.org/10.1016/j.crfs.2020.11.004
Schopf, M. & Scherf, K.A. (2021). Water absorption capacity determines the functionality of vital gluten related to specific bread volume. *Foods*, 10 (2). https://doi.org/10.3390/foods10020228
Selga, L. (2023). *Wheat flour quality for baking : Linking flour components and dough performance to predict loaf volume*. Swedish University of Agricultural Sciences. https://doi.org/10.54612/a.6fk4lfac5g
Selga, L., Johansson, E. & Andersson, R. (2024). Prediction models to evaluate baking quality instruments for commercial wheat flour. *Cereal Chemistry*, 101 (3), 681–691. https://doi.org/10.1002/cche.10772
Shewry, P. (2019). What Is Gluten—Why Is It Special? *Frontiers in Nutrition*, 6. https://doi.org/10.3389/fnut.2019.00101
Singh, H. & MacRitchie, F. (2001). Application of Polymer Science to Properties of Gluten. *Journal of Cereal Science*, 33 (3), 231–243. https://doi.org/10.1006/jcrs.2000.0360
Singh, S. & Singh, N. (2013). Relationship of polymeric proteins and empirical dough rheology with dynamic rheology of dough and gluten from different wheat varieties. *Food Hydrocolloids*, 33 (2), 342–348. https://doi.org/10.1016/j.foodhyd.2013.04.007
Sissons, M.J., Ames, N.P., Hare, R.A. & Clarke, J.M. (2005). Relationship between glutenin subunit composition and gluten strength measurements in durum wheat. *Journal of the Science of Food and Agriculture*, 85 (14), 2445–2452. https://doi.org/10.1002/jsfa.2272

Southan, M. & MacRitchie, F. (1999). Molecular Weight Distribution of Wheat Proteins. *Cereal Chemistry*, 76 (6), 827–836. https://doi.org/10.1094/CCHEM.1999.76.6.827
Stauffer, C.E. (1990). *Functional Additives for Bakery Foods*. 1. ed. Van Nostrand Reinhold.
Van Der Borght, A., Goesaert, H., Veraverbeke, W.S. & Delcour, J.A. (2005). Fractionation of wheat and wheat flour into starch and gluten: overview of the main processes and the factors involved. *Journal of Cereal Science*, 41 (3), 221–237. https://doi.org/10.1016/j.jcs.2004.09.008
Veraverbeke, W.S. & Delcour, J.A. (2002). Wheat Protein Composition and Properties of Wheat Glutenin in Relation to Breadmaking Functionality. *Critical Reviews in Food Science and Nutrition*, 42 (3), 179–208. https://doi.org/10.1080/10408690290825510
Wadhawan, C.K. (1988). Fundamental studies on vitality of gluten for breadmaking. (Doctoral dissertation). University of Manitoba. http://hdl.handle.net/1993/16740 [2025-01-30]
Wadhawan, C.K. & Bushuk, W. (1989). Studies on Vitality of Commercial Gluten. I. Physical, Chemical, and Technological Characteristics. *Cereal Chemistry*, 66 (6), 456–461
Wieser, H. (2007). Chemistry of gluten proteins. *Food Microbiology*, 24 (2), 115–119. https://doi.org/10.1016/j.fm.2006.07.004
Wieser, H., Koehler, P. & Scherf, K.A. (2023). Chemistry of wheat gluten proteins: Qualitative composition. *Cereal Chemistry*, 100 (1), 23–35. https://doi.org/10.1002/cche.10572
Zhang, P., He, Z., Zhang, Y., Xia, X., Chen, D. & Zhang, Y. (2008). Association Between % SDS-Unextractable Polymeric Protein (%UPP) and End-Use Quality in Chinese Bread Wheat Cultivars. *Cereal Chemistry*, 85 (5), 696–700. https://doi.org/10.1094/CCHEM-85-5-0696

Popular science summary

Boosting industrial bread with vital gluten – and why the quality of vital gluten matters

Ever wondered why some breads turn out big and tall, while others do not rise to the occasion? A lot of it comes down to the properties of their gluten – the stretchy and pliable network of wheat flour proteins that allows bread dough to hold its shape, trap gas and expand during proofing. Differences in the composition and size of these gluten proteins influences how the dough behaves during breadmaking, which in turn influences the quality of the resulting bread.

Vital gluten is a gluten protein concentrate that is produced from wheat flour. It is mainly used within the baking industry to get fluffier loaves of bread with a better crumb structure. Adding vital gluten to the bread recipe gives the bread a "boost", as it contributes to a stronger gluten network with better gas retention. Surprisingly, vital gluten is sold with very little information about its quality, including the composition and size of its gluten proteins, and it can therefore be difficult for industrial bakeries to predict its effects on the properties of the dough and bread. Industrial bakeries may opt to test the quality of the vital gluten through practical baking trials; however this is a time-consuming method that requires plenty of raw material. Therefore, it would be beneficial to find another method of evaluating vital gluten quality which is better adapted to industry needs.

In this study, different types of commercial vital gluten was added to the same wheat flour to see how they affected the overall composition and size of gluten proteins, as well as dough behaviour and bread volume. A secondary goal was to find suitable methods for evaluating the quality of vital gluten quality in the baking industry. It was found that adding vital gluten to the wheat flour increased the loaf volume of the resulting bread, regardless of vital gluten type. However, the vital glutens varied in their ability to increase loaf volume, and this could be linked to differences in the composition and size of their gluten proteins, as well as differences in dough behaviour. Vital glutens with a higher content of large elastic gluten proteins produced stronger doughs, which resulted in fluffy free-form bread. Tin pan loaves, on the other hand, seemed to benefit from weaker doughs with greater extensibility. These results emphasise the need to provide industrial bakeries with information about the quality of their vital gluten, as the effects of the commercial vital glutens on dough and bread properties was shown to vary.

Of the methods evaluated in this project, the extensograph test appeared most promising for describing vital gluten quality. In this test, a sample of dough is stretched until breaking, and after each stretch, the dough is reshaped and allowed to rest before being stretched again. In total, the dough sample is stretched three times, and the force required to stretch the dough is monitored each time. Both free-form loaf volume and tin pan loaf volume were positively associated with dough properties measured in this test. The next step is to use the extensograph method to develop quality criteria for vital gluten, which would not only be beneficial for vital gluten manufacturers

aiming to improve their product, but also for industrial bakeries to better see which vital gluten is right for their application.

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