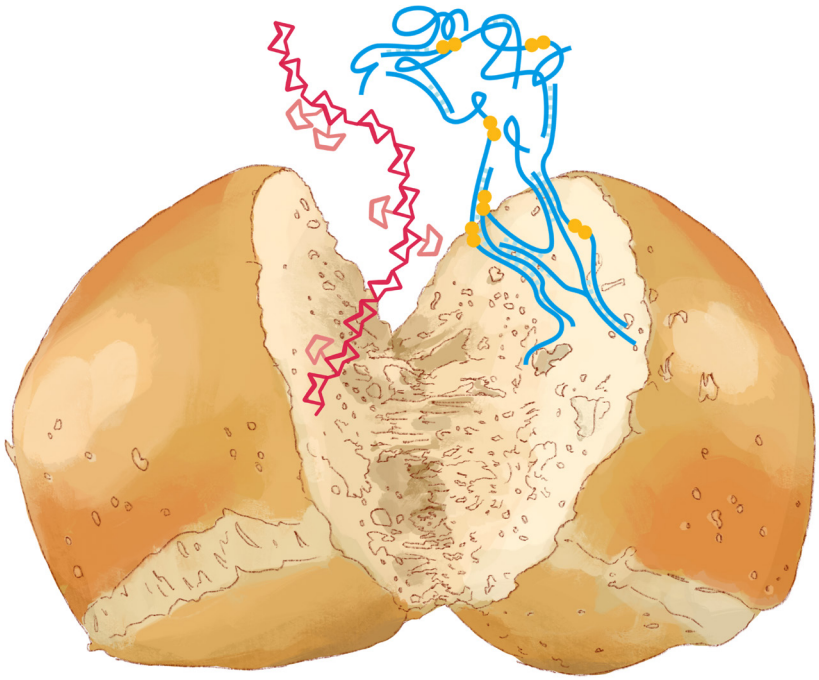
Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2023:79
Faculty of Natural Resources and Agricultural Sciences

Wheat flour quality for baking

Linking flour components and dough performance to predict loaf volume

Louise Selga



Wheat flour quality for baking

Linking flour components and dough performance to predict loaf volume

Louise Selga
Faculty of Natural Resources and Agricultural Sciences (NJ)
Department of Molecular Sciences
Uppsala

SWEDISH UNIVERSITY
OF AGRICULTURAL
SCIENCES

DOCTORAL THESIS
Uppsala 2023

Acta Universitatis Agriculturae Sueciae
2023:79

Cover: Bread with schematic drawings of arabinoxylan and gluten proteins.
(Illustration: L. Selga)

ISSN 1652-6880
ISBN (print version) 978-91-8046-210-5
ISBN (electronic version) 978-91-8046-211-2
https://doi.org/10.54612/a.6fk4lfac5g

Swedish University of Agricultural Sciences, Department of Molecular Sciences, Uppsala, Sweden

Print: SLU Grafisk service, Uppsala 2023

Wheat flour quality for baking

Abstract

The main target of industrial mills is to supply flour of stable quality. Mills therefore perform rigorous quality control, but may still overlook parameters with significant effects on baking quality. This thesis explored: (1) how flour components affect baking quality; (2) the best instruments for use in quality control; and (3) whether test baking can be reduced by implementing prediction models. During 2018-2019, two mills in southern and central Sweden collected 197 wheat flour samples, including winter wheat, spring wheat and blends thereof. Test baking and routine quality control data were supplied by the mills, with inclusion of additional quality control instruments. Key flour components were also analysed: water-extractable and -unextractable arabinoxylan, protein composition by size exclusion HPLC, and damaged starch content. Principal component analysis (PCA) was used to rapidly assess relationships between parameters and samples. Partial least square regression (PLS) was used to predict loaf volume and evaluate parameter contributions.

The protein content and size distribution of polymeric protein were found to be linked to loaf volume, while protein composition in dough was further linked to dough performance. Protein content did not influence water absorption, which was mainly determined by damaged starch content and total arabinoxylan content. Among the quality control instruments evaluated, NIT and SDmatic were most efficient in measuring protein content and damaged starch, both linked to loaf volume. Alveograph parameters were strongly linked to different flour components measured, but the interpretability of parameter extensibility and tenacity was limited by the fixed amount of water used in the standard protocol. Farinograph and Glutomatic data contributed to loaf volume predictions, but both instruments displayed notable measurement errors. Mixolab proved unsuitable for quality control, due to its inability to differentiate flours when all were of acceptable quality. Winter wheat loaf volume was accurately predicted based on selected instrument parameters.

Keywords: wheat, flour, gluten, arabinoxylan, rheology, PCA, PLS, loaf volume.

Vetemjölskvalitet för bakning

Abstract

En industriell kvarn har som huvudsaklig målsättning att producera mjöl med stabil produktkvalitet. Kvarnar utför därför en grundlig kvalitetskontroll, men det är möjligt att denna missar parametrar av betydande effekt för bakningskvalitet. Den här avhandlingen utforskade (1) hur mjölets beståndsdelar påverkar bakningskvalitet, (2) vilka instrument som är bäst för kvalitetskontroll, och (3) om provbakning kan reduceras genom att implementera prediktionsmodeller. Under 2018–2019 samlade två kvarnar, i Malmö och Strängnäs, in 197 prover av vetemjöl, bestående av höstvete, vårvete och blandningar av dessa. Resultat från provbakning och rutinmässig kvalitetskontroll samlades också in. Ytterligare instrument för kvalitetskontroll testades. Mjölkomponenter analyserades också: arabinoxylan, skadad stärkelse och proteinsammansättning, genom size exclusion HPLC. Principialkomponent-analys (PCA) användes för att få en snabb överblick över sambanden mellan parametrar och provgrupper. Partial least square regression (PLS) användes för att prediktera brödvolym och att utvärdera bidraget från olika parametrar.

Proteinhalten och storleksfördelningen av polymer-proteiner kunde kopplas till brödvolym. Vid analys av proteinsammansättningen i deg kunde denna även kopplas till deg-egenskaper. Proteinhalt påverkade inte vattenabsorption, som i stället avgjordes av halterna skadad stärkelse och arabinoxylan. I utvärderingen av kvalitetsinstrument stod NIT och SDmatic ut som effektiva för att mäta proteinhalt och skadad stärkelse, mått som båda påverkade brödvolym. Alveograf-parametrarna kunde tydligt länkas till mjölets komponenter, men tolkningen av dessa försvårades av att standardprotokollet använde en konstant vattenhalt. Farinograf och Glutomatic bidrog till att prediktera brödvolym, men instrumenten hade en påtaglig felmarginal. Mixolab framstod inte som användbar för kvalitetskontroll i kvarnar, då den ej kunde differentiera mellan prover av acceptabel kvalitet. För höstvete kunde brödvolym predikteras väl baserat på en begränsad uppsättning instrument.

Nyckelord: vete, mjöl, gluten, arabinoxylan, reologi, PCA, PLS, brödvolym.

Dedication

To Stefan

Explanations have existed for all time; there is always a solution to every complex problem — neat, simple, and wrong.
H. L. Mencken

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Selga, L., Andersson, A.A.M, Moldin, A. & Andersson, R. (2023). Determining levels of water-extractable and water-unextractable arabinoxylan in commercial Swedish wheat flours by a high-throughput method. *Journal of Cereal Science* 109, 103608.

II. Selga, L., Johansson, E. & Andersson, R. Prediction models to evaluate baking quality instruments for commercial wheat flour (submitted).

III. Selga, L., Andersson, R., Berghtén, I. & Johansson, E. Protein aggregation behaviour – from wheat flour to mixed dough (manuscript).

Paper I is reproduced with the permission of the publisher.

The contribution of Louise Selga to the papers included in this thesis was as follows:

I. Performed data collection and data analysis. Wrote the final version of the manuscript, with input from the co-authors.

II. Designed the study together with the co-authors, performed data collection and data analysis. Wrote the final version of the manuscript, with input from the co-authors.

III. Designed the study together with the co-authors, contributed to data collection and performed data analysis. Wrote the final version of the manuscript, with input from the co-authors.

List of tables

List of figures

Abbreviations

AX	Arabinoxylan
CIMMYT	Centro Internacional de Mejoramiento de Maíz y Trigo
GC	Gas chromatography
HMW-GS	High molecular weight glutenin subunits
LMW-GS	Low molecular weight glutenin subunits
PC	Principal component
PCA	Principal component analysis
PLS	Partial least square regression
RMSEP	Root mean square error of prediction
SE-HPLC	Size exclusion high performance liquid chromatography
SS	Disulphide
SW	Spring wheat
TAXI	*Triticum aestivum* endoxylanase inhibitor
TOTE	Total extractable protein
TOTU	Total unextractable protein
%UPP	Percentage of unextractable polymeric protein in total polymeric protein
VIP	Variable importance in projection
WE-AX	Water-extractable arabinoxylan
WU-AX	Water-unextractable arabinoxylan
WW	Winter wheat

Instrument parameter abbreviations

Alveograph

Dmin	Minimum derivate
EI	Elasticity index
L	Extensibility
P	Tenacity
W	Strength

Amylograph

T	Temperature

Farinograph

DDT	Dough development time
DoS	Degree of softening
S	Stability
WA	Water absorption

Glutomatic

GI	Gluten index
WG	Wet gluten

Mixolab

C1	Consistency at absorption
CS	Consistency at 8 min
C2	Protein weakening
C3	Starch gelatinisation
C4	Hot gel stability
C5	Starch retrogradation

NIT	Near-infrared transmittance
PC	Protein content

RheoF4

CO2 ret.	CO_2 retention
V	Volume

RVA	Rapid Visco Analyser

SDmatic

DS	Damaged starch

SRC-Chopin

SRC	Solvent retention capacity
LAc	Lactic acid
$NaCO_3$	Sodium carbonate
Suc	Sucrose

1. Introduction

Wheat (*Triticum aestivum*) is the most traded crop globally (FAOSTAT 2020), due to high global consumption and varying quality requirements. Wheat may be suitable for a wide range of products, so mills and bakeries use quality specifications when trading wheat grain or flour. However, quality control differs between mills and is partly influenced by tradition. The Swedish milling industry is therefore unsure whether all relevant quality fluctuations are captured by its current quality control system. For this reason, flours are commonly test-baked to evaluate baking quality, but this is laborious and the results vary with the method used (Thanhaeuser et al. 2014).

In industrial bakeries, consistent quality is preferable to high but varying quality, as flour with unpredictable functionality can lead to unstable product quality, process interruptions and food waste (Carson and Edwards, 2009). Protein content and composition appear to be the main determinants of baking quality (Hamer et al. 2009), but other flour components such as starch and fibre can also have an effect (Goesaert et al. 2005). This thesis explored different aspects of baking quality, in particular the influence of different flour components, the relevance of different quality control instruments and whether test baking results can be predicted using quality control data.

2. Background

This background chapter begins by outlining aspects of baking quality related to dough rheology and loaf volume (section 2.1), which are the main quality aspects quantified in this research. Section 2.2 describes different wheat flour components affecting baking quality, while section 2.3 explains why some of these compounds may fluctuate in composition, leading to variations in baking quality.

2.1 Dough rheology

Dough is a liquid-gas foam, which transitions to a solid-gas foam during baking. The unique air-holding capacity comes from formation of a gluten network, which entraps gas cells. Starch and fibres are embedded in the gluten network and affect it by competing with the proteins for water. Non-polar lipids may weaken the gluten network by binding to hydrophobic regions of the protein polymers. Gas cell stability may also be affected by soluble fibre, polar lipids and smaller proteins, as these may affect the surface tension or viscosity surrounding air cells.

Wheat dough is viscoelastic, meaning that it holds its shape but does not maintain its shape when force is applied. Dough rheology is generally measured by applying controlled deformation over time and measuring the resulting force (Dobraszczyk & Morgenstern 2003). Descriptive empirical rheological measurements are generally used in the cereal industry and differ from fundamental rheology measurements in that the stress and strain rates applied are uncontrolled (Dobraszczyk & Morgenstern 2003). As a result, the rheological parameters obtained are purely descriptive and specific to the type of instrument used. However, these tests are easy to perform compared

with fundamental rheology tests and are designed to yield results which are easily interpretable and applicable in the cereal industry.

Farinograph and Mixolab tests measure dough consistency during mixing, to evaluate water absorption by the flour and dough mixing properties. Water absorption is measured by determining the amount of water needed to reach a predetermined consistency during initial mixing. Water absorption changes depending on flour composition and particle size, and is important since water availability in the dough is key during breadmaking. The amount of water added when baking is often adjusted according to water absorption. Dough development time is the timepoint at which the maximum consistency is obtained. Related parameters include stability and degree of softening. The Mixolab test also measures dough consistency during heating, yielding information on the viscosity during gelatinisation, gel degradation and retrogradation. These parameters can also be evaluated using a rapid visco analyser (RVA) or amylograph, but then the tests are performed on flour slurries instead of doughs.

Alveograph tests measure biaxial extensibility by inflating a small circular sheet of dough and measuring the air pressure and dough bubble size. This mimics the air-holding capacity needed to obtain high bread volume. Dough extensibility (L) is determined from the bubble size at rupture and is dependent on both viscous and elastic properties (Jødal & Larsen 2021). Dough tenacity (P) is determined from the maximum pressure needed to inflate the dough. The amount of water added during alveograph tests is not adjusted according to water absorption, and the tenacity is partially influenced by this. Dough strength (W) is determined by the area under the curve measuring pressure and bubble size. Dough strength is commonly used to classify wheat varieties and wheat flour, as it is influenced by protein quality, and different bakery products require different degrees of protein quality (Jødal & Larsen 2021).

2.2 Wheat flour components

The chemical composition of wheat flour is affected by the variety/varieties from which it is produced, the environment in which it is grown, and the milling process. In combination, these factors can result in wide variation in wheat flour quality. Breadmaking quality differences are primarily caused by variations in gluten protein content and composition (Hamer et al. 2009),

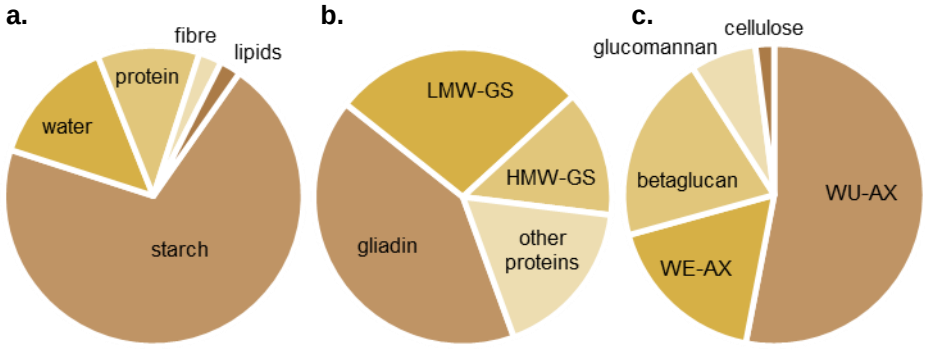


Figure 1: Composition of (a) wheat flour, (b) endosperm proteins and (c) endosperm fibre (sources: Goesaert et al. 2005; Delcour et al. 2012; Mares and Stone 1973). LMW-GS = low molecular weight glutenin subunits, HMW-GS = high molecular weight glutenin subunits, WU-AX = water-unextractable arabinoxylan, WE-AX = water-extractable arabinoxylan.

which can differ markedly depending on variety and environment and often change between harvest years (Johansson et al. 2013). These quality differences in protein may in turn mask the effect of other components, such as fibres, lipids and enzymes, on baking quality (Figure 1a).

2.2.1 Gluten proteins

Gluten proteins impart unique viscoelastic properties to wheat dough. They are a complex group of proteins with a wide range of size and functionality. These consist of gliadins, which are monomeric, and glutenins, which can form polymers by disulphide (SS) bonds through cysteine residues (Tatham et al. 1990) (Figure 1b). Gluten proteins are quite difficult to study, as they vary widely in molecular weight, which also results in a wide range of solubility. The tendency of glutenin polymers to aggregate can result in issues with polymer solubilisation and analysis (Shewry & Lafiandra 2022).

Glutenins consist of low molecular weight glutenin subunits (LMW-GS), with molecular weights of 30,000 – 50,000, and high molecular weight glutenin subunits (HMW-GS), with molecular weights of 60,000 – 100,000 (Shewry & Lafiandra 2022). The HMW-GS are rich in glutamine, glycine and proline, which are largely located in repeat regions (Tatham et al. 1990). These repeat regions may form β-turns and β-sheets, depending on water availability (Tatham et al. 1990). However, the overall structure appears to be intrinsically disordered (Rasheed et al. 2013), meaning that it does not

hold a set structure (Markgren et al. 2022), and is extended without being rigid (Blanch et al. 2003).

Each wheat genotype contains three to six HMW-GS (Johansson et al. 2013). The combination of these present in a genotype is a main determinant of flour quality (Guzmán et al. 2022) partly as they differ in their number of cysteine residues which can form interchain SS bonds (Shewry et al. 1992). Multiple wheat varieties are usually blended during storage and milling, producing combinations of HMW-GS which may be difficult to characterise. Wheat genotypes contain 7-16 LMW-GS, depending on gene silencing. However, the LMW-GS protein group is heterogeneous and complex and for this reason is not well characterised (Johansson et al. 2013). Structure predictions indicate that LMW-GS have a more globular structure than HMW-GS (Blanch et al. 2003). Wheat generally contains approximately twice as much LMW-GS as HMW-GS by weight (Delcour et al. 2012). The exact ratio of HMW-GS to LMW-GS may affect glutenin size distribution (Gupta et al. 1993), and therefore dough strength (Veraverbeke & Delcour 2002).

During dough formation, flour is hydrated and mixed, and glutenin subunits are de-aggregated and re-aggregated into a viscoelastic gluten network (Weegels et al. 1997). While additional formation of intermolecular SS bonds increases polymer size, they are not essential for explaining dough properties, as gluten protein polymers of sufficient size are already present in wheat flour (Hamer et al. 2009). In fact, the molecular weight distribution does not increase from flour to dough. The actual mechanisms behind the elastic properties of gluten proteins are not known, but may be a combined effect of (i) SS bonds providing structural continuity by connecting HMW-GS to form large protein polymers (Delcour et al. 2012; Tatham et al. 1990) (ii) the intrinsic elasticity of loose β-spirals formed by HMW-GS repeat regions (Haward et al. 2011) and (iii) formation of hydrogen bonds between protein polymers, which align upon stretching (Belton, 1999; Wellner et al. 2005) (Figure 2). These mechanisms can be illustrated using the entangled polymer model (Singh & MacRitchie 2001), according to which glutenin polymers may be connected through entanglement points consisting of SS bonds or hydrophilic regions aligned by hydrogen bonds. These coiled, or spiral-shaped, polymers may be initially stretched, and further stretching occurs through polymers slipping through entanglement nodes (Hamer et al. 2009). Elasticity arises from the polymers being interconnected by SS bonds

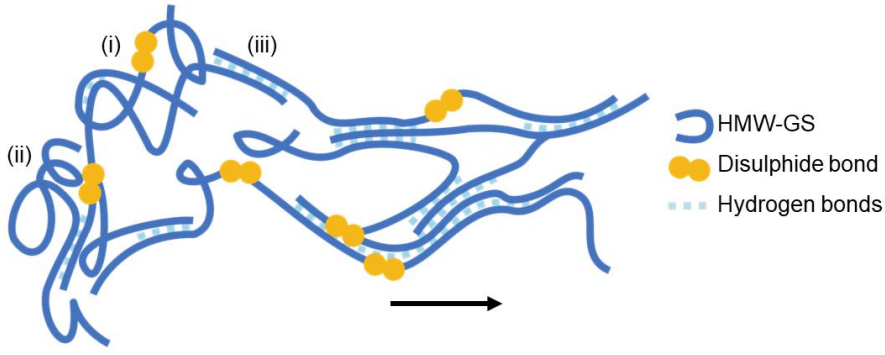


Figure 2: Schematic illustration of the mechanisms behind glutenin viscoelasticity: (i) disulphide bonds, (ii) loose β-spirals and (iii) hydrogen bond arrays. The network stretches when a force is applied.

and their tendency to reconfigure back to a state of lower free energy (Hamer et al. 2009). Viscosity arises from entanglement nodes allowing the polymers to slip under stress (Hamer et al. 2009). Sufficient water levels, as evaluated by flour water absorption, are necessary for polymer mobility and reconfiguration (Belton 1999).

Gliadins can be categorised into α-, γ- and ω-gliadins (Delcour et al. 2012). The primary and secondary structures of α-and γ-gliadins are similar to those of LMW-GS, while ω-gliadins are larger (molecular weight 46,000-74,000) than the other gliadins (30,000-45,000) and are rod-shaped (Shewry et al. 2009). Gliadin acts as a plasticiser by weakening the interactions between glutenin polymers (Delcour et al. 2012). This increases viscosity and decreases elasticity, with the ratio of gliadins to glutenins determining the balance between viscosity and elasticity (Veraverbeke & Delcour 2002). Some studies suggest that while gliadins do not form polymers in flour, they may do so during dough formation, as a decrease in extractability of gliadins caused by sodium dodecyl sulphate (SDS) has been seen during this step in breadmaking (Lee et al. 2002; Kuktaite et al. 2004). However, it is possible that not all non-covalent interactions are broken during quantification, as differences in solubility rates in SDS have been reported for different qualities of flour (He et al. 1991; Veraverbeke & Delcour 2002). Nonetheless, α-and γ-gliadin genes encoding an additional cysteine have been identified (Shewry et al. 2009) and one of the encoded proteins has been quantified in a polymeric fraction (Ferrante et al. 2006). In addition, wheat flour contains 15-20 % water-soluble, non-gluten proteins, which generally have a more structured secondary structure compared with gluten. While

monomeric in general, some albumins and globulins can form interchain SS bonds, possibly to glutenin subunits (Veraverbeke & Delcour 2002).

2.2.2 Arabinoxylan

Arabinoxylan (AX) originates from wheat cell walls and is the main cell wall polysaccharide in the endosperm. Overall, the endosperm cell wall consists of 70 % AX, 20 % β-glucan, 7 % glucomannan, and 2 % cellulose (Mares & Stone 1973) (see Figure 1c). Thus, the β-glucan level in wheat endosperm is low compared with the AX level (Andersson et al. 1993; Mares & Stone 1973; Saulnier et al. 2012). In sieved wheat flour, the AX content is typically around 2 %, of which ~25 % consists of water-extractable AX (WE-AX) (Barron et al. 2007). However, the size of these fractions can vary, with total AX content of 1.4-2.7 % and WE-AX content of 0.3-1.4 % being observed (Saulnier et al. 2007). Among the dietary fibre types present in sieved wheat flour, AX is considered the most important for baking properties. Cellulose and β-glucan are only present in low levels in sieved wheat flour (Saulnier et al. 2012). Wheat β-glucan has a low solubility (Rakha et al. 2011; De Paula et al. 2017) and remains unextractable in water at 65 °C (Beresford & Stone 1983).

In structural terms, AX originating from wheat endosperm is composed of a backbone of β-D-xylopyranosyl residues connected by 1→4 glycosidic linkages, which are partly substituted by α-L-arabinofuranosyl (Izydorczyk & Biliaderis 1994). The substitution level determines the solubility of AX, with lower substitution in WU-AX, and higher substitution and higher heterogeneity in WE-AX (Stone and Morell, 2009). Substitutions of ferulic acid also occur, but the level of ferulic acid in wheat endosperm is low (Saulnier et al. 2012).

Water absorption by flour is affected by both WE-AX and WU-AX, but their effect on baking properties appears to differ (Courtin et al. 1999). Upon water absorption by WE-AX, the viscosity of free water in the dough increases (Courtin et al. 1999) and WE-AX with higher molecular weight causes a larger increase in water absorption (Biliarderis et al. 1995). The WU-AX component instead traps the water it absorbs, as it is insoluble, limiting the water available to gluten protein during gluten network development (Wang et al. 2003). The impact of AX on gas retention is more difficult to study (Courtin & Delcour 2002) and some conflicting results have been reported. Reconstruction studies of wheat flour performed by

fractionating the flour and enzymatically treating the fractions rich in AX have demonstrated that removal of WU-AX significantly improves loaf volume and decreases absorption, while solubilising AX improves loaf volume even further (Courtin et al. 1999). Degrading WE-AX has a negative impact on loaf volume, in agreement with results showing that addition of WE-AX increases loaf volume (Courtin & Delcour 1998). Contrary to this, Shogren et al. (1987) observed in correlation studies that water-extractable pentosans were correlated negatively with loaf volume, although this could potentially be caused by a negative correlation between water-extractable pentosans and protein content in the sample set used in that study.

There are multiple theories on how AX influences gas retention. Hoseney (1984) suggested that WE-AX contributes to gas retention by slowing the diffusion rate of carbon dioxide, while Gan et al. (1995) suggested that the dough is stabilised by a liquid film surrounding gas cells. When gas cells expand beyond the capacity of the gluten network, this liquid film maintains the integrity of the gas cells (Gan et al. 1995). The liquid film in turn is stabilised by WE-AX, increasing its viscosity and lowering the surface tension (Gan et al. 1995). In contrast, WU-AX is present as fragments in the dough which, in addition to blocking gluten network formation, may rupture the liquid film and thus have a negative impact on loaf volume (Courtin and Delcour 2002). It has also been suggested that WE-AX forms a network which enforces the gluten network (Jelaca and Hlynca 1972). However, since AX originating from the starchy endosperm has very a low ferulic acid substitution level (Barron et al. 2007; Saulnier et al. 2012), this level of cross-linking seems unlikely in sieved wheat flour.

The impact of AX on baking properties has mainly been studied to date by addition of AX, enzymatic treatment and correlation studies (Gan et al. 1995). However, the source of the added AX or the enzyme levels tested may not be suitable for uncovering the impacts of AX on baking properties when present at endogenous levels in wheat flour. Results from correlation studies may be more directly applicable, but robust characterisation of the material studied is necessary as parameters other than those studied may vary. For example, Hernández-Espinosa et al. (2020) observed a positive correlation between WE-AX and alveograph strength in CIMMYT wheat breeding lines, but a stronger correlation between WE-AX and results of an SDS sedimentation test, indicating that quality differences may mainly be caused by differences in protein composition.

2.2.3 Starch

Starch is the main component in wheat flour, but its influence on baking quality is limited. Starch consists of two different glucose polysaccharides: amylose, which is smaller and linear, and amylopectin, which is larger and highly branched. Wheat starch contains 65-82 % amylopectin and 18-35 % amylose (Rhazi et al. 2021). Amylose and amylopectin are organised in semi-crystalline granules that are classified as: A-type granules, which are greater than 15 µm and of lenticular shape; B-type granules, which are spherical with diameter 5-15 µm; or C-type granules, which are smaller than 5 µm (Rhazi et al. 2021). Rhazi et al. (2021) found the average granule content to be 75 % A-type, 21 % B-type and 4 % C-type in wheat, with variations mainly being genetically determined. A-type granules are reported to contain slightly more amylose (Zhang et al. 2016). The impact of granule size distribution on baking quality is not well studied and test baking with varying granule type ratios have yielded contradictory results (Wilson et al. 2006).

There may be competition for water between starch and gluten proteins in dough, as the water content is limited. At room temperature, starch granules absorb water to up to 50 % of their weight (Goesaert et al. 2005). However, around 8 % of the starch in grain is damaged during milling, which drastically increases its water absorption capacity (Goesaert et al. 2005). Damaged starch is more susceptible to hydrolysis, which increases the amount of sugar available during fermentation. When starch granules are heated at sufficient water levels, they gelatinise. The crystalline structure begins to melt and absorb more water, which leads to swollen distorted granules and leaching of amylose. A-type granules have a higher gelatinisation temperature and higher peak viscosity (Zhang et al. 2016). Upon cooling the amylose partially recrystallises, a process known as retrogradation. Amylopectin retrogrades during bread storage which, in combination with water migration from crumb to crust, leads to bread staling.

2.2.4 Lipids

Lipids comprise ~2-3 % of wheat flour and originate from the endosperm, the aleurone and the germ (Figure 3). The lipid composition in flour is therefore affected by milling procedures (Chung et al. 2009). Approximately 40 % of the lipids in flour are phospholipids bound inside starch granules, and do not have a direct effect on baking properties. The non-starch lipids in

flour comprise around 57 % non-polar lipids, 25 % galactolipids and 18 % phospholipids (Melis & Delcour 2020), with composition varying slightly between varieties and years (McCormack et al. 1991).

Endogenous lipids are known to affect baking quality, *e.g.*, lipid reconstitution studies on defatted flour have revealed non-linear relationships between loaf volume and the polar lipid content in flour (Gan et al. 1995). This non-linear relationship may be explained by applying liquid film theory to gas cell stabilisation, where polar lipids impede the stabilising impact of surface-active proteins and *vice versa* (Gan et al. 1995). Correlation studies have also indicated that endogenous lipids affect baking quality, as the ratio of non-polar lipids to polar lipids has been found to be correlated negatively with loaf volume in several studies (McCormack et al. 1991; Melis & Delcour 2020).

Test baking methods differ in terms of whether fat, or shortening, is included in the recipe. Fats that are solid at room temperature are suggested to adsorb to the gas cell surface. When these fats melt during baking, this increases the amount of liquid at the gas cell surface, which prevents coalescence between gas cells (Pareyt et al. 2011). The interaction mechanisms between shortening, endogenous polar lipids and surface-active proteins are not known. The degree of impact that endogenous lipids have on baking quality in commercial flour is therefore likely to vary with bakery products, milling practices, wheat varieties and wheat growing environments.

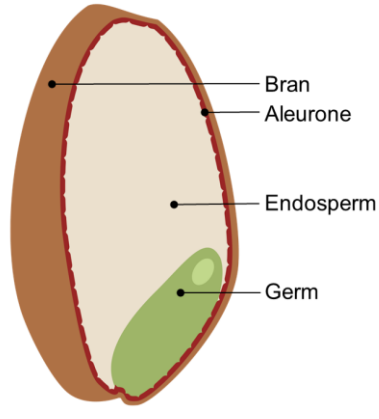


Figure 3: Illustration of a wheat grain. Sieved wheat flour is mainly composed of endosperm material, but traces of other material are also incorporated during milling.

2.2.5 Enzymes

Wheat flour contains enzymes, which can affect baking quality by modifying flour components upon dough production. α-Amylase has the most well-studied effect on baking quality, and α-amylase levels in wheat grain may increase due to sprouting (Brijs et al. 2009). Wheat with high α-amylase activity is not commonly used for bread production, as it leads to high starch degradation and sticky doughs. Moderate α-amylase activity is desired to produce reducing sugars, which are more easily fermented by bread yeast, and α-amylase levels are therefore sometimes increased in flours by adding malt. Falling number is routinely used to measure α-amylase activity indirectly (Olaerts et al. 2016). β-Amylase activity has a weak correlation with falling number, due to its stable levels during grain maturation (Olaerts et al. 2016). The activity of peptidases is also correlated poorly with falling number, as they form at a much slower rate than α-amylases during germination (Brijs et al. 2009). Lipases have also been detected in wheat flour (Mousavi & Kadivar 2018).

The enzymes present in wheat flour may be of endogenous or microbial origin. On analysing wheat kernels harvested at peak maturity, Olaerts et al. (2016) found that around 90 % of endoxylanases were of microbial origin. However, the impact of these on breadmaking is unclear, due to the presence of *Triticum aestivum* endoxylanase inhibitor (TAXI) (Courtin and Delcour, 2002). Endogenous endoxylanase activity is strongly correlated with α-amylase activity and falling number (Olaerts et al. 2016). Assays of enzyme activity are available for multiple enzymes, but it should be noted that the conditions in these assays are completely different from dough conditions, where water and substrates are present in limited amounts (Brijs et al. 2009).

2.3 Quality fluctuations

Stable flour quality is of high importance to industrial bakeries. Fluctuations in baking quality are primarily caused by differences in wheat genotype and growing environment. For protein quality, genotype and environment play an approximately equal role (Johansson et al. 2013). The protein polymer structure is heavily affected by environmental factors such as nitrogen availability, precipitation and temperature during both plant development and grain maturation (Johansson et al. 2013). In a study of different CIMMYT wheat varieties, Guzmán et al. (2022) linked several LMW-GS

and HMW-GS to different quality traits and found that while environment affected the quality, the correlations between glutenin subunit genes and quality persisted across varieties. Differences in plant development time between varieties also affect protein polymer structure (Malik et al. 2011).

In sieved wheat flour, the AX content is primarily determined by genotype (Shewry et al. 2010; Marion & Saulnier 2020), with the HEALTHGRAIN screening of wheat flour showing that genotype has twice as much influence as environment on the WE-AX content (Shewry et al. 2010). For WU-AX, the influence of genotype is even greater (Shewry et al. 2010). According to Rhazi et al. (2021), the amylose:amylopectin ratio in bread wheat is mainly determined by the growing environment. Labuschagne et al. (2007) observed some correlations between amylose:amylopectin ratio and baking quality aspects, but these were not replicable between environments, despite the amylose:amylopectin ratio being stable across the environments included in that study.

3. Aims

Research on wheat flour baking quality has shown that many different aspects may influence the quality, but the aspects that are most relevant in different commercial contexts are not always clear. When studying one aspect at a time, other unmonitored parameters may mask the effect of the parameter of interest. The aim in this thesis was therefore to monitor a large number of parameters. A large sample set was used to ensure that relevant quality fluctuations were captured. The research questions addressed were as follows:

- How do flour components affect baking quality?
- What are the best instruments for use in quality control?
- Can test baking be reduced by implementing prediction models?

The effects of flour components on baking quality were investigated in the studies described in Papers I-III. Arabinoxylans were investigated in Papers I and II, while the protein composition in flour was examined in Paper II and the protein composition in dough in Paper III. Quality control instruments were evaluated in Paper II, as was the question of reducing test baking using prediction models.

4. Materials and methods

4.1 Project outline

Material was collected continuously during harvest years 2018 and 2019 at two Swedish flour mills (Lantmännen Cerealia), located in Malmö in southern Sweden and Strängnäs in central Sweden, to obtain samples representing relevant quality variations (Table 1). The material used is described in detail in Paper I. Routine quality control data, including test baking data, were supplied by the mills. All wheat material collected during this thesis work was of acceptable quality according to the quality control results. Additional quality control instruments of potential interest to the mills were applied to this sample set (Table 2), with the aim of evaluating both the instruments and the samples. Damaged starch content was analysed using one of these instruments. Instruments and test baking methods are described in detail in Paper II. The AX composition (Paper I) and protein composition (Paper II) in flour was analysed at the Swedish University of Agricultural Sciences (SLU). Protein composition was also analysed in doughs prepared in an alveograph for a subset of samples, to further understand the changes that proteins undergo during mixing and to link protein composition to baking quality. This is described in detail in Paper III.

Table 1: Number of samples collected from the mill in Malmö and that in Strängnäs (in brackets)

Harvest year	Winter wheat	Spring wheat	Flour blends	Total
2018	46 (11)	18 (6)	29 (0)	93 (17)
2019	59 (12)	21 (7)	24 (6)	104 (25)
Total	105 (23)	39 (13)	53 (6)	197 (42)

Table 2: Instruments applied during the work in this thesis

Instrument	Metric
Alveograph	Biaxial extension
Amylograph	Pasting
Falling number	α-Amylase activity
Farinograph	Mixing characteristics
Glutomatic	Wet gluten
Mixolab	Mixing characteristics during heating and cooling
Near infrared transmittance	Protein content and water content
Rapid visco analyser	Pasting
Rheo F4	Fermentation
SDmatic	Damaged starch
SRC-Chopin 2	Solvent retention
Test baking	Loaf volume

4.2 Limitations of the work

The focus in this thesis was on the baking quality of commercially milled and sieved wheat flour. Only pure wheat flours were included in the analyses, *i.e.*, without addition of malt or ascorbic acid. Spelt and durum flours were considered beyond the scope of the work. The specific varieties or growing environments were not known for the samples, due to their commercial nature. The two mills in Sweden selected for sample collection were chosen because they have the same quality control routine, with the same methods of test baking. Additional quality control instruments tested were limited to those available at collaborating sites that could be allocated to the research in this thesis for a sufficient time. Due to time restrictions, analysis of flour components was limited by the availability of sufficiently high-throughput methods and equipment availability. As a result, this work focused on arabinoxylan composition, protein composition and damaged starch content.

4.3 Quantification of components

Arabinoxylans were quantified using a high-throughput method developed within this thesis work (Paper I). It is based on the Uppsala method for determining dietary fibre content described by Theander et al. (1995), with modifications by Andersson et al. (1999). The main adjustment is that the

method in this thesis solely quantifies AX and excludes other dietary fibre types. This was deemed necessary for the present context as it reduced the number of days needed for extraction. The main consequence of this was that β-glucan and cellulose were not quantified. The impact of these compounds on baking quality is likely to be lower than that of AX, as cellulose and β-glucan originating from wheat have low solubility (Beresford and Stone 1983; De Paula et al. 2017; Rakha et al. 2011) and are present in lower amounts (Saulnier et al. 2012). In addition to these adjustments, smaller volumes were analysed in order to reduce the test tube size, allowing more samples to fit in the centrifuge and autoclave. This reduction in sample size was deemed appropriate as sieved wheat flour is relatively homogeneous compared with wholegrain material. Depending on the workload, the method could be further adjusted by quantifying the samples using high performance liquid chromatography (HPLC) instead of gas chromatography (GC), as this would remove the acetylation step, which would reduce the workload by half a working day.

The protein composition in flour (Paper II) and dough (Paper III) was analysed by extraction by sonication in SDS buffer and quantification using size exclusion (SE)-HPLC. This method was selected as it is high-throughput and well-used. Glutenin polymers are stabilised by SS bonds and interact strongly with other polymers and gliadins through arrays of hydrogen bonds. There is no consensus on which extraction method is best for separating polymers without disrupting SS bonds (Shewry & Lafiandra 2022).

4.4 Statistical approach

The dataset was primarily analysed using principal component analysis (PCA) and partial least square regression (PLS) modelling in SIMCA 17, alongside with visual examination of the data. PCA and PLS are multivariate methods which compress the variation of individual variables in principal components (PC) or latent variables describing the overall variation in the dataset. This provides an overview of the relationships between multiple variables. A major advantage of multivariate modelling is that the variables analysed can be dependent, which applied to most variables in the dataset in this thesis. The PLS model optimisation process is described in Paper II. Correlations were calculated using Minitab and all correlations presented in this thesis are statistically significant ($p<0.05$).

5. Results and discussion

5.1 Effects of flour components on baking quality

Stable quality is the most important aspect when supplying flour to industrial bakeries. The variation in the commercial flours analysed in this thesis was therefore low compared with the variation that may be observed in other research projects. In fact, all samples collected during this thesis work were of acceptable quality. However, there were still some distinct variation patterns in the sample set (Figure 4), which could be linked to the different flour components.

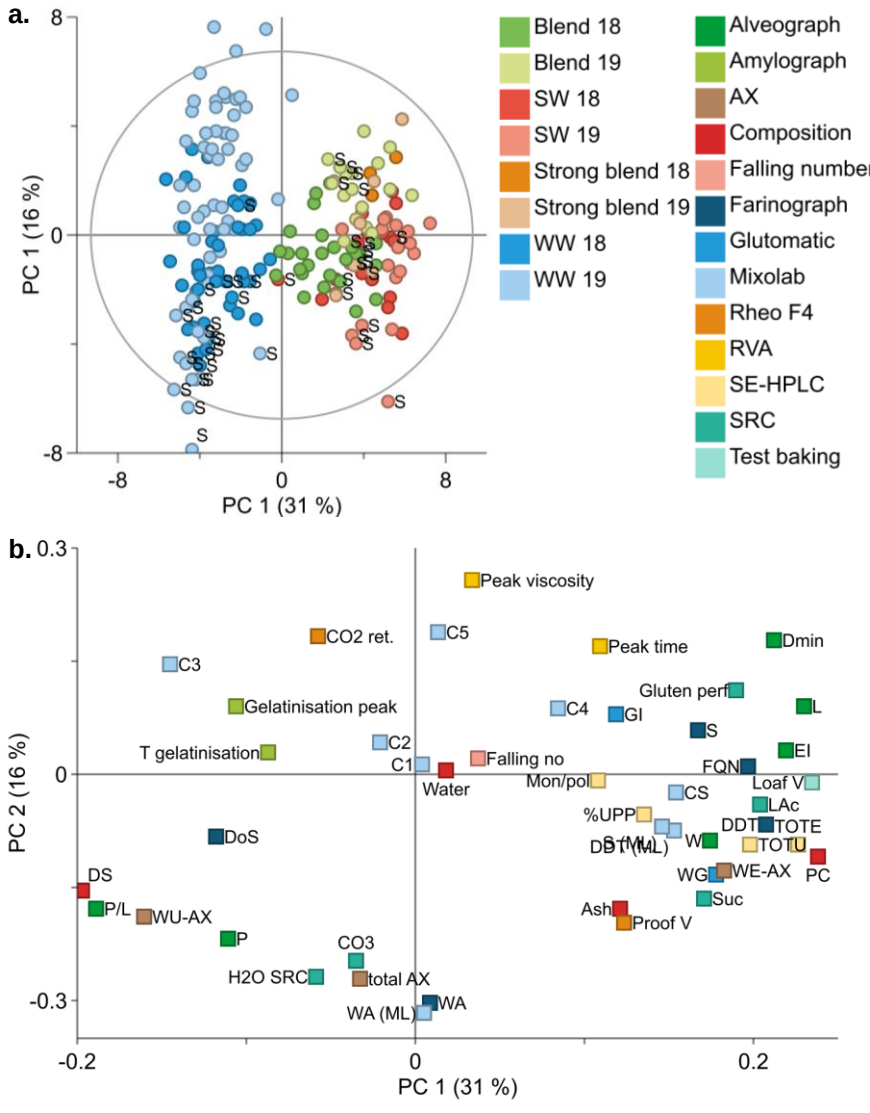


Figure 4: Principal component analysis (a) score plots and (b) loadings plots for the full sample set. Scores for flours milled in Strängnäs are marked with S, circle indicates 95 % Hotelling's T2 distribution. Score abbreviations: SW = spring wheat, WW = winter wheat, 18 = 2018, 19 = 2019. Loading abbreviations for alveograph: P = tenacity, L = extensibility, W = strength, EI = elasticity index, Dmin = minimum derivate. Arabinoxylan abbreviations: WU-AX = water-unextractable arabinoxylan, WE-AX = water-extractable arabinoxylan. Composition abbreviations: DS = damaged starch, PC = protein content. Farinograph abbreviations: WA = water absorption, DDT = dough development time, S = stability, DoS = degree of softening. Glutomatic abbreviations:

GI = gluten index, WG = wet gluten. Mixolab (ML) abbreviations: C1 consistency at absorption, CS = consistency at 8 min, C2 = protein weakening, C3 = starch gelatinisation, C4 = hot gel stability, C5 = starch retrogradation, S (ML) = Mixolab stability, WA (ML) = Mixolab absorption, DDT (ML) = Mixolab dough development time. RheoF4 abbreviations: Proof V = proofing volume, CO2 ret. = CO_2 retention. SE-HPLC abbreviations: TOTE = total extractable protein, TOTU = total unextractable proteins, %UPP = percentage of unextractable polymeric protein in total polymeric protein, Mon/Pol = monomeric-to-polymeric protein ratio. Solvent retention capacity (SRC) abbreviations: CO3 = sodium carbonate SRC, LAc = lactic acid SRC, Suc = sucrose SRC, Gluten perf = SRC gluten performance. Test baking abbreviation: Loaf V = loaf volume.

5.1.1 Differences between sample groups

The winter wheat and spring wheat samples differed in terms of both genetics and environment, with different wheat varieties, harvest seasons and different soils represented in the dataset. Winter wheat has multiple end-uses, while spring wheat is only grown for bread wheat. Spring wheat is a less commonly farmed crop in Sweden and is grown in fewer fields using fewer varieties. These factors may have contributed to spring wheat displaying lower variation in the dataset (Figure 4a). Winter wheat and spring wheat differed significantly in terms of most parameters measured (Paper II), with protein content being the leading difference between the flour types (Figure 4). The AX composition and damaged starch content also differed between winter and spring wheat (Figure 5b-c), as reflected in the score placement along PC1 in Figure 4a. On average, the total AX content did not differ between the flour types, but AX content and composition was more varied in winter wheat (Paper I). As a result of these distinct differences, the flour types were investigated separately when evaluating quality instruments and loaf volume predictions (Paper II).

There was a drought in Sweden during 2018, causing the lowest harvest seen for 25 years. Plant nitrogen uptake was low due to dry soils and temperatures reached 30 °C during grain maturation (Lama et al. 2022). Kernel size was small, with low moisture content. The low nitrogen uptake led to fertiliser remaining in the fields after harvest, resulting in high protein content in grain in 2019 despite the higher harvest. This led to protein content not differing significantly between the harvest years (Paper II).

Due to the low harvest of spring wheat during 2018, the flour blend contained more winter wheat in that year (Paper I). As a result, the AX composition in the flour blend differed between harvest years (Paper I), but

this did not cause any apparent differences in protein composition between harvest years for the flour blend. There were fewer links between composition and quality for the blend compared with winter and spring wheat. Overall, the flour blend displayed weak correlations between the parameters measured and low variability within harvest years. As a result, loaf volume could not be predicted for the flour blend. This thesis therefore generally focused more on winter and spring wheat rather than flour blends.

5.1.2 Flour components affecting water absorption

Samples were collected from two mills: Malmö, located in southern Sweden, and Strängnäs, located in central Sweden. The varieties grown in the two regions were similar for spring wheat but differed somewhat for winter wheat (Paper I). Flours from Malmö and Strängnäs mainly differed in water absorption, as seen by the score distribution along PC2 in Figure 4. Winter wheat milled in Strängnäs had a higher proportion of damaged starch, AX and ash content compared with the corresponding flour from Malmö. Spring wheat from Strängnäs also had a higher amount of damaged starch than that from Malmö. These different levels of components indicated possible differences in milling practices between the two locations. However, the Strängnäs flour mainly had higher levels of WE-AX, which originates from the endosperm (Marion & Saulnier 2020) and was therefore not caused by bran inclusion during milling. Thus, differences in growing environment and possibly also varieties probably explained the composition difference between Malmö and Strängnäs (Paper I), in addition to milling practices.

Concentrations of damaged starch, AX and protein all affect water absorption (Courtin et al. 1999; Greer and Stewart, 1959). The differences in these parameters between Strängnäs and Malmö thus contributed to major variation in water absorption in winter wheat flour, as seen by the large score distribution along PC2 in Figure 4. Damaged starch, AX and protein content were positively correlated in winter wheat (Paper II), making it difficult to discern whether any of these components had a larger effect on water absorption. In spring wheat, water absorption was correlated with damaged starch content ($R = 0.36$), but not protein content or AX content (Figure 5). Compared with winter wheat, the AX content was more consistent in spring wheat, while the protein content varied more in spring wheat. The lack of correlation between water absorption and protein content in spring wheat, despite the high variation in protein content, indicated that protein content

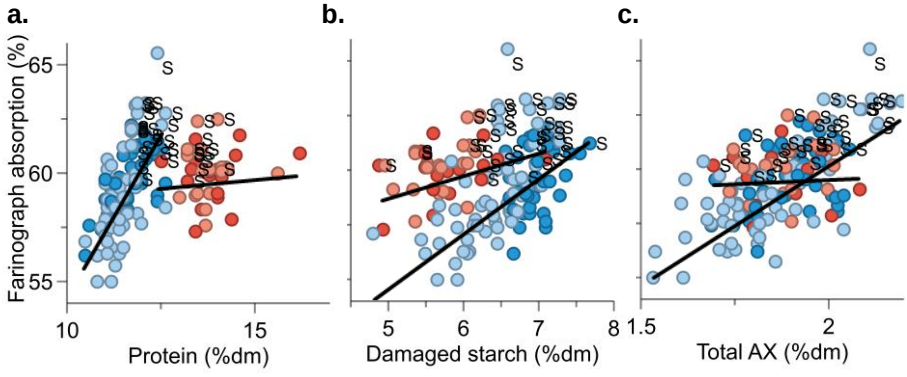


Figure 5: Farinograph water absorption plotted against (a) protein content, (b) damaged starch content and (c) total arabinoxylan (AX) content. Winter wheat is coloured blue and spring wheat is coloured red, with harvest year 2018 in darker shades. Separate trendlines for winter and spring wheat are shown in black. Samples milled in Strängnäs are marked with S.

had a low effect on water absorption in these samples. However, protein content might have had a larger effect on water absorption in other harvest years, as the protein content did not differ between 2018 and 2019, which limited the sample set variation.

Lower variation in quality was observed for winter wheat during 2018 compared with 2019 (Papers I and II), as seen by lower score distribution when modelling baking quality or AX composition (Figure 4a). This could have been caused by a more consistent environment during 2018 compared with 2019, as the drought in 2018 affected all of Sweden. The WE-AX content was somewhat higher during 2019 for winter wheat milled in Strängnäs, despite similar varieties being supplied to Strängnäs in both harvest years (Paper I). This agrees with observations by Rakszegi et al. (2014) of lower WE-AX content in winter wheat under drought conditions. However, in Malmö the WE-AX levels were higher during 2018 (Paper I). This inconsistency could possibly be due to differences between harvest years in the varieties supplied to Malmö.

5.1.3 Protein composition in flour

Winter wheat differed in quality between harvest years, with significantly higher dough strength and percentage of unextractable polymeric protein in total polymeric protein (%UPP) during 2018 (Figure 6a). In general, %UPP increases under drought conditions, as the low moisture content in the grain

promotes hydrogen bond formation between protein polymers (Johansson et al. 2008; Lama et al. 2022; Shewry & Lafiandra 2022). Low nitrogen uptake is also associated with lower formation of monomeric protein (Johansson et al. 2001). For spring wheat, dough strength did not differ between harvest years, but dough stability was higher during 2018 for spring wheat from Strängnäs (Figure 6b). %UPP content did not differ for spring wheat between harvest years at either location.

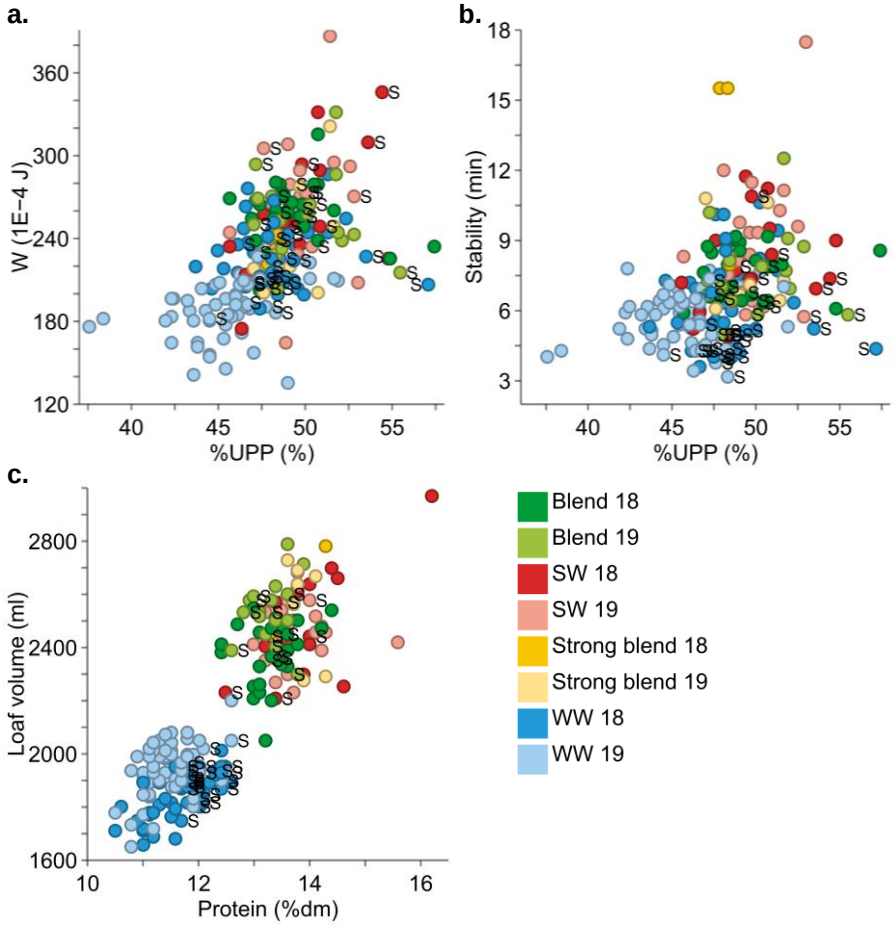


Figure 6: Percentage of unextractable polymeric protein in total polymeric protein (%UPP) in flour plotted against (a) alveograph strength (W) and (b) farinograph stability, and (c) protein content plotted against loaf volume. Samples milled in Strängnäs are marked with S.

On comparing rheological parameters and protein content and composition, surprisingly few correlations were consistent between winter and spring wheat. Overall, %UPP was correlated with strength, while total extractable protein (TOTE) was correlated with Mixolab dough development time. Protein content, as determined by near-infrared transmittance (NIT), did not display any significant consistent correlations between winter and spring wheat for any of the rheological instruments tested. This lack of links between rheology and protein composition was likely caused by the low variation in the set of commercial flours analysed. More consistent trends were noted when using PCA modelling in Paper II, with %UPP clustering with stability and strength, and monomer-to-polymer ratio placing orthogonally to tenacity. However, the spring wheat PCA model developed in Paper II was quite unstable, with SE-HPLC loading placements changing depending on the parameters included. The flour blends displayed very weak correlations between protein composition and rheology, possibly due to differences between flour blend samples mainly arising from the spring wheat inclusion ratio differing between harvest years. Protein composition did not differ greatly between winter wheat and spring wheat, except for winter wheat from Malmö in 2019 (Figure 6a). This allowed winter and spring wheat to be investigated jointly in Paper III.

5.1.4 Protein composition in dough

Gluten composition differs between flour and dough, as the hydration levels increase and forces are applied during mixing. To better understand why the rheological behaviour aligned poorly with flour protein composition, 26 doughs were prepared in the alveograph and dough protein composition was analysed (Paper III). Samples were selected from a fixed range of farinograph absorption, to exclude the link found between tenacity and water absorption (Paper II).

On analysing the protein composition in dough, higher variation was observed for the monomer-to-polymer ratio. For this parameter, a difference was seen between winter wheat and spring wheat, which was not visible for the corresponding flour. In dough, spring wheat displayed a proportionally higher content of monomeric protein than winter wheat. This agrees well with reported proportionally higher production of monomeric protein in wheat supplied with more nitrogen (Johansson et al. 2013). This may indicate

that not all monomeric protein was released during protein extraction in flour and that non-covalent interactions remained during quantification.

Compared with the protein composition in flour, dough protein composition was more strongly correlated with the alveograph parameters measured. This was observed on combining winter and spring wheat, and also when only considering winter wheat. Dough strength was correlated strongly with dough total unextractable proteins (TOTU) and dough %UPP. Dough tenacity was correlated negatively with monomer-to-polymer ratio in dough and TOTE, and positively with dough %UPP.

It should be noted that these observations were made on a small subset of samples, selected to exclude the effect of other flour components. Stronger correlations can be expected between alveograph parameters and dough prepared in that alveograph, compared with flour. The main outcomes from these tests in this thesis work were therefore a better understanding of alveograph parameters, and indications of the potential and limitations of wheat protein extraction and SE-HPLC.

5.2 Screening of instruments for quality control

Quality control tests are routinely performed on flour and dough to ensure that the flours milled are of acceptable quality. The actual tests used vary between mills and countries, while selection of tests is also partly influenced by tradition. There is some uncertainty regarding whether all relevant fluctuations in baking quality are captured by the quality control instruments applied in Swedish mills. Paper II used prediction models to evaluate the most suitable instruments for use in measuring baking quality in the two Swedish mills from which materials were obtained for this thesis work. Partial least square regression (PLS) models predicting loaf volume were generated and optimised against the variable importance in projection (VIP) scores to increase the predictive capacity while limiting the number of instruments included (Paper II). A high VIP score indicates strong predictive capacity of a parameter, while exclusion of parameters with lower VIP scores has a smaller effect on the PLS model. The selected sets of instruments for the different flour types can be seen in Figure 7. These comprised: protein content determined by NIT, alveograph, farinograph, Glutomatic, SDmatic and Mixolab. Protein composition and AX composition did not improve loaf volume predictions, except for TOTE in spring wheat flour. Inclusion of

%UPP yielded a high VIP score in the winter wheat PLS model, but did not improve predictions. The other strength-related parameters included appeared to fully capture the information carried by %UPP relevant for predicting loaf volume.

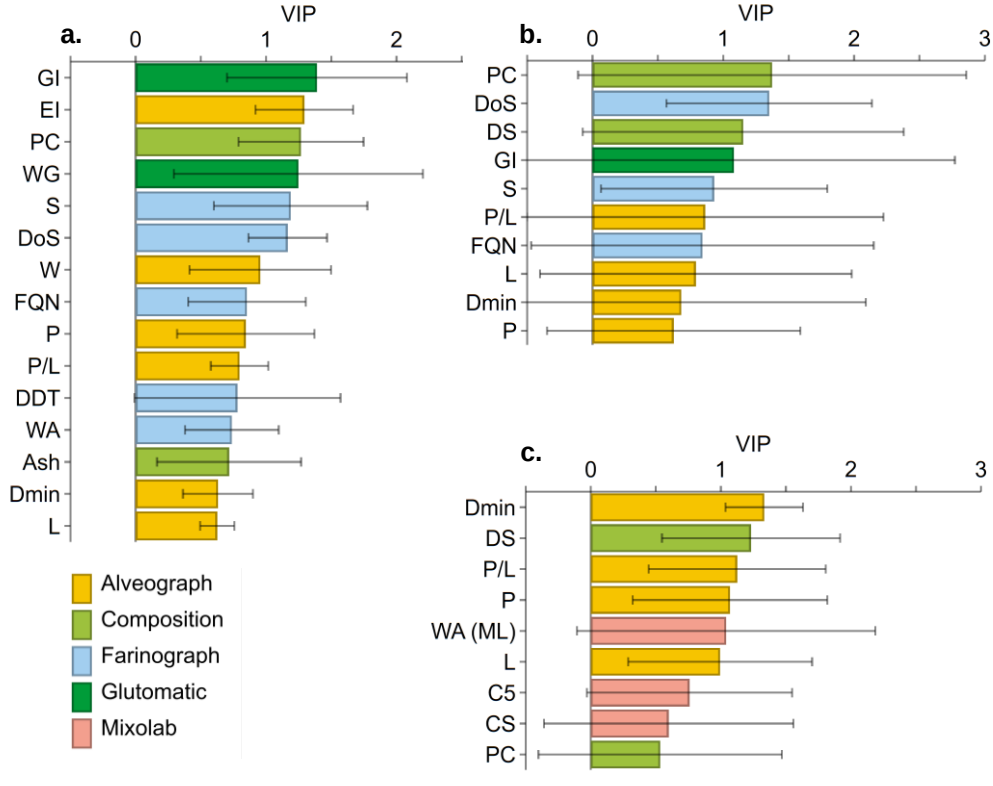


Figure 7: Variable importance in projection (VIP) scores of partial least square regression (PLS) models predicting loaf volume (presented in Paper II) for (a) winter wheat, (b) spring wheat and (b) flour blends. Confidence intervals indicate VIP score stability during cross-validation. Alveograph abbreviations: P = tenacity, L = extensibility, W = strength, EI = elasticity index, Dmin = minimum derivate. Composition abbreviations: PC = protein content, DS = damaged starch. Farinograph abbreviations: WA = water absorption, DDT = dough development time, S = stability, DoS = degree of softening. Glutomatic abbreviations: GI = gluten index, WG = wet gluten. Mixolab (ML) abbreviations: CS = consistency at 8 min, C5 = starch retrogradation.

5.2.1 Instruments for measuring flour quality

The NIT method was used to determine protein content. The values obtained were well correlated with total protein content (TOTE+TOTU) as analysed by SE-HPLC ($R = 0.93$ and $R^2 = 0.87$, respectively). The samples that deviated from the trend had a higher protein content determined by SE-HPLC, likely caused by errors during TOTU extraction.

The Glutomatic system quantifies wet gluten and gluten index, which gives a rough estimate of gluten strength. In both winter and spring wheat, gluten index was correlated with farinograph stability ($R = 0.34$ and 0.38, respectively) and degree of softening ($R = -0.37$ and -0.43, respectively), but not with other strength parameters. Wet gluten and gluten index were quite unstable across cross-validations, as indicated by the VIP error bars in Figure 7a-b, but the coefficient of variation was not especially large (Paper II). The Glutomatic was experienced as having a relatively high error rate, with operators often having to repeat measurements. The significant difference in gluten index between samples from Malmö and Strängnäs identified in Paper II could therefore be due to different practices by those performing the measurements.

An SDmatic device was used to estimate damaged starch content. Damaged starch content was found to be correlated with loaf volume in spring wheat ($R = -0.50$) and in the flour blend ($R = -0.56$), but not in winter wheat. These strong correlations may in part be caused by both loaf volume and damaged starch differing between harvest years for the flour blend, and between locations for spring wheat. The differences in loaf volume may have been caused *e.g.*, by the flour blend containing less spring wheat during 2018, which could have resulted in lower loaf volume and higher damaged starch content. However, the correlations persisted when considering the harvest years separately. The SDmatic was highly effective in quantifying the amount of damaged starch, so it would be useful to implement in routine testing, especially at the start of new harvest years.

An SRC-Chopin 2 device was used to measure solvent retention capacity (SRC) and thus rapidly estimate flour components present based on absorbance of different solvents, with lactic acid SRC being associated with glutenin, sodium carbonate SRC with damaged starch, and sucrose SRC with pentosans (AACC 56-15.01). For winter wheat, these SRC parameters were correlated significantly with the corresponding flour components, but for spring wheat and the flour blend no correlations were found between SRC

and flour components. This might be due to the lower variation in these flour types. None of the parameters improved loaf volume predictions. Routine implementation of the SRC-Chopin 2 device is therefore not recommended for commercial Swedish flours at this time. However, sucrose SRC may be useful for evaluating quality deviations, as changes in this parameter may be caused by irregular AX content.

5.2.2 Rheological quality instruments

The RVA device measures starch gelatinisation behaviour. In this thesis, RVA peak time was correlated positively with loaf volume in winter wheat ($R = 0.35$), which was similar to the strength of correlation between protein and loaf volume ($R = 0.36$). However, on considering the harvest years separately, no linear trend remained for RVA peak time, while the linear trend became slightly stronger for protein. For this reason, RVA was not included in the winter wheat PLS model presented in Paper II. Adding peak time to the winter wheat PLS model decreased root mean squared error of prediction (RMSEP) from 74 to 70 ml and increased Q^2Y from 0.50 to 0.56, indicating an increase in model performance. However, this improvement was not deemed to be of sufficient significance for inclusion of an additional instrument for routine quality control. The RVA peak viscosity and peak time were strongly correlated with AX content in winter wheat flour and the flour blend, which should be kept in mind when interpreting these parameters. For comparison, the amylograph parameters were not correlated with AX content, which may be an advantage if one is only interested in starch behaviour.

Fermentation volume and CO_2 retention were measured using a RheoF4 device. In winter wheat and the flour blend, alveograph elasticity index (EI) was correlated positively with CO_2 retention, and protein content was correlated positively with fermentation volume. There was no correlation with loaf volume and no contribution to loaf volume predictions for any flour type. The RheoF4 instrument was thus not deemed suitable for routine quality control of Swedish commercial flours.

The alveograph measures biaxial extensibility, and multiple alveograph parameters were included in each loaf volume prediction model (Figure 7). Tenacity (P) was mainly determined by damaged starch content and AX content (Paper II) and was significantly correlated with water absorption. On examining the protein composition in dough using a subset of samples with

fixed water absorption, it was found that monomer-to-polymer ratio, TOTE and %UPP also influenced tenacity (Paper III). Extensibility (L) was positively correlated with TOTE in spring wheat and the flour blend, and in the winter wheat subset with fixed water absorption (Paper III). Strength (W) was positively correlated with %UPP in both winter and spring wheat. Overall, the alveograph results were strongly affected by the different flour components, but parameter interpretation was obstructed by the dough analysed not being optimally hydrated. The alveograph has an alternative protocol using adapted hydration, but operators found the dough from this protocol to be sticky and difficult to handle.

The farinograph uses adjusted hydration, giving a reliable measurement of water absorption corresponding to the damaged starch and AX content (Figure 5). This may mean that the farinograph is more suited for Swedish winter wheat compared with the alveograph, since water absorption varied greatly in the winter wheat samples analysed in this thesis. As found for the parameters related to mixing properties, the coefficient of variation was quite high for the farinograph parameters, especially for stability (Paper II). This may indicate low precision of the farinograph device since *e.g.*, the stability did not differ significantly between harvest years, despite the drought in 2018, and none of the farinograph parameters was correlated with protein content or protein composition. Despite this, in accordance with model optimisation steps, farinograph parameters were included in winter wheat and spring wheat PLS models predicting loaf volume (Figure 7), which might be related to the fixed mixing time used during test baking.

Mixolab measures torque during mixing of a dough that is gradually heated and cooled. This yields several parameters similar to those obtained by the farinograph. However, the results obtained for the test materials in this thesis were quite different, with winter wheat displaying a very uneven distribution for stability and dough development time when analysed in Mixolab (Figure 8). In addition, Mixolab dough development time had a much higher coefficient of variation than the corresponding farinograph parameter (Paper II). Because of this, Mixolab parameters were excluded from the winter and spring wheat PLS models (Figure 7a-b). The flour blend PLS model included parameters CS and C5 measuring protein and starch behaviour (Figure 7c). However, the interpretability of these parameters was low, with small numerical differences being significant (Paper II). These parameters are commonly evaluated visually using the Mixolab curve, an

approach ill-fitted for routine quality evaluations. To increase understanding of the consistency parameters, the Mixolab approach was further evaluated.

5.2.3 Potential use of the Mixolab for quality control

Mixolab is commonly used in recipe formulation and improver selection. Its applicability in wheat breeding has also been studied (Koksel et al. 2009; Dhaka et al. 2012; Vazquez & Veira 2015). The suitability of Mixolab tests for quality control in Swedish mills was evaluated further in this thesis by examining the Mixolab parameters and raw data curve obtained for test samples. Theoretically, the curve maxima and minima consistencies (C1-C5) and timepoints (t1-t5) correspond to curve regions linked to absorption, protein weakening, starch gelatinisation, hot gel stability and starch retrogradation. First, PCA modelling combining Mixolab parameters and flour component data was used to examine whether this parameter interpretation corresponded to composition differences. Similar approaches have been used by Dhaka et al. (2012) and Singh et al. (2019). Winter wheat and spring wheat were modelled separately.

Damaged starch content was the only flour component with distinct and consistent loading placement, correlating negatively with C3 (starch gelatinisation). Other component parameters (protein content, protein composition, AX composition and falling number) did not appear to strongly affect Mixolab parameter loading placement. Mixolab measures dough

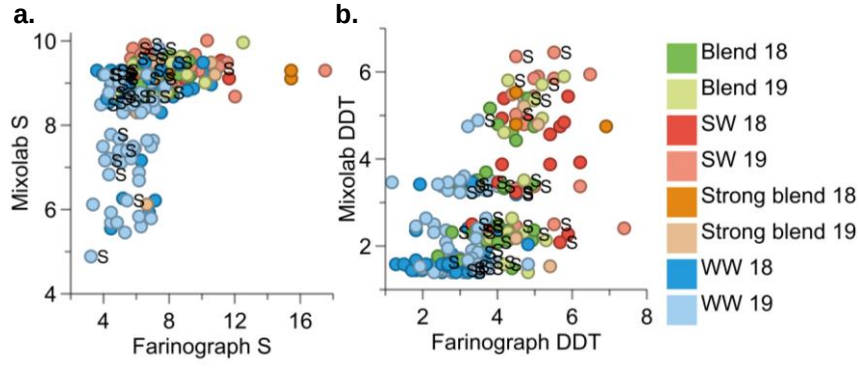


Figure 8: Comparison of (a) dough stability (S, minutes) and (b) dough development time (DDT, minutes) analysed by farinograph compared with Mixolab. Samples milled in Strängnäs are marked with S. Note different scale on the x- and y-axis.

behaviour during heating, which should primarily be affected by starch and enzymes. These components were not thoroughly analysed in this thesis, which limited interpretability of the results. A study by Dhaka et al. (2012) observed similar damaged starch loading placement, but also a negative correlation between falling number and C4 loadings and a positive correlation between glutenin-to-gliadin ratio and Mixolab loadings related to dough strength. A later study by Singh et al. (2019) observed similar trends. Both those studies used wheat breeding material with distinct characteristics, with a much higher variation than the commercial flour samples used in this thesis, so stronger results could therefore be expected.

Next, numerous PLS models were generated to evaluate whether the Mixolab data could predict flour components, quality parameters or loaf volume. Winter wheat and spring wheat were modelled separately, with both Mixolab parameters and the data curve tested as X-matrices. The data curve performed consistently worse than the Mixolab parameters, as continuous data with peaks shifting in time are difficult to quantify in a PLS model. Protein content and damaged starch content were accurately predicted, but not any other flour components. These predictions are of low interest, as the content of damaged starch and protein can be rapidly measured. Loaf volume could not be predicted using Mixolab data alone. Overall, quality parameters from other instruments were not well predicted, except for alveograph parameters. Prediction models of alveograph parameters are also built into the Mixolab software.

To compare Mixolab and other instruments, separate PCA models were built for Mixolab, alveograph, farinograph, RVA and SRC-Chopin. These PCA models used winter wheat flour and were limited to only include parameters from one instrument. On comparing the PCA models, it was found the Mixolab R_2 and Q_2 values were by far the lowest and score distributions indicated the other PCA models were better at distinguishing flour milled in Strängnäs. This indicates that Mixolab was unable to differentiate wheat flours that were all of acceptable quality. Overall, it appears that the Mixolab device is not suitable for quality control of commercial flours. However, the instrument may be of use in plant breeding, where the quality variations are higher, or for evaluating flour of deviating quality in mills.

5.3 Can test baking be reduced by prediction models?

The PLS models predicting loaf volume presented in section 5.2 and Paper II were also used to evaluate whether the test baking performed in the two mills could be reduced by implementing similar prediction models. The PLS models for spring wheat and the flour blend did not perform reliably and are therefore not included in this discussion. The PLS model for winter wheat performed well, with a loaf volume RMSEP of 74 ml. While this was low, the model did not appear to be overfitted, as the prediction error remained in the same range when the number of parameters or latent variables were changed. This model RMSEP can also be viewed in relation to the results of linear regression of loaf volume against protein content, which gave an RMSEP of 102 ml. These low RMSEP values were likely partly due to winter wheat displaying the lowest range of measured loaf volume.

Test baking generally has a notable measurement error, which RMSEP cannot fall below without the model being overfitted. The measurement error of the test baking method used is not known, and may vary slightly between products, as the test baking method used differs somewhat between products. Theoretically, if model prediction error equal to the measurement error is achieved, the error generated by the model is equal to zero. If this were the case, predictive modelling would be a more reliable method than test baking. This could be further evaluated by quantifying the measurement error of the test baking method and expanding the test set to include samples from additional harvest years. The alveograph would also need to be included in routine quality control. Mixolab is not recommended for inclusion, for reasons described in section 5.2.3. Including Mixolab parameters in the winter wheat PLS model (Figure 7a) only decreased the RMSEP from 74 to 72 ml, which is of no consequence compared with the workload involved in including another instrument. It should again be noted that all samples analysed in this thesis were of acceptable quality. Upon implementation, any new quality control routine should therefore apply test baking if a measured parameter is outside the set specifications.

6. Conclusions

The following answers were obtained for the three main research questions addressed in this thesis:

How do flour components affect baking quality?

- Water absorption was mainly affected by the content of damaged starch and AX.
- The 2018 drought affected the quality of winter wheat by increasing %UPP and dough strength.
- Dough protein composition was more strongly correlated with alveograph results than flour protein composition.

What are the best instruments for use in quality control?

- Near-infrared transmittance and SDmatic were efficient in measuring protein content and damaged starch.
- Alveograph parameters were strongly linked to the flour components measured.
- Farinograph and Glutomatic measurements contributed to loaf volume predictions. However, both instruments displayed notable measurement errors.

Can test baking be reduced by implementing prediction models?

- Loaf volume was well predicted for winter wheat.
- Quantification of the test baking measurement error is recommended.
- Prediction model utilisation would require routine implementation of the alveograph in mill quality control.

7. Outlook

This thesis explored different aspects of baking quality using commercial wheat flour, so the conclusions are limited to this context. Within the cereal industry, there many historical datasets that remain unexplored, but which could yield insights of aid in optimisation of different steps of the grain value chain. This thesis showed that quantification of wheat flour components can assist in interpretation of different quality parameters but is of no commercial interest to mills. The results of empirical rheological tests agreed better with test baking results, but correlations between measurements produced by the different instruments tested were generally weak. Some of these instruments were developed based on wheat varieties no longer in use and some could benefit from an update of the protocol or parameter extraction.

It was difficult to find any link between wheat flour components and test baking results, possibly due to limited variation in quality in the sample set used in this thesis. Despite this, the approach of analysing multiple flour components proved promising in determining flour components affecting different aspects of baking quality. This requires continued development of high-throughput methods for quantifying flour components and of extraction methods for glutenins. Enzymes and lipids were not suitable for analysis in the sample set due to the test baking method used, but these components could be further explored using an approach similar to that described in this thesis.

References

Andersson, A.A.M., Merker, A., Nilsson, P., Sörensen, H. & Åman, P. (1999). Chemical composition of the potential new oilseed crops Barbarea vulgaris, Barbarea verna and Lepidium campestre. Journal of the Science of Food and Agriculture, 79 (2), 179–186. https://doi.org/10.1002/(SICI)1097-0010(199902)79:2<179::AID-JSFA163>3.0.CO;2-N

Andersson, R., Westerlund, E., Tilly, A.-C. & Åman, P. (1993). Natural Variations in the chemical composition of white flour. Journal of Cereal Science, 17 (2), 183–189. https://doi.org/10.1006/jcrs.1993.1018

Barron, C., Surget, A. & Rouau, X. (2007). Relative amounts of tissues in mature wheat (Triticum aestivum L.) grain and their carbohydrate and phenolic acid composition. Journal of Cereal Science, 45 (1), 88–96. https://doi.org/10.1016/j.jcs.2006.07.004

Belton, P.S. (1999). Mini Review: On the elasticity of wheat gluten. Journal of Cereal Science, 29 (2), 103–107. https://doi.org/10.1006/jcrs.1998.0227

Beresford, G. & Stone, B.A. (1983). (1→3), (1→4)-β-D-glucan content of Triticum grains. Journal of Cereal Science, 1 (2), 111–114. https://doi.org/10.1016/S0733-5210(83)80028-9

Blanch, E.W., Kasarda, D.D., Hecht, L., Nielsen, K. & Barron, L.D. (2003). New insight into the solution structures of wheat gluten proteins from Raman optical activity. Biochemistry, 42 (19), 5665–5673. https://doi.org/10.1021/bi027059y

Carson, G.R. & Edwards, N.M. (2009). Criteria of wheat and flour quality. In: Wheat: Chemistry and Technology. 4th. ed. American Association of Cereal Chemists, Inc (AACC). 97–118. https://www.cabdirect.org/cabdirect/abstract/20093327186 [2022-12-13]

Courtin, C.M. & Delcour, J.A. (1998). Physicochemical and bread-making properties of low molecular weight wheat-derived arabinoxylans. Journal of Agricultural and Food Chemistry, 46 (10), 4066–4073. https://doi.org/10.1021/jf980339t

Courtin, C.M. & Delcour, J.A. (2002). Arabinoxylans and endoxylanases in wheat flour bread-making. Journal of Cereal Science, 35 (3), 225–243. https://doi.org/10.1006/jcrs.2001.0433

Courtin, C.M., Roelants, A. & Delcour, J.A. (1999). Fractionation−reconstitution experiments provide insight into the role of endoxylanases in bread-making. Journal of Agricultural and Food Chemistry, 47 (5), 1870–1877. https://doi.org/10.1021/jf981178w

De Paula, R., Abdel-Aal, E.-S.M., Messia, M.C., Rabalski, I. & Marconi, E. (2017). Effect of processing on the beta-glucan physicochemical properties in barley and semolina pasta. Journal of Cereal Science, 75, 124–131. https://doi.org/10.1016/j.jcs.2017.03.030

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 (1), 469–492. https://doi.org/10.1146/annurev-food-022811-101303

Dhaka, V., Gulia, N. & Khatkar, B. (2012). Application of Mixolab to assess the bread making quality of wheat varieties. Open Access Scientific Reports, 1 (2), 1–8. https://doi.org/10.4172/scientificreports.183

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

FAOSTAT (2020). FAOSTAT: Statistical database of production quantity from Crops and livestock products. FAO, Rome, Italy. https://www.fao.org/faostat/en/#compare [2022-12-14]

Ferrante, P., Masci, S., D'Ovidio, R., Lafiandra, D., Volpi, C. & Mattei, B. (2006). A proteomic approach to verify in vivo expression of a novel γ-gliadin containing an extra cysteine residue. PROTEOMICS, 6 (6), 1908–1914. https://doi.org/10.1002/pmic.200500236

Gan, Z., Ellis, P.R. & Schofield, J.D. (1995). Gas cell stabilisation and gas retention in wheat bread dough. Journal of Cereal Science, 21 (3), 215–230. https://doi.org/10.1006/jcrs.1995.0025

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 (1–3), 12–30. https://doi.org/10.1016/j.tifs.2004.02.011

Greer, E.N. & Stewart, B.A. (1959). The water absorption of wheat flour: Relative effects of protein and starch. Journal of the Science of Food and Agriculture, 10 (4), 248–252. https://doi.org/10.1002/jsfa.2740100409

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

Guzmán, C., Crossa, J., Mondal, S., Govindan, V., Huerta, J., Crespo-Herrera, L., Vargas, M., Singh, R.P. & Ibba, M.I. (2022). Effects of glutenins (Glu-1 and Glu-3) allelic variation on dough properties and bread-making quality of CIMMYT bread wheat breeding lines. Field Crops Research, 284, 108585. https://doi.org/10.1016/j.fcr.2022.108585

Hamer, R.J., MacRitchie, F. & Weegels, P.L. (2009). Structure and functional properties of gluten. In: Wheat: Chemistry and Technology. 4th. ed.

American Association of Cereal Chemists, Inc (AACC). 153–178. https://www.cabdirect.org/cabdirect/abstract/20093327177 [2022-12-13]

He, H., Feng, G.H. & Hoseney, R.C. (1991). Differences between flours in the rate of wheat protein solubility. Cereal Chemistry, 68 (6), 641–644

Hernández-Espinosa, N., Posadas Romano, G., Crespo-Herrera, L., Singh, R., Guzmán, C. & Ibba, M.I. (2020). Endogenous arabinoxylans variability in refined wheat flour and its relationship with quality traits. Journal of Cereal Science, 95, 103062. https://doi.org/10.1016/j.jcs.2020.103062

Izydorczyk, M.S. & Biliaderis, C.G. (1994). Studies on the structure of wheat-endosperm arabinoxylans. Carbohydrate Polymers, 24 (1), 61–71. https://doi.org/10.1016/0144-8617(94)90118-X

Jødal, A.-S.S. & Larsen, K.L. (2021). Investigation of the relationships between the alveograph parameters. Scientific Reports, 11 (1), 5349. https://doi.org/10.1038/s41598-021-84959-3

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 Journal, 90 (4), 367–376. https://doi.org/10.1094/CCHEM-08-12-0105-FI

Johansson, E., Prieto-Linde, M.L. & Gissén, C. (2008). Influences of weather, cultivar and fertiliser rate on grain protein polymer accumulation in field-grown winter wheat, and relations to grain water content and falling number. Journal of the Science of Food and Agriculture, 88 (11), 2011–2018. https://doi.org/10.1002/jsfa.3312

Johansson, E., Prieto-Linde, M.L. & Jönsson, J.Ö. (2001). Effects of wheat cultivar and nitrogen application on storage protein composition and breadmaking quality. Cereal Chemistry, 78 (1), 19–25. https://doi.org/10.1094/CCHEM.2001.78.1.19

Koksel, H., Kahraman, K., Sanal, T., Ozay, D.S. & Dubat, A. (2009). Potential utilization of Mixolab for quality evaluation of bread wheat genotypes. Cereal Chemistry, 86 (5), 522–526. https://doi.org/10.1094/CCHEM-86-5-0522

Kuktaite, R., Larsson, H. & Johansson, E. (2004). Variation in protein composition of wheat flour and its relationship to dough mixing behaviour. Journal of Cereal Science, 40 (1), 31–39. https://doi.org/10.1016/j.jcs.2004.04.007

Labuschagne, M.T., Geleta, N. & Osthoff, G. (2007). The influence of environment on starch content and amylose to amylopectin ratio in wheat. Starch - Stärke, 59 (5), 234–238. https://doi.org/10.1002/star.200600542

Lama, S., Vallenback, P., Hall, S.A., Kuzmenkova, M. & Kuktaite, R. (2022). Prolonged heat and drought versus cool climate on the Swedish spring wheat breeding lines: Impact on the gluten protein quality and grain

microstructure. Food and Energy Security, 11 (2), e376. https://doi.org/10.1002/fes3.376

Lee, L., Ng, P.K.W. & Steffe, J.F. (2002). biochemical studies of proteins in nondeveloped, partially developed, and developed doughs. Cereal Chemistry, 79 (5), 654-661. https://doi.org/10.1094/CCHEM.2002.79.5.654

Mares, D.J. & Stone, B.A. (1973). Studies on wheat endosperm I. Chemical Composition and ultrastructure of the cell walls. Australian Journal of Biological Sciences, 26 (4), 793–812. https://doi.org/10.1071/bi9730793

Marion, D. & Saulnier, L. (2020). Minor components and wheat quality: Perspectives on climate changes. Journal of Cereal Science, 94, 103001. https://doi.org/10.1016/j.jcs.2020.103001

McCormack, G., Panozzo, J., Bekes, F. & MacRitchie, F. (1991). Contributions to breadmaking of inherent variations in lipid content and composition of wheat cultivars. I. Results of survey. Journal of Cereal Science, 13 (3), 255–261. https://doi.org/10.1016/S0733-5210(09)80004-0

Melis, S. & Delcour, J.A. (2020). Impact of wheat endogenous lipids on the quality of fresh bread: Key terms, concepts, and underlying mechanisms. Comprehensive Reviews in Food Science and Food Safety, 19 (6), 3715–3754. https://doi.org/10.1111/1541-4337.12616

Mousavi, B. & Kadivar, M. (2018). Effect of brine solution as a wheat conditioner, on lipase, amylase, and lipoxygenase activities in flour and its corresponding dough rheological properties. Journal of Food Processing and Preservation, 42 (6), e13631. https://doi.org/10.1111/jfpp.13631

Olaerts, H., Roye, C., Derde, L.J., Sinnaeve, G., Meza, W.R., Bodson, B. & Courtin, C.M. (2016). Evolution and distribution of hydrolytic enzyme activities during preharvest sprouting of wheat (Triticum aestivum) in the field. Journal of Agricultural and Food Chemistry, 64 (28), 5644–5652. https://doi.org/10.1021/acs.jafc.6b01711

Pareyt, B., Finnie, S.M., Putseys, J.A. & Delcour, J.A. (2011). Lipids in bread making: Sources, interactions, and impact on bread quality. Journal of Cereal Science, 54 (3), 266–279. https://doi.org/10.1016/j.jcs.2011.08.011

Rakha, A., Åman, P. & Andersson, R. (2011). Dietary fiber in triticale grain: Variation in content, composition, and molecular weight distribution of extractable components. Journal of Cereal Science, 54 (3), 324–331. https://doi.org/10.1016/j.jcs.2011.06.010

Rakszegi, M., Lovegrove, A., Balla, K., Láng, L., Bedő, Z., Veisz, O. & Shewry, P.R. (2014). Effect of heat and drought stress on the structure and composition of arabinoxylan and β-glucan in wheat grain. Carbohydrate Polymers, 102, 557–565. https://doi.org/10.1016/j.carbpol.2013.12.005

Rasheed, F., Newson, W.R., Plivelic, T.S., Kuktaite, R., Hedenqvist, M.S., Gällstedt, M. & Johansson, E. (2013). Structural architecture and solubility of native

and modified gliadin and glutenin proteins: non-crystalline molecular and atomic organization. RSC Advances, 4 (4), 2051–2060. https://doi.org/10.1039/C3RA45522J

Rhazi, L., Méléard, B., Daaloul, O., Grignon, G., Branlard, G. & Aussenac, T. (2021). Genetic and environmental variation in starch content, starch granule distribution and starch polymer molecular characteristics of French bread wheat. Foods, 10 (2), 205. https://doi.org/10.3390/foods10020205

Saulnier, L., Guillon, F. & Chateigner-Boutin, A.-L. (2012). Cell wall deposition and metabolism in wheat grain. Journal of Cereal Science, 56 (1), 91–108. https://doi.org/10.1016/j.jcs.2012.02.010

Saulnier, L., Sado, P.-E., Branlard, G., Charmet, G. & Guillon, F. (2007). Wheat arabinoxylans: Exploiting variation in amount and composition to develop enhanced varieties. Journal of Cereal Science, 46 (3), 261–281. https://doi.org/10.1016/j.jcs.2007.06.014

Shewry, P.R., D'Ovidio, R., Lafiandra, D., Jenkins, J.A., Mills, E.N.C. & Békés, F. (2009). Wheat grain proteins. American Association of Cereal Chemists, Inc (AACC). https://www.cabdirect.org/cabdirect/abstract/20093327180 [2022-12-14]

Shewry, P.R., Halford, N.G. & Tatham, A.S. (1992). High molecular weight subunits of wheat glutenin. Journal of Cereal Science, 15 (2), 105–120. https://doi.org/10.1016/S0733-5210(09)80062-3

Shewry, P.R. & Lafiandra, D. (2022). Wheat glutenin polymers 1. structure, assembly and properties. Journal of Cereal Science, 106, 103486. https://doi.org/10.1016/j.jcs.2022.103486

Shewry, P.R., Piironen, V., Lampi, A.-M., Edelmann, M., Kariluoto, S., Nurmi, T., Fernandez-Orozco, R., Ravel, C., Charmet, G., Andersson, A.A.M., Åman, P., Boros, D., Gebruers, K., Dornez, E., Courtin, C.M., Delcour, J.A., Rakszegi, M., Bedo, Z. & Ward, J.L. (2010). The HEALTHGRAIN Wheat Diversity Screen: Effects of genotype and environment on phytochemicals and dietary fiber components. Journal of Agricultural and Food Chemistry, 58 (17), 9291–9298. https://doi.org/10.1021/jf100039b

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, N., Gujral, H.S., Katyal, M. & Sharma, B. (2019). Relationship of Mixolab characteristics with protein, pasting, dynamic and empirical rheological characteristics of flours from Indian wheat varieties with diverse grain hardness. Journal of Food Science and Technology, 56 (5), 2679–2686. https://doi.org/10.1007/s13197-019-03756-z

Tatham, A.S., Shewry, P.R. & Belton, P.S. (1990). Structural studies of cereal prolamins, including wheat gluten. Advances in cereal science and technology, 10, 1–78

Thanhaeuser, S.M., Wieser, H. & Koehler, P. (2014). Correlation of quality parameters with the baking performance of wheat flours. Cereal Chemistry, 91 (4), 333–341. https://doi.org/10.1094/CCHEM-09-13-0194-CESI

Theander, O., Åman, P., Westerlund, E., Andersson, R. & Pettersson, D. (1995). Total dietary fiber determined as neutral sugar residues, uronic acid residues, and Klason lignin (The Uppsala Method): Collaborative Study. Journal of AOAC INTERNATIONAL, 78 (4), 1030–1044. https://doi.org/10.1093/jaoac/78.4.1030

Vazquez, D. & Veira, M.C. (2015). Applicability of Mixolab test with local wheat flours. International Journal of Food Studies, 4 (1). https://doi.org/10.7455/ijfs/4.1.2015.a6

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

Wang, M., Oudgenoeg, G., van Vliet, T. & Hamer, R.J. (2003). Interaction of water unextractable solids with gluten protein: effect on dough properties and gluten quality. Journal of Cereal Science, 38 (1), 95–104. https://doi.org/10.1016/S0733-5210(03)00018-3

Weegels, P.L., Hamer, R.J. & Schofield, J.D. (1997). Depolymerisation and re-polymerisation of wheat glutenin during dough processing. II. Changes in composition. Journal of Cereal Science, 25 (2), 155–163. https://doi.org/10.1006/jcrs.1996.0082

Wilson, J.D., Bechtel, D.B., Todd, T.C. & Seib, P.A. (2006). Measurement of wheat starch granule size distribution using image analysis and laser diffraction technology. Cereal Chemistry, 83 (3), 259–268. https://doi.org/10.1094/CC-83-0259

Zhang, Y., Guo, Q., Feng, N., Wang, J., Wang, S. & He, Z. (2016). Characterization of A- and B-type starch granules in Chinese wheat cultivars. Journal of Integrative Agriculture, 15 (10), 2203–2214. https://doi.org/10.1016/S2095-3119(15)61305-3

Popular science summary

Wheat flour is the main ingredient used in bakeries and needs to be of consistently high quality for work in the bakery to run smoothly. At the same time, wheat flour is a biochemical material that is strongly influenced by the cultivar of wheat grown and conditions in the growing environment. This is why Swedish mills devote much effort to quality control of the flours they produce. Mills can check flour quality in many different ways, *e.g.,* by measuring protein content, water absorption capacity, dough strength, and dough changes during kneading. Different mills use different measurements, and this is partly guided by tradition. However, experience shows that even if all stages of quality control produce good results, the wheat flour can underperform when it is baked. For this reason, Swedish mills must commit resources to test baking of their wheat flour. However, the results of test baking vary greatly depending on the method used, which can mean that the flour works well for some bakery products but causes problems for others.

This thesis examined the baking quality of wheat flour from several perspectives. To assess whether the chemical composition of the flour affects baking quality, three important components were measured: the composition of gluten proteins, the content of arabinoxylan (which is the main fibre type in wheat flour) and the amount of damaged starch in the flour. It was found that a high content of gluten proteins of larger size led to larger loaf volume. The other components in the flour mainly affected its water absorption capacity. Tests were performed with different instruments used internationally for quality control, to see if the quality control system of the participating Swedish mills could be improved. Tests were also performed to investigate whether test baking is a necessary step in quality control or whether baking test results could be predicted based on the results of other analyses. Promising results were obtained for winter wheat, the main crop in

Sweden. Reducing the amount of test baking needed for winter wheat by using prediction models instead could potentially lead to significant savings.

Populärvetenskaplig sammanfattning

Vetemjöl är den huvudsakliga råvaran för bagerier och behöver hålla en hög och jämn kvalitet för att arbetet i bageriet ska löpa på enligt plan. Samtidigt är vetemjöl ett biokemiskt material som påverkas starkt av vilken vetesort som odlats samt hur miljö och odlingsförhållanden sett ut. Svenska kvarnar arbetar därför hårt med kvalitetskontroll av det vetemjöl som produceras. Flera olika aspekter kan mätas, till exempel proteinhalt, vattenupptagningsförmåga, degstyrka, och hur mycket degen tål att knådas. Vilka mått som används varierar mellan olika kvarnar och är delvis styrt av tradition. Erfarenhet visar dock att även om alla steg av kvalitetskontrollen ser bra ut, så kan vetemjölet underprestera när man bakar på det. Svenska kvarnar lägger därför stora resurser på att provbaka sitt vetemjöl. Provbakningsresultat varierar dock mycket med vilken metod som används, vilket kan leda till att mjölet fungerar bra för vissa bageriprodukter, men leder till oförutsedda problem för andra bagerier.

Denna avhandling har undersökt bakningskvaliteten hos vetemjöl ur flera perspektiv. För att se om mjölets kemiska sammansättning påverkar bakningskvaliteten har tre viktiga parametrar mätts: glutenproteinernas sammansättning, mängden arabinoxylan (som är den huvudsakliga kostfibern i vetemjöl) och mängden skadad stärkelse i mjölet. Här syntes det att en hög halt och större storlek på glutenproteinerna ledde till en högre brödvolym. De andra komponenterna i mjölet påverkade främst vatten-upptagningsförmågan. Ett stort antal instrument som används internationellt för kvalitetskontroll testades också för att se om kvarnens arbetssätt kunde förbättras. Slutligen undersöktes det om provbakning är ett nödvändigt steg i kvalitetskontrollen, eller om resultaten kunde förutsägas utifrån de andra analyserna som görs. Här syntes lovande resultat för höstvete, som är den

största grödan i Sverige. Att minska provbakningen för höstvete genom att använda prediktionsmodeller kan därför leda till stora besparingar.

Acknowledgements

I would like to extend my warmest thanks to all the people who helped me throughout this work. First and foremost, I want to acknowledge my supervisors at SLU. Roger Andersson, thank you for compiling such a fun research project for me and for being a calm presence in this occasionally chaotic work. Eva Johansson, you always raised the quality of my work by challenging me just the right amount. Annica Andersson, I would not have got through all the lab work without following your great work ethic.

A massive thank you to all the Master's students who contributed to this thesis work: Edvin Nåbo, Assar Sundholm, Josefine Dahlman, Stephanie Laurent-Hedlund and Ida Berghtén. Thank you for all your hard work and your insights. You have all been great company and brought so much energy into this project.

Thank you to everyone I met at Lantmännen R&D, you have been such fun colleagues! Christian Malmberg, thank you for giving me such a great start at Lantmännen and for keeping me ambitious in the project design. Annelie Moldin, thank you for helping me find my confidence and for being a great support. To everyone I met at the mill, thank you for all the practical help and all the cosy coffee breaks. Special thanks to Jan Poulsen and Thony Hedin for making the project possible and teaching me so much, and to Christer Nordh for all the breads baked. Thank you also to Tina Henriksson, Pernilla Vallenback and Anders Lindgren at Lantmännen for being patient guides to the world of wheat. Thank you to everyone in the Unibake research team, especially Seija Sulanto and Rune Gerner Möller for being great travel companions.

At SLU, thank you Anders Ekholm and Sabine Samples for all the help in the lab. Thank you to everyone in LivsID for being such great company. Thank you to everyone at the Department of Molecular Sciences for the fun

lunch breaks and Christmas parties. To everyone at my adoptive department, the Department of Plant Breeding, thank you for making me feel so welcome in Alnarp.

At the University of Copenhagen, thank you to Sören Balling Engelsen and Tomasz Czaja for bringing your enthusiasm to this project and to Rasmus Bro for being an excellent teacher in chemometrics.

Finally, a wholehearted thank you to my parents and sisters for their unwavering love and support. Anna and Jörgen, thank you for being a part of my life. Stefan, thank you for your unending kindness throughout my PhD studies. No achievement will ever be greater than starting my life with you.

I

Journal of Cereal Science 109 (2023) 103608

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

Determining levels of water-extractable and water-unextractable arabinoxylan in commercial Swedish wheat flours by a high-throughput method

Louise Selga [a,b,*], Annica A.M. Andersson [a], Annelie Moldin [b], Roger Andersson [a]

[a] Department of Molecular Sciences, BioCenter, Swedish University of Agricultural Sciences, P.O. Box 7015, SE-750 07, Uppsala, Sweden
[b] Lantmännen, Sankt Göransgatan 160A, Stockholm, 112 17, Sweden

ARTICLE INFO

Keywords:
Arabinoxylan
Wheat flour
Principal component analysis

ABSTRACT

A high-throughput method for quantification of water extractable arabinoxylan (WE-AX) and water unextractable arabinoxylan (WU-AX) was adapted for and evaluated on 197 commercial Swedish wheat flours, collected continuously during harvest years 2018 and 2019. In the method, starch was hydrolysed by alpha-amylase and WE-AX was precipitated with 80% ethanol. AX residues were quantified by gas chromatography after acid hydrolysis. The method had a good repeatability (2.1% RSD_r for total AX). Spring wheat flour had a higher WE-AX content (0.68%) and lower WU-AX content (1.19%) than winter wheat flour (0.56% and 1.31%). The variation of total AX content was high for winter wheat flour (1.5–2.2%), with no correlation to ash or protein content. Total AX content differed significantly both between harvest years and locations, indicating an impact from environment on AX composition. Overall, the method enabled high-throughput analysis of wheat flour and can be further used to study how endogenous AX impacts baking quality.

1. Introduction

Wheat is unique in its capacity to yield well aerated bread. Gluten is the main factor impacting loaf volume (Goesaert et al., 2005), but multiple studies indicate that arabinoxylan (AX) also is of importance (Courtin et al., 1999; Gan et al., 1995). While the impact of AX on baking properties may be less than that of gluten, it is still worthwhile to study. Gluten consists of a wide arrange of large insoluble proteins (Goesaert et al., 2005) which makes it difficult to quantify. As AX is easier to quantify, it would be a great asset to the milling and baking industries if AX could improve quality evaluations of wheat flour. Gluten content and structures are heavily influenced by environment and genotype and can vary considerably between harvest years (Johansson et al., 2013). This can lead to fluctuations in quality which are difficult to predict and manage. This in turn may mask the influence of flour components of a smaller quantity, such as fibres, enzymes, and lipids, and makes it difficult to assess their impact on baking quality.

AX is the main cell wall polysaccharide in the endosperm constituting 70% of the cell wall (Mares and Stone, 1973). Other cell wall polymers include beta-glucan, 20%, glucomannan, 7% and cellulose, constituting 2% of the cell wall in wheat endosperm (Mares and Stone, 1973). The total AX content in sieved wheat flour is typically around 2.2%, out of which about 25% is water-extractable AX (WE-AX) (Barron et al., 2007). Still, quite a varying range has been observed with total AX contents of 1.4–2.7% and WE-AX contents of 0.3–1.4% (Saulnier et al., 2007).

AX in wheat is composed of β-d-xylopyranosyl residues linked through 1 → 4 glycosidic linkages, partially substituted with α-L-arabinofuranosyl at position 2 or positions 2 and 3 (Izydorczyk and Biliaderis, 1994). AX can also be substituted with ferulic acid, but the levels of ferulic acid are low in the wheat endosperm (Saulnier et al., 2012). The degree of arabinofuranosyl substitution determines the solubility of the AX, with a lower substitution degree in water-unextractable AX (WU-AX) and higher heterogeneity in WE-AX (Stone and Morell, 2009).

Both WE-AX and WU-AX contributes to increasing the water absorption, but the influence on baking properties appears to differ (Courtin et al., 1999). The insolubility of WU-AX causes it to trap water, limiting the water available for gluten development (Wang et al., 2003). WE-AX instead increases the viscosity of the free water in the dough upon absorption (Courtin et al., 1999). Gan et al. (1995) has theorized that when dough expands beyond the capacity of the gluten network the structure is stabilized by a liquid film that surrounds the gas cells and

* Corresponding author. Department of Molecular Sciences, BioCenter, Swedish University of Agricultural Sciences, P.O. Box 7015, SE-750 07, Uppsala, Sweden.
E-mail address: louise.selga@slu.se (L. Selga).

https://doi.org/10.1016/j.jcs.2022.103608
Received 7 July 2022; Received in revised form 7 November 2022; Accepted 30 November 2022
Available online 30 November 2022

Table 1
Mean values (% of dry matter) and standard deviations for total arabinoxylan content (total AX), water-unextractable arabinoxylan (WU-AX), water-extractable arabinoxylan (WE-AX), unextractable Ara/Xyl-ratio (ara/xyl U), extractable Ara/Xyl-ratio (ara/xyl E), Ara/Xyl-ratio (ara/xyl), WE-AX/total AX, unextractable mannose residues (man U), unextractable galactose residues (gal U), extractable galactose residues (gal E), ash and protein in different wheat flour products, harvest years and milling locations, with n number of samples.

Content (% flour dm)	Product			Harvest year		Milling location	
	Winter wheat	Spring wheat	Flour blend	2018	2019	Malmö	Strängnäs
	n = 104	n = 49	n = 43	n = 92	n = 104	n = 154	n = 42
Total AX[a]	1.87 ± 0.14a[b]	1.86 ± 0.10a	1.88 ± 0.08a	1.89 ± 0.09a	1.85 ± 0.14b	1.86 ± 0.11b	1.93 ± 0.14a
WU-AX[a]	1.31 ± 0.11a	1.19 ± 0.10b	1.22 ± 0.09b	1.28 ± 0.10a	1.25 ± 0.13a	1.25 ± 0.11a	1.30 ± 0.14a
WE-AX[a]	0.56 ± 0.06b	0.68 ± 0.07a	0.66 ± 0.05a	0.61 ± 0.06a	0.61 ± 0.10a	0.60 ± 0.09a	0.63 ± 0.06a
ara/xyl U	0.59 ± 0.01a	0.56 ± 0.02c	0.58 ± 0.01b	0.58 ± 0.02a	0.57 ± 0.02b	0.58 ± 0.02a	0.57 ± 0.02a
ara/xyl E	0.57 ± 0.05a	0.55 ± 0.03b	0.57 ± 0.03 ab	0.57 ± 0.03a	0.56 ± 0.05a	0.57 ± 0.04a	0.54 ± 0.04b
ara/xyl	0.59 ± 0.02a	0.55 ± 0.02c	0.57 ± 0.01b	0.58 ± 0.02a	0.57 ± 0.03b	0.58 ± 0.02a	0.56 ± 0.03b
WE-AX/total AX	0.30 ± 0.02b	0.36 ± 0.03a	0.35 ± 0.03a	0.32 ± 0.03a	0.32 ± 0.05a	0.32 ± 0.04a	0.33 ± 0.04a
man U	0.08 ± 0.01a	0.07 ± 0.01b	0.07 ± 0.01b	0.07 ± 0.01b	0.08 ± 0.01a	0.08 ± 0.01a	0.08 ± 0.01a
gal U	0.04 ± 0.01a	0.04 ± 0.01a	0.04 ± 0.00a	0.04 ± 0.00a	0.04 ± 0.01b	0.04 ± 0.01b	0.05 ± 0.00a
gal E	0.14 ± 0.02b	0.15 ± 0.03a	0.14 ± 0.02 ab	0.14 ± 0.02a	0.14 ± 0.03a	0.14 ± 0.02a	0.15 ± 0.02a
Ash	0.58 ± 0.05c	0.64 ± 0.04a	0.60 ± 0.03b	0.60 ± 0.05a	0.59 ± 0.05a	0.59 ± 0.05b	0.64 ± 0.03a
Protein	11.6 ± 0.5c	13.8 ± 0.6a	13.3 ± 0.4b	12.6 ± 1.1a	12.4 ± 1.2a	12.5 ± 1.2a	12.6 ± 0.7a

[a] Calculated from arabinose, xylose and galactose residues assuming that the arabinose to xylose ratio was 0.69 in arabinogalactan.
[b] Different letters in the same row indicate significant differences ($p < 0.05$) within categories product, harvest year and milling location.

retains their integrity. WE-AX could stabilise this film by lowering the surface tension and increase its sturdiness by increasing its viscosity (Gan et al., 1995). WE-AX with a higher molecular weight increases the water absorption further (Biliaderis et al., 1995). For these reasons, AX is of interest in breadmaking both through modification using xylanases (Courtin et al., 1999) and as an ingredient (Pietiäinen et al., 2022). The effect of differences in endogenous AX levels are more difficult to study.

The levels of total AX and WE-AX in wheat endosperm are mainly affected by genotype (Finnie et al., 2006), but can also be influenced by the environment (De Santis et al., 2018; Li et al., 2009; Rakszegi et al., 2014). Decreases in arabinose substitution have been observed during grain maturation (Toole et al., 2010) and differences in AX composition may reflect different grain developmental rates among varieties (Marion and Saulnier, 2020). Lower WE-AX levels have been observed in winter wheat after induced drought (Rakszegi et al., 2014). Xylanase activities in wheat are higher after cold and rainy summers compared to hot and dry summers, but the correlation to WE-AX content is low (Dornez et al., 2009). Studies of AX variation have mainly been done on grain from field trials, and the AX variation has to our knowledge not been studied in industrially produced flours to any significant extent.

The method for AX quantification is based on the analytical procedure AOAC 994.13 by Theander et al. (1995), which was developed to accurately determine the total dietary fibre content in a wide range of foods. The method was modified by Andersson et al. (1999) to quantify soluble and insoluble fibres separately. Further modifications are presented in this study to speed up the method for sieved wheat flour. Cellulose and lignin were not quantified as they are only present in low levels in sieved wheat flour (Saulnier et al., 2012). Beta-glucan in wheat has a low solubility (De Paula et al., 2017; Rakha et al., 2011) and remains water-unextractable at 65 °C (Beresford and Stone, 1983). For this reason, beta-glucan was not quantified as it is unlikely to have an impact on baking properties. Additionally, the beta-glucan levels present in wheat endosperm are low compared to AX (Andersson et al., 1993; Mares and Stone, 1973; Saulnier et al., 2012).

The impact of AX on baking properties has mainly been studied by addition of AX, enzymatic treatment, and correlation studies (Gan et al., 1995). However, the source of the added AX or the enzyme levels studied may not be suitable for discovering what impact AX has at endogenous levels. For this, correlations studies on large samples set are needed, to ensure sufficient variation in AX levels. This in turn requires a high-throughput method. The aims of this study were to evaluate an adapted high-throughput method for WE-AX and WU-AX quantification, and to screen the AX levels in commercial sieved wheat flours.

2. Experimental

2.1. Material

Commercial Swedish wheat flours produced by Lantmännen Cerealia in Malmö and Strängnäs, Sweden, were collected during harvest years 2018 and 2019 (Table 1). A total of 197 samples were collected continuously during this period to give a sample set fully representative of Swedish flour production. Three different products were analysed: i) flour composed of winter wheat, ii) flour of spring wheat, and iii) a high protein blend composed of 0–15% winter wheat, 15–35% spring wheat and 50–70% of a high protein German winter wheat. All products used were sieved flours without any additions, such as malt or ascorbic acid, and all samples were stored at −20 °C. Protein content (determined by NIT measurements) and ash content were provided by the mill.

The variety or blend of varieties used in individual products were not known. Information on winter wheat supplied to the mills showed that varieties used in Strängnäs did not differ much between years but varied more for Malmö. The variety Julius was the most common winter wheat in both Malmö and Strängnäs during both harvest years. Julius constituted roughly half of the winter wheat milled in Strängnäs and the composition and proportion of varieties milled were similar for 2018 and 2019. For Malmö, about one fourth of milled winter wheat was Julius, h Brons was second most common in 2018 and Linus was second most common in 2019.

2.2. Determination of water-extractable and water-unextractable AX

AX analysis was based on the method for dietary fibre analysis by Theander et al. (1995) with modifications by Andersson et al. (1999) to separate soluble and insoluble fractions. The protocol was modified to speed up fibre extraction and allow for analysis of a larger number of

samples. Reagents and apparatus used are listed by Theander et al. (1995). Dry matter was determined by oven drying for 16 h at 105 °C.

Duplicate samples of approximately 200 mg were weighed in test tubes, noting the exact weight. These were vortexed with 5 ml 0.1 M acetate buffer of pH 5.0 and 40 μl thermostable α-amylase (3000 U/mL, purified from *Bacillus licheniformis*, product code E-BLAAM, Megazyme, Bray, Ireland). The samples were capped and placed in a boiling water bath, vortexed to disperse the sample particles, and incubated for 1 h with occasional mixing. The samples were cooled and centrifuged for 10 min at 1000×*g*. The pellet was kept for determination of water-unextractable AX, while the supernatant was used for determination of water-extractable AX.

Two ml of the supernatant were transferred to a new test tube and 8 ml 99.5% ethanol was added. The samples were mixed and kept at 4 °C for 1 h to precipitate water-extractable AX. After refrigeration the samples were centrifuged for 10 min at 1000×*g*. The supernatant was discarded, and the pellet was resuspended in 5 ml acetone using a spatula. The samples were centrifuged for 15 min at 1000×*g*, and the acetone was removed.

The pellet obtained after the amylase treatment was washed with 5 ml 0.1 M acetate buffer and centrifuged for 10 min at 1000×*g* to obtain water-unextractable AX. The pellet was washed twice with 5 ml acetone by vortexing and centrifugation for 15 min at 1000×*g*. Acetone was removed by suction to keep the pellet intact. All samples were air dried. Glass rods were placed in each sample for stirring to prevent formation of compact residue.

Hydrolysis was conducted once the samples had dried. For water-unextractable fibres, 0.3 ml 12 M sulfuric acid was added and distributed with the glass rod. Samples were then placed in a 30 °C water bath for 1 h. After this 7.8 ml H_2O and 0.6 ml *myo*-inositol standard solution (2.0 mg/ml) were added. For the water-extractable fibres 2.6 ml H_2O was added with 0.1 ml 12 M sulfuric acid and 0.2 ml *myo*-inositol standard solution (2.0 mg/ml). All samples were then covered with aluminium foil and hydrolysed at 125 °C for 1 h in autoclave. Acetylation and quantification by GC were done in accordance with Theander et al. (1995). Extractable and unextractable residues of the sugars arabinose, xylose, galactose and mannose were quantified.

2.3. Statistical analysis

Significant differences were calculated at 95% confidence by Tukey's pairwise comparisons in Minitab. Principal component analysis was done using SIMCA 16.

3. Results and discussion

3.1. Modification of the method

Adjustments to Theander et al. (1995) and Andersson et al. (1999) were done to allow a high throughput of samples while still achieving a reliable quantification of water-extractable and water-unextractable AX. The repeatability of the method can be observed by the relative standard deviation of repeatability (RSD_r). The RSD_r was calculated for the method by repeat analysis of a sample at 15 separate occasions. The RSD_r was 3.4% for WU-AX, 2.5% for WE-AX and 2.1% for total AX, respectively. These values are in the same range as those obtained by Theander et al. (1995). Further, the results obtained agreed well with those obtained by Andersson et al. (1993) when analysing Swedish wheat flour using the method of Theander et al. (1995).

The main adjustment to the method was that the overnight incubation with amyloglucosidase was removed. This led to residual dextrins originating from starch remaining in the samples throughout quantification. This excluded the quantification of cellulose and beta-glucan from the method. This exclusion was negligible as the overall project aim was to study AX in relation to baking properties. The impacts on baking properties of wheat endosperm cellulose and beta-glucan are likely not of significance, as the solubilities of both cellulose and beta-glucan are low (Beresford and Stone, 1983; De Paula et al., 2017; Rakha et al., 2011), and both are very low in content compared to AX (Saulnier et al., 2012).

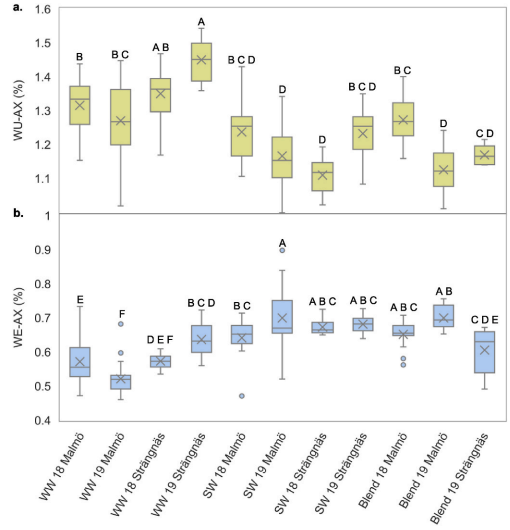


Fig. 1. WU-AX content (% dry matter) (a) and WE-AX content (% dry matter) (b). "WW" refers to winter wheat flour and "SW" to spring wheat flour milled during harvest years 2018 (18) or 2019 (19). Whiskers span highest and lowest samples, boxes span 25–75% percentiles, circles mark outliers (50% higher or lower than these percentiles), and cross marks show the mean values. Different letters indicate significant differences within figures ($p < 0.05$).

3.2. Differences in products, harvest years and milling locations

The total AX content was around 1.9% and did not differ between products but differed significantly both between harvest years and milling locations (Table 1). Total AX content showed no correlation with ash content, indicating that AX content was not affected by differences in milling conditions between locations. It was unexpected that winter wheat and spring wheat did not differ in total AX content, as they differ considerably in genetics. While Finnie et al. (2006) observed total AX to be more impacted by genetics than environment, Li et al. (2009) and Tremmel-Bede et al. (2020) observed environment to have a greater impact. Total AX content was significantly ($p < 0.05$) higher during the drought affected harvest year 2018 (1.89%) than during 2019 (1.85%) and varied more during 2019. Winter wheat flour had a significantly higher WU-AX content (1.31%) and lower WE-AX content (0.56%) than the other products. The Ara/Xyl-ratios calculated all differed significantly between products, with low variations. This could indicate that the AX structure is more influenced by the different genetic origin of spring and winter wheat. However, Tremmel-Bede et al. (2020) observed genotype to have a lower effect on extractable Ara-Xyl ratio than on WE-AX. The extractable Ara/Xyl-ratio (ara/xyl E) showed a slightly higher variation than the other ratios calculated, which is in line with WE-AX having a higher heterogeneity, as suggested by Stone and Morell (2009). The content of mannose and galactose varied very little. Ash content differed significantly between products and between milling locations, with higher contents for spring wheat and products milled in Strängnäs.

During harvest years 1987 and 1988 Andersson et al. (1993)

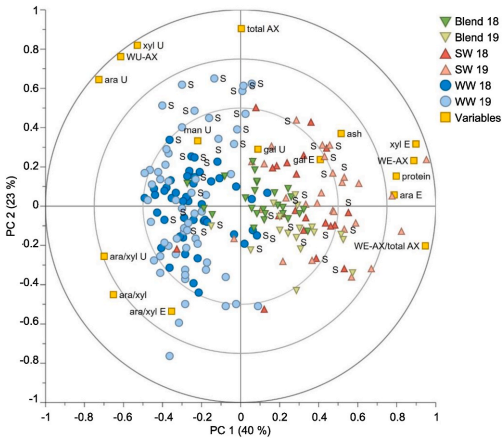


Fig. 2. PCA biplot displaying variables (yellow, ■) together with the analysed samples: flour blend (Blend, green, ▼), spring wheat (SW, red, ▲), and winter wheat (WW, blue, ●). Harvest year 2018 is coloured darker and harvest year 2019 is coloured lighter. Letters denote milling location Strängnäs (S), while all other samples were milled in Malmö. Total AX = total arabinoxylan content, WU-AX = water-unextractable arabinoxylan, WE-AX = water-extractable arabinoxylan, ara U = unextractable arabinose residues, xyl U = unextractable xylose residues, man U = unextractable mannose residues, gal U = unextractable galactose residues, ara E = extractable arabinose residues, xyl E = extractable xylose residues, gal E = extractable galactose residues, ara/xyl U = unextractable Ara/Xyl-ratio, ara/xyl E = extractable Ara/Xyl-ratio. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

collected 49 flours of Swedish spring wheat and winter wheat and the WE-AX and WU-AX contents determined (0.60% and 1.24%) agree well with those obtained in this study. They did however observe a higher variance in AX content compared to this study, likely due to their use of pure varieties, field trial design and sample selection (Andersson et al., 1993). As in this study, they observed higher WE-AX in spring wheat flour but the contrast between spring and winter wheats were not as clear (Andersson et al., 1993). Harvest year 1987 had unfavourable weather conditions during the summer, which yielded lower WU-AX and higher WE-AX, and a higher variation of AX content between different varieties (Andersson et al., 1993). This can be compared to the lower variation in AX content observed during harvest year 2018, during which there was a drought. Overall, Swedish wheat appears to be unchanged in regard to AX content and composition since 1988, but it is possible that atypical varieties could be masked in this study due to the wheat being blended during milling.

3.3. Water-extractable and water-unextractable AX in products, years and locations

There were significant differences in WE-AX and WU-AX contents between the products, years, and locations (Fig. 1). For winter wheat flour, the WU-AX content was highest in flours from Strängnäs during 2019 (Fig. 1a). Harvest year 2019 had significantly ($p < 0.05$) lower WE-AX in flours from Malmö and higher WE-AX in the Strängnäs flours (Fig. 1b). The flours from Malmö showed a higher variation in WU-AX content during 2019 and a higher variation in WE-AX content during 2018.

For spring wheat flour, WU-AX content did not differ significantly but was somewhat lower in flours from Strängnäs during 2018 (Fig. 1a). The variation in WE-AX content was highest for flours from Malmö during 2019 (Fig. 1b). The flour blends differed in WU-AX content, with highest levels during 2018. This was probably caused by a higher proportion of winter wheat being added to the blend in 2018, due to a lower spring wheat harvest. The WE-AX content was significantly lower for the flour blend from Strängnäs than those from Malmö during 2019.

Previous studies have shown that while environment and genotype both influence AX content in grain (Marion and Saulnier, 2020), AX content in the endosperm is mainly influenced by genotype (Finnie et al., 2006; Li et al., 2009). WE-AX content is mainly influenced by genotype but can also be affected by the environment (De Santis et al., 2018), and differences have been observed between harvest years (Andersson et al., 1993). Rakszegi et al. (2014) observed lower WE-AX content in winter wheat after induced drought. There was a high temperature drought in Sweden during the summer in 2018, which led to a low harvest. Flours milled in 2018 mainly seemed to have a lower variation in AX content (Table 1). One reason could be that the drought generated a consistent environment which led to more similar wheat quality and AX levels nationally. The higher variation in AX content during 2019 could be caused by a more varied environment with local weather differences.

3.4. Overall variation patterns

The overall variation of the data set was explored using Principal Component Analysis (PCA) (Fig. 2). Principal component 1 (PC 1) accounted for 40% of the overall variation in the data set and Principal component 2 (PC 2) for 23%, leaving 37% of the variation unexplained. It could be noted that PC 1 separated samples according to the genetic and seasonal differences between spring and winter wheat, while PC 2 distributed winter wheat samples based on differences in harvest year and location. WE-AX, WE-AX/total AX and protein all had high values on PC 1 and were therefore the main source of variation in the data set. Winter wheat formed a separate cluster with negative values on PC 1 and there was very little overlap between winter wheat and the other products, displaying distinct product differences in WE-AX and protein content.

Total AX had the highest value on PC 2 and differences between winter wheat samples was the main pattern identified. Total AX content showed no correlation with ash content, which can be seen by their orthogonal placement in Fig. 2. The lack of a correlation to ash content indicates that the total AX content was not influenced by varying levels of aleurone layer or bran being incorporated during milling. Winter wheat flours milled in Strängnäs during 2019 formed a group with high PC 2 values and had notably high WU-AX and WE-AX content (Fig. 1). These differences between winter wheat milled in Strängnäs during 2018 and 2019 appear to be caused by differences in weather conditions between harvest years rather than genetic differences, as the wheat varieties supplied to Strängnäs barely differed between the years.

The high PC 1 values for WE-AX content and protein indicated that they could be correlated, but this was caused by them both being present in higher concentrations in spring wheat. Correlations between protein and AX were not observed when further studying winter wheat and spring wheat separately. It could be expected that a higher protein content would lead to a relatively lower total AX content, in the same way as a higher starch content in the grain can lead to a relatively lower amount of protein. This was however not the case, which could be an indication of stable cell sizes. Dunstone and Evans (1974) have studied endosperm cell sizes in wild and cultivated wheats and they did not detect a larger endosperm cell size in larger grain. Increases in starch or protein content seemed to influence the number of endosperm cells, not the cell sizes. This would then not influence the AX content in the endosperm.

The mill in Malmö receives wheat from southern Sweden where there is a lower demand for winter hardiness in cultivars and longer daylight hours during winter, which allows for cultivars with earlier grain development. Marion and Saulnier (2020) speculate that different

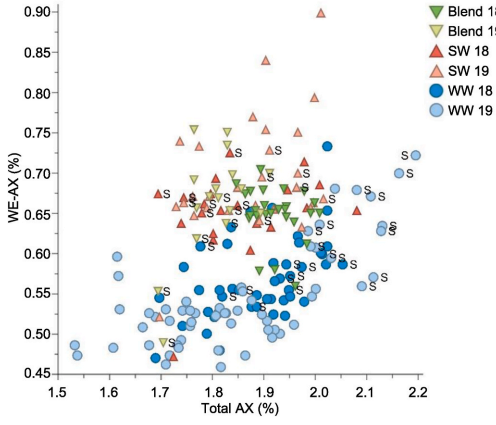


Fig. 3. Total arabinoxylan content (% dry matter) plotted against WE-AX content (% dry matter): flour blend (Blend, green, ▼), spring wheat (SW, red, ▲), and winter wheat (WW, blue, ●). Harvest year 2018 is coloured darker and harvest year 2019 is coloured lighter. Letters denote milling location Strängnäs (S), all other samples were milled in Malmö. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

grain developmental rates could lead to differences in arabinose substitution between cultivars. During grain development, the substitution of arabinose decreases and this occurs more rapidly in plants under drought conditions (Toole et al., 2007). This could be seen in samples milled in Malmö, where the extractable Ara/Xyl-ratio was somewhat lower in 2018 in all product categories. This was however not seen for samples milled in Strängnäs.

According to the PCA, the WE-AX content and total AX content cover the overall variation in the dataset, as they align with PC 1 and PC 2. The WE-AX content and total AX content appear to be inherently different in winter wheat and spring wheat (Fig. 3). Both the spring wheat and the flour blend show a low variation in total AX. Winter wheat had a larger variation in total AX, especially flours milled in Malmö. This could be due to a higher diversity in varieties being grown in the Malmö area compared to Strängnäs, as previous studies indicate that differences in genotype have a larger influence on AX content compared to differences in environment (Gebruers et al., 2010; Marion and Saulnier, 2020). The flour blend showed the lowest variation out of the different products, which is in line with the stable quality of this product.

4. Conclusions

A high-throughput analysis of AX was used to screen a large number of commercially produced Swedish wheat flours. The method showed a good repeatability, and the obtained results were in line with those obtained by Andersson et al. (1993) when using the original method by Theander et al. (1995) on sieved wheat flour. Continuous sampling ensured an accurate representation of Swedish flours during harvest years 2018–2019. Milling conditions did not seem to have an effect on the total AX content, as there was no correlation between this and ash content. WE-AX and WU-AX levels were similar to those measured on Swedish wheat during harvest years 1987–1988. According to previous studies, genotype influences AX content and composition more than environment. There was however a drought during 2018 which appears to have reduced the variation in AX content. In addition to this, flours milled in Strängnäs displayed large differences in total AX content between years, despite a similar assortment of varieties being used. Overall, a larger variation was seen for winter wheat compared to spring wheat, and further studies will examine if this variation can be linked to fluctuations in baking quality. This screening of AX content may be useful for evaluating the impact of xylanases, which are commonly used in bakeries to affect the baking properties.

Author statement

Louise Selga: Investigation, Formal analysis, Visualization, Writing - Original Draft, Review & Editing. Annica A.M. Andersson: Methodology, Validation, Writing - Review & Editing. Annelie Moldin: Supervision, Resources, Writing - Review & Editing. Roger Andersson: Conceptualization, Methodology, Supervision, Writing - Review & Editing.

Data availability

Data will be made available upon project completion.

Acknowledgements

Funding: This study was financed through an industry PhD program of the Swedish Government (governmental decision N201703895), SLU (grant number SLU.ua.2017.1.1.1–2416) and Lantmännen. Louise Selga and Annelie Moldin are employed by Lantmännen. Annica Andersson and Roger Andersson have no interests to declare.

References

Andersson, A.A.M., Merker, A., Nilsson, P., Sörensen, H., Åman, P., 1999. Chemical composition of the potential new oilseed crops Barbarea vulgaris, Barbarea verna and Lepidium campestre. J. Sci. Food Agric. 79, 179–186. https://doi.org/10.1002/(SICI)1097-0010(199902)79:2<179::AID-JSFA163>3.0.CO;2-N.

Andersson, R., Westerlund, E., Tilly, A.-C., Åman, P., 1993. Natural variations in the chemical composition of white flour. J. Cereal. Sci. 17, 183–189. https://doi.org/10.1006/jcrs.1993.1018.

Barron, C., Surget, A., Rouau, X., 2007. Relative amounts of tissues in mature wheat (Triticum aestivum L.) grain and their carbohydrate and phenolic acid composition. J. Cereal. Sci. 45, 88–96. https://doi.org/10.1016/j.jcs.2006.07.004.

Beresford, G., Stone, B.A., 1983. (1→3), (1→4)-β-D-glucan content of Triticum grains. J. Cereal. Sci. 1, 111–114. https://doi.org/10.1016/S0733-5210(83)80028-9.

Biliaderis, C.G., Izydorczyk, M.S., Rattan, O., 1995. Effect of arabinoxylans on bread-making quality of wheat flours. Food Chem. 53, 165–171. https://doi.org/10.1016/0308-8146(95)90783-4.

Courtin, C.M., Roelants, A., Delcour, J.A., 1999. Fractionation−Reconstitution experiments provide insight into the role of endoxylanases in bread-making. J. Agric. Food Chem. 47, 1870–1877. https://doi.org/10.1021/jf981178w.

De Paula, R., Abdel-Aal, E.-S.M., Messia, M.C., Rabalski, I., Marconi, E., 2017. Effect of processing on the beta-glucan physicochemical properties in barley and semolina pasta. J. Cereal. Sci. 75, 124–131. https://doi.org/10.1016/j.jcs.2017.03.030.

De Santis, M.A., Kosik, O., Passmore, D., Flagella, Z., Shewry, P.R., Lovegrove, A., 2018. Comparison of the dietary fibre composition of old and modern durum wheat (Triticum turgidum spp. durum) genotypes. Food Chem. 244, 304–310. https://doi.org/10.1016/j.foodchem.2017.09.143.

Dornez, E., Gebruers, K., Delcour, J.A., Courtin, C.M., 2009. Grain-associated xylanases: occurrence, variability, and implications for cereal processing. Trends Food Sci. Technol. 20, 495–510. https://doi.org/10.1016/j.tifs.2009.05.004.

Dunstone, R.L., Evans, L.T., 1974. Role of changes in cell size in the evolution of wheat. Funct. Plant Biol. 1, 157–165. https://doi.org/10.1071/pp9740157.

Finnie, S.M., Bettge, A.D., Morris, C.F., 2006. Influence of cultivar and environment on water-soluble and water-insoluble arabinoxylans in soft wheat. Cereal Chem. 83 (6), 617–623. https://doi.org/10.1094/CC-83-0617.

Gan, Z., Ellis, P.R., Schofield, J.D., 1995. Gas cell stabilisation and gas retention in wheat bread dough. J. Cereal. Sci. 21, 215–230. https://doi.org/10.1006/jcrs.1995.0025.

Gebruers, K., Dornez, E., Bedő, Z., Rakszegi, M., Frás, A., Boros, D., Courtin, C.M., Delcour, J.A., 2010. Environment and genotype effects on the content of dietary fiber and its components in wheat in the HEALTHGRAIN diversity screen. J. Agric. Food Chem. 58, 9353–9361. https://doi.org/10.1021/jf100447g.

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 Food Sci. Technol. 16, 12–30. https://doi.org/10.1016/j.tifs.2004.02.011.

Izydorczyk, M.S., Biliaderis, C.G., 1994. Studies on the structure of wheat-endosperm arabinoxylans. Carbohydr. Polym. 24, 61–71. https://doi.org/10.1016/0144-8617(94)90118-X.

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 Journal 90, 367–376. https://doi.org/10.1094/CCHEM-08-12-0105-FI.

Li, S., Morris, C.F., Bettge, A.D., 2009. Genotype and environment variation for arabinoxylans in hard winter and spring wheats of the U.S. Pacific northwest. Cereal Chem. 86, 88–95. https://doi.org/10.1094/CCHEM-86-1-0088.

Mares, D.J., Stone, B.A., 1973. Studies on wheat endosperm I. Chemical composition and ultrastructure of the cell walls. Aust. Jnl. Of Bio. Sci. 26, 793–812. https://doi.org/10.1071/bi9730793.

Marion, D., Saulnier, L., 2020. Minor components and wheat quality: perspectives on climate changes. J. Cereal. Sci. 94, 103001 https://doi.org/10.1016/j.jcs.2020.103001.

Pietiäinen, S., Moldin, A., Ström, A., Malmberg, C., Langton, M., 2022. Effect of physicochemical properties, pre-processing, and extraction on the functionality of wheat bran arabinoxylans in breadmaking – a review. Food Chem. 383, 132584 https://doi.org/10.1016/j.foodchem.2022.132584.

Rakha, A., Åman, P., Andersson, R., 2011. Dietary fiber in triticale grain: variation in content, composition, and molecular weight distribution of extractable components. J. Cereal. Sci. 54, 324–331. https://doi.org/10.1016/j.jcs.2011.06.010.

Rakszegi, M., Lovegrove, A., Balla, K., Láng, L., Bedő, Z., Veisz, O., Shewry, P.R., 2014. Effect of heat and drought stress on the structure and composition of arabinoxylan and β-glucan in wheat grain. Carbohydr. Polym. 102, 557–565. https://doi.org/10.1016/j.carbpol.2013.12.005.

Saulnier, L., Guillon, F., Chateigner-Boutin, A.-L., 2012. Cell wall deposition and metabolism in wheat grain. J. Cereal. Sci. 56, 91–108. https://doi.org/10.1016/j.jcs.2012.02.010.

Saulnier, L., Sado, P.-E., Branlard, G., Charmet, G., Guillon, F., 2007. Wheat arabinoxylans: exploiting variation in amount and composition to develop enhanced varieties. J. Cereal. Sci. 46, 261–281. https://doi.org/10.1016/j.jcs.2007.06.014.

Stone, B., Morell, M., 2009. Carbohydrates. In: Khalil, K., Shewry, P. (Eds.), Wheat: Chemistry and Technology, fourth ed. American Association of Cereal Chemists, St. Paul, Minnesota, pp. 322–323.

Theander, O., Åman, P., Westerlund, E., Andersson, R., Pettersson, D., 1995. Total dietary fiber determined as neutral sugar residues, uronic acid residues, and Klason lignin (the uppsala method): collaborative study. J. AOAC Int. 78, 1030–1044. https://doi.org/10.1093/jaoac/78.4.1030.

Toole, G.A., Le Gall, G., Colquhoun, I.J., Nemeth, C., Saulnier, L., Lovegrove, A., Pellny, T., Wilkinson, M.D., Freeman, J., Mitchell, R.A.C., Mills, E.N.C., Shewry, P. R., 2010. Temporal and spatial changes in cell wall composition in developing grains of wheat cv. Hereward. Planta 232, 677–689. https://doi.org/10.1007/s00425-010-1199-5.

Toole, G.A., Wilson, R.H., Parker, M.L., Wellner, N.K., Wheeler, T.R., Shewry, P.R., Mills, E.N.C., 2007. The effect of environment on endosperm cell-wall development in Triticum aestivum during grain filling: an infrared spectroscopic imaging study. Planta 225, 1393–1403. https://doi.org/10.1007/s00425-006-0448-0.

Tremmel-Bede, K., Szentmiklóssy, M., Tömösközi, S., Török, K., Lovegrove, A., Shewry, P.R., Láng, L., Bedő, Z., Vida, G., Rakszegi, M., 2020. Stability analysis of wheat lines with increased level of arabinoxylan. PLoS One 15. https://doi.org/10.1371/journal.pone.0232892.

Wang, M., Hamer, R.J., van Vliet, T., Gruppen, H., Marseille, H., Weegels, P.L., 2003. Effect of water unextractable solids on gluten formation and properties: mechanistic considerations. J. Cereal. Sci. 37, 55–64. https://doi.org/10.1006/jcrs.2002.0478.

Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2023:79

The main target of industrial mills is to supply flour of stable quality. Loaf volume is the main indicator of wheat flour quality, but test baking has major limitations. In this thesis, prediction models were used to evaluate which quality instruments best captured baking quality in Swedish commercial wheat flour and if quantification of flour components increased prediction accuracy. The results have the potential to modernise and simplify wheat flour quality control.

Louise Selga received her doctoral education at the Department of Molecular Sciences, Swedish University of Agricultural Sciences (SLU), Uppsala. She received her MSc in Engineering at Lund University.

Acta Universitatis Agriculturae Sueciae presents doctoral theses from the Swedish University of Agricultural Sciences (SLU).

SLU generates knowledge for the sustainable use of biological natural resources. Research, education, extension, as well as environmental monitoring and assessment are used to achieve this goal.

ISSN 1652-6880
ISBN (print version) 978-91-8046-210-5
ISBN (electronic version) 978-91-8046-211-2