Baking properties and sensory evaluation of the perennial intermediate wheatgrass Kernza (*Thinopyrum intermedium*) cultivated in Sweden

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Bakegenskaper och sensorisk bedömning av det fleråriga gräset intermediate wheatgrass Kernza (Thinopyrum intermedium) odlad i Sverige

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Abstract

To develop a climate-smart and nutritious perennial crop for flour production, the forage grass intermediate wheatgrass (IWG, *Thinopyrum intermedium*), has been domesticated to produce grain (Kernza®). Understanding the plant characteristics and developing baking techniques are crucial for using the grain crop for baking. The main aim of this study was to scientifically evaluate the baking properties and composition of Kernza. A second aim was to investigate the consumer preference of bread with Kernza and to carry out a descriptive analysis to obtain sensory attributes. Neither baking tests nor sensory evaluation have been studied on Kernza cultivated in Sweden before. Baking bread was done with 0%, 15%, and 45% Kernza flour and with or without the addition of vital wheat gluten. Kernza had a higher water holding capacity and requires a higher amount of water at baking. The dough development time, stability, maximum resistance to extension, and extensibility measured by farinograph respectively extensograph decreased with higher percentage of Kernza. Compared to annual wheat, Kernza flour significantly affected the baking properties and bread characteristics by lowering the specific volume and height. Vital wheat gluten addition compensated for Kernza being deficient in high-molecular-weight glutenin subunits. The colours of Kernza were darker, tending towards red and yellow. Finally, the consumer test revealed consumers choosing bread baked with 15% and 45% Kernza over a conventional wheat bread. Kernza was described as having a wholegrain, cereal, rye, and a slightly bitter taste. The findings suggest that Kernza has great potential as an innovative, healthy, and sustainable ingredient in bread.

Keywords: baking properties, bread, bread quality, consumer test, descriptive analysis, gluten, Kernza, perennial, sensory attributes, Thinopyrum intermedium

Sammanfattning

För att utveckla en klimatsmart och näringsrik gröda för mjölproduktion har det fleråriga vallgräset intermediate wheatgrass Kernza® (IWG, *Thinopyrum intermedium*) domesticerats. Förståelse för växtens egenskaper och utveckling av bakningstekniker är avgörande för att använda spannmålsgrödan för bakning. Huvudsyftet med denna studie var att vetenskapligt utvärdera bakningsegenskaperna och sammansättningen hos Kernza som har odlats i Sverige. Ett andra syfte var att undersöka konsumenters preferenser för bröd med Kernza och att genomföra en deskriptiv analys för att erhålla sensoriska attribut. Varken baktester eller sensoriska tester har genomförts och studerats på Kernza odlad i Sverige tidigare. Bröd bakades med 0%, 15% och 45% Kernza och med eller utan tillsats av rent vetegluten. Kernza har en högre vattenhållande förmåga och kräver mer vatten än annuellt vete vid bakning. Degens utvecklingstid, stabilitet, maximalt motstånd vid uttänjning och töjbarhet, minskade med högre andel tillsatt Kernza, undersökt med hjälp av farinograf respektive extensograf. Jämfört med vanligt ettårigt vete, påverkade mjöl från Kernza bakegenskaperna och brödets egenskaper tydligt genom att minska den specifika volymen och höjden på brödet. Tillsatt gluten kompenserade för att Kernza har ett lågt innehåll av glutenin-subenheter med hög molekylvikt. Färgen på Kernza var mörkare än vanligt vetemjöl och färgen gick åt rött och gult. Slutligen visade konsumenttestet att konsumenterna valde både bröd bakat på 15% och 45% Kernza framför konventionellt vetebröd. Kernza beskrevs ha en smak av fullkorn, korn, råg och en lätt bitterhet. Resultaten tyder på att Kernza har mycket goda förutsättningar att användas som en innovativ, hälsosam och hållbar komponent i bröd.

Nyckelord: bakegenskaper, bröd, brödkvalitet, konsumenttest, deskriptiv analys, gluten, Kernza, perenn, sensoriska egenskaper, Thinopyrum intermedium

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Abbreviations

ANOVA	Analysis of variance
BU	Brabender units
C5	The fifth selection cycle
DDT	Dough development time
EFSA	European Food Safety Authority
EU	Extensograph unit
FU	Farinograph unit
IWG	Intermediate wheatgrass
QDA	Quantitative descriptive analysis
RH	Relative humidity
SLU	Swedish university of agricultural sciences
WHC	Water holding capacity
0	Wheat control bread (0% Kernza)
0+G	Wheat control bread with 5% vital wheat gluten (0% Kernza)
15	15% Kernza
15+G	15% Kernza with 5% vital wheat gluten
45	45% Kernza
45+G	45% Kernza with 5% vital wheat gluten

1. Research issue

Throughout an extended period, the focus of grain domestication and breeding has been to achieve high yields rather than other factors like nutrient density or adaptability to climatic change. Due to the prevalence of annual (one-year) grains, the current food systems are vulnerable. Vulnerable to high temperatures, insufficient water, and the accessibility of nutrients during the growth phase (Chapman et al. 2022; Guardia-Velarde et al. 2023). Annuals are planted and harvested, every year. After plowing the soil, the procedure is repeated each year. This requires a high addition of fertilizer and can lead to soil erosion, decreased soil quality, and the release of greenhouse gases (Chapman et al. 2022).

Nowadays, annual wheat crops stand for 20% of the human dietary consumption (Urade et al. 2018). Even though refined wheat flour contains lower values of nutrients in the form of fibres, minerals, and vitamins, annual wheat flour is chosen to a higher extent than whole grain wheat (Guardia-Velarde et al. 2023). The Nordic Nutrition Recommendations 2023 advocate at least 25 grams of dietary fibre per day for females and 30 grams per day for males (Blomhoff et al. 2023). Whole grain cereals should provide some of the fibre and eating a diet high in whole grains is advised. Consequently, instead of choosing refined cereal products, whole grain cereal products should be chosen (Blomhoff et al. 2023). A study from 2023 found that Swedish consumers prioritize eating whole grains, particularly in bread (Brödinstitutet & United Minds 2023). Bread was consumed twice a week or more frequently by 93% of the respondents. In a higher context, elderly respondents consumed bread daily. Since 2017, eating more bread has been a trend (Brödinstitutet & United Minds 2023).

A solution to both the research issue regarding fibre intake and grains resistant to climate change, is adding the grain (Kernza®) from the perennial intermediate wheatgrass (IWG, *Thinopyrum intermedium* [Host] Barkworth & D. R. Dewey) as a novel ingredient in bread making. Kernza flour contains more nutrients than refined wheat flour. It contains increased levels of dietary fibre, minerals, vitamins, and proteins (Rahardjo et al. 2018). Not only the fact that Kernza contains more nutrients, but it is also adapted to climate changes since it is perennial with a several meters deep root system. The crop is also more resistant against water stress, drought, and common diseases for annual wheat. Thus, Kernza offers plenty of positive effects on the environment and constitutes a valuable opportunity for a climate-friendly, resistant, and more nutritious ingredient in bread making.

1.1. Purpose of the thesis

The aims of this thesis were to investigate the baking properties of Kernza, harvested from intermediate wheatgrass (IWG), *Thinopyrum intermedium*), and its nutritional content and impact on sensory attributes and consumer preference. Three different percentages of Kernza flour (0%, 15%, and 45%), as well as with or without added vital wheat gluten, were evaluated for bread baking and dough properties. Two sensory evaluations, both a consumer test and a descriptive analysis, were performed to obtain consumer preference and sensory attributes. Bread baked on sourdough was also investigated in the descriptive analysis. This project was carried out in collaboration with researchers at SLU and Axfoundation. The following questions were answered:

1. How do the baking properties of Kernza differ from the baking properties of wheat flour?
2. Could an addition of gluten lead to a higher bread volume and make it possible to include a higher percentage of Kernza in the dough?
3. Can the addition of Swedish cultivated Kernza be used to bake a bread that consumers prefer?
4. How can the Kernza bread be described from a sensorial point of view?

2. Background

2.1. Intermediate wheatgrass (IWG)

IWG (*Thinopyrum intermedium*) is a perennial grass originally native to Europe, parts of Western Asia, and Siberia. It is primarily known in agricultural and environmental circles for its prospects as a sustainable grain crop under the commercial name Kernza®.

2.1.1. Cultivar

Ordinary annual wheat *(Tricum aeestivum L.)* was domesticated twelve thousand years ago, and plant breeding during the last 150 years has resulted in large improvements for various cultivars (Kiszonas & Morris 2017).

In contrast, the domestication of a perennial relative of wheat, IWG, is very recent. IWG has been used as a perennial forage grass in the USA, Canada, and in its native range in Eurasia (DeHaan et al. 2018). It is a perennial relative of wheat, rather than doing crosses between IWG and wheat. The continued domestication towards a perennial cereal grain crop has been through *de novo* domestication (Chapman et al. 2022). Plant breeders working at the Rodale Institute in Pennsylvania, USA, carried out the first steps of domestication. Under the direction of Dr. Lee DeHaan, scientist at The Land Institute, Kansas, USA, further domestication and advanced breeding have been carried out since 2003. This is to improve the grain yield of intermediate wheatgrass under the brand name Kernza® (DeHaan et al. 2018).

The Swedish University of Agricultural Sciences (SLU) improved the populations to select the greatest plants. Selection was evaluated based on characteristics such as measurements of seeds, seed quantity, yield, and other functional characteristics. Because it is a forage grass, it is easy to cultivate (Chapman et al. 2022).

There are both advantages and drawbacks to the plant material used in this study. The crop grows yearly by itself, greatly lowering the input of agricultural processing needed. Due to the minimized level of soil cultivation, it does improve the health of the soil and does not disturb the communities of microorganisms, which enhances the biodiversity (Chapman et al. 2022). The development of a deeper and more resilient root system enables several advantages (Hayes et al. 2012; Banjade et al. 2019; Dai et al. 2021). The roots are increasing resistance against drying periods, water stress, and climate changes with dry summers. Also, it makes the plant more stress resistant, more resistant to common diseases for wheat, and reduces later seasonal stress (DeHaan et al. 2018). Additionally, perennial wheatgrass is linked to improved nutrient uptake related to larger root volume (Marti et al. 2015; Rahardjo et al. 2018).

The drawbacks, particularly with Kernza are due to the early domestication state. For example, it is complex to dehull the kernels, because the kernels are smaller than regular wheat (Banjade et al. 2019). Another drawback is that the current yield is half the yield of wheat cultivated in similar conditions. Kernza is still in an early stage and therefore does not result in a high yield. Further studies focus on the development of high yielding Kernza. Still, there is a considerable decrease of the values in Kernza output over time, due to increased plant competition and increased carbon storage by the roots. Other challenges with Kernza are the fertility of the offspring and the stability of the plant over time (Chapman et al. 2022).

2.1.2. Nutritional values for Kernza

Kernza is a nutritious crop. The smaller grain size results in a greater bran: endosperm ratio, and a higher protein content than in bread wheat, but this does not mean that the proteins are highly functional in the form of gluten proteins (Rahardjo et al. 2018). Kernza flour exhibited considerably lower levels of total starch, immediately digested starch and lipids (Zhong et al. 2019). As the starch amount lowers, damaged starch increases. Damaged starch increases the water absorption. A higher damaged starch content affects the amount of water developing the gluten network; due to damaged starch, less water can participate in the gluten network, making the bread specific volume smaller. In total, dietary fibre is increased in Kernza (range between 17-19.5%), of which 32% was soluble dietary fibre. Other nutritional values in Kernza, are an enhanced ash content, indicating enhanced mineral levels (Rahardjo et al. 2018). The content of Kernza grains obtained from IWG cultivated in Sweden has not yet been analysed. Although showing promising nutritional values the Kernza grain is still not approved as food in the European Union (EU), but needs to get a novel food approval according to the European Food Safety Authority (EFSA). Since the application was submitted in 2019, the approval procedure has been prolonged several times and is still ongoing (Directorate-General for Health and Food Safety n.d.).

2.2. Breadmaking and bread quality

Bread is an essential food source that has been consumed since the Neolithic period (Mondal & Datta 2008). For more than 12,000 years ago, bread originated for the first time. Despite the fact that baking is a long-standing tradition, the process is complex due to fundamental processes like expansion of volume, water evaporation, starch gelatinization, protein denaturation, and crumb- respectively crust formation (Mondal & Datta 2008).

The two fundamental ingredients in bread are basically flour and water (Mondal & Datta 2008). Those two ingredients have the largest impact on the crumb and texture of the bread. A higher water content in yeast-leavened breads results in the formation of more CO_2 bubbles, resulting in larger pores. Additional ingredients can be added, such as sugar and salt. The addition of sugar starts the fermentation process. Salt is blended into the dough to help limit its expansion and to enhance the gluten network through the activity of yeast. These days, there is a wide variety of different breads; finely textured, low density, and light breads, as well as more compact, darker breads. They can also differ in the way they are leavened, by yeast or sourdough (Mondal & Datta 2008).

White or refined flour is defined as flour that has had the majority of both the germ and bran eliminated (Finnie & Atwell 2016). Roughly 72% of the kernels are left, which corresponds to 72% in flour yield. Since minerals are most abundant in the bran and germ, the mineral concentration in the refined flour decreases. To determine how much bran and germ the flour contains, a visual examination can be performed, because simply bran and germ are darker in colour, while the endosperm appears white (Finnie & Atwell 2016).

Recent developments in mechanization, mass manufacturing, and rising customer demands for convenience, high quality, and extended shelf life have resulted in the use of functional food additives. Preservatives, emulsifiers, and other additives are now considered necessary to produce bread with the right quality (Mondal & Datta 2008). One of the most common additives is vital wheat gluten.

2.2.1. Vital wheat gluten

The bread quality is mainly affected by the gluten proteins. Gluten is made up of two functional proteins named glutenin and gliadin, which are creating a polymeric elastic gluten network during the addition of water and mixing. Glutenins are separated into the high- and low-molecular weight glutenin subunits (HMW-GS and LMW-GS). The size of the glutenin polymers has a clear connection to the rheological properties of the flour. HMW-GS are predominant in wheat flour, but LMW-GS also have a role in baking properties, especially elastic properties in dough (Lafiandra et al. 2004).

Therefore, vital gluten can be added to improve the gluten quality. Vital wheat gluten are the proteins separated from wheat starch and other components of the grain (Finnie & Atwell 2016). In the bread industry, vital wheat gluten is often added to develop fluffy and likable bread. Most of the common bread companies in Sweden add vital wheat gluten into their breads. The table below illustrates what proportion of the total amounts of soft breads does contain vital wheat gluten (Table 1). It is most common in light-colour breads, which might depend on the consumer preference, when buying a light bread, they expect it to be fluffy.

Table 1. An investigation performed on Swedish bread labels and percentage of soft bread products with added vital wheat gluten

Company	**Proportion of their total number of soft bread products containing added vital wheat gluten**
Polarbröd	100%
Pågen	Light breads: 87%, Darker breads: 86%
Skogaholm	100%
Fazer	Light breads: 100%, Darker breads: 64%

Kernza does contain gluten, but previous studies reported Kernza to be poor in high-molecular-weight glutenin subunits (HMW-GS), nevertheless abundant in gliadins (Rahardjo et al. 2018; Tyl & Ismail 2019). It is highlighted how the addition of Kernza lowers the gluten quality, weakens the gluten network, and thereby the dough characteristics (Marti et al. 2015; Rahardjo et al. 2018; Cetiner et al. 2023). Consequently, a dough from Kernza may have a limited capacity to hold gas. Hence, the molecular weight distribution regarding HMW-GS varied throughout different Kernza lines, affecting the gluten network differently (Rahardjo et al. 2018; Tyl & Ismail 2019).

2.3. Analysis methods

2.3.1. Farinograph

The rheological characteristics and behaviours of a dough are most commonly measured by a farinograph (Mondal & Datta 2008). This is performed by adding an adjusted amount of flour and water to reach a target consistency (water absorption), whereas the farinograms register the resistance of the dough during mixing (Mondal & Datta 2008). The farinograms display some of the quality characteristics of the examined flour. Firstly, the dough development time (DDT) signifies the time in minutes until the dough reaches a maximum level of resistance, identified as the ideal amount of time to mix the dough. At the maximum level of DDT, all protein and starch particles are hydrated, which means they are unable to absorb additional water. Stability is another quality parameter that measures how long the dough keeps its maximum consistency. The stability time is directly correlated with the strength of the flour. A long stability time requires more time for mixing but does result in a bread adapted for bread production. Furthermore, continuously mixing causes the gluten proteins to break down, which lowers the resistance. The decrease in resistance after a certain period of time is called the degree of softening, which is measured in Brabender units (BU) (Finnie & Atwell 2016).

2.3.2. Extensograph

To measure the stretching characteristics of the dough, namely its extensibility and resistance to stretching during dough extension, an extensograph is a useful tool for obtaining accurate data (Abbasi et al. 2012). The extensibility (cm) measures how long the dough can be stretched before breaking. The maximum resistance is the height of the extensogram curve in Extensograph units (EU), measuring the strength of the dough (Abbasi et al. 2012). Figure 1 illustrates weak, medium, strong, and very strong doughs.

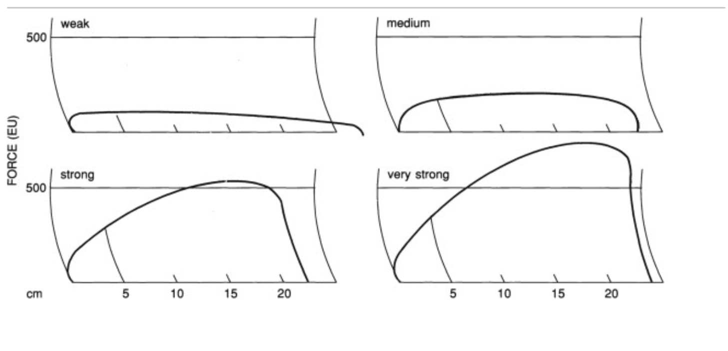

Figure 1. Illustration of extensograms showing weak, medium, strong and very strong doughs (Finnie & Atwell 2016).

2.3.3. Specific volume measurement

One of the most important measurements of the quality of the flour is bread volume. Specific volume measurement is essential for the product quality and tells the efficiency of the leavening process. For example, one of the factors that limits the ability to expand is the creation of the bread crust (Mondal & Datta 2008).

2.3.4. Colour measurement

The science of colour measurement is named colorimetry and is widely used within the food industry. L* means lightness (0-100), where 0 is black and 100 is white. The letter a* stands for negative (-) green or positive (+) red. The last letter, b* stands for negative (-) blue and positive (+) yellow. Colour is therefore three dimensional and the incorporation of air can affect the lightness and colour measurement in the sample of interest (Nielsen 2019). Interestingly, wheat contains pigments named xanthophylls and tricins, but wheat lacks carotene totally, which otherwise contributes to a yellow colour. During baking, moisture content, baking temperature, and baking time all affect colour development. The surface colour calculates the reflected light. Browning of the crust depended on the temperature in the oven and weight loss under baking. Colorimetric analysis can be used to determine the baking end point of bread (Mondal & Datta 2008).

2.3.5. Measurement of sensory attributes

The doctrine of sensory evaluation measures examines, elicits, and interprets responses to properties of food. By using the five senses of smell, touch, taste, sight, and hearing, the sensory qualities are assessed, named sensory evaluation (Stone et al. 2021).

2.3.6. Consumer test

A consumer test is a hedonic, subjective, and affective test (Stone et al. 2021). Consumer tests are separated into acceptance and preference tests (Gustafsson et al. 2014). Of every sensory program, acceptance testing is essential since it measures the liking of the product (Stone et al. 2021). For assessing the degree of liking, often a nine-point hedonic scale is used. The nine-point hedonic scale requires fewer instructions than other scale methods. Meanwhile, preference is a statement indicating which product is preferred or has received a higher rating (more liked) than the other. Preference and acceptance have a clear and direct relationship. Because of this, the sensory acceptance measure is a good indicator of potential rather than a guarantee of commercial success. The number of respondents in small tests is based on the location performing the consumer test. Using a laboratory, twenty-five to fifty respondents are enough. On the other hand, marketing tests require twice the respondents. The choice of respondents is crucial for the outcome of the consumer test. For example, employers might not be an optimal illustration of their intended market since they are biased (Stone et al. 2021).

2.3.7. Descriptive Analysis

Quantitative Descriptive Analysis (QDA) is one kind of descriptive analysis under the category of analytical tests. The process is based on the assessors, developing during tasting several products, deciding which characteristics fit the product in focus. That is, trying to map the sensory attributes and their differences and similarities resulting in the availability to relate the characteristics to one ingredient or process. Those characteristics are then estimated on a scale (Gustafsson et al. 2014). The goal is to gain a full sensorics profile of the product. Between ten and twelve respondents are recommended. In comparison to a consumer test, the descriptive analysis contains training during a short period of time (2-3 days with 90-minute sessions) to know where to place the different products on the scale. The results are usually analysed by ANOVA and illustrated in the form of spider plots or sensory pictures (Stone et al. 2021).

3. Materials and methods

3.1. Origin of kernels, flours and used materials

The domestication of the grains came originally from The Land Institute, Kansas, USA. Grains were further developed in a several-year ongoing breeding program at the Swedish University of Agricultural Sciences (SLU) by researchers at the Department of Plant Biology, Uppsala. The crops used in this study were from the fifth selection cycle (C5) of the Land Institute breeding. The plants were planted in 2019 at Torsåkers farm, Upplands-Väsby, Sweden, together with nitrogen-fixing legumes, as an example white clover (*Trifolium repens L.).* Kernza® grains were harvested with the help of a combine harvester for three years. The kernels used were from the harvest year 2023. They were cleaned, dehulled, grounded, sieved, and used in this thesis. Since Kernza has smaller grains than ordinary wheat, processing may include specific equipment and further knowledge about the unique grain.

The utilized control flour (special wheat from Limabacka Kvarn, Väröbacka Sweden) was used since bakers, who have been baking at Kernza before, had been using the same control flour. The focus was to find a control flour that will not affect the result and effect of Kernza, which was the purpose of this study. The control flour was therefore a Swedish wheat flour special (organic) without any additives and a mix between spring- and winter wheat (measured moisture content 9.86%). Vital wheat gluten was from Lantmännen. The remaining bread ingredients were bought from the supermarket: compressed fresh yeast (Kronjäst för matbröd, Jästbolaget), powdered sugar (ICA), rapeseed oil (Zeta Kallpressad svensk rapsolja ekologisk), table salt (Finkornigt salt med jod, NaCl 99.9% and iodine (5 mg/100 g), ascorbic acid (Askorbinsyra Santa Maria), and tap water (37°C). All ingredients were used at room temperature, except for the water (37°C) and the yeast, which came straight out of the fridge.

3.2. Nutritional values of Kernza grain and flour

Analysis of nutritional values was performed for three different harvesting years for kernels from Kernza with help of Eurofins in May 2024. Additionally, the sieved Kernza flour used in this study was analysed with additional aspects such as mineral and fibre content.

3.3. Experimental design

The experimental design aimed to study the replacement of perennial wheatgrass, Kernza flour, with ordinary (control) wheat flour in yeast-leavened bread, together with the addition of vital wheat gluten, to see the interactions and effects on different dough and bread quality parameters. This was done with a completely randomized design (CRD) at three levels with two separate crossover components: 1) Replacement of control wheat flour with Kernza flour (0%, 15%, or 45%), 2) Addition of 5% vital wheat gluten. Triplicates of every combination and level were baked. The experimental design is illustrated in Figure 2.

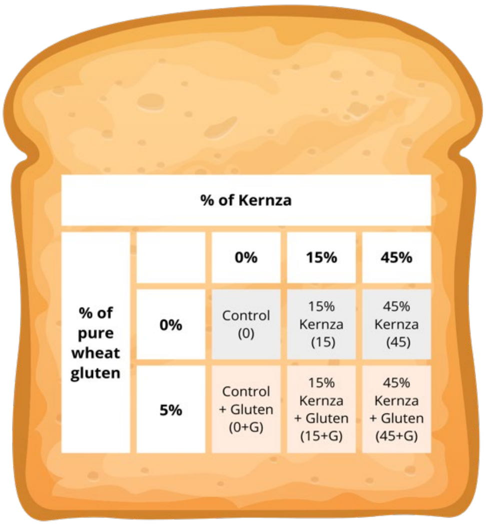

Figure 2. A brief explanation of the experimental design of the study. G is the abbreviation for vital wheat gluten.

3.4. Grinding and sieving method

A stone mill (Fidibus Classic) was used to mill the Kernza kernels into flour. All samples were grinded two times through the stone mill on a fine mode. Separation of particles was based on the diversity of particle size of flour using standard test sieves (Retsch Test sieve, 200 mm DIA x 25 mm, ISO 3310-1, 1 mm, Serial No. 08041089). Resulting in no whole grain flour. The bran fraction was not used further in this study. The final flour was stored dark and cold in plastic bags until the flour was used for the experiments. The flour was always allowed to rest for at least a week before baking.

3.5. Water holding capacity (WHC)

The water holding capacity (WHC) was calculated using the method "Hydration Capacity of Pregelatinized Cereal Products", provided by AACC 56-20. First, a 100 ml centrifuge tube with a stopper that was weighted and filled with 1.0 gram of sample. After weighing the tubes again, 20 ml of deionized water (pH 6-7) were added. To suspend the sample, the tube was firmly shaken after securing the lid. At the conclusion of the 10- and 5-minute periods, the suspension was kept in room temperature and was reversed three times. Thereafter, the samples were centrifuged at 1000 x g for 15 minutes. Finally, the supernatant was suctioned and then weighted. Each type of flour and gluten used in the experiment was analysed in triplicates. The water holding capacity could be calculated using Equation 1:

Equation 1. Hydration capacity

$$WHC = (Weight\ of\ tube + sediment) - (Weight\ of\ tube) \div (Sample\ weight, dry\ basis)$$

3.6. Baking pre-trials

Pre-trials were carried out to determine the level of yeast. Both tests with 2% and 3.5% yeast were carried out. Additionally, the control flour did not contain any ascorbic acid, therefore, a baking trial with and without ascorbic acid was conducted. Lastly, an additional trial of washing out eventual gluten of pure Kernza flour was performed. See results from the baking pre-trials in Appendix III.

3.7. Baking trials

Doughs were prepared in the farinograph mixing bowl in accordance with the method 10-10.03 (Optimized Straight-Dough Bread-Making, AACC 2009b) with certain adjustments. There were triplicates of each dough, resulting in nine breads in total per sample. The dough contained the following ingredients: wheat flour with 0.01 g/kg added ascorbic acid, 225 g flour in total (including the percentage of Kernza and added vital wheat gluten, based on the flour basis and the experimental design in Figure 2), yeast (8 g), and lastly 10 ml of a sugar- and salt solution (50 g sugar, 19 g salt in 100 ml water).

Subsequently, to produce a dough with ideal consistency, data on water absorption was used and the amount of water added was therefore adjusted to reach a consistency of 400±20 BU. The same amount of water was added to all three replicates. Firstly, the flour mix was placed into the heated (30°C) farinograph bowl. At the same time, the yeast was dissolved in 65 ml of 37°C water and 10 ml of the sugar- and salt solution. The flour mix was mixed for 1 minute, and thereafter the dissolved yeast-sugar-salt solution was added into the farinograph bowl together with the rest of the water until the farinograph reached 400±20 BU. Mixing continued for an additional 10 minutes. The dough was then transferred to a ceramic bowl, covered with a clean kitchen towel, and placed into a fermentation oven (EKL 832, Italy, 35-37° C, relative humidity (RD) between 50-70%). The dough was allowed to ferment for 1 hour. After fermentation, four 100 g portions of the dough were formed into loaves using an extensograph rounder, rotating 20 times (Brabender GmBH & Co KG, Duisburg, Germany). The round dough was thereafter placed in small, oiled baking pans and allowed to ferment for an additional 1 hour in the fermentation oven. Then they were baked in an oven for 10 minutes at 230°C. The bread was steamed for 5 seconds in the beginning of the baking process, and the same procedure was repeated after 5 minutes in the oven. During the night, the breads were placed under a kitchen towel, until further measurements the next following day.

3.8. Dough properties

3.8.1. Farinograph

Water absorption, DDT, dough stability, and the degree of softening were measured using a Farinograph-TS (C.W Brabender, Duisburg, Germany) with a large farinograph bowl. The flour amount (including vital wheat gluten and Kernza) was adjusted to 14% moisture content, resulting in values of the different flours and gluten, shown in Table 2. Dry matter content of the flours and gluten was determined by oven-drying at 105°C for 16 hours.

Table 2. Moisture content of different flour mixtures with wheat, Kernza (at 0,15 or 15% addition) and gluten (G) and added water amount in the farinograph during mixing

	Moisture content (%)	Amount of flour (g)	Amount of each flour/ gluten			
			Wheat flour (g)	**Kernza (g)**	**Gluten (g)**	**Added water (ml)**
0	9.84	286.15	286.15	0.00	0.00	168
0+G	9.61	285.42	271.15	0.00	14.27	174
15	9.93	286.45	243.48	42.97	0.00	168
15+G	9.70	285.72	228.58	42.86	14.29	178
45	10.12	287.05	157.87	129.17	0.00	174
45+G	9.89	286.31	143.16	128.84	14.32	186

Target moisture content= 14% in amount of flour (flour/gluten/Kernza mixes).

The rest of the method was performed according to method 54.21.02 (AACC 2009a). The waterbath for the farinograph was turned at 30°C on 1 hour prior to use to heat up. The adjusted flour amount was added into the large farinograph bowl. Deionized water was then titrated into the flour, during mixing until the consistency of the dough reached 500± 20 FU. The DDT, stability, and degree of softening in Brabender units (BU) were measured for the farinogram, found in Appendix I.

3.8.2. Extensograph

The extensograph test was performed according to AACC method 54-10. A dough was initially prepared using a Brabender Farinograph® with a large farinograph bowl (C.W. Brabender, Duisburg, Germany). The amount of flour was corrected to 14% moisture content based on 300 g of flour (Table 2). The amount of water was based on the water absorption from the farinograph, adjusted to be 2% less to compensate for the 6 g of salt added. Deionized water was added together with the salt and flour and mixed for 1 minute, then resting for 5 minutes and finally mixed again until reaching a resistance of 500 BU. The dough was split into two equal halves, each weighing exactly 150 g. The dough pieces were rolled in an extensograph rounder for 20 rotations and thereafter shaped in the shaping unit into a cylindrical dough. Afterwards, pinching, each cylindrical piece of dough was placed on a dough holder. The dough was then allowed to rest in the humidified chamber in the extensograph for 45 minutes. The extensograph test was done by stretching the dough until it broke. The maximum resistance to extension and the extensibility were measured from the graph produced during the test, called an extensogram.

3.9. Colorimetry

A colorimeter/spectrophotometer (CM-600D, Konica Minolta Sensing, Osaka, Japan) was utilized for measuring the colour dimensions within the L* a* b* colour spectrum on the Kernza flour, control wheat flour, bread crumb, and bread crust. To make the colour analysis of the breads possible, the loaves were cooled down to room temperature. They were then cut into two slices, whereas both slices were measured at three different measuring points to give a reliable mean value. The colorimeter was calibrated prior to every use.

3.10. Specific loaf volume and height

Between 3-12 hours after baking, the loaf volume and weight were measured. The loaf volume was determined by using the Rapeseed Displacement method, according to the method 10-05.01 (AACC 2009a). Further, to calculate the specific volume (ml/g), the total volume was divided with the weight. This was performed for all the triplicates. Finally, the height of two loaf halves was metered.

3.11. Sensory Analysis

3.11.1. Consumer test

The consumer test contained an intern, untrained panel of 20 participants. The age of the participants differed between 24 and 61 years (mean age 38 years), of which 80% were women and 20% were men. The locals for the consumer test were in a classroom in BioCenter, SLU, Uppsala. The respondents had to pre-book the session of the consumer test. The invited were students, researchers, and other staff at SLU. The requirements for the consumer test were that the participants needed to like soft bread and eat bread frequently, at least several times a month. Per email, the participants were informed to not eat, use tobacco products, chew gum or drink coffee within 30 minutes before the consumer test.

Three samples were provided in the consumer test: 1) control without any Kernza or gluten (0), 2) 15% Kernza with added gluten (15+G), and 3) 45% Kernza with added gluten (45+G). It was a blind test, were the breads where coded with anonymous three-digit numbers. The participants did not receive any information about the samples before the session. The serving order were randomized and balanced according to a randomized complete block design (RCBD). The respondents had a glass of water to rinse the mouth between the samples along with a scoring sheet and a pen. Every respondent had their own table to not be disturbed. The samples were prepared equal in size and placed on a white paper plate. Firstly, respondents answered some questions about their bread habits and then an affective test with a nine-graded hedonic scale was performed (Figure 3). Lastly, a preference test was answered. All questions needed to be answered.

Sample code: ____________

What do you think of the sample?

- ❑ Like extremely
- ❑ Like very much
- ❑ Like moderately
- ❑ Like slightly
- ❑ Neither like nor dislike
- ❑ Dislike slightly
- ❑ Dislike moderately
- ❑ Dislike very much
- ❑ Dislike extremely

Figure 3. The nine- point hedonic scale used in the consumer test.

3.11.2. Descriptive Analysis

Additionally, a shortened descriptive analysis was performed on an intern untrained panel at Axfoundation, Torsåkers Farm. The panel was composed of six respondents who all worked at Axfoundation. The respondents differed in age between 27 and 63 years (mean age 49 years), of which 67% were women and 33% were men. The locals for the descriptive analysis were in a test kitchen.

Samples provided in the descriptive analysis were as follows: 1) control without any Kernza, sourdough, or added gluten (0), 2) A 15% Kernza bread without sourdough or added gluten (15), 3) A 45% Kernza bread without sourdough or added gluten (15), 4) A sourdough control without any Kernza or added gluten (0+S), 5) A 15% Kernza bread, baked on sourdough, but none gluten added (15+S), 6) A 45% Kernza bread baked on sourdough, but none gluten added (45+S). In comparison to the consumer test, the respondents had some knowledge about Kernza. All the breads were coded with anonymous three-digit numbers, and the serving order was randomized and balanced according to a randomized complete block design (RCBD). The respondents had a napkin, a spit cup, and a glass of water to rinse the mouth between the samples along with a scoring sheet and a pen. Respondents were required to answer on twelve different sensory attributes, one sample at a time. The respondents had to choose a value between 1 and 9 on a structured (exposed numbers) unipolar or bipolar scale. Table 3 highlights the twelve attributes and the minimum respective maximum levels on the scale used.

Table 3. Sensory attributes of the tested breads. Minimum on the scale, maximum on the scale

Attribute	**Minimum on the scale (0)**	**Maximum on the scale (9)**
Colour (*appearance)*	Light	Dark
Porosity (*appearance*)	Small pores	Many pores
Sweetness *(taste)*	Low intensity of sweetness	High intensity of sweetness
Saltiness *(taste)*	Low intensity of saltiness	High intensity of sweetness
Acidity *(taste)*	Low intensity of acidity	High intensity of acidity
Bitterness *(taste)*	Low intensity of bitterness	High intensity of bitterness
Yeast/ Sponginess *(taste)*	Low intensity of sponginess	High intensity of sponginess
Roasted *(taste)*	Low intensity of roasted	High intensity of roasted
Cereals *(taste)*	Low intensity of cereals	High intensity of cereals
Density *(texture)*	Fluffy	Compact
Juiciness *(texture)*	Low intensity of juiciness	Low intensity of juiciness
Elasticy *(texture)*	Slightly elastic	Very elastic

3.12. Statistical Analysis

Minitab® version 19.2020.1 was used for statistical analysis of all raw data. For physical results on dough and bread. Analysis of Variance (ANOVA) with a General Linear Model (GLM) was performed to study the effect of gluten addition and addition level of Kernza, as well as interactions between the two factors. Tukey's pairwise comparison was used to find possible significant differences between the samples. Only significant interactions were included in the final models. Values are reported as mean values for with or without gluten and for addition of levels of Kernza, respectively, and P-values <0.05 are considered significant. The answers from the descriptive analysis, were also analysed with GLM and Tukey´s pairwise comparison as described above. In order to evaluate the results from the consumer test, a result management table for a paired test, with a significance level of 5% was utilized to assess the consumer test findings between the tested breads (Gustafsson et al. 2014).

4. Results

The following section presents all the results from the study. Firstly, the flour characteristics, such as water holding capacity, colour and moisture content, were examined, then the dough characteristics from the farinograph and extensograph. Lastly, the bread was measured in the form of weight, the mean height, volume, specific volume, and L*a*b values on bread crumb, respectively, bread crust. Following, two sensory evaluations have been conducted on different breads. All raw data have been analysed statistically by ANOVA to find any significant differences between samples.

4.1. Flour

By milling Kernza and comparing the amount of flour with the fraction removed, the flour yield could be calculated. The calculated flour yield differed significantly based on how large batches were grinded at the same time. The final flour yield differed between 77-92%. The removed coarse fraction was not further used in this study. The two milled flours (one milled at SLU with the stone mill and one milled at Axfoundation, using a whirling mill, 100% Kernza) were compared in colour to the control wheat flour. With the aid of a Konica Minolta spectrophotometer, the flours did undergo a colour measurement, represented in Table 4. It is apparent from the table that wheat flour is the lightest flour. Interestingly, the 100% Kernza flour, sieved in the whirling mill was lighter than the sieved Kernza flour. Even though there are no significant difference between the two of them (Table 4). Regarding the a* value, the colour of all flours tends towards red. Thus, it was a significant difference between the Kernza-samples and the control flour, but not between the two different kinds of Kernza flours. Kernza had more similar a* values and was more alike in red scale. Analysing the b* value, it was the value with the lowest standard derivation of all of them. All flours were more yellow than blue. The mostly used flour in this study, sieved Kernza flour, is the darkest flour which goes towards red and yellow. Also, here there is a significant difference in colour between Kernza and control flour. This is also seen in Figure 4, showing the three flours.

Table 4. Colour properties of the three used flours. Sieved Kernza, 100% Kernza and wheat control flour L, a*, b* values. Significant differences in values are indicated by different letters (p<0.05). The L*-value represents lightness (0=black, 100=white). The a*-value represents green (-a) to red (+a). The b*value represents blue (-b) to yellow (+b)*

Colour	Sieved Kernza Flour			100% Kernza Flour			Wheat Flour		
L*	76.2[b]	±	5.18	81.18[b]	±	0.89	87.72[a]	±	2.46
a*	1.75[a]	±	0.89	1.50[a]	±	0.80	0.20[b]	±	0.27
b*	17.66[a]	±	0.42	14.15[a]	±	0.16	10.79[b]	±	0.52

± confidence interval (n=3).

Figure 4. The three different flours used in the study. From the left:1) Control wheat flour, 2) A 100% Kernza flour (used only in the descriptive analysis), lastly 3) The sieved Kernza flour, mostly used.

4.2. Nutritional values of Kernza grain and flour

In Table 5, the results from an analysis of the minerals in Kernza from different cultivation years, in kernels, sieved Kernza flour, and in common wheat are shown. Kernza had clearly higher levels of potassium, magnesium, and calcium than common wheat.

Table 5. Minerals analysed in Kernza from three different harvesting years and in the used flour in the study (kernels, sieved Kernza flour and wheat flour). Analysed in fresh weight. The values for reference wheat flour are taken from the Swedish Food Agency's database. Unit: Mg/ 100 g. All plant materials are from the fifth selection cycle (C5)

Mineral	Kernza 2021 (mg)	Kernza 2022 (mg)	Kernza 2023 (mg)	Sieved Kernza (mg)	Wheat flour (mg)
Potassium (K)	600	500	560	480	166
Selenium (Se)	<0.005	<0.005	<0.005	<0.005	10.1
Iron (Fe)	7.9	6.2	5.9	6.6	7.0
Magnesium (Mg)	180	150	160	140	21
Calcium (Ca)	140	120	130	120	21
Zinc (Zn)	6.0	4.0	4.5	4.2	8.0

Water content, specific weight, falling number, and crude protein content are factors that affect the pricing of ordinary wheat. The water content for Kernza differed greatly between the years (Table 6). For wheat, the maximum level of allowed water content is 14.5%, to prolong shelf life. Regarding specific weight, it was lower for Kernza than for common wheat. The minimum specific weight for wheat is 740 g/l, which is higher than all values for Kernza. Even the falling number for Kernza is low, especially from the harvest year 2023 used in this study. Furthermore, Kernza had remarkably higher values of protein than common wheat (Table 6).

*Table 6. Water content, specific weight, falling number, crude protein and dietary fibre three different years and the used flour in the study. DM represents dry matter; the rest values are analysed in fresh weight. The values for reference wheat flour are taken from information from Lantmännen "before harvest 2024". All plant materials are from the fifth selection cycle (C5) * means not analysed in this test*

Parameters	Kernza 2021	Kernza 2022	Kernza 2023	Sieved Kernza (g)	Wheat flour
Water content	9.9%	13.3%	11.7%	11.7%	14.5%
Specific weight	729 g/l	694 g/l	652 g/l	652 g/l	780 g/l
Falling number	197 seconds	214 seconds	137 seconds	137 seconds	250 seconds
Crude protein	19.9% DM	17.4% DM	18.8% DM	18.8% DM	11% DM
Dietary fibre	*	*	*	15.7 g	3.6 g
soluble	*	*	*	1.8 g	0.62 g
insoluble	*	*	*	13.9 g	7.22 g
Wet gluten	*	*	*	<15% DM	30-32% DM

4.3. Water holding capacity (WHC)

A test was done to determine the water holding capacity (WHC) of Kernza. Kernza had a higher water holding capacity (WHC) than the control wheat. The measured value for the water holding capacity in units of g/g for Kernza was 2.55±0.22 g/g (mean value± standard deviation, n=3), meanwhile the control wheat had 1.85±0.03 g/g (mean value± standard deviation, n=3).

4.4. Dough properties

4.4.1. Farinograph

Data in Table 2 shows a positive correlation between the percentage of Kernza in the bread and the added amount of water needed to reach a dough resistance of 400±20 BU. Kernza binds more water, the more Kernza added, the more water is needed. Also, gluten binds extra water, and the amount of water needed to be adjusted further when both gluten and Kernza were added. Since the same amount of water was used for all replicates when preparing doughs in the farinograph, no statistics based on the amount of water supplied could be performed. Instead, the first replicate determined the amount of water added. The DDT, stability, and degree of softening were measured from the farinograms (Table 7) (Appendix 1).

To analyse those results further, ANOVA was used to investigate if there were any significant differences (Table 7). The first variable, with or without the addition of gluten, did not have any effect on DDT, but it did affect both stability and degree of softening. The second variable, the addition of Kernza, did have an effect on all factors. Interestingly, there was an interaction between added vital wheat gluten and the addition of Kernza on both DDT and degree of softening.

Table 7. ANOVA- Analysis of variance. The p-values for significant effects of gluten addition (with or without gluten, w/wo gluten) and level of Kernza addition, and possible interactions for the measured parameters from extensograph and extensograph

Parameters	w/wo gluten	% Kernza	w/wo*%Kernza
DDT (min)	ns*	0.020	0.006
Stability (min)	0.012	0.000	ns*
Degree of softening	0.000	0.000	0.007
Max. resistance	0.005	0.010	ns*
Extensibility (cm)	ns*	0.000	ns*

**ns= not significant*

As illustrated in Table 8, increased addition of Kernza resulted in a shorter DDT. Also, the addition of gluten resulted in a shorter DDT. Regarding stability, added gluten affected the dough to be stable for longer, regardless of which dough. However, there was no significantly difference between 0% and 15% of Kernza regarding the stability. On the 45% addition level, the stability was significantly lowered. The degree of softening increased significantly in direct proportion to the amount of Kernza, whereas the degree of softening decreased when gluten was added, indicating a more stable dough (Table 8).

Table 8. Mean values for dough development time (DDT), stability, degree of softening, maximum resistance to extension and extensibility of doughs prepared with (W) or without (Wo) 5% vital wheat gluten, and with different levels of Kernza added (0, 15 and 45%). Significant differences between values are indicated by different letters (vertical division)

Parameters	w/wo gluten		% Kernza		
	0%	**5%**	**0%**	**15%**	**45%**
DDT (min)	6.25[a]	5.67[a]	6.38[a]	6.25[a]	5.25[b]
Stability (min)	10.17[b]	11.33[a]	12.25[a]	11.50[a]	8.50[b]
Degree of softening	100[a]	63.33[b]	50[c]	90[b]	105[a]
Max. resistance (BU)	396[b]	555.42[a]	595[a]	438[b]	394[b]
Extensibility (cm)	33.79[a]	32.71[a]	39.25[a]	36.38[a]	24.13[b]

4.4.2. Extensograph

The dough was stretched until it broke to conduct the extensograph test. Maximum resistance to extension value (BU) and extensibility (cm) were measured from the extensograms. Information from Table 8 is illustrated in Figure 5.

The maximum resistance was significantly different based on vital wheat gluten, but not the extensibility (Table 8). There was no interaction between added gluten and Kernza according to maximum resistance and extensibility (Table 8). Gluten had a positive effect on dough strength regardless of the amount of Kernza added, as evidenced by the higher values of maximum resistance, indicating a stronger dough (Table 8). The effect of gluten is shown in Figure 5. Doughs with 15% and 45% Kernza were similar regarding maximum resistance, while the 0% and 15% Kernza doughs were more similar in extensibility (Table 8).

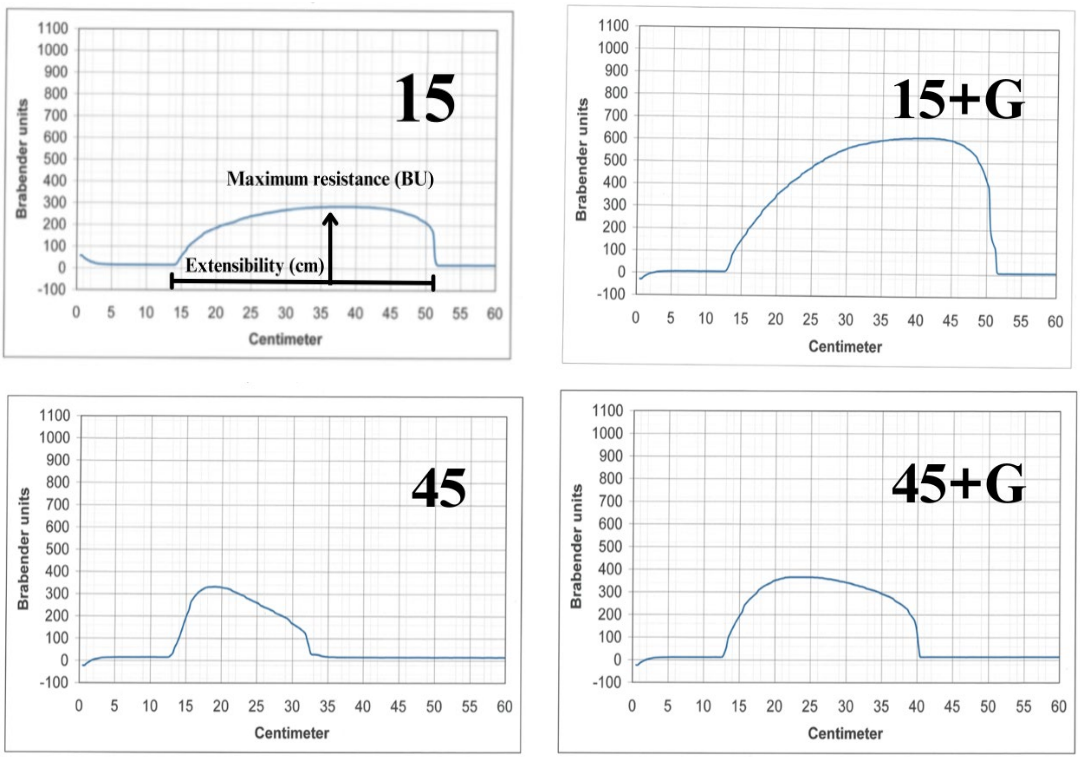

Figure 5. Extensograms for the four samples with addition of Kernza (15% and 45%) and with (G) without 5% vital wheat gluten.

4.5. Specific loaf volume and height

Kernza and gluten addition did have a significant difference on the specific volume and height of the loafs. Hence, there were no interaction between the two variables (Table 9).

Table 9. ANOVA- Analysis of variance. The p-values for significant effects of gluten (with or without gluten, w/wo gluten) and level of Kernza addition and possible interactions for specific bread volume, bread height and colour parameters (L, a, b*) of bread crumb and crust*

Parameters	w/wo gluten	% Kernza	w/wo*%Kernza
Specific volume	0.003	0.000	ns*
Height	0.000	0.000	ns*
L* crumb	0.003	0.000	ns*
a* crumb	ns*	0.000	ns*
b* crumb	ns*	0.000	ns*
L* crust	0.010	0.001	ns*
a* crust	0.000	0.000	ns*
b* crust	ns*	ns*	ns*

**ns= not significant*

Table 10 contains all mean values, regarding specific volume and height measurements along with colour measurement of the crumb and crust. Generally, as illustrated in Table 10, at a higher percentage of Kernza, there was a trend of lower specific volume. However, both 15% of Kernza (15) and the addition of gluten with 15% of Kernza (15+G) resulted in bread with a specific volume that was not significantly different from the control (0) (Table 10). In general, gluten had a trend of giving a higher specific volume (Table 10), regardless of the amount of Kernza added, e.g., effect on both 15% and 45% Kernza. Between breads baked with 0% and 15% of Kernza there was no significant difference (Table 11), which means 15% Kernza can be added without affecting the volume to a large. However, the level of 45% Kernza resulted in a significantly lower specific volume (2.5 compared to 4.2 and 3.6 g/g). Regarding the addition of vital wheat gluten, there was a significantly higher specific volume and height when gluten was added (3.8 and 8.3 g/g, respectively) compared to no gluten added (3.1 and 6.7 g/g) (Appendix II).

Table 10. The mean values of specific volume (g/g), bread height (cm) and colour measurements of crumb and crust. Significant differences in values are indicated by different letters (vertical division)

Parameters	w/wo gluten		% Kernza		
	0%	**5%**	**0%**	**15%**	**45%**
Specific volume	3.07^{b}	3.78^{a}	4.19^{a}	3.63^{a}	2.45^{b}
Height	6.74^{b}	8.28^{a}	8.96^{a}	8.12^{b}	5.46^{c}
L* crumb	62.76^{b}	67.23^{a}	75.53^{a}	65.55^{b}	53.90^{c}
a* crumb	3.12^{a}	3.41^{a}	6.40^{a}	2.88^{b}	0.51^{c}
b* crumb	18.91^{a}	19.27^{a}	22.63^{a}	19.12^{b}	15.53^{c}
L* crust	51.48^{a}	48.27^{b}	52.39^{a}	49.34^{b}	46.88^{b}
a* crust	10.53^{b}	12.72^{a}	12.87^{a}	12.08^{a}	9.92^{b}
b* crust	27.38^{a}	27.77^{a}	30.53^{a}	27.50^{a}	24.69^{a}

Connected to the specific volume is the height of the bread loaf (Table 10). It showed that adding vital wheat gluten resulted in a higher bread loaf. Higher addition of Kernza resulted in significantly lower heights (8.96, 8.12 and 5.46 cm for 15 and 45% Kernza, respectively). Generally, a higher level of Kernza results in a lower height of the bread (Table 10).

4.6. Colour of breads

The colour of the crumb and crust of the bread was affected by the percentage of Kernza added. All the various addition levels were different from each other, regarding L* crumb, a* crumb, b* crumb (Table 10). The L* value for the crumb, L*, and a* values of the crust also differed significantly if gluten was added or not, and between different levels of Kernza added (Table 10). The L* value for crust was significantly higher without added gluten, while the a* value was higher with added gluten (Table 10). The L* value was lower for breads baked with 15 and 45% Kernza than for breads with no addition of Kernza, while the a* value was lower only for the breads baked with 45% Kernza (Table 10).

In Figure 6, the coordinates from the colour analysis are displayed. The colour of the bread crumb was mainly influenced by the percentage of Kernza, and not that much by gluten addition. The more Kernza, the darker bread. The control bread was the lightest one, closest to white (100). The colour of the crumb and crust of the bread was affected by the percentage of Kernza added. All the various addition levels were different from each other, regarding L* crumb, a* crumb, b* crumb. Thus, the values of the crust were more similar, and the Kernza percentage does not seem to have the same effect. No clear patterns can be observed regarding crust. The more Kernza added, the more the bread tends towards red and yellow in bread crumb.

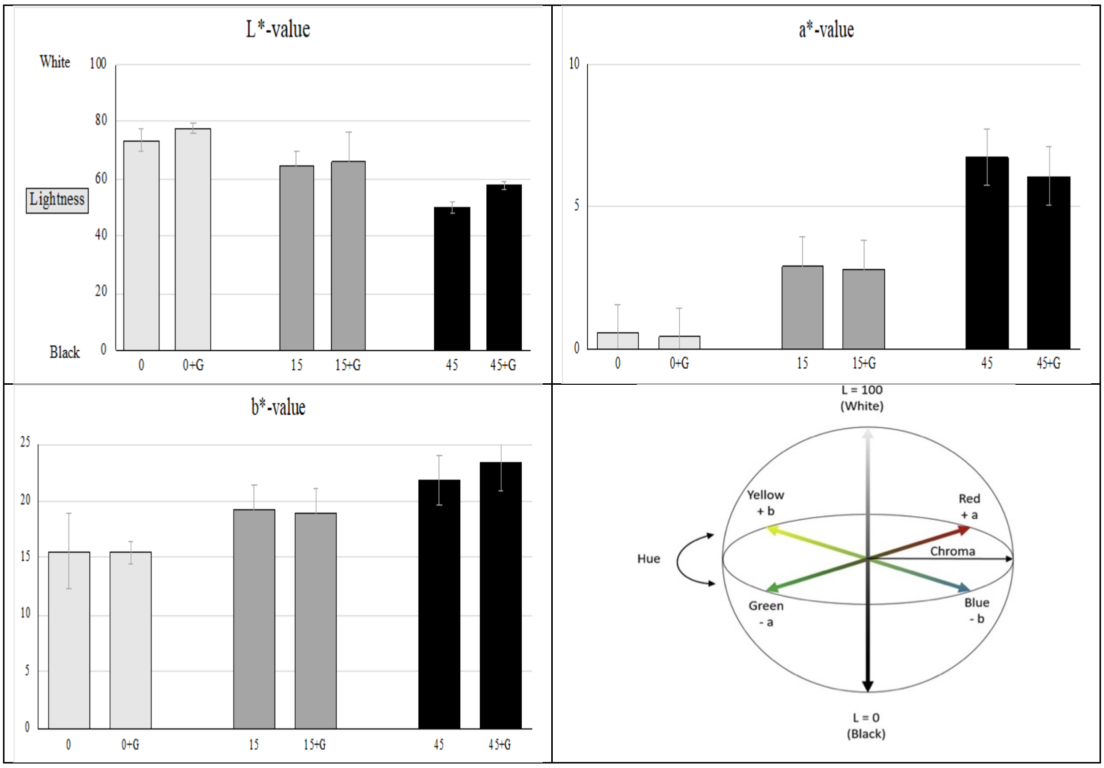

Figure 6. L values, a* values, b*values for the crumb. The data are displayed as mean values. The figure is included to facilitate the interpretation of the staple diagrams besides, reference: (Ly et al. 2020).*

4.7. Sensory Analysis

4.7.1. Consumer test

The consumer test respondents ranged in age from 24 to 61 years (mean age 37 years). 40% ate bread once a day or several times a day, and 50% ate bread several times a week. Only one respondent ate bread once a month. The respondents were able to select their preferred bread texture and bread colour. Fluffy bread was preferred by 40% of respondents, while 35% of the respondents preferred compact bread. 25% of the respondents answered that the bread texture was based on cravings or mood. On the other hand, when choosing preference about bread colour, 60% of respondents preferred a darker loaf over a lighter one. This was brought on by increased nutrition, taste, texture, and the sensation of being fuller for longer.

Regarding the acceptance test and rating of the different breads, the control bread (with 0% Kernza), earned a mean score of 5.55 (± 1.43), which represents the criterion “Neither like nor dislike”. According to the respondents, the bread makes them think of a regular white bread or toast bread. The bread with 15% Kernza was accepted at a higher rate of 6.55 (± 1.43), or "Like moderately". This bread tasted slightly nutty and reminded of whole grain toast bread. Some even find it slightly bitter. Applicable to the 45% Kernza bread, the acceptance level was comparable to the bread with 15% Kernza, the mean value landed at 6.45 (± 1.64) which indicates "Like slightly". As a result, this sample exhibited the largest standard deviation, indicating a panel that disagreed. The responders were reminded of rye bread and sourdough bread in general by the bread. There was a significant difference (significance level 0.05) between the control bread and Kernza bread. Fifteen out of twenty respondents must prefer one type of bread for there to be a substantial difference. Nineteen individuals chose the bread with Kernza in this case, whereas only one preferred the control bread without Kernza.

4.7.2. Descriptive Analysis

The initial results from the descriptive analysis are shown below in Table 11 and 12. Concerning the attribute colour, all factors were significant different, even the interaction between gluten and Kernza. For porosity, sourdough affects the porosity more than the percentage of Kernza, which did not result in any significantly difference. Sourdough influenced the saltiness, but not the amount of Kernza. Bitterness differed based on sourdough or the amount of Kernza, and additionally had an interaction. Cereals were affected by the addition of Kernza but not the amount of sourdough. Density was affected by sourdough and not by the percentage of Kernza. The attributes sweetness, acidity, yeast/sponginess, roasted, juiciness, and elasticy did not result in any significant difference. All those results can be illustrated in a spider plot, Figure 7, for it to be easier visualized.

*Table 11. ANOVA- Analysis of variance. The p-values for significant differences and possible interactions for sensorics attributes from the descriptive analysis. w/wo gluten illustrates the variable with or without added gluten. *ns=not significant*

Parameters	w/wo sourdough	% Kernza	w/wo sourdough*%Kernza
Colour	0.000	0.000	0.000
Porosity	0.000	ns*	ns*
Sweetness	ns*	ns*	ns*
Saltiness	0.007	ns*	ns*
Acidity	ns*	ns*	ns*
Bitterness	0.018	0.022	0.039
Yeast/Sponginess	ns*	ns*	ns*
Roasted	ns*	ns*	ns*
Cereals	ns*	0.000	ns*
Density	0.000	ns*	0.046
Juiciness	ns*	ns*	ns*
Elasticy	ns*	ns*	ns*

Table 12. Mean values and organisation for sensory attributes by ANOVA. Significant differences in values are indicated by different (vertical division)

Parameters	**w/wo sourdough**		**% Kernza**		
	-	**+**	**0%**	**15%**	**45%**
Colour	3.88^b	5.10^a	2.15^c	4.66^b	6.67^a
Porosity	6.87^a	4.94^b	6.48^a	5.75^a	5.50^a
Sweetness	4.29^a	4.00^a	3.93^a	4.67^a	3.83^a
Saltiness	5.38^a	4.00^b	4.06^a	4.50^a	5.50^a
Acidity	4.61^a	4.55^a	4.00^a	4.92^a	4.83^a
Bitterness	3.82^a	2.89^b	4.00^a	3.42^{ab}	2.65^b
Yeast/Sponginess	5.78^a	5.34^a	5.52^a	5.42^a	5.75^a
Roasted	4.75^a	4.94^a	4.47^a	4.66^a	5.41^a
Cereals	4.90^a	4.44^a	3.10^b	5.08^a	5.83^a
Density	3.38^b	6.61^a	4.68^a	4.92^a	6.00^a
Juiciness	6.08^a	6.33^a	5.78^a	6.41^a	6.41^a
Elasticy	6.20^a	5.27^a	6.13^a	5.91^a	5.17^a

Significant differences in values are indicated by different letters (vertical division). The data are displayed as mean values.

Figure 7 shows that the sample with 45% Kernza without sourdough (45) was the darkest one. Interestingly, the 45% with sourdough (45+S) were lighter. The control bread (0) was the lightest bread. The control (0) had the most porosity, then the 15% Kernza without sourdough (15). The sourdough breads had in general lower values, and less porosity. Regarding sweetness, in general, there were the same values, but the sourdough bread with 45% Kernza (45+S) was less sweet. 45% of Kernza without sourdough (45) were the saltiest ones. The sourdough breads with the largest content of Kernza were more compact and denser. The 45% Kernza without sourdough (45) were the most bitter, but not the 45% with sourdough. Kernza gives a taste of roasted and cereals. The rest of the attributes were hard to assess, since they were not significant differences and therefore complex to sum up.

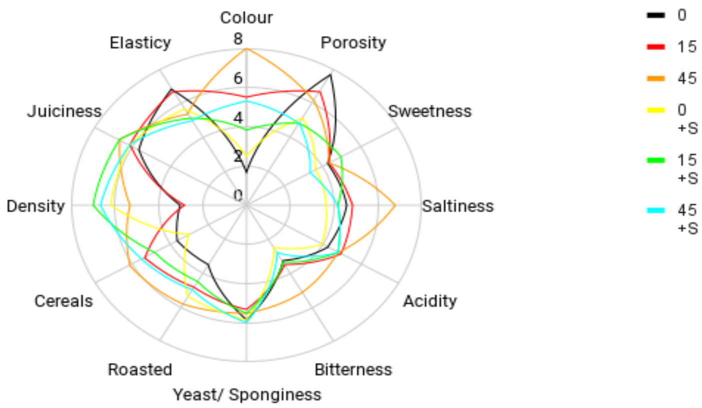

Figure 7. A graphic representation of the mean values from the descriptive analysis) findings (n=6 respondents). Evaluation of the attributes according to maximum/ minimum on the scale. The relative intensity of each attribute rises with increasing distance from the central point.

5. Discussion

The aim of the study was to investigate the baking properties of intermediate wheatgrass (IWG, *Thinopyrum intermedium*) and their impact on sensory attributes and consumer preference. Three different percentages of Kernza flour (0%, 15%, and 45%) and the addition of vital wheat gluten were evaluated for dough and bread properties. Two sensory evaluations, both one consumer test and one descriptive analysis, were performed to obtain consumer preference and sensory attributes. Kernza bread baked on sourdough was also investigated in the descriptive analysis. This will be discussed in the coming section.

5.1. Flour

The flour yield of refined Swedish Kernza flour was significantly higher than the flour yield of refined ordinary wheat flour. Refined white wheat flour has a flour yield of 72% or lower (Finnie & Atwell 2016). Meanwhile, refined Kernza flour had a flour yield differing between 77-92%, even though studies show that Kernza has a higher endosperm: germ ratio (Rahardjo et al. 2018). It is obvious based on the darker colour (Figure 4 and Table 4) that the colour of Kernza is darker, representing bran and germ since they are darker in colour, and the endosperm is white (Finnie & Atwell 2016). The whirling milled 100% Kernza was lighter according to the measurements, even though it contains all the germ and bran. The explanation might be that there are smaller particles coming out of the whirling mill. Regarding the sifted wheat flour, it is expected to be the lightest flour because it does not contain any bran or germ. Additionally, wheat flour contains pigments named tricins and xanthophylls and lacks carotene (Mondal & Datta 2008).

In comparison with other studies performing colour measurements of the Kernza flour, their L* value was a lot higher than in the present study. Their flour was lighter (95.6 versus 76.2), meanwhile the a* value and b* value were in the similar range (Table 4) (Cetiner et al. 2023).

If the study were to be repeated with more time in the beginning, the whole grain flour produced by the whirling mill should have been used for the whole study instead, since it is more nutritious. This is a drawback in the study since whole grain flour may result in other baking properties.

5.2. Water holding capacity (WHC)

The water holding capacity (WHC) was higher for the Kernza flour in comparison to the control wheat flour. Previous studies have reported that Kernza contains more dietary fibre, resulting in an increased water holding capacity (Cetiner et al. 2023). The key factor, fibre, influencing such ability is the abundance of hydroxyl groups, which interact with the water through bonds with hydrogen (Rahardjo et al. 2018).

5.3. Dough properties

The dough and general baking properties differed significantly between Kernza flour and the control wheat flour.

5.3.1. Farinograph

The amount of water required to accomplish 400±20 BU seems reasonable based on the water holding capacity test, the higher amount of dietary fibre in Kernza, and the positive association between addition level Kernza and amount of water addition. According to Rahardjo et al. (2018), Kernza had both a higher amount of dietary fibre (between 17-19.5%) and a higher amount of damaged starch than common wheat, increasing the water absorption (Rahardjo et al. 2018).

The results from the farinograph test on Kernza indicate an exceptionally short dough development time, a substantially shorter stability time, and a high degree of softening. Longer durations of dough development time (DDT), stability, and a lower degree of softening, are usually characteristics of robust, strong doughs. Thus, reduced DDT and stability times, as well as high degree of softening are generally undesirable. The study therefore revealed that the Kernza dough is a weaker dough, but can be improved with the help of gluten, regarding the parameter's stability and the degree of softening. Most interestingly, there were a significant interaction between the addition of gluten and percentage of Kernza concerning both the DDT and the degree of softening. Regarding all parameters except from degree of softening 15% of Kernza can be added without giving a significant difference on the dough. The results of Rahardjo et al. (2018) are in line with these findings. The authors revealed that an addition of Kernza resulted in a reduction in DDT and stability. The weakened impact caused by Kernza addition on both the rheological characteristics and gluten network, was highlighted by Marti et al. (2015). Further interesting studies found no difference in DDT between 30% and 45% Kernza, but regarding stability (Cetiner et al. 2023).

5.3.2. Extensograph

The percentage of Kernza added affected both the maximum resistance and the extensibility of the doughs. Meanwhile, the addition of gluten only affected the maximum resistance. As can be seen in Figure 1, 15% Kernza gave a medium strong dough, but when added gluten, the dough was very strong. On the other hand, for the 45% Kernza, the dough was also medium, but when gluten was added (45+G), it was not improved (Figure 5). No other studies have investigated the extensograph values on wheat doughs with different percentages of Kernza added to and with the addition of vital wheat gluten. However, Rahardjo et al. (2018) investigated the extensibility for different lines of Kernza, thus with 100% Kernza in the doughs. The extensibility was lower than in this study (with a range between 4.4 and 13.5 cm). The lowest extensibility in this study was for 45% Kernza, with 24.13 cm, which greatly differs from the study by Rahardjo et al. (2018). Thus, the theory is given that wheat has a more balanced gluten-forming composition of gliadin and glutenin (Rahardjo et al. 2018).

5.4. Colour of breads

All colour values were affected by Kernza addition, except the b* value on the crust. Gluten did only affect the lightness (L* values) and the a* value on the crust. This might depend on vital wheat gluten addition, resulting in lighter bread due to incorporation of air bubbles into the bread. Bigger holes and air do affect the colour measurement (Nielsen 2019).

Similarly, Cetiner et al. (2023) found the colour of the bread crumb tending towards red and yellow, which is the same result as in this study. Cetiner et al. (2023) explained that bread containing Kernza showed notably higher levels of antioxidant activity, yellow pigments, and total phenolic contents. Those characteristics are affecting the colour and health benefits of Kernza. Regarding the crumb, they found significant differences between a*values and b*values compared to common wheat, which this study also showed.

The reflected light is calculated from the colour of the surface. Mondal & Datta (2008) suggested that weight loss during baking could be a factor in crust browning, as well as oven temperature. According to some studies, colorimetric analysis can be used to figure out when bread is done baking (Mondal & Datta 2008). In terms of the crust values, adding gluten made the crust darker. This might depend on a higher oven temperature higher up in the oven since heat rises upward. The Kernza addition showed no discernible changes in colour. This view is supported by Cetiner et al. (2023), who stated that there was no significant difference between the 15% and 45% addition of Kernza. Additionally, the 15% Kernza resulted in approximately 27 in b* value for the bread crust (Cetiner et al. 2023).

5.5. Specific volume and height

One of the primary factors used to assess the bread quality is measuring the bread volume. Makes it reasonable to answer one of the questions of issue: Could an addition of gluten lead to a higher bread volume and is it possible to include a higher percentage of Kernza in the dough when also gluten is added? Clearly, vital wheat gluten led to a higher bread volume (Table 10). There was a significant difference between the breads with or without vital wheat gluten. As seen for the 45% Kernza, when vital wheat gluten was added, bread volume increased. The bread height also differed significantly between the samples; there was a significant difference between the substitution of Kernza.

Previous studies have found that 15% of Kernza even had a higher volume than the control sample (Banjade et al. 2019; Cetiner et al. 2023), but the highest level of Kernza still received the lowest volume, which was similar for Banjade et al. (2019) and Certiner et al. (2023), who tested higher percentages of Kernza (60%, respectively 100%). There is less starch overall and more damaged starch, which enhances the absorption of water. Fewer water molecules may engage in the gluten network when there is less damaged starch around, which reduces the specific volume of the bread. Another explanation is that the bread volume is affected negatively by insoluble dietary fibre by disrupting the gluten network (Rahardjo et al. 2018). Interestingly, at the 45% addition level, the Kernza bread collapsed and received a deformed loaf shape. Positively, the deformed loaf shape was not a problem when adding vital wheat gluten, which shows that it was the weak gluten proteins, not able to hold the CO_2 that was produced when the dough fermented.

5.6. Sensory evaluation

5.6.1. Consumer test

The consumer test resulted in high acceptability values from the respondents, as well as preference for the two breads containing Kernza in front of the control wheat bread. Indicating that the addition of Kernza can lead to a bread that consumers prefer. Due to the big standard deviation regardless of the 45% of Kernza bread, it meant a dissenting panel about the darkest bread. Dark bread is bread that you either like or dislike, which might explain the results. There was a significant difference (significance level 0.05) between the control bread and Kernza bread. Fifteen out of twenty respondents must prefer one type of bread for there to be a substantial difference. Nineteen individuals chose the bread with Kernza in this case, whereas only one chose the control bread without Kernza. Commenting on the preference of bread texture, it was quite similar between compact versus fluffy bread, but clearly, the respondents prefer a darker bread (60%). A 15% Kernza bread tasted like wholegrain toast bread and was slightly bitter, while on the other hand, the 45% Kernza tasted like rye bread or sourdough. Comparing the study where vital wheat gluten was added to different bread label companies, it seemed like lighter bread had added gluten to a greater extent. This might depend on the expectations of the consumer, who expects fluffy bread when buying light bread.

5.6.2. Descriptive analysis

Depending on the proportion of Kernza and the leavening agent (yeast or sourdough), the Kernza bread can be classified and described differently (Table 12). An essential feature of breads that influences consumer perception and acceptance of foods is the colour. The colour was lighter for Kernza when using the sourdough leaving agent since there was a significant difference in the interaction, which might depend on the lower levels of total starch that are immediately digested (Zhong et al. 2019) faster when sourdough and microorganisms are present, affecting the colour. Additionally, the higher the percentage of Kernza, the darker the bread was received. Porosity was lower based on sourdough, which might depend on a tired sourdough not leaving the bread in an optimal way. Porosity was the same regardless of the amount of Kernza. Sweetness showed no significant difference, meaning Kernza did not contribute to any additional sweetness. Sourdough affected the saltiness, the samples with Kernza and sourdough were most salty. This might depend on different amounts of salt in different recipes. Kernza does not contribute to acidity. The sourdough seems to take away the bitterness from the most bitter Kernza bread. This might depend on the lower content of starch being degraded, contributing to sugars, and balancing the bitterness. Yeast and sponginess were the sensory attributes with the biggest standard derivation, indicating the attributes were complex to assess. Density was affected by sourdough and the interaction of Kernza and the sourdough. Regarding juiciness and elasticity, there were no significant differences. Kernza gave a taste of cereal. In the consumer test, some respondents associated Kernza bread with a slightly bitter taste. Meanwhile, in the descriptive analysis, the sourdough samples with Kernza were less bitter, which might mean that sourdough either compensates for or takes away the taste of bitterness. The higher the level of Kernza, together with sourdough, the less bitter the bread tasted.

5.7. Further research

Considering the current research, many research questions remain. This study was only performed on one perennial grain crop, from one cycle, from one harvesting year, and at one location in Sweden. There are plenty of breeding cycles for Kernza.

It would be possible to draw conclusions regarding genetic effects and location effects if this were performed.

In the present study, one fraction was removed by sieving. The removed fraction was not further studied in the present study, and research could see if the fraction could be used for anything. Thus, it seemed like Kernza had a higher flour yield than annual crops, this would be interesting to investigate. More addition levels of Kernza would be interesting, like 30%, 60%, and 100%. The same study can be performed on whirling milled 100% Swedish cultivated Kernza.

Regarding the vital gluten addition, a life cycle analysis (LCA) could be performed to interpret whether a gluten addition and a higher percentage of Kernza would be more environmentally friendly than using annual crops. Gluten proteins for Kernza have been studied in the US, thus not in Swedish-cultivated Kernza. This would be interesting to see if the gluten proteins differ.

6. Conclusion

The purpose of the study was to investigate the baking properties of Kernza, an intermediate wheatgrass (IWG), *Thinopyrum intermedium*, and their impact on sensory attributes and consumer preference. Three different percentages of Kernza flour (0%, 15%, and 45%) in yeast-leavened bread with or without the addition of vital wheat gluten were evaluated for bread baking and dough properties. Two sensory evaluations, a consumer test and a descriptive analysis were performed to obtain consumer preference and sensory attributes. Kernza bread baked on sourdough was also investigated in the descriptive analysis. Kernza has a higher water holding capacity and therefore requires more water than annual wheat. The fact that Kernza is deficient in high-molecular-weight glutenin subunits was compensated by adding vital wheat gluten. With a higher percentage of Kernza, the dough's extensibility, maximum resistance, stability, and development times were all reduced. Kernza flour has a substantial impact on the baking qualities and features of bread when compared to annual wheat, by lowering the specific volume, and height. Regarding the colour, Kernza flour tends to be more red and yellow in colour. It was also darker than regular wheat flour. Consumers chose both 15% and 45% of Kernza over conventional bread, according to the results of the consumer test. The bread was described as having a slightly bitter taste, rye, and a taste of wholegrain or cereal. In conclusion, the results indicate that using Kernza as a novel, healthy, and sustainable ingredient in bread-making has a lot of potential. Could Kernza be the starting point for a revolution in agriculture?

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8. Popular science summary

Could Kernza be the starting point for a revolution in agriculture?

Methods: The study was conducted by baking bread with different proportions of Kernza flour: 0%, 15%, and 45%, both with and without the addition of vital wheat gluten. Various baking characteristics, such as water holding capacity, specific volume, height, colour and overall bread quality, were measured using an extensograph and a farinograph.

Background and purpose: 20% of the human diet comes from annual wheat. Meanwhile, bread is the most consumed food historically, and bread is also climate smart. Kernza originates from the perennial grass intermediate wheatgrass, with a lot of advantages, both nutritionally and climate-smart. Thus, Kernza does have a weaker gluten network; therefore, the effect of added wheat gluten is investigated. The aim of the current study was to scientifically evaluate the baking properties and characteristics of Kernza grown in Sweden. A secondary objective was to investigate consumer preferences for bread containing Kernza and to conduct a descriptive analysis to identify sensory attributes.

Main findings: Kernza was found to have higher water absorption and water holding capacity compared to regular wheat flour, which affects dough consistency and bread texture. Doughs with a higher proportion of Kernza had shorter development times and stability and reduced maximum resistance and extensibility. The addition of gluten improved these properties by compensating for Kernza flour, which lacks in high molecular weight glutenin subunits. The flour from Kernza gave the bread a darker colour, tending towards red and yellow, which is different from the lighter traditional wheat bread. The consumer test revealed that bread with 15% and 45% Kernza was preferred over conventional bread. The bread was described as having a whole grain, cereal, and rye flavor with a slightly bitter aftertaste.

Applications: The study shows that Kernza has great potential as an innovative, healthy, and sustainable ingredient in bread baking. Its higher water-holding capacity and unique flavor may provide new opportunities for bread producers to create different types of bread that appeal to conscious consumers. Although Kernza affects the baking properties of dough, the addition of gluten can improve the quality of bread, demonstrating that it is possible to adapt recipes to achieve the desired texture and taste. This is particularly important in the development of breads that are not only nutritious but also sensorially appealing.

Final words: Kernza represents a significant step towards a more sustainable agricultural system and offers exciting opportunities for the future of bread baking.

By integrating this crop on a larger scale, we can reduce the environmental impact of agriculture while offering consumers nutritious and tasty alternatives to traditional breads. Continued research and development will be crucial to maximizing its potential and ensure its place in the food chain of the future.

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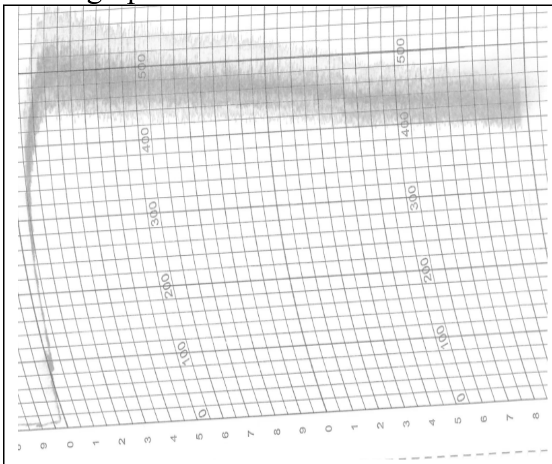
Thank you all for making this possible!

Appendix I

Farinograph results



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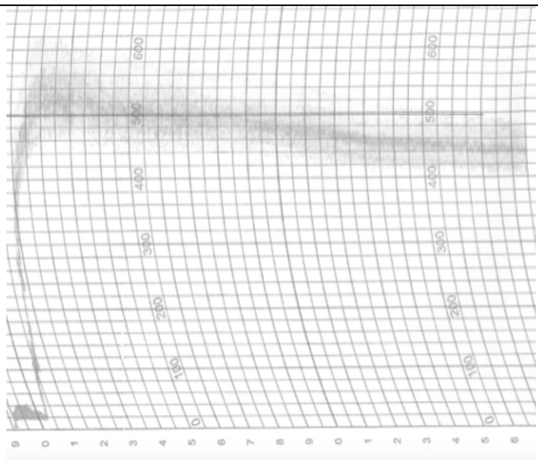

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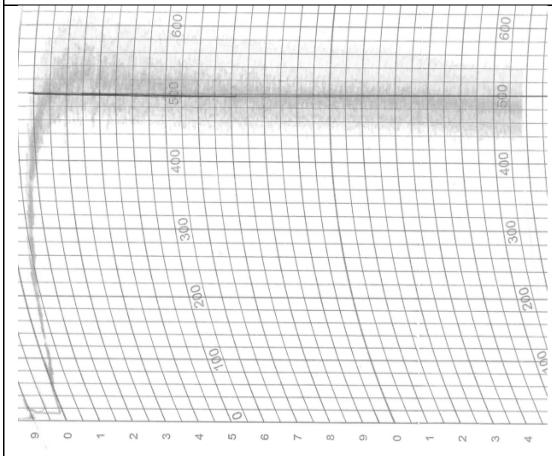
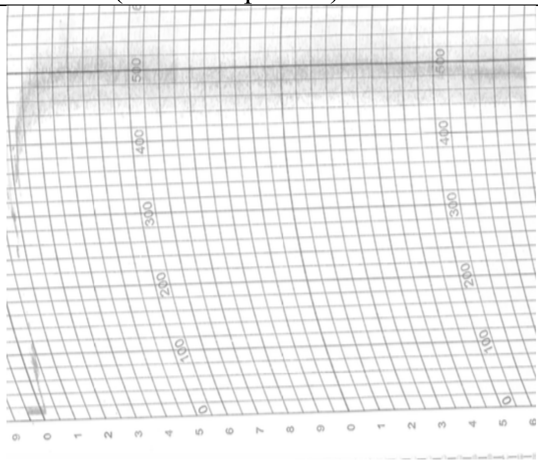
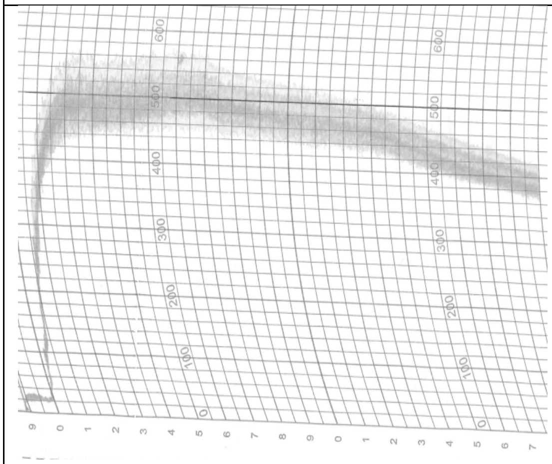


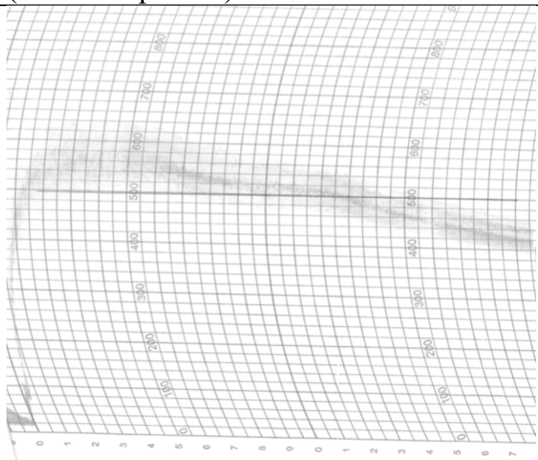
Control with 5% vital wheat gluten



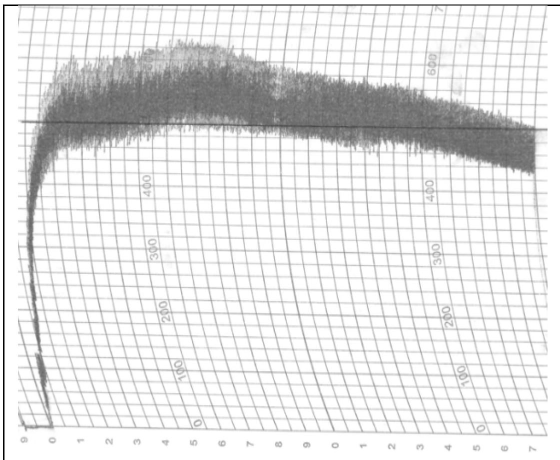
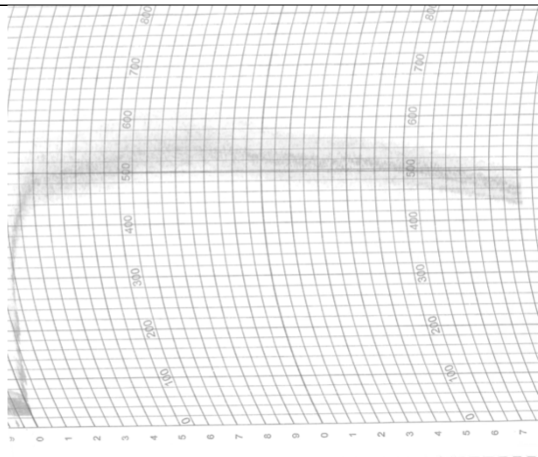
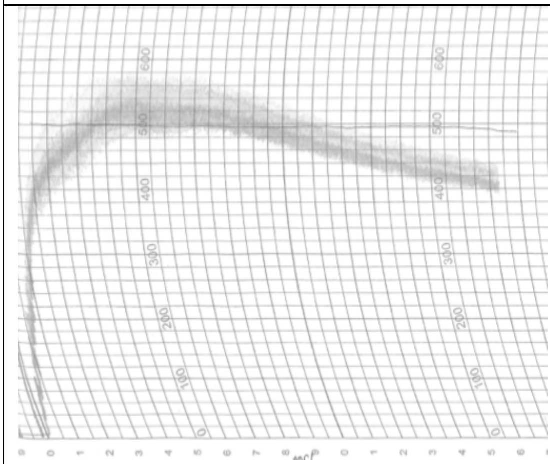
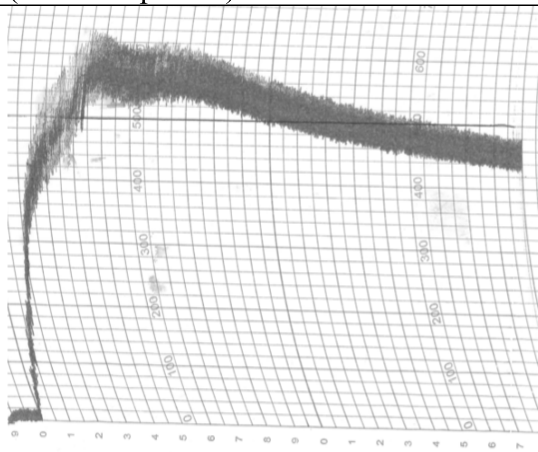
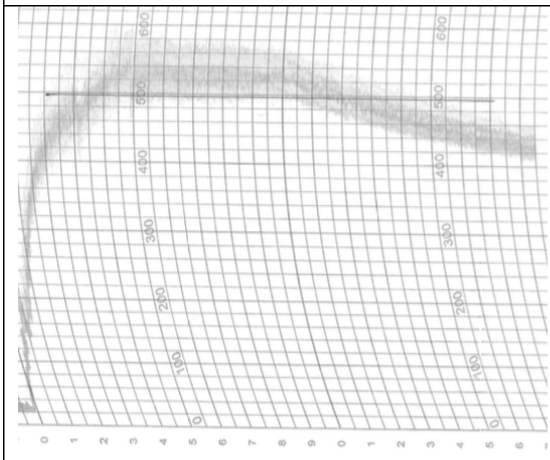
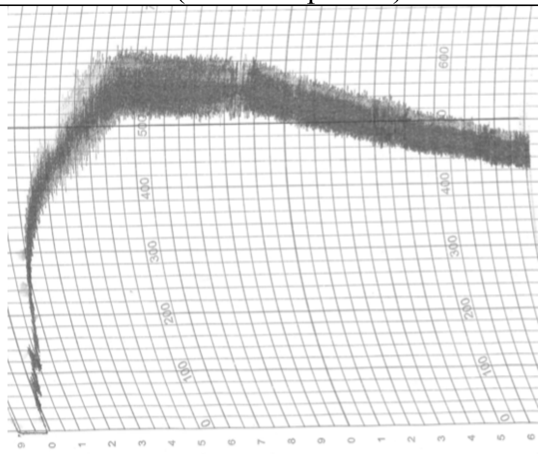
Control with 5% vital wheat gluten (second replicate)



15% Kernza



15% Kernza (second replicate)

 15% Kernza with 5% vital wheat gluten	 15% Kernza with 5% vital wheat gluten (second replicate)
 45% Kernza	 45% Kernza (second replicate)
 45% Kernza with 5% vital wheat gluten	 45% Kernza with 5% vital wheat gluten (second replicate)

Appendix II

Appendix III Baking pre-trials

Pre-trials were done to figure out how much yeast there was. Both the 3.5% and the 2% yeast tests were run. Furthermore, ascorbic acid was absent from the control flour; hence, both an ascorbic acid-containing and an ascorbic acid-free baking trial were carried out. Finally, a second experiment was conducted to remove the remaining gluten from pure Kernza flour.

Results

The control bread baked with 2% yeast was visually lower than the bread baked with 3.5% yeast. Due to the addition of Kernza further on and gluten, the extra leavening agent is desirable and needed. Therefore, 3.5% yeast of the total dough was decided, corresponding to 8 grams of yeast.

Secondly, a test with and without ascorbic acid was performed. However, this did not result in any visual difference, which could be decided based on one preliminary baking trial. But with the fact that the Kernza flour was freshly milled, ascorbic acid can be used as a maturation agent. Therefore, ascorbic acid was added directly to the control flour.

Lastly, since previous studies indicated weak gluten in Kernza, the test of washing out Kernza was tried. Unfortunately, no gluten could be washed out of the used flour. Left were the fibres and other components. Although, the starch was easily washed out.

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