

Article

The Influence of Milling Granulation on The Proportion of Fermentable and Non-Fermentable Ingredients in Mash Obtained from Mixed Endosperm Wheat

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Abstract: The use of purpose-selected wheat in brewing is limited because it is either not suitable for the application of usual agrotechnical measures or, more often, is not adapted to specific climatic conditions. As unmalted wheat in brewing can cause process problems and problems with the quality composition of mash/wort and consequently beer, by using the appropriate type and variety, but also by adjusting the malting process, these problems can be completely avoided or significantly reduced at the start. Two varieties were selected for the research, which in previous research showed good brewing/malting properties and which have significantly different starting values for the share of total protein (TP) in the grain. The goal of this research was to determine the effect of grain milling granulation on the quality of mash obtained from a hard type of wheat, i.e., the changes in the fermentable and non-fermentable extract ratio during mashing. Varieties with higher and lower initial contents of total protein (TP) in the grain were tested. It was found that regardless of the initial difference in TP concentration, the concentrations of total soluble proteins (TSP) and high molecular protein fraction (HMW N) are similar for varieties with higher and lower initial TP and that the difference in initial TP concentrations is shown as a difference in the concentration of insoluble protein fractions, which are eliminated during fermentation. By increasing the milling granulation in both kinds of wheat, there is a significant increase in the medium molecular protein fraction (MMW N), which is more pronounced in wheat with a lower starting concentration of TP, even though the starting concentrations of TSP in fine ($\varnothing$ 0.2 mm) and the superfine milling ($\varnothing$ < 0.2 mm) were similar. A significant drop in fermentability at the superfine granulation of the grain was noted.

Keywords: wheat; wheat proteins; grist milling degree; mash fermentability



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1. Introduction

Wheat was utilized for the production of beer perhaps longer than barley grain, either as unmalted raw material or as malt, and, due to the emergence of new types of beer (especially craft), its use has even increased [1] because its use has both economic and sensory advantages [2]. As a raw material, wheat has both advantages and disadvantages compared to barley, with the biggest advantages being significantly more extract (2–6%), higher fermentability (up to 5%), and a significantly lower proportion of β -glucan (–20 and more %). The main disadvantages are that wheat and wheat malt burden the wort with an increased content of protein (+0.5–2.5%), a longer saccharification time (+2–15 min), an increased content of high molecular weight (HMW N) and medium molecular weight (MMW N), and, in certain cases, by an increase in the viscosity of the wort (+0.1–1.5 mPa·s) and a lower proportion of α -amino N (FAN) [3,4]. Wheat beer has more unique aromatic compounds compared to lager beer (4-vinyl guaiacol) for the phenolic aroma, i.e., isoamyl acetate and ethyl acetate for the ester aroma [3]. Furthermore, the main process problems

caused by wheat are the additional burden of mash and wort HMW N protein fraction and difficult filtration of mash due to the formation of residual protein gel and/or protein-pentosan gel [5–7] and a negative impact on colloidal stability, which indirectly affects the minimum shelf life due to the increased presence of so-called “haze active” proteins, mostly from gliadin fractions of wheat proteins [8,9]. Groups of macromolecules that can cause process problems when using wheat are, in addition to proteins and arabinoxylans (pentosans), dextrans, which, according to some authors, have the greatest effect on the increase in viscosity, especially if they are prone to cyclization [10–12].

Wheat, when used in brewing, is divided into four qualitative groups based on the TSP and mash viscosity values. The first group is the best; these are the varieties that give a low proportion of soluble proteins (0.65–0.78 g/100 g malt) with a good value for wort viscosity (<1.65 mPa·s) [13], although it should be noted that different authors claim different values. The second group consists of varieties that give an increased proportion of soluble proteins with a satisfactory viscosity, the third group consists of varieties that have an increased viscosity and a satisfactory proportion of soluble proteins, and the fourth group consists of varieties that have unsatisfactory values for both indicators and are not suitable for the use in brewing.

Wheat used in brewing usually has a TP between 10 and 13%. The protein complex of wheat is called gluten and is divided into four categories: glutenin, gliadin, albumin, and globulin. Gluten is a complex mixture of 50–100 individual proteins and is usually divided into two groups: polymorphic gliadins (α -type gliadins, γ -gliadins, and ω -gliadins with a molecular weight range of 30–80 kDa) soluble in 70% ethanol and polymeric glutenin (HMW N and LMW N fraction with the molecular weight range of 75–120 kDa and 30–74 kDa). Glutenins and gliadins are recognized as the major wheat storage proteins, constituting about 60–85% of the total grain proteins [14,15]. Generally, the TP share in wheat kernels is not much higher than in barley but it has a higher amount of HMW N proteins whose breakdown products end up in mash and indirectly in beer and have a positive and negative impact on its quality. The positive influence is reflected primarily in the taste and stability of the beer foam [16], while the negative influence primarily refers to the colloidal stability of the beer, where in addition to the molecular weight of the protein (MW), the nature of the protein, i.e., its amino acid composition (especially “haze active” gliadin MMW N fractions rich in proline and hydroxyproline that easily form complexes with polyphenols in mash and beer) [17–19]. Even when, due to the combination of raw materials in the infusion of barley malt (50%):unmalted wheat (18%):corn grist (32%), the concentrations of TSP and their HMW N and MMW N fractions are equal or even lower in the beer produced by the partial substitution of barley malt with unmalted wheat, those beers have significantly lower colloidal stability and minimal self-life compared to beers produced from barley malt [20]. The positive effect of HMW N protein and its degradation fractions is crucial for the formation of stable foam, where both high-molecular (HMW N) and low-molecular (LMW N) proteins are important, including lipid transport proteins LTP1 (a protein that survives mashing but becomes foam active when denatured in the boil), hordeins, glutelins, and albumins (protein Z). Some proteins in the beer have a hydrophobic end that is attracted to the gas and a hydrophilic end that is attracted to the liquid surrounding the gas. These proteins align with each other to form a bubble. Proteins with lower surface hydrophobicity promote beer foam stability more, especially protein Z [21,22].

The second group of macromolecules that influence the brewing quality of wheat consists of arabinoxylans AXs (pentosans), along with β -glucans, the most important non-starch polysaccharide of wheat, which make up 6.5–12.2% of the dry matter of the grain, the largest part being in wheat bran [5,23]. They affect milling performance and starch-gluten separation. According to solubility, they are divided into two categories: water-soluble AXs and water-insoluble AXs. The content of water-soluble AXs in grains is generally 1.5–3.0%, AXs in mash and beer originate mainly from the water-soluble fraction [5]. The solubility of AXs depends on the backbone substitution patterns as well as the enzymatic degradability

of the AX polymers [23]. When considering water-solubility, AXs with extended regions of unsubstituted xylose residues are inclined to form aggregates and are therefore more likely to be water-unextractable AXs. Arabinoxylans with greater backbone substitution density are more likely to be water-extractable AXs. Furthermore, AXs prolong the filtration of wheat starch hydrolysis products [24]. Recently, some studies have reported that AXs can affect the amylase in starch granules, which obstructs the filtration of wort and beer [23]. During the membrane filtration operation of draft beer, it can cause membrane blockage [25] and turbidity of finished beer [9].

When wheat is used as an unmalted raw material, it must be combined with barley malt in the mixture (ratio 50:50) and the malting process itself is aided by enzymes from barley malt. A prerequisite for successful mashing is milling or mechanical preparation of the grain for the availability of the endosperm of the grain to the action of enzymes. During this process, all macromolecules (proteins, starch, dextrins, and non-starch polysaccharides arabinoxylans) go through mechanical and physical–chemical changes (cytolysis, proteolysis, and amylolysis), whereby the mechanical (milling) is the first key step because it affects all later stages in production, (sugar reduction, speed of wort separation, etc.), and thus also the quality of beer as a final product [26]. Despite the importance of the influence of the milling fineness on mashing and further production processes, there are few papers on this topic.

Research on the influence of milling granulation on the quality of mash, wort, and beer have been carried out but they were primarily focused on the examination of mashing at different temperature regimes and monitoring the change in the solubility of grain hemicelluloses (Erdal and Gjertsen) [27], where they reported that during mashing at lower temperatures, hemicelluloses do not come from water-soluble fractions in the malt but they are a result of enzymatic decomposition during mashing as well as the relationship between the quality of malt and the intensity of mashing to the maximum breakdown of protein and hemicellulose compounds (Narziß and Litzenburger) [28]. Einsiedler et al. developed a kinetic model of the mashing process in relation to the fineness of the milled grain, i.e., the increase in the enzyme–substrate contact surface. They concluded that an increase in the contact surface with an increase in milling fineness increases the degree of proteolysis, which leads to a significant increase in α -amino N (FAN), where the optimum for proteolysis is 47 °C and the concentration of FAN does not increase significantly [29]. They also concluded that the increase in grist fineness has a minor role in amylolysis and does not lead to a faster release of low molecular sugars because increasing the milling fineness does not increase the number of enzymatic reaction sites [30]. When it comes to cytolysis, they found that the β -glucan content of the fine grist was 1.5- to 3-fold compared to the coarse grist [31]. The conclusion of the research by Einsiedler et al. was that grain fineness did not make a difference in terms of starch degradation but it did in terms of β -glucan and protein degradation. More β -glucan and FAN were obtained by grinding the finer ground in the first 10–20 min due to the greater number of available reaction sites. Although enhanced proteolysis might be desirable to increase the concentration of amino acids (FAN), the use of finer granulation does not make it the optimal measure to achieve a shortening of the mashing process. Schneider investigated the influence of the milling fineness on the mashing process. The finer the mill, the higher the rate of decomposition of high-molecular substances, which was especially true for the decomposition of proteins. When fine milling was used, concentrations of FAN were high already at the beginning of mashing. This value was reached only at the end of mashing for less fine granulation. The extracted content was initially higher in fine milling but the difference between fine and coarse granulation decreased over time. Therefore, he concluded that finer grinding primarily shortens the mashing time but does not increase the yield of the fine extract. Furthermore, the increase in the grist:liquor ratio was limited only by α -amylase activity. However, when using a finer granulation, mass exchange was increased so that almost no inhibition of amylolysis could be observed even with a grist:liquor ratio of 1:2.5.

As for β -glucan, essentially no difference can be observed if different degrees of granulations are used when a grist:liquid ratio of 1:3.5–4 was applied [32]. Kühbeck et al. found that malt modification had a major impact on the β -glucan release in contrast to a variation in the milling procedure and of the grist:liquor ratio. Prolongation of cytolysis led to an increase in the final values only for the slightly modified malt. Similarly, FAN release was predetermined by malt modification, while variation in milling and the grist:liquid ratio was not relevant in contrast to prolonging the proteolytic pause. None of the applied variations affected the extract yield until the gelatinization temperature was reached [26].

In this work, we wanted to establish how increasing the degree of granulation of grist affects the quality of mash produced from unmalted wheat and barley malt, i.e., how increasing the contact surface by increasing the fineness of milling affects the ratio of fermentable and non-fermentable ingredients in the fine extract of mash and thus the success of the overall process of preparing mash for application of unmalted wheat in brewing.

2. Materials and Methods

2.1. Samples

Wheat (*Triticum aestivum* L.), cultivar Ana and Žitarka (Agricultural Institute Osijek, Osijek, Croatia), harvested in June 2023 was used for this study. They are typical wheat varieties (hard, red, and winter varieties) that have a relatively high hardness and a high degree of transient vitreosity of the grain and which, during processing, show some typical characteristics of soft wheat varieties and have proven to be suitable for the production of light wheat malt [33–35]. The crop was harvested and stored in a dry and cold place for a month in order to overcome the post-harvest dormancy of the grain. The grain collected (10 kg) was treated as described in [35]. Sampling was performed on cleaned and processed wheat grains (according to the EBC 3.3.1. method) and the samples were packed into double-walled paper bags and kept in dry conditions.

2.2. Mash Preparation and Analysis

The mash was prepared in the ratio of unmalted wheat:barley malt 50:50% and was subjected to mashing according to the standard MEBAK procedure (R-206.00.002 Congress Mash Method) [36]. Since the same barley malt of fine granulation (EBC-standard malt) was used during the preparation of the mixture, the differences that appear in the values for the specified protein fractions in the obtained mash are the result of the particular tested variety and the particular degree of wheat milling/granulation.

Analyses of wheat and barley malt were performed according to the approved methods described by MEBAK [36] and EBC [37]. Wheat quality parameters were determined [38,39] as described in [35]. Total and soluble pentosans (arabinoxylans) were analyzed as described by [40]. This method also provides information on the total/soluble ratio of pentosans, which is an important indicator of their susceptibility to dissolution and degradation by the cytolytic enzyme complex. Listed methods (Table 1) are determined according to [41].

The vitreosity of wheat is determined by the ICC visual method Standard 129 [39], which is determined using Pohl's grain cutter (Farinotom, Sadkiewicz Instruments, Bydgoszcz, Poland), which is modified in a way to avoid false marbling of the grain. After the cut had been made, a visualization and designation of gray (hard and glassy) areas of the endosperm was performed by three trained analysts [42]. The vitreosity of the cut grains was expressed as a percentage as described by [43]. Prior to wheat mash production, wheat grain was milled into three granulations, $\varnothing = 1$ mm, $\varnothing = 0.2$ mm, and $\varnothing < 0.2$ mm, using an IKA (IKA, Staufen, Germany) laboratory mill (Model MF10); separation was performed as described by [42].

Two L of wheat mash was produced mash of each variety by a standard MEBAK procedure and analyzed as described by [35].

Table 1. List of used methods.

Indicator	Unit	MEBAK®	EBC®
Moisture (grain)	%		3.2/4.2
Extract (grain)	%	3.1.4.2.2	
Proteins	%	1.5.2.1	
Soluble N	mg/L		4.3.1
Total N	%dm	4.1.4.5.1.1	
Kolbach index	%	3.1.4.5.3	
Total N	mg/100 mL	3.1.4.5.1.1	
HM N	mg/100 mL	2.9.3.1	
MM N	mg/100 mL	2.9.3.12.9.3.2	
LM N	mg/100 mL	2.9.3.2	
α -amino N	mg/L		4.10
Spec, gravity	g/mL		8.2.2
Final attenuation	%		4.11
Fine extract	%		4.5.1
Saccharification time	min	4.1.4.2.4	
Filtration time	min	R-205.04.730	
Viscosity	mPa s	4.1.4.4.2	
Hartong VZ45°	%	4.1.4.11	

2.3. Statistical Analysis

Results were subjected to analysis of variance (ANOVA) and Fisher's least significant difference test (LSD). The p -value was designated to be significant at $p < 0.05$. Analysis was conducted using Statistics 13.1 (TIBCO Software Inc., Palo Alto, CA, USA).

3. Results and Discussion

3.1. Quality of Wheat

The results of determining the initial quality of wheat are shown in Tables 2 and 3. When considering the initial quality of used wheat (Table 2), it should be emphasized that the selected kinds of wheat (varieties/lines) have significantly different initial shares of total protein TP (boldly marked in Table 2). They were chosen because they proved to be a good raw material basis for the production of light wheat malt, with both belonging to the second qualitative group according to Narziss [13] and resulting in malts with the best properties under application of a moderately restrictive malting process (slight increase in temperature from 14.5 °C according to 18.5 °C on the last day of germination with air humidity adjusted to 43%) [33,34].

The initial TP, its solubility and susceptibility to enzymatic degradation, and the distribution of their fractions (HMW N, MMW N, and LMW N) are of crucial importance for the quality of certain types and varieties of wheat for use in brewing [44] and are especially considered for the quality of the obtained mash.

If we consider the results for the hardness and vitreousity of the endosperm, it is noticeable that both varieties can be considered medium hard and medium vitreous varieties. Most authors consider a variety as hard if it has $\geq 85\%$ of vitreous grains [44,45]. The hardness of the endosperm is essentially related to its vitreousity, which was almost entirely of a transitory nature, both for wheat with a higher and lower TP content, regardless of the significant initial difference (Table 2). These values are good from the brewing point of view because a hard endosperm disables water absorption and grain modification caused

by malting and leads to lower results of mash quality indicators when using wheat as an unmalted raw material [3].

Table 2. Basic raw material quality parameters used for the production of wheat mash.

No.	Mass 1000 Grains (g)	Hardness NIR-HD	Total Vitreous (%)	Permanent Vitreous (%)	Transient Vitreous (%)	Protein Content (%)	Starch (%)	Total Pentosans (%)	Soluble Pentosans (%)
1	44.1 ^a ± 0.10	78.0 ^b ± 0.12	34 ^b ± 0.5	6 ^b ± 0.25	94 ^a ± 0.31	10.4 ^b ± 0.09	71.7 ^a ± 0.12	6.43 ^b	0.67 ^a ± 0.05
2	43.5 ^b ± 0.15	78.7 ^a ± 0.21	56 ^a ± 0.31	12 ^a ± 0.11	88 ^b ± 0.42	12.7 ^a ± 0.10	68.4 ^b ± 0.28	7.22 ^a ± 0.17	0.59 ^b ± 0.10

^{a,b} Means ± SD within columns with different superscripts are significantly different ($p < 0.05$); 1 = Ana; 2 = Žitarka.

Table 3. Separated values for the degree and nature of vitreosity of wheat endosperm (before and after soaking of 24 h).

No.		Degree of Grain Endosperm Surface Vitreosity (%)						
		100	100–80	80–60	60–40	40–20	20–0	0
1	Before soaking	20 ^a ± 0.50	16 ^b ± 0.50	4 ^d ± 0.50	27 ^a ± 1.00	10 ^b ± 0.50	22 ^b ± 1.50	1 ^c ± 0.50
	After soaking	0 ^d ± 0.00	0 ^d ± 0.50	6 ^c ± 0.50	21 ^b ± 1.50	30 ^a ± 1.50	33 ^a ± 1.00	10 ^a ± 0.50
2	Before soaking	16 ± 0.5 ^b	42 ^a ± 1.00	24 ^a ± 1.5	10 ^c ± 0.50	6 ^c ± 0.50	2 ^d ± 0.50	0 ^c ± 0.00
	After soaking	2 ^c ± 0.50	10 ^c ± 1.00	8 ^b ± 0.50	28 ^a ± 1.50	31 ^a ± 1.00	15 ^c ± 1.00	6 ^b ± 0.50

^{a–d} Means ± SD within columns with different superscripts are significantly different ($p < 0.05$); 1 = Ana; 2 = Žitarka.

Along with proteins, arabinoxylans from wheat are another component that can cause process problems in case of excessive concentrations. Wheat arabinoxylans mainly originate from the endosperm cell wall and their share in it as well as the relationship is a strongly varietal-determined property. Namely, numerous works state that arabinoxylans from wheat lead to higher viscosity of mash and reduced filtration volume [5]. The increased filtration time, even if the values for the viscosity of wheat wort are satisfactory, is probably caused by the formation of a protein or protein–polysaccharide gel on the filter. This was also noted when using unmalted wheat as a partial substitute in the grist [5,20]. From (Table 2), it can be seen that the share of total and soluble pentosans is at the lower limit for this type of wheat, while the share of soluble in total AXs (soluble/total AXs ratio) is lower than expected (9.3 for cultivar 1 and; 8.2 for cultivar 2). A lower proportion of soluble AXs in total AXs is also characteristic of southern European kinds of wheat [46].

Considering the results for permanent and transient vitreosity (Table 2), a high degree of transient vitreosity is noted. Table 3 shows the results of the share of vitreous and starchy zones of the endosperm in its total surface before and after soaking and shows a similar distribution of the results but different behavior in terms of retaining the number of vitreous grains during soaking, regardless of the very similar initial grain hardness for both cultivars. This indicates that the nature of the vitreosity, not only its share in the surface of the endosperm, is important for the amount of water that the grain can absorb.

3.2. Quality of Mash

The results obtained in this research (Table 4) show that the extract (grain) is expectedly lower in wheat compared to barley malt. Interestingly, the lowest extract was found in the superfine grinding in wheat with a lower proportion of total proteins. In wheat with a significantly higher proportion of total proteins in the superfine fraction, the extract and total N were significantly higher than in the fine, which indicates that by reducing the granulation of the grist, more protein fractions are transferred in the mash, which consequently leads to a drop in the Kolbach index, i.e., that the degree of their solubility increases.

Table 4. Results of the quality indicators of wheat wort for different endosperm granulations.

Granulation		Superfine <0.2 mm		Fine 0.2 mm		Coarse 1 mm		Barley Malt
Parameter	Samples/Unit	1	2	1	2	1	2	
Moisture (grain)	%	10.11 ^a ± 0.10	10.10 ^a ± 0.08	10.00 ^a ± 0.10	10.10 ^a ± 0.12	10.10 ^a ± 0.10	10.20 ^a ± 0.10	4.0
Extract (grain)	%	71.20 ^d ± 0.22	74.11 ^{ab} ± 0.34	73.91 ^b ± 0.48	70.75 ^e ± 0.22	74.41 ^a ± 0.21	72.50 ^c ± 0.58	79.2
Proteins	%	10.41 ^c ± 0.10	12.72 ^a ± 0.10	10.00 ^d ± 0.10	11.92 ^b ± 0.12	10.22 ^d ± 0.18	12.05 ^b ± 0.10	9.9
Soluble N	mg/L	542 ^e ± 2.50	590 ^b ± 2.50	541 ^f ± 1.80	584 ^c ± 3.1	575 ^d ± 1.70	620 ^a ± 2.90	760
Soluble N	%dm	0.54 ^{bc} ± 0.10	0.57 ^b ± 0.11	0.52 ^c ± 0.09	0.55 ^{bc} ± 0.08	0.56 ^b ± 0.10	0.60 ^a ± 0.11	0.66
Kolbach index	%	27.60 ^c ± 0.27	24.6 ^e ± 0.41	29.00 ^b ± 0.22	26.8 ^d ± 0.78	31.2 ^a ± 0.19	27.6 ^c ± 0.32	41.6
Total N	mg/100 mL	54.20 ^d ± 0.91	59.0 ^b ± 0.66	54.10 ^d ± 0.25	58.4 ^b ± 0.33	57.51 ^c ± 0.49	62.2 ^a ± 0.18	76.0
HM N	mg/100 mL	20.92 ^f ± 0.16	23.63 ^c ± 0.27	21.86 ^e ± 0.55	24.41 ^b ± 0.33	22.47 ^d ± 0.57	25.33 ^a ± 0.61	20.9
MM N	mg/100 mL	4.92 ^e ± 0.18	7.13 ^b ± 0.18	2.16 ^f ± 0.32	5.15 ^d ± 0.16	5.87 ^c ± 0.22	7.51 ^a ± 0.43	7.0
LM N	mg/100 mL	28.43 ^d ± 0.39	28.3 ^d ± 0.22	30.21 ^a ± 0.33	28.91 ^c ± 0.44	29.33 ^b ± 0.27	29.41 ^b ± 0.15	48.1
α-amino N	mg/L	64.13 ^f ± 0.39	65.72 ^e ± 0.55	66.32 ^d ± 0.41	67.88 ^c ± 0.57	85.15 ^a ± 0.42	76.19 ^b ± 0.24	132.8
Spec, gravity	g/mL	1.0327 ^{ab} ± 0.10	1.0321 ^{ab} ± 0.10	1.0329 ^b ± 0.11	1.0319 ^{ab} ± 0.10	1.0330 ^a ± 0.12	1.0323 ^{ab} ± 0.10	1.0340
Final attenuation	%	83.21 ^d ± 0.54	88.42 ^c ± 0.33	94.01 ^a ± 0.67	88.94 ^b ± 0.71	83.11 ^d ± 0.22	78.75 ^e ± 0.41	85.8
Fine extract	%	77.40 ^a ± 0.38	75.65 ^d ± 0.49	77.31 ^b ± 0.27	75.17 ^e ± 0.41	77.48 ^a ± 0.37	76.03 ^c ± 0.62	79.34
Saccharification time	min	20 ^b ± 0.50	20 ^b ± 0.50	25 ^a ± 1.00	20 ^b ± 0.50	25 ^a ± 0.50	20 ^b ± 0.50	10
Lautering time	min	25 ^a ± 1.00	25 ^a ± 0.50	25 ^a ± 0.50	25 ^a ± 0.50	25 ^a ± 0.50	20 ^b ± 1.00	65
Viscosity	mPa s	1.453 ^a ± 0.15	1.407 ^c ± 0.20	1.451 ^a ± 0.18	1.402 ^d ± 0.12	1.451 ^a ± 0.10	1.415 ^b ± 0.15	1.478
Hartong VZ45°	%	31.22 ^a ± 0.50	30.13 ^c ± 0.23	29.75 ^d ± 0.36	29.23 ^e ± 0.39	30.41 ^b ± 0.15	28.77 ^f ± 0.42	40.8
Appearance of the wort		slight opalescent	slight opalescent	clear	clear	clear	slight opalescent	slightly opalescent

^{a–f} Means ± SD within rows 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 N; LMW N—low molecular weight 1 = Ana; 2 = Žitarka.

At the same time, with this wheat, the proportion of fine extract in the finest and in the fine granulation did not differ significantly, nor did the final attenuation limit. The obtained results regarding the fine extract in the mash and the final attenuation limit show that, with relatively similar values for the fine mash extract (even higher), there is a significant drop in the final attenuation limit in the superfine milling for wheat with a lower initial share of total proteins, while for wheat with a higher starting proportion of total proteins, similar values were obtained for the extract of mash and the final attenuation limit in the case of the superfine and fine granulations. This agrees with the findings of Einnsiedler et al. who reported that an increase in milling fineness has a minor effect on amylolytic activity (it does not increase it) [30].

As similar values for viscosity were obtained for the fine and superfine milling and did not represent a problem from the point of view of their absolute values, it can be assumed that if the starting share of total AXs (pentosans) is low with a low share of soluble in total AXs (which is, as already mentioned, together with a lower enzymatic strength, a characteristics of southern European wheat). Numerous authors believe that there is a positive correlation between the proportion of soluble AXs in mash and beer and the viscosity and rate of membrane filtration. Recently, there have been publications in which no correlation between AXs (total and soluble) was found but a positive correlation was established with the reduction in filtration rate. This is not, however, related to viscosity [27,46,47]. The results obtained in this work showed that increasing the milling fineness did not lead to an increase in viscosity.

The results for FAN and indicators of protein degradation (HMW N, MMW N, and LMWN) show that increasing the milling fineness does not lead to an increase in FAN but it is slightly lower in the superfine fraction (Table 4), which is in accordance with the

findings of Kuhbeek who believes that the release of FAN during mashing is predominantly determined by the initial FAN value in the grain and the degree of grain modification and that the degree of grain granulation and the grist:liquor ratio play a small role [26]. On the other hand, the indicator of proteolytic degradation is related to the increase in milling finesse. This agrees with the results obtained by Einnsiedler et al. [29] who found that the degree of protein hydrolysis increases with the increase in the grist granulation fitness. The results obtained in this work show a significantly different starting TP and even when they have the same starting share of TSP, different wheats can have different ratios of the mentioned protein groups, which results in different shares of HMW N, MMW N, and LMW N. Furthermore, by reducing the granulation of the grist, it exceeds more protein fractions in the mash, which consequently leads to a drop in the Kolbach index, i.e., to an increase in their solubility. The values for HMW N concentrations for the fine and superfine granulation of the grist were similar. MMW N concentrations were significantly higher in wheat with a lower and higher initial total protein content, while TSP concentrations were similar with similar values for LMW N concentrations for fine and superfine granulation for both wheats.

A significant reduction in the final attenuation limit for the superfine granulation for wheat with less initial total protein and a higher fine extract for superfine granulation compared to fine (Table 2) failed to compensate for the greater transfer of non-fermentable soluble protein compounds of the ingredients (primarily MMW N) and a significant reduction in the final attenuation limit. In wheat with a higher initial protein content, this phenomenon is less pronounced, although similar values were found for total soluble N in both mash. As it has already been established that viscosity is not a problematic factor when using this type of wheat in brewing, the main emphasis will be on solving the problem of removing MMW N fractions from the mash in order to increase fermentability, especially since the proteins of this fraction make up the bulk of the so-called “haze active” proteins, which is problematic from the point of view of colloidal stability and minimum shelf life of beer [17].

So, from the results of the research, it can be seen that the increase in the fineness of the grist results in a decrease in the fermentability of the mash (expressed as the final attenuation limit), which is certainly an economic disadvantage caused by the increase in the non-fermentable extract in the total fine extract. This decrease in fermentability is caused by an increase in the concentration of the MMW N protein fraction. If the advantages of increasing the fineness of grain milling are to be utilized, further research should be focused on the selective separation (precipitation) of the MMW N fraction. In this way, it would be possible to increase the proportion of unmalted wheat and/or wheat malt in the mash/wort without adversely affecting the other quality indicators of the mash and consequently the beer.

4. Conclusions

The paper found that, regardless of the initial difference in the TP concentration, the concentrations of total soluble proteins (TSP) and high molecular weight protein fraction (HMW N) are similar for varieties with higher and lower initial TP, i.e., for varieties with higher initial TP it expresses as a difference in the concentration of insoluble protein fractions (which are removed by mashing) because the concentration of total soluble proteins was similar for both tested varieties. It was also established that by increasing the degree of grain granulation, a significant increase in the medium molecular protein fraction (MMW N) was observed, both in wheat with a higher and lower initial TP, which is associated with the fact that it was more pronounced in wheat with a lower initial concentration of total proteins. Consequently, this was followed by a significant decrease in fermentability (final attenuation limit) in the finest granulation of the mash for wheat with a lower initial (TP), while in wheat with a higher initial TP, the increase in grain milling fineness did not lead to an increase in mash fermentability. It can be concluded that the efficient removal of MMW N protein fractions from the mash will be a more efficient way

to increase the fermentability if, by increasing the milling fineness, one wants to increase the proportion of fine extract in the mash, especially for wheat with a lower initial TP. An increase in the fineness of the milled grain did not cause an increase in viscosity either in wheat with a lower or higher TP content.

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