



Article The Influence of Texture Type and Grain Milling Degree on the Attenuation Limit, Protein Content, and Degradation in Wheat Wort

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Featured Application: The results of the research should help in the application of wheat with a transitional (marbled) type of endosperm texture as a raw material for brewing purposes.

Abstract: Wheat of medium hardness with marbled endosperm (transitional form between hard and soft wheat) in which glassy and floury zones alternate, form almost exclusively available assortment for brewing needs in Southeastern Europe. The aim of this work is to establish the influence of the grain texture and the degree of milling on the attenuation limit of wheat wort obtained from this type of wheat. Wheat worts using hard, soft transitional, or marbled endosperm texture were produced. The indicators of proteolysis, cytolysis, and amylolysis were determined, with regard to the parameter attenuation limit (AL) or fermentability. From the results for the tested parameters, it was established that despite similar starting values for the most important quality parameters, transitional wheat produces significantly different wort, both among themselves and in comparison with hard and soft wheat, and also when looking at the results for different milling degree (fine or coarse). The obtained values for the attenuation limit for transition wheat are similar or even better when compared to soft wheat, with satisfactory values for almost all examined quality parameters of wort. It can be concluded that a transitional type of wheat can be used just as well as unmalted raw material for the production of wort, as well as a raw material for malting.

Keywords: hardness and vitreosity of endosperm; grist milling degree; fermentability of wheat wort

1. Introduction

Wheat (*Triticumm aestivum*) has been used for beer production for probably as long as barley. Wheat varieties selected for brewing are very rare and exist in only a few countries (mostly Germany). These varieties are commonly not favorable for growth in Southeast Europe [1]. Soft varieties are commonly suitable for brewing, particularly because they have lower protein content. Desirable characteristics for brewing wheat are given in a review paper by Faltermaier et al. [2], and the main remark is that a high protein content, which may be a benefit for farmers and bakers, is a fault for brewers, since it can prolong lautering times, cause filtration difficulties and fermentation problems in the brewery, as well as decreased flavor stability in the finished beer [3]. Ultimately, wheat's overall protein content is not much higher than barley's, but it contains a higher amount of high-molecular-weight (HMW) proteins which end up in wort [4,5] and contain a higher content of albumin and gluten [6,7].

European winter wheat varieties display a decreased content of proteins than spring varieties [8]. Red, hard wheat varieties commonly grown in the Pannonian Basin with typical continental climate which is often causing "forced maturation" phenomenon, are formally characterized as "hard". Regardless, they have many characteristics of soft wheat



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). varieties. These varieties are characterized by a pale reddish color, moderate hardness, almost completely transient vitreous grain, and the absence of awn. The forced maturation phenomenon is a result of a combination of increased air humidity and high temperatures during the grain-filling phase. This displays unsuitable effects on many cereal quality indicators, mostly affecting the protein content [9]. The hardness of wheat endosperm represents the main indicator of the wheat's suitability for its use for various end products and is closely related to its glassiness and protein content and structure, whereby glassy wheat can be considered to have an initial glassy grain content of $\geq 80\%$ [10]. According to the hardness of wheat grains, they are divided into hard and soft, and transitional or marbled. Transitional types of hardness occupy the largest share on the market and have determined hardness as hard, while in the industrial process, they behave like soft wheat, which is characterized by a high proportion of false or transient glassiness, i.e., that which is lost by soaking the wheat in water. These transitional forms of wheat are characterized by the so-called marbled endosperm, whose texture alternates between hard (glassy) and soft (floury) surfaces. Such wheat varieties typically belong to the II qualitative malting group. They are portrayed by increased total and soluble proteins and preferable wort viscosity) [11].

Unlike barley, there are no reference wheat varieties that would be used as standards. Narciss and Back [11], when assessing the quality of a certain variety for use in brewing, use a classification into 4 qualitative groups, of which 1 group is acceptable for brewing, and it is characterized by obtaining wort with a low proportion of soluble proteins (over 750 mg/L of soluble N in wort) and low viscosity (not over 1.6 mPa×s 8.6%e), for the so-called soft wheat varieties. Such wheat is rarely present in Europe, so for the purposes of malt production or the use of wheat as unmalted raw material, suitable hybrids are used such as red hard wheat. They are characterized by their initial indicators (hardness, vitreosity) being more similar to true red hard wheat, while their behavior during milling is more similar to soft wheat (very high transitory vitreosity). As they have a significant proportion of glassy parts on the surface of the endosperm, the aim of this research was to establish how an increase in the degree of grain granulation can affect fermentability. On one hand, an increase in the degree of milling/granulation increases the ability of the enzyme complex (amylolysis, proteolysis, and cytolysis) to act on the cells of the endosperm, which would have a positive effect, and on the other hand, more non-fermentable ingredients (especially proteins) pass into the wort, which has a negative effect on the same.

Fermentability is usually affected by many agents such as variety, genetics, and phenotype. Process parameters during malting and mashing also contribute to the fermentability. All these factors complexly interlace and affect one another [12]. Thus, it is not easy to absolutely and doubtlessly connect fermentability with any of the quality indicators. The goal of the investigation was to establish how these transitional types of endosperm will behave during the milling process (fine and coarse granulation), that is, how the mentioned differences will affect the attenuation limit or fermentability of wheat wort.

2. Materials and Methods

2.1. Sample Preparation and Analysis

In order to avoid the influence of location and agrotechnical measures on the tested wheat, all samples were obtained from the same varietal experiment from the same location belonging to the Institute of Agriculture Osijek, and under the same agrotechnical growing conditions. The grain collected from these experiments was refined and untreated, separated, packaged, and stored in a dry and dark place for 3 months in order to overcome the so-called grain dormancy. For this experiment, two transitional or marbled varieties of medium hardness with similar hardness values, permanent and transient glassiness, and different proportions of starch and total proteins, were selected for testing. These are typical bread varieties (sample 2 = Tika-Taka; sample 3 = Bezostaja). Both varieties showed a very high proportion of transient vitreosity with the highest value for the glassy surface of the endosperm in the range between 20–60%. Along with them, one hard (sample 1 = Golubica)

and one soft variety (sample 4 = Indira) were tested. Golubica is designated as a hard wheat variety, with high protein content and high hardness, while Indira is a variety with higher starch share and significantly lower hardness.

Wheat quality parameters were determined (for the vast majority of parameters) according to Analytica-EBC [13] methods: 3.4/4.4. thousand corn weight; 3.2/4.2 moisture; NIRhardness; NIR-protein content (AACC Method 39–70A) [14] and starch (ICC method 169) using Infratec 1241 Grain Analyzer (Foss, Hilleroed, Denmark).

The vitreosity of wheat is determined by the ICC visual method Standard 129 [15], which is determined using Pohl's grain cutter (Farinotom, Sadkiewicz Instruments, Byd-goszcz, Poland). After the cut had been made, a visualization and designation of gray (hard, glassy) areas of the endosperm was performed by three trained analysts. The vitreosity of the cut grains was expressed as a percentage as described by [16].

Prior to wheat mash production, wheat malt was milled to two granulations, 1 mm and 0.2 mm using an IKA (Staufen, Germany) laboratory mill (Model MF10). Granulations were separated using a vibratory Sieve Shaker with a standard series of sieves (Retch, Haan, Germany).

Wheat mash was produced using a 50:50% wheat:barley malt ratio (2 L mash of each variety), by a standard mashing program (EBC[®] method 4.5.1.) and analyzed. The following EBC[®] methods were used for parameter determination: 4.1.4.5.1.1. total N; 49.1. soluble N; 4.1.4.11. Hartong number; 4.10 α -amino N (FAN); 4.5.1. the specific gravity of mash/wort, fine extract, saccharification time, appearance of mash/wort, and filtration time; 4.8. viscosity 4.11. attenuation limit of mash/wort (AL). High molecular N (HMW N) was determined according to MEBAK[®] [17] method 2.9.3.1., medium molecular N (MMW N) was determined as the difference between HMW N and LMW N and low molecular N (LMW N) MEBAK[®] method 2.9.3.2.

2.2. Statistical Analysis

Results were subjected to analysis of variance (ANOVA) and Fisher's least significant difference test (LSD). The *p*-value was set to be significant at <0.05. Statistica 13.1. (TIBCO Software Inc., Palo Alto, CA, USA) was the software of choice for this analysis.

3. Results and Discussion

The results of the initial analysis of the tested wheat are shown in Table 1. The hardness of the endosperm is an extremely important parameter for the use of wheat as a raw material in brewing because the harder endosperm delays the rate of hydration and enzyme modification during the malting process [18]. It is observed that only sample 1 can be considered as hard wheat with a borderline vitreosity (\geq 80%). All tested wheat have a very high transition vitreosity, even in the case of, as can be seen from the results for NIR-hardness, the hardest wheat (sample 1), which, as expected, also had the highest proportion of protein amounting to 13.3%.

ID No.	Mass 1000 Grains (g)	Hardness NIR-HD	Total Vitreosity (%)	Permanent Vitreosity (%)	Transient Vitreosity (%)	Protein Content (%)	Starch (%)
1	39.9 ^c	94.1 ^a	64 ^a	30 ^a	70 ^d	13.3 ^a	68.8 ^c
2	44.9 ^a	77.2 ^b	32 ^c	0 ^c	100 ^a	10.2 ^c	71.7 ^b
3	43.6 ^b	77.0 ^b	58 ^b	10 ^b	90 ^c	12.6 ^b	68.4 ^c
4	43.6 ^b	48.0 ^c	12 ^d	2 ^c	98 ^b	9.7 ^d	72.7 ^a

Table 1. Basic raw material quality indicators used for the production of wheat wort.

^{a-d} Means within columns with different superscripts are significantly different (p < 0.05); sample 1 = Golubica (hard); sample 2 = Tika-Taka (medium hard); sample 3 = Bezostaja (medium hard); sample 4 = Indira (soft).

Similarly, as expected, soft wheat (sample 1) had the highest proportion of starch (72.7%) with the lowest proportion of protein (9.7%). In general, the results for the proportion of

starch were very high for all tested varieties. A very high proportion of high transition vitreosity indicates what was already said in the introduction, that transitional wheats will behave more like soft wheats regardless of the relatively high initial (total) hardness, which is favorable for the production of mash [19].

This is confirmed by the results shown in Table 2 displaying the relationship between permanent and transition vitreuos vitreosity ity, which is particularly pronounced for the transitional type of endosperm hardness (samples 2 and 3). Vitreosity is a property easily tested by soaking the grains for 24 h period. Truly hard wheats show little changes in the results after 24 h of soaking, while transitional wheats show large changes in vitreosity, i.e., a decrease in it. From Table 2 it is visible that designated hard wheat (sample 1) had, before soaking, the highest share of 100–80% vitreous grains, 52%, which was lowered to 26% after 24 h soaking. Transitional wheats (samples 2 and 3) showed a significant shift towards lower vitreosity (below 60%) after soaking. This indicates that they are truly transient and have more soluble proteins which can cause problems during filtration of wort and beer. Similarly, this was the case with soft wheat (sample 4), where the vitreosity shifted towards the lower end after soaking for 24 h. However, further analysis is to show whether hard, soft, and transient wheats can be utilized for brewing with regards to grain milling degree. To test this thesis grain was milled into two granulations, coarse (1 mm) and fine (0.2 mm).

Table 2. Separated values for the degree and nature of vitreosity of wheat endosperm (before and after soaking of 24 h).

ID No		Degree of Grain Vitreosity (%)							
ID NO.		100	100-80	80–60	60–40	40-20	20–0	0	
1	before soaking after soaking	12 ^b 4 ^c	52 ^a 26 ^c	20 ^a 22 ^a	12 ^d 20 ^a	4 ^f 20 ^b	0 ^f 8 ^e	0 d 0 d	
2	before soaking after soaking	18 ^a 0 ^d	14 ^d 0 ^f	6 ^{cd} 4 ^d	26 ^b 26 ^b	12 ^{cd} 32	24 ^c 38 ^b	0 d 0 d	
3	before soaking after soaking	18 ^a 2 ^c	40 ^b 8 ^e	20 ^a 8 ^c	8 ^e 30 ^a	10 ^d 32 ^a	2 ^f 14 ^d	2 ^d 6 ^c	
4	before soaking	0 d	12 ^d	14 ^b	20 ^c	16 ^c	24 ^c	14 ^b	
7	after soaking	2 ^c	0 ^f	8 c	8 e	8 e	52 ^a	22 ^a	

^{a–f} Means within columns with different superscripts are significantly different (p < 0.05); sample 1 = Golubica (hard); sample 2 = Tika-Taka (medium hard); sample 3 = Bezostaja (medium hard); sample 4 = Indira (soft).

Reference or target values when it comes to wheat as a raw material do not actually exist, but general requirements are set for the weight of 1000 grains as high as possible, hardness as low as possible, glassiness as low as possible, and of a transient character as low as possible, proteins as low as possible. The low share of proteins simultaneously increases the proportion of starch because it is about the indicators that are in the so-called formal correlation (together they add up to 100) so decreasing one increases the other. Reference values in terms of target values or certain limits for the values of individual indicators exist when it comes to wheat malt and we often use them when presenting the results for the quality of wheat malt in our works; we have attached one such table with the specified values below. However, in this paper, we used unmalted wheat as a raw material for obtaining wheat wort and could not use those values for wheat malt for comparison with the values for wort obtained in this paper.

The results in Table 3 show the influence of the type of endosperm texture and differences in milling degree on the main quality indicators of wheat wort. When considering the influence of the type of endosperm texture as the main indicator in fine granulation samples, a very clear and significant difference was observed between hard and soft wheat, i.e., the corresponding wort. The main indicators of proteolysis are the Kolbach index, soluble proteins, and FAN values [19,20]. Hard wheat has significantly higher values for soluble protein and FAN, but also for HM N and MM N, with a significantly lower filtration time and wort viscosity. All tested kinds of wheat have very good values for soluble protein for both fine and coarse granulation.

Comula No	Granulations								Barley
Sample No.	1		2		3		4		Malt
Parameters	Fine 0.2 mm	Coarse 1 mm	Fine 0.2 mm	Coarse 1 mm	Fine 0.2 mm	Coarse 1 mm	Fine 0.2 mm	Coarse 1 mm	
Moisture (grain) (%)	10.4 ^a	10.0 ^{bc}	9.9 ^c	10.2 ^{abc}	10.2 abc	10.1 ^{bc}	10.3 ^{ab}	10.2 abc	3.9
Extract (grain) (%)	71.3 ^e	71.9 ^{cd}	74.6 ^a	74.8 ^a	70.3 ^f	72.1 ^c	72.7 ^b	71.9 ^d	79.4
Total N (mg/100 mL)	59.5 ^b	55.0 ^d	53.5 ^f	56.0 ^c	59.4 ^b	61.6 ^a	53.1 ^g	54.2 ^e	75.4
HM N (mg/100 mL)	24.8 ^{ab}	24.1 ^c	20.8 ^e	22.4 ^d	24.5 ^{bc}	25.2 ^a	19.2 ^g	20.4 ^f	21.1
MM N (mg/100 mL)	5.2 ^d	2.1 ^g	2.6 ^f	4.8 ^e	5.7 ^b	6.9 ^a	5.6 ^{bc}	5.4 ^{cd}	6.7
LM N (mg/100 mL)	29.5 ^b	28.7 ^d	30.1 ^a	28.8 ^d	29.2 ^c	29.5 ^b	28.4 ^e	28.4 ^e	47.6
FAN α -amino N (mg/L)	76.8 ^d	78.3 ^b	66.2 ^g	84.3 ^a	68.7 ^e	77.5 ^c	66.6 ^f	77.4 ^c	133.8
Proteins (%)	12.7 ^a	12.5 ^b	10.1 ^d	10.0 ^d	11.9 ^c	12.0 ^c	9.8 ^d	9.6 ^e	9.8
Soluble N (mg/L)	595 ^b	550 ^d	535 ^f	560 ^c	594 ^b	616 ^a	531 ^g	542 ^e	754
Soluble N (%dm)	0.57 ^a	0.52 ^{bc}	0.51 ^c	0.54 ^b	0.57 ^a	0.59 ^a	0.51 ^c	0.52 ^c	0.67
Kolbach index (%)	25.2 ^f	23.7 ^g	28.4 ^c	30.4 ^a	26.8 ^e	27.6 ^d	29.4 ^b	30.5 ^a	41.0
Hartong 45° (%)	29.6 ^b	30.1 ^a	29.6 ^b	29.5 ^{bc}	29.2 ^c	28.7 ^d	28.2 ^e	28.8 ^d	40.8
Viscosity (mPa×s)	1.426 ^{bc}	1.430 ^b	1.451 ^a	1.451 ^a	1.401 ^d	1.417 ^c	1.423 ^{bc}	1.401 ^d	1.478
Specif. gravity (g/mL)	1.0321 ^{cd}	1.0323 ^{bc}	1.0329 ^a	1.0329 ^a	1.0319 ^d	1.0323 ^{bc}	1.0324 ^b	1.0322 ^{bc}	1.0340
Attenuation limit (%)	86.3 ^d	82.4 ^g	93.3 ^a	82.9 ^f	88.9 ^c	78.7 ^h	92.9 ^b	84.1 ^e	86.0
Extract (%)	75.67 ^e	75.92 ^d	77.27 ^b	77.40 ^a	75.17 ^f	76.03 ^d	76.34 ^c	75.94 ^d	79.41
Sacchar. time (min)	20	20	25	25	20	20	25	25	10
Filtration time (min)	35	20	30	25	25	20	45	25	70
Appearance of wort	clear	clear	clear	clear	clear	slightly opales- cent	slightly opales- cent	slightly opales- cent	slightly opales- cent

Table 3. Results of the quality indicators of wheat wort for different types of hardness and granulations.

^{a–g} Means within columns with different superscripts are significantly different (p < 0.05); dm—dry matter; N—nitrogen; HMW N—high molecular weight N; MMW N—medium molecular weight; LMW N—low molecular weight N; sample 1 = Golubica (hard); sample 2 = Tika-Taka (medium hard); sample 3 = Bezostaja (medium hard); sample 4 = Indira (soft).

For the attenuation limit (AL), a very clear difference between fine and coarse granulation was established, in such a way that fine granulation has a significantly higher AL compared to coarse in all types of wheat except soft, where this difference for AL is the smallest. The values for AL were very good for all tested types of wheat with the expected lowest value for hard wheat (coarse granulation, 82.4%), but it is interesting that both transition wheats have values for AL similar to soft wheat (both granulations). In the case of transitional types (samples 2 and 3), the only significant difference between these wheats is in the proportion of total proteins (Table 1) where sample 2 had 10.2%, and sample 3 had 12.6% of proteins.

The distribution of the glassiness of the endosperm surface after soaking (Table 2) is also very similar, with the final vitreosity being entirely transient. Compared to hard wheat, both worts from transitional types of wheat had a significantly lower proportion of protein and soluble N and FAN (Table 3). Sample 3 had the highest content of soluble N (616 mg/L) in coarse granulation, while FAN content was highest in sample 2 (84.3 mg/L for coarse granulation). In sample 2, which had the highest extract in the grain (74.8% in coarse granulation) and fine extract (77.27% in fine granulation) in the wort, a value obtained for AL was (higher than in soft wheat), together with values for total soluble proteins, HMW N, MMW N LMW N, and FAN, similar or even better in compared to soft wheat.

In this investigation, significant differences between fine and coarse granulation were found in all types of wheat. When it comes to the difference between fine and coarse milling granulation, Einsiedler et al. [21–23] found that an increase in milling degree results in an increase in the contact surface available for enzyme action, as a result of which,

during isothermal mashing, the release of amino acids in mash occurs much faster and more complete, even in wheat with lower proteolytic power. He also established that when it comes to amylolysis, the increase in the milling degree has very little effect on the faster release of low molecular sugars, because the availability of places for the enzymatic reaction does not increase with the increase in the milling degree, while the activity of α -and β -amylase is halved in poorly modified endosperm.

When it comes to cytolysis, the authors concluded that the fineness of the granulation does not significantly affect starch degradation, but it does significantly affect the breakdown (transfer into mash) of β -glucan and protein degradation. Schneider [24] investigated the influence of the milling degree on the mashing process and found that the increase in the milling degree leads to an increase in the breakdown of high-molecular protein fractions, which consequently leads to an increase in the concentration of their degradation products in wort, with the largest increase of FAN concentration already at the very beginning of the mashing process. When using wheat as unmalted raw material (16% substitution for malt), it was found that the concentrations of soluble proteins, HMW, MWM, and LWM are similar to those of the control worts produced from barley malt, and the concentrations of formol N and FAN are even lower [25], while in the case of using wheat malt, the degree of grain modification (as a consequence of the applied malting procedure) has a very significant influence on their concentration in the wort [26].

Kühbeck et al. [24] examined the influence of different milling procedures (upward/ isothermal) and preparation procedures (milling procedure, grits modification, grits: liquor ratio), from where indicators of cytolysis, proteolysis, and amylolysis were measured β -glucan, FAN and extract. He concluded that malt modification is the most important factor responsible for the breakdown of β -glucan and the release of FAN, while starch modification is responsible for extract yield, and for milling degree, he did not find that it significantly affects the mentioned indicators except in the case of poorly modified malt. From the results in (Table 3), it can be seen that in hard wheat (sample 1), the concentration of total N, medium molecular weight N (MM N), and soluble N increases significantly with the increase of milling degree, and with a significant increase of filtration time. For the attenuation limit (AL), a very clear difference between fine and coarse granulation was established, in such a way that fine granulation has a significantly higher AL compared to coarse in all types of wheat, except for soft, where this difference for AL is the smallest.

As for the main indicators of protein breakdown (Kolbach index, soluble proteins, and FAN), no significant difference was found between fine and coarse milling, nor for extract, viscosity, and Hartong 45°, so this significant increase in AL can be attributed to the increase in milling degree. The same trend of a significant increase in AL is also observed for transitional wheats (samples 2 and 3), although with them, with an increase in the milling degree, a significant decrease in MM N and FAN and a slightly less pronounced increase in the filtration time were observed. Compared to barley, wheat has a lower proportion of β -glucan and a higher arabinoxylan content, which should result in higher mash viscosity and reduced filtration volume [27,28]. As a reason for increasing the filtration time and in the case of very good values for the viscosity of wheat wort authors [25] state the possibility of forming a protein or protein-polysaccharide gel on the filter, which was also observed when unmalted wheat was used as a substitute for part of the malt in the infusion [25].

When the results for viscosity and Hartong number are observed, the already mentioned negative influence of the "forcing maturation" effect caused by the climate, characterized by high temperatures and high humidity towards the end of the growing season, results in a lower enzymatic strength compared to northern European wheats. This effect could be responsible for the lower proportion of soluble in total pentosans also compared to Northern European wheats [29], which also affects the viscosity of wort. In the case of soft wheat (sample 4), AL is also significantly higher for fine granulation with a very high, practically unacceptable, filtration time and a significant increase in viscosity for the same. The best values for AL, but also almost all tested parameters, were shown by sample no. 2 (Tika-Taka) with fine grit granulation (ø 0.2 mm). It can be concluded that the transitional type of wheat, which meets the criteria of classification into 2 qualitative groups, according to Narziss [11], can be just as good unmalted raw material for the production of wort, and raw material for malting, as soft wheat.

4. Conclusions

From the results for the tested transition wheats, it is evident that, despite similar starting values for the most important quality parameters, they behave very differently when it comes to mash obtained from them, both among themselves and in comparison with hard and soft wheat, and also when observing the results for different milling degree. The obtained values for the attenuation limit for transition wheats (fine grind—sample 2, 93.3% and sample 3, 88.9%) are similar or better when compared to soft wheats (92.9% for fine grist), with satisfactory values for almost all examined quality parameters of wort. It can be concluded that transitional type wheat can be utilized, as unmalted and even malted raw material for the production of wort, as soft wheat. Further research should be focused on obtaining hybrid, winter varieties that would display values for primarily proteolytic quality indicators as similar as possible to soft wheat. For breweries, this could mean an easier way to obtain wheat beer since the raw material would be suitable for brewing.

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