

Effect of quality properties of added gluten on the texture and sensory properties of rye and buckwheat breads

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Abstract. Bread producers use vital wheat gluten to enhance the quality of their products. However, commercial isolated glutens could have different properties and therefore influence the properties of the final products. As studies on the quality properties of glutens and their effect on the final baking products are limited, the aim of this study was to characterize commercial isolated glutens and the effect of their addition on the textural and sensory properties of rye and buckwheat breads. Three glutens were tested for water binding capacity (WBC), gluten index (GI), protein sedimentation, and resistance using modified methods. Afterwards, three different bread recipes were developed and commercial glutens were tested in each model bread. The commercial glutens had different physicochemical and viscoelastic properties, which were below the typical values of native glutens (GI was 36–46%, extensibility- 48–78 mm). Breads also had different sensorial and textural properties, which diverged more during storage. The sour taste intensity and springiness of the rye bread increased, while its moistness, adhesiveness, and typical odour intensity decreased. Fresh and staled rye toast breads were softer and more porous. The buckwheat bread was the most stable, though it was drier and springier after storage. The effect of gluten was specific to the bread recipe and was uncorrelated with the gluten quality properties individually. However, gluten with the intermediate values of WBC, sedimentation, and extensibility, also resulted in breads with intermediate sensory properties. Thus, it is possible to enhance specific properties of bread using commercial glutens with different quality attributes.

Key words: commercial gluten, rye and buckwheat bread, sensory profile, texture profile, viscoelastic properties.

INTRODUCTION

Gluten is a native wheat storage protein complex (Wieser, 2007). Gluten proteins can be divided into two main fractions according to their solubility in alcohol-water solutions - soluble gliadins and insoluble glutenins. During bread dough making from wheat flour gluten forms a sponge-like three-dimensional structure with high gas holding capacity and other important rheological properties (Kieffer, 2006). Gliadins contribute to viscosity and extensibility, glutenins - to dough strength and elasticity (Wieser, 2007).

Due to the weak properties or absence of gluten in other cereal flours their use for bread baking can be difficult or cause quality problems. For example, it is not possible to make bread from buckwheat flour only, because the quality of baking is low, and the sensory properties are also quite poor (Gimenez-Bastida et al., 2015). Nevertheless, due to the high antioxidant capacity, high amount of protein with well-balanced amino-acids composition, dietary fibres, and peculiar, pleasant flavour buckwheat flour is still used for production of gluten-free bread (Wronkowska et al., 2009; Krupa-Kozak et al., 2011). In this case, hydrocolloids must be used to enhance the dough and bread structure due to the absence of gluten fractions (Mariotti et al., 2013).

Rye flour is popular for bread making in the Baltic States, Finland, Germany, Denmark, Poland, and Russia (Mihhalevski et al., 2013). However, due to the inability of rye flour to form a gluten network during dough production, it is necessary to apply additional technological steps such as making sourdough (Hansen, 2006; Poutanen et al., 2009), or add emulsifiers, hydrocolloids, or wheat flour to facilitate dough structure formation (Katina et al., 2014).

Alternatively, the baking properties can be enhanced by the addition of isolated glutens. Commercial glutens are produced as a by-product during the isolation of starch from wheat flour by various wet separation and controlled drying processes (Day, 2011). Thus, the properties of glutens receive less attention compared to starch, but viscoelastic and physicochemical properties of added glutens can influence the quality of the final product.

Commercial glutens have different quality properties stemming from the quality and genotype of the raw material, the production procedures and processing parameters (Sayaslan et al., 2010). Drying of a gluten extract is the final and most critical step of gluten production (Kaushik et al., 2015). Negative effect of drying on gluten functionality was reported. Esteller et al. (2005) and Day (2011) observed degradation of gluten viscoelasticity even at a relatively low drying temperature.

Commercial gluten is available in two forms - vital and non-vital. Usually, bakeries use vital gluten; its viscoelastic properties are restored by hydration and are similar to the native wheat gluten. Non-vital gluten can be used for the enhancement of protein content, as it was irreversibly denatured (Esteller et al., 2005). Vital gluten can be added to the dough to enhance the quality and sensory characteristics of wheat flour-based products (Dhaka & Khatar, 2015; Selaković et al., 2021) or bread made from the frozen dough (Giannou & Tzia, 2016), but also as a replacement of wheat flour in non-gluten breads (Esteller et al., 2005; Marconi et al., 2007). Moreover, the addition of gluten may enrich the bread flavour profile due to the Maillard reaction between sugars and amino acids released during the gluten drying process (Cho & Peterson, 2010; Day, 2011). The optimal added gluten amount is 1–5% (w/w) of the flour weight (Codina et al., 2008; Giannou & Tzia, 2016).

Gluten quality can be evaluated by measurement of gluten index, water absorption, and rheological properties such as resistance to extension and extensibility during stretching (Kaushik et al., 2015; Wang et al., 2021). Gluten characteristics such as degree of extensibility and elasticity are more important for bread quality than gluten content in flour and they correlate well with baking properties (Dhaka & Khatar, 2015; Kaushik, et al., 2015). Water absorption, analysis of viscoelastic properties, and sedimentation test are the most used methods for the characterization of native glutens. The Zeleny sedimentation test describes the quality of gluten proteins according to their ability to swell and settle in a slightly acidic medium (Hruškova & Faměra, 2003). Gluten index and extensibility

parameters give information about the viscoelastic properties. However, bread producers occasionally observe differences in the baking performance between glutes from different batches or producers, even if their certified characteristics are the same. Therefore, bakery manufacturers need clear criteria for gluten quality properties for choosing the best gluten for their demands.

Although commercial isolated glutes are widely used in the production of high-quality breads, studies on the quality properties of glutes and their effect on the final baking products are limited. Recent research pays more attention to the partial substitution of wheat flour with gluten (Giannou & Tzia, 2016; Tebben et al., 2018; Quian et al., 2022) or quality improvement of gluten-free products (Salehi, 2019), but the effect of the addition of gluten to breads from other flours is overlooked. Therefore, the aim of this study was to characterize commercial isolated glutes and evaluate the effect of their addition on the textural and sensory properties of breads made from rye and buckwheat flours to improve the stability of baking products and expand the assortment.

MATERIALS AND METHODS

Materials

Two types of rye flour (R1800 and R815) and buckwheat flour were used in this study. Rye flours were obtained from Tartu Grain Mill Ltd. (Tartu, Estonia), and buckwheat flour was obtained from Balti Veski Ltd. (Jüri, Estonia). Vital wheat glutes were obtained from three different commercial producers, specifications of all the glutes were as follows: moisture max 8%, protein content min 75%, ash content max 1.5%. Malt was obtained from Estonian Malt OÜ (Võru, Estonia). Flour conditioner (emulsifiers mono- and diglycerides of fatty acids, diacetyl tartaric acid ester of mono- and diglycerides, thickener xanthan gum, enzymes) was obtained from a local distributor. The rest ingredients used for the preparation of bread samples, such as sugar, salt, pressed yeast, seeds mix, and margarine (80% fat), were obtained from a local market.

Acetic acid (99.8%, Sigma-Aldrich), isopropanol (99.8%, Sigma-Aldrich), MilliQ water (Millipore Corp., Molsheim, France), and sodium chloride (99.8%, Fluka) were used for the determination of gluten quality properties.

Methods

Moisture content

The moisture content was measured with Halogen Moisture Analyser HR83 (Mettler Toledo, USA) at 105 °C. The sample weight was 1 g.

Water binding capacity of gluten

Gluten samples of 0.5000 g were put into 50 mL tubes and vortexed with 25 mL MilliQ water for 10 sec. The suspensions were held for 15 min and after that centrifuged by Hettich D-78532 centrifuge for 15 min at 1,100 × g. The supernatant was decanted and the tube with sludge was weighed. Water binding capacity (WBC, %) was calculated according to Eq. 1.

$$WBC(\%) = \frac{C - B - A}{A} \times 100\% \quad (1)$$

Here, A is the mass of the gluten sample (g), B is the tube weight (g), and C is the wet gluten sample with the tube (g).

Gluten index

Gluten index (GI) was measured according to ICC 155/AACC 38-12 by weighing 1.5 g of gluten and using a Glutomatic 2200 Gluten washing system (Perten, Sweden) and centrifuge ($2,200 \times g$) with standardized cassettes. The gluten index (%) was calculated according to Eq. 2.

$$GI(\%) = \frac{a}{b} \times 100\% \quad (2)$$

Here, a is the gluten mass (g), which remained on the centrifuge rest after centrifugation and b is the total gluten mass (g).

Gluten sedimentation test

The sedimentation test was carried out according to the Zeleny test (Hrušková et al., 2004; ISO 5529:2007) with some modifications. The extraction solution was prepared by mixing 80 mL 2% (v/v) acetic acid with 20 mL isopropanol. Weighed gluten samples (0.2000 g) with 25 mL of extraction solution in 50 mL measuring cylinders were sedimented for 20 min after intensive shaking. The results were recorded as mL of precipitated gluten.

Viscoelastic properties of gluten

Viscoelastic properties (resistance to extension, extensibility) of glutes were evaluated by Texture analyser TA.XT2i (Stable Micro Systems, Godalming, England) with the A/TG probe. The dough was made by mixing 3.00 g of gluten with 3.00 g of MilliQ water and was immediately fixed on the A/TG probe with a distance of 10 mm between the grips. The maximum force (resistance to extension) and distance of gluten extension at which the peak force occurs (extensibility) were measured using tension mode at 3 mm s^{-1} .

Bread baking

Three different recipes (Table 1) were developed for making wheat-flour-free breads without the sourdough process. Water absorption of raw flours was used to find the optimal gluten-to-water ratio for the best buckwheat and rye dough consistency. Rye and buckwheat model breads were prepared by mixing base bread ingredients and gluten. The rye toast bread recipe was supplemented with flour conditioner and additional ingredients such as malt, seeds, and sugar to simulate an industrial baking process. All three commercial glutes were tested in each model bread.

The ingredients were weighed with 0.1 g precision and mixed in a spiral dough mixer OASE 48477 (Diosna GMBH, Germany) for 2 min at low speed and 4 min at high speed.

Table 1. Recipes of the tested breads, the amounts are given as a percentage of the total dough weight

Ingredient	Buckwheat bread, %	Rye bread, %	Rye toast bread, %
Buckwheat flour	22.5	-	-
Rye flour 1800	-	25.1	21.1
Rye flour 815F	22.5	25.1	21.1
Gluten	6.8	10.1	7.3
Yeast	1.1	2.5	1.9
Fat	1.1	1.2	1.3
Salt	1.0	0.8	0.9
Water	45.0	35.2	35.4
Malt, seeds mix, flour conditioner, sugar	-	-	11

The dough (3 kg) was divided into 300 g pieces, dough (3 kg) was divided into 300 g pieces, moulded, and proofed for 45 min in a Metos Chef 200 (Metos OY, Finland) proofing chamber at 37 °C and 70% relative humidity. After that, the products were baked for 15 min at 225 °C and then for 15 min at 200 °C. Baked and cooled loaves of bread were wrapped in food film and stored at room temperature until analysis.

Texture profile analysis

The texture profile analysis of fresh (1 day after baking) and stale (4 days after baking) breads was measured according to the modified procedure from standard method AACC 74-09 with TA.XT2i (Stable Micro Systems, Godalming, England) Texture analyser with 40% penetration depth with a 36 mm diameter probe and 5 s gap between double compression cycles on bread slice with 25 mm thickness.

Sensory analysis

Sensory analysis was carried out by seven trained assessors. The appearance (crumb colour, porosity), odour (sour odour, typical odour intensity, off-odour), texture (elasticity, resilience, softness, crumbliness, adhesiveness, moisture), and taste (typical taste intensity, sour taste, sweet taste, off-taste) of fresh (1 day after baking) and stale (4 days) bread were evaluated on a 0–14 scale. On the scale, 0 corresponds to the minimum intensity and 14 – the maximum.

Finn Crisp Original and Finn Crisp Multigrain crispbreads (Lantmännen Cerealia Oy, Finland) were used as rye bread and rye toast bread flavour reference, respectively. Buckwheat bread's typical odour and taste were evaluated by comparing with Dr. Nature buckwheat crackers (Kora Ltd., Latvia).

Porosity and crumb colour were evaluated visually using a photo scale and a colour palette.

Statistical analysis

At least triplicate measurements were carried out for the gluten quality properties, bread texture measurements, and sensory analyses, and the results were averaged. Statistical analysis and visualization were performed in R 4.0.2 software (The R Foundation for Statistical Computing, Austria, Vienna). Statistically significant difference ($p < 0.05$) was calculated using pairwise *t-test* with adjustment for multiple comparisons. Partial least squares discriminant analysis (PLS-DA) of instrumental and sensory data was performed using the R package 'mixOmics' 6.11.33. The correlation coefficient is Pearson's *r*.

RESULTS AND DISCUSSION

Properties of the glutens

Gluten quality parameters such as water binding capacity (WBC), Zeleny sedimentation, and gluten index (GI) as well as rheological properties such as resistance to extension and extensibility during stretching are the main parameters for the characterization of baking properties of flours that contain native gluten. In our study, we applied the same methods to describe isolated commercial glutens. Thus, we distinguish hereinafter commercial isolated vital glutens and flour native gluten.

The quality properties of the three analysed commercial isolated glutes are presented in Table 2. Results showed that the moisture of the studied glutes was in the range of 5.5–6.5%, which is below the maximum recommended threshold of 10% (CODEX STAN 163-1987). The moisture content correlated with WBC ($r = 0.99$).

Table 2. Properties of the commercial isolated glutes. Standard deviations are shown ($n = 3-7$)

Gluten	Moisture, %	Water binding capacity, %	Gluten index, %	Sedimentation test, mL	Resistance to extension, N	Extensibility, mm
Gluten 1	5.56 ± 0.06 ^a	87.76 ± 6.17 ^a	36.6 ± 1.43 ^a	12.6 ± 0.4 ^a	3.79 ± 0.40 ^a	78.32 ± 2.74 ^a
Gluten 2	6.16 ± 0.11 ^b	125.57 ± 2.99 ^b	46.4 ± 1.43 ^b	11.9 ± 0.4 ^b	3.77 ± 0.19 ^a	62.95 ± 2.35 ^b
Gluten 3	6.44 ± 0.05 ^c	135.76 ± 8.63 ^b	42.1 ± 1.98 ^c	9.4 ± 0.5 ^c	3.73 ± 0.22 ^a	48.90 ± 3.16 ^c

The values in a column not sharing a letter are statistically significantly different ($p < 0.05$).

The highest WBC was observed in Gluten 3. The water binding capacity of flour can predict the quality of baked products (Unbehend et al., 2006, Kaushik et al., 2015). Bread made from flour with higher WBC is usually softer (Salmenkallio-Marttila et al., 2004), moister, and stales more slowly (Fadda et al., 2014). High water absorption is needed for optimal swelling of the protein matrix that in turn leads to the formation of a three-dimensional network and good mixing tolerance of the dough, resulting in high volume and porosity of the bread (Hruškova & Faměra, 2003; Kaushik et al., 2015). Water absorption of flour depends on protein amount and its properties; starch and wheat polysaccharides such as pentosans and β -glucans also contribute (Unbehend et al., 2006; Okuda et al., 2016). According to Peters et al. (2017), the type of water binding depends on the protein structure and water can be bound internally (within the structures or network) or interstitially (between the structures or within the pores of the network). The hydrophilicity of vital gluten as well as its ability to swell can be affected by the technological process of isolation and production (Day, 2011; Peters et al., 2017). In addition, other components like starch, lipids, and fibres incorporated in isolated gluten, may affect the water binding properties of vital gluten (Day et al., 2006; Schopf & Scherf, 2020).

The gluten index of flour is an important flour quality parameter that characterizes wheat protein, especially the viscoelastic properties of glutenin fraction, and has been correlated with the strength of the protein network (Oikonomou et al., 2013). The GI of flour is considered acceptable when it is above 30% (Oikonomou et al., 2013) and is optimal in the range of 70–90% (Ćurić et al., 2001). The studied vital glutes were within 36–46% range, which is acceptable but below optimal. Low GI values of the commercial samples could be the result of the isolation and drying processes during production, which were shown to affect the properties of gluten (Popper et al., 2006) due to the thermal denaturation into less ordered unfolded structures (Kaushik et al., 2015). GI showed moderately positive correlation with moisture ($r = 0.72$) and with WBC ($r = 0.79$).

The higher is wheat protein content and its quality, the higher the sedimentation test value is (Hruškova & Faměra, 2003; Hruškova et al., 2004). The sedimentation test correlates with water binding and dough formation properties (Hruškova et al., 2004). Gluten 1 had the highest sedimentation test value, but the lowest gluten index, which could result in poorer baking properties.

Results showed that the resistance to extension of all the glutes was identical. However, significant differences in the extensibility of the gluten-water mixture and a moderately negative correlation between the GI and extensibility ($r = -0.58$) were observed. Gluten 1 and 2 showed longer extensibility than Gluten 3, which correlated with higher sedimentation test values ($r = 0.94$). Typical extensibility of native wheat gluten was reported to be higher in the range of 130–160 mm, although the measurement method was different (Wang et al., 2021).

Wheat flour usually shows a positive correlation between WBC and sedimentation test (Hrušková et al., 2001; Hrušková & Faměra, 2003), but in our case, it was negative ($r = -0.81$). These parameters mainly depend on the structure and properties of native gluten, so the negative correlation highlighted the difference in behaviour between native gluten and isolated, dried, and rehydrated gluten. That means that native and isolated commercial glutes are very different, and the production stages of isolated commercial gluten affect its properties. Furthermore, the methods used for the characterization of native glutes in flour might not be applicable to commercial isolated glutes.

According to Table 2, Gluten 1 had the highest sedimentation test value and extensibility, which potentially leads to good baking properties. Yet, it had the lowest and worst gluten index and water absorption. Due to the ambiguity of these results, we decided to bake three different bread types to investigate how added glutes performed in the final products by assessing their textural and sensorial properties.

Textural and sensorial properties of breads

The model breads were made from rye and buckwheat flours according to the recipes in Table 1 and each gluten was tested in each recipe. Photos are in Supplementary materials.

The three different bread types had distinct sensory properties. The results of the sensory analysis summarized with the PLS-DA method are shown in Fig. 1, A. Three clear clusters formed by the recipe: the buckwheat bread had a darker crumb and intense specific taste; the rye bread demonstrated stronger sourness, overall odour intensity, and springiness; the rye toast bread showed higher moistness, adhesiveness, porosity, and softness due to the use of flour conditioner. Furthermore, rye and rye toast breads were more closely located to each other, indicating their similarity, while buckwheat bread was more distinct.

Changes in the sensory properties during storage were also observed, seen in Fig. 1, A as the shift of the clusters grouped by the day of storage. The largest difference was observed in the rye bread. The sour taste intensity and springiness of the rye bread increased, while its moistness, adhesiveness, and typical odour intensity decreased during storage. Furthermore, it became harder and in the case of Gluten 3 also less porous. The moistness of the rye toast bread decreased, except for Gluten 3, and some sour taste and odour notes appeared after four days. The buckwheat bread was the most stable, but still, it was drier and springier after storage. However, overall, Gluten 3 provided the highest bread stability over time.

Texture profile analysis of the breads is summarized by PLS-DA in Fig. 1, B. Analogous clustering by recipe and storage time is also observed here. Buckwheat bread here also forms the most distinct cluster, which is separated from the rye and rye toast bread by higher moistness and adhesiveness due to the higher water content in the recipe (Table 1). In comparison to the stale rye toast bread, cohesiveness, elasticity, and

resilience of the fresh bread were higher, while chewiness and hardness were lower. The rye bread behaved similarly to the rye toast bread. Changes in the buckwheat bread were minor, corroborating the sensory analysis.

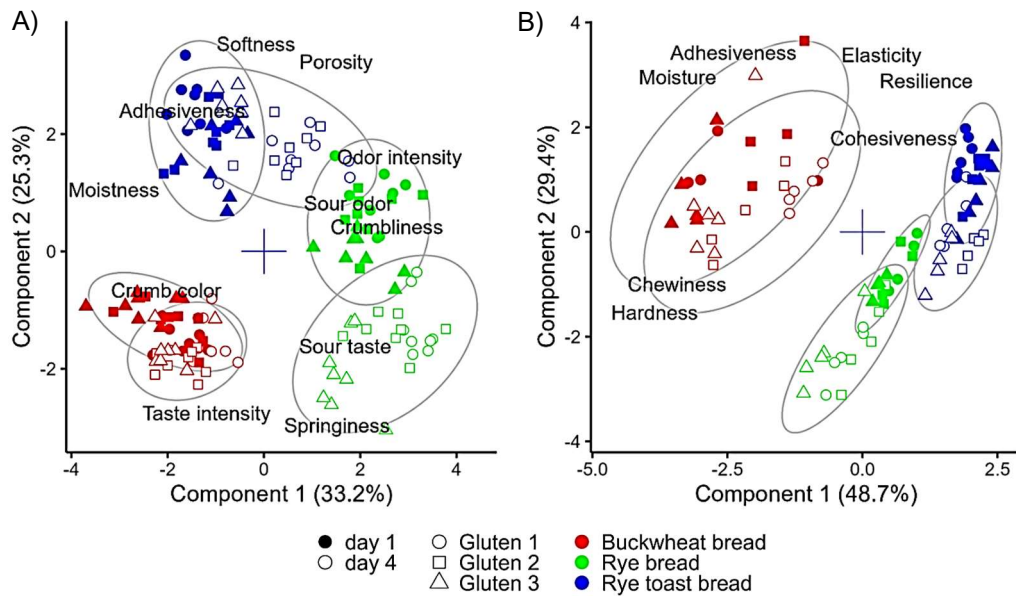


Figure 1. PLS-DA biplots are grouped by the bread recipes and the days of storage. A) Sensory properties. B) Instrumental properties. Explained variance by the component is shown in parentheses. Ellipses indicate a 95% confidence region for the group means.

Comparison of Fig. 1, A and 1, B reveals that sensory moistness and instrumental moisture were not correlated. This may happen because sensory moistness depends on how strongly the water is absorbed by macromolecules such as proteins, starch, and other polysaccharides. Even if the water amount mixed into the dough is high, but it is bound strongly and little is released during chewing, this bread can still be evaluated sensorially as dry (Nilova et al., 2017).

In general, gluten addition had a weaker influence on the sensory and textural properties of breads in comparison to the overall recipe composition.

Effect of gluten on the sensory properties

Because the studied recipes were different, we did not observe any general trends attributable to the glutes, thus, we decided to investigate the effect of gluten addition on the sensory properties of each bread type separately. PLS-DA models in Fig. 2 show some clustering according to the used gluten. However, the total explained variance of two components is around 30% and the clusters are overlapping, confirming that glutes had a relatively minor impact.

In the buckwheat bread, Gluten 3 increased crumb colour, sour taste, moistness, and adhesiveness, but decreased springiness, while Gluten 1 was the opposite. In the rye bread, Gluten 1 increased porosity and sour odour, Gluten 3 increased springiness and adhesiveness, and Gluten 2 bread had the lowest springiness and slightly elevated

crumbliness. Gluten 3 in the rye toast bread increased crumb colour, porosity, springiness, and moistness. The rye toast bread with Gluten 1 had the highest sour odour and with Gluten 2 it was the driest.

The breads made with Gluten 1 had a stronger sour aroma. The sedimentation test value of Gluten 1 was higher, and in flours this test was shown to be related to higher protein content (Hrušková et al., 2004). Amino acids are important for odour formation, particularly free leucine, isoleucine, and phenylalanine which could be released during gluten production (Cho & Peterson, 2010; Gioia et al., 2017; Rohleder et al., 2019).

The only effect universally observed in all the breads was the enhancement of moistness and adhesiveness by Gluten 3, which also had the highest WBC (Table 2). The glutes had no substantial effect on the overall odour and taste intensities and softness and crumbliness of the breads. The effect on springiness depended on the recipe and was not related to the measured properties of the glutes. Likewise, porosity was enhanced in different breads by different glutes. The rye toast bread recipe contained dough conditioner with proteolytic enzymes that could partially degrade gluten. Furthermore, the diastase enzyme from the conditioner can also affect gluten by its side proteolytic activity (Mihhalevski et al., 2013). This could explain why the effects of the glutes on some bread properties like porosity and springiness were inconsistent across the bread recipes and were not correlated with the properties of the isolated glutes.

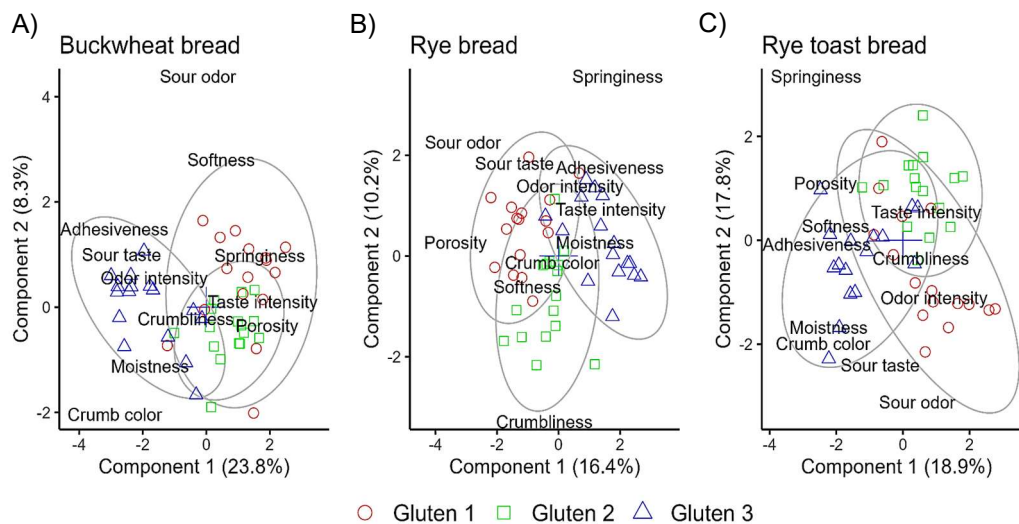


Figure 2. PLS-DA biplots of sensory properties grouped by the glutes. A) Buckwheat bread. B) Rye bread. C) Rye graham bread. Explained variance by the component is shown in parentheses. Ellipses indicate 95% confidence regions for the group means.

Interestingly, Gluten 2, which had intermediate values of WBC, sedimentation, and extensibility, also resulted in breads with intermediate sensory properties. Visually this is seen in Fig. 2 by the position of the clusters, where Gluten 2 is largely positioned between Glutes 1 and 3. This observation can mean that the effect of isolated glutes on the bread properties cannot be reliably predicted by assessing just one gluten quality attribute. Instead, multiple parameters need to be taken into account and their relation to

the final bread quality is complex and depends on the recipe. Further research could investigate other functional properties of commercial isolated glutes and effect of their chemical structure on the sensory attributes of bread.

CONCLUSIONS

The results showed that physicochemical and viscoelastic properties of commercial isolated glutes are significantly different, and this could stem from the raw material and production technology. Other studies typically focus on substituting wheat flour or developing gluten-free products. Uniquely, this study considered the addition of commercial isolated glutes to improve the quality and stability of bread made from rye and buckwheat flours. The results showed that adding gluten to bread could influence its sensory and instrumental properties. Therefore, it is possible to optimize final products by selecting glutes that enhance the required specific properties, but the effect depends on the bread recipe and is relatively weak. The quality parameters of isolated glutes which are typically measured for native glutes of flours were poorly correlated with the sensory attributes of the baked breads, when assessed individually. The exception was water binding capacity that correlated with moistness and adhesiveness of the breads. Partial least squares discriminant analysis, however, suggested that a complex assessment of the quality attributes of glutes is needed to predict their effect on different breads.

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Supplementary material for the manuscript 'Effect of quality properties of added gluten on the texture and sensory attributes of bread made from rye and buckwheat flour'



Figure 1. Rye toast bread with Glutens 1, 2, 3 (left to right).



Figure 2. Rye bread with Glutens 1, 2, 3 (left to right).



Figure 3. Buckwheat bread with Glutens 1, 2, 3 (left to right).