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Analysis of Haze Susceptibility in Beers with Unmalted Barley Addition Under Varying Storage Conditions

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Beer consumption constitutes a significant portion of alcoholic beverage consumption in Poland. The growing interest in this product has prompted the exploration of solutions that not only affect production technology and costs but also influence the sensory and physicochemical attributes of the beverage. Incorporating unmalted raw materials is one such solution. This research aims to conduct a comparative analysis of haze formation in malted beers and beers with unmalted barley additions, considering diverse storage conditions and production scales. The results revealed that light has a more significant effect on turbidity formation than temperature. In the variants in which the impact of light on haze formation was investigated, the values of the average number of total particles were in the range of 130 (unsweetened laboratory under ultraviolet) to 1025 (unsweetened commercial under ultraviolet). The effect of temperature on haze formation was significantly less. In most cases, the average number of total particles was no greater than 200, with the highest result obtained being 300 (commercial malted under forced aging). Based on the study, it was concluded that, regardless of the scale of production, ultraviolet radiation causes significant haze formation in beer.

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1. Introduction

Malted barley in beer production serves as a source of nutrition and energy for yeast, which carries out alcoholic fermentation [23]. The use of unmalted raw materials stems from their lower cost compared to malt, thus impacting production economics [8, 10]. It should be noted that these raw materials also influence the flavor-aroma profile and stability of beer. Depending on climatic conditions and raw material

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availability, various options such as barley, white rice, wheat, corn, or sorghum are utilized [9, 34], albeit not exceeding 45% of the grist (PN-A-79098:1995) [24]. Soluble unmalted raw materials include sugar, caramel, and glucose, which can be dissolved in water [22].

The stability of the final beer product consists of four components: flavor stability, foam stability, physicochemical stability, and microbiological stability. Different factors can influence each component, such as light, polyphenol and α,β-glucan content, temperature, mechanical interactions, or oxygen exposure [1]. The use of unmalted raw materials in brewing as a substitute for malt leads to a reduction in nitrogen compounds, polyphenols, β-glucan, and lipids. These compounds impact foam stability and the occurrence product of haze in the final [14, 15, 3, 32]. The use of unmalted raw materials decreases the content of enzymes required during mashing. To improve beer filtration and colloidal stability in the presence of unmalted raw materials, the addition of enzymatic additives such as bacterial α-amylases, β-glucanases, or proteases is beneficial [35, 16, 30]. Prolyl endopeptidase isolated from Aspergillus niger, as well as other proteolytic enzymes, reduce the beer's susceptibility to haze [2, 18].

Haze formation in beer can be attributed to the presence of microorganisms, including fungi such as Fusarium, Pichia, Candida, and bacteria such as Lactobacillus brevis [33, 6]. Microbial contamination poses a risk to the safety of the final beer product. Additionally, particles originating from raw materials can contribute to haze formation through the creation of protein-polyphenol complexes. Notably, proline-rich proteins with a molecular weight ranging from 15 to 30 kDa play an active role in the generation of colloidal sediments [18].

Factors such as the presence of production yeast due to inadequate removal methods and the presence of calcium oxalate can also influence beer haze. The presence of β-glucan and inadequate enzymatic activity during the mashing process can further contribute to haze formation. When unmalted raw materials are employed, the β-glucan-rich cell walls remain undegraded during germination, potentially impacting beer clarity [27].

Beer stabilization methods involve the utilization of various substances, including enzymes, diatomaceous earth, polyvinylpolypyrrolidone (PVPP), carrageenan, chitosan, and bentonite. These additives aid in the elimination of excess proteins and polyphenols, thereby promoting beer clarity [28, 26, 5].

Furthermore, inorganic contaminants, including filtration and colloidal stabilization agents, dust particles, or other non-raw material particles, can penetrate damaged filters, thereby reducing beer clarity [7].

Various methods are used to assess haze in beer: the determination of polyphenols and proteins [17], the use of turbidity meters [4], microscopic analysis, gel chromatography [31], Raman microspectroscopy [12], and the use of a Coulter Counter particle size analyzer [20].

The process of forced aging involves subjecting beer to alternating storage temperatures to promote aging. For non-stabilized beers, temperatures of 0 °C and 40 °C are utilized, while pasteurized beers undergo aging at temperatures of 0 °C and 60 °C. Throughout the entire process, the beer is shielded from light exposure. Haze measurements are conducted after each cycle of forced aging, and a haze increase of 2 EBC units compared to the control sample indicates the completion of beer aging.

The aim of the conducted research is to perform a comparative analysis of susceptibility to haze formation in malted beers and those with an unmalted raw material addition, considering different storage conditions and production scales.

2. Materials and methods

The research materials consisted of commercial beers available on the Polish market and beers produced on a laboratory scale using a Speidel Braumeister brewing kettle. The study included malted beers and beers with an unmalted raw material addition in the form of barley. The influence of sample storage on haze formation was also considered, including storage at temperatures of 4 ± 2 °C and 21 ± 2 °C, exposure to darkness, and exposure to ultraviolet radiation (UV). The beers were also subjected to a forced aging process according to PN-A-79093-9 [25].

The laboratory beers were produced following a specific technological scheme (Figure 1). The production utilized process water from a local source, Pale Ale malt from a domestic malt house, the Quench spring barley variety, Fermentis SafAle S-04 yeast, as well as Lubelski and Magnat hops.

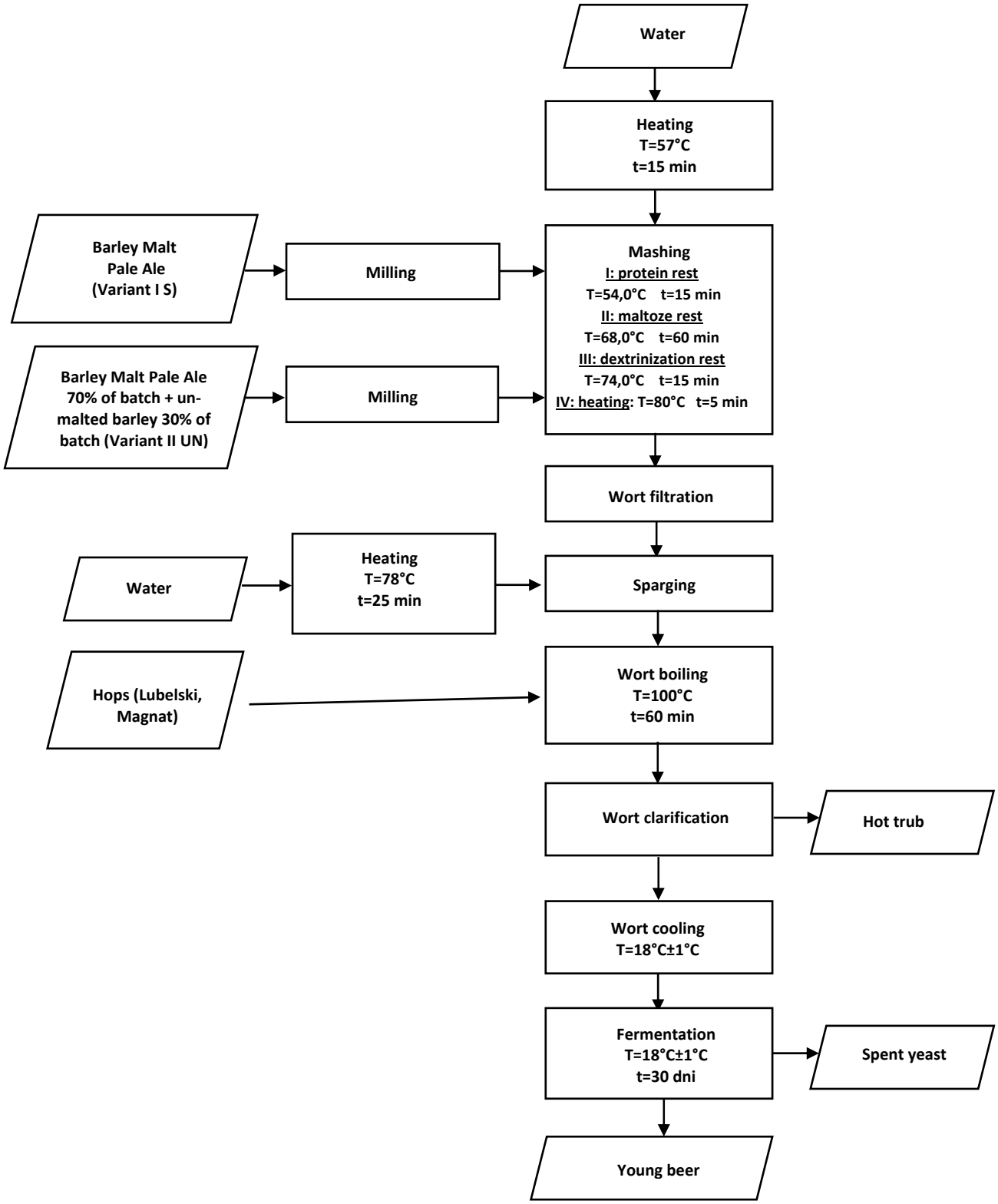

Fig. 1. Scheme of beer production on a laboratory scale

The characteristics of the researched beers were as follows:

Commercial beer variants:

- M COM: commercial beer without unmalted raw material addition; ingredients: water, barley malt, hops; packaging: green glass bottle.
- UN COM: commercial beer with unmalted raw material addition; ingredients: water, barley malt, brewing barley, hops, hop extract; packaging: green glass bottle.
- Laboratory beer variants:
- M LAB: laboratory beer without unmalted raw material addition; ingredients: water, barley malt, hops; packaging: green glass bottle.
- UN LAB: laboratory beer with unmalted raw material addition (30% of the grist); ingredients: water, barley malt, brewing barley, hops; packaging: green glass bottle.

The control sample consisted of beers that were tested immediately after purchase (commercial beers) or manufacture (laboratory beers).

The particle size analysis of beer haze was conducted using the Shadow Sizing method. A FlowSense 2M PIV camera equipped with a sensor having a resolution of 1600 × 1200 pixels was employed. The macro lens Nikkor 50 mm f/1.8, along with a set of three intermediate rings (12, 20, 36 mm) attached to the camera, allowed significant image magnification. Each beer variant was analyzed in 4 replicates, with 10 images taken for each replicate. The image analysis for particle distribution and quantity was performed using DynamicStudio 6.7 software.

Color determination was carried out by means of the spectrophotometric method with a UV-3100PC spectrophotometer. The methodology involved placing a filled cuvette with a capacity of 1 cm3 in the working space of the spectrophotometer and measuring the absorbance at a wavelength of 430 nm. The results were then converted to the EBC unit according to the following formula:

$$Abs_{430} \cdot 25 = color\ [EBC] \quad (1)$$

where:

Abs430 is the absorbance read from the spectrophotometer display, and 25 is the conversion factor [19].

The turbidity of the samples was also measured using a TB 300 IR turbidimeter, and the results were reported in nephelometric turbidity units (NTU). The color and turbidity measurements were performed in three replicates for each variant. The significance of the effects of the studied variables on the color and turbidity values was determined using one-way analysis of variance (ANOVA). The significance of the differences between the means was verified using Tukey's test ($p<0.05$). The statistical analysis was conducted using Statistica 13 software by StatSoft.

3. Results and discussion

The turbidity of the laboratory beers (Fig. 2) was significantly higher even in the zero sample (143-156 NTU) compared to the commercial beers (3-6 NTU). This is because the laboratory-scale beers were not subjected to the filtration process. All the storage methods and forced aging resulted in increased turbidity in the commercial beer samples. Similar results were obtained for the laboratory beers, except for the sample without unmalted raw material, which showed a decrease in turbidity during storage at 21 ± 2 °C and under dark conditions. It should be noted that the commercial malted beers were more sensitive to different storage conditions in terms of turbidity compared to those with the addition of barley. It was demonstrated that ultraviolet radiation has the strongest impact on the formation of turbidity.

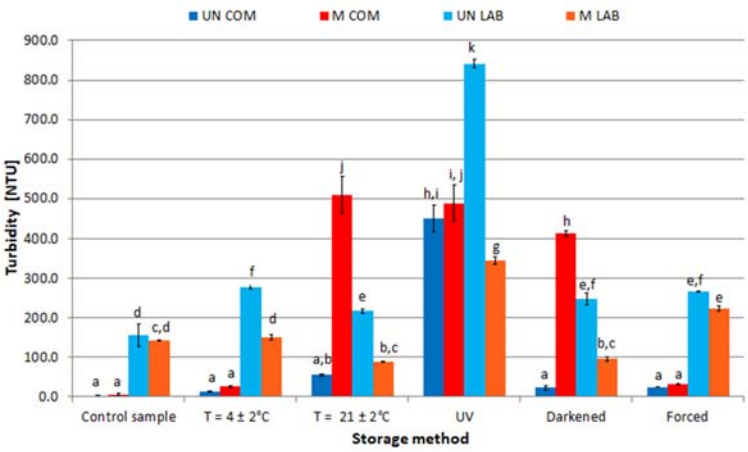

Fig. 2. Turbidity values in NTU for studied beers stored under different conditions (n=3, α=0.05; homogeneous groups within each parameter denoted by the same letters)

The laboratory beers exhibited a higher color intensity (18-31 EBC), including the control sample, compared to the commercial beers (7.7 EBC). This could be due to the type of malt, hops used, or the lack of filtration in the case of the LAB samples. Storage at different temperatures and light exposure caused an increase in color intensity below 10 EBC in the case of the commercial beers. However, in these samples, forced aging resulted in an increase in color intensity above 10 EBC (13-14 EBC). It should be noted that the UN LAB beers exhibited a significantly higher color intensity compared to the malted beer. It was observed that ultraviolet radiation had the greatest impact on color intensity.

The results for the color measurement of the investigated beers are presented in Figure 3. The average number of all particles (Fig. 4) was highest for the samples stored in darkness and under ultraviolet radiation. The commercial beers exhibited greater sensitivity, as they showed larger differences in the particle count depending on the storage conditions. In the case of the laboratory samples, the particle count was less than 300.

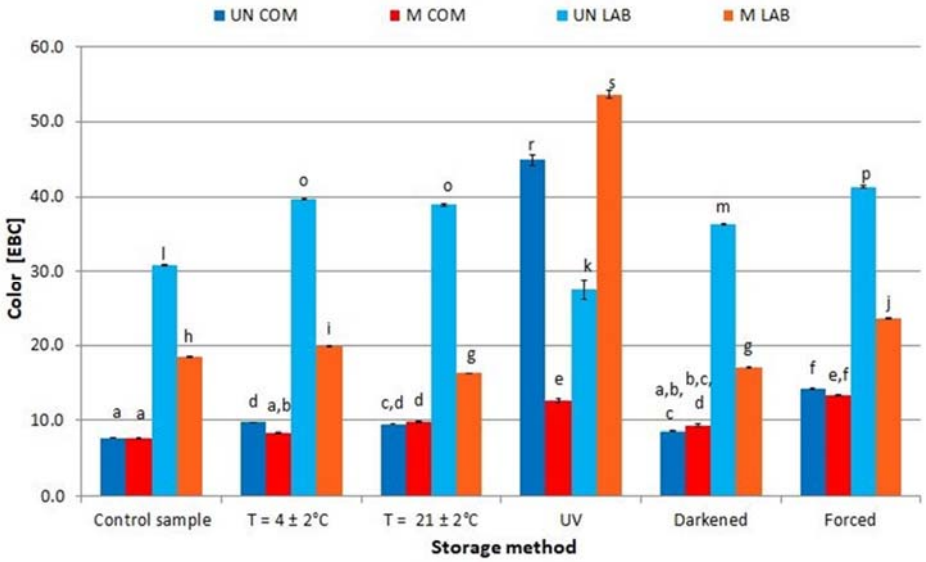

Fig. 3. Color values in EBC units for studied beers stored under different conditions (n=3, α=0.05; by the same letters denote homogeneous groups of results within each parameter)

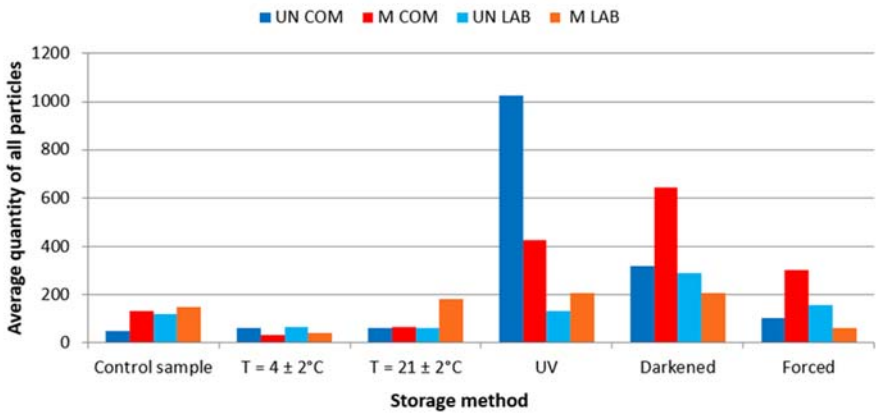

Fig. 4. Average particle count in studied beers stored under different variants

In the control samples of the researched beers (Fig. 5a), the dominant fraction consists of particles ranging in size from 0 to 0.05 mm. The laboratory beers exhibited a higher presence of fine particles, while the beers with the unmalted adjuncts exhibited lower levels of identifiable particles compared to the malted beers. Particles larger than 0.1 mm were only detected in two variants.

The beers stored at a temperature of 4 ± 2 °C (Fig. 5b) exhibited a decrease in the particle count compared to the non-stored beers. The addition of the unmalted adjuncts had a significant impact on

increasing the particle count. This can be attributed to the occurrence of cold haze formation, a phenomenon where polypeptides and polyphenols interact through non-covalent bonding. This process is reversible, and the haze disperses when the temperature is increased. Nevertheless, if covalent bonding occurs between these compounds, the resulting aggregates become stable and insoluble (Steiner et al., 2010). This low-temperature phenomenon is utilized in brewing during beer lagering, where the formed hazes settle to the bottom, resulting in increased beer clarity (Munroe, 2006).

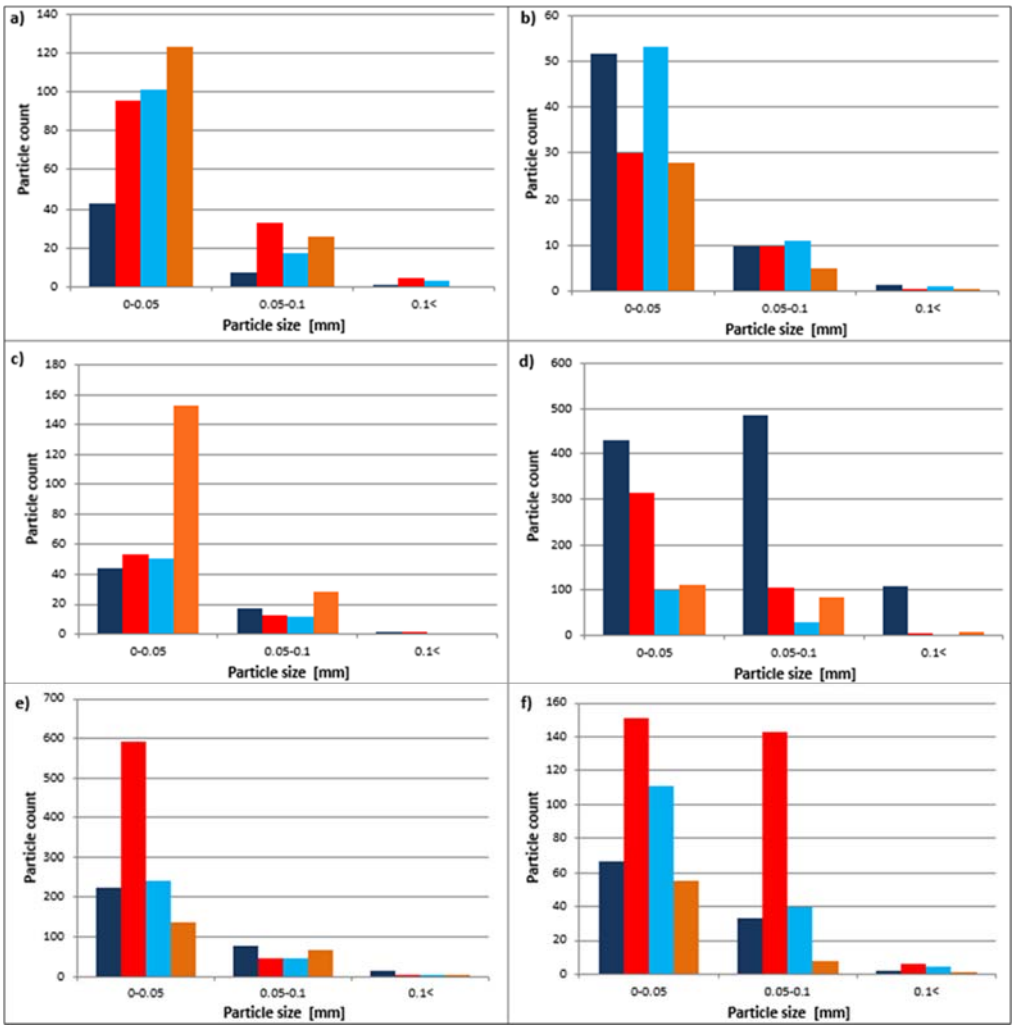

Fig. 5. Particle count according to their size for: a) control sample, b) beers stored at 4 ± 2 °C, c) beers stored at 21 ± 2 °C, d) beers stored under ultraviolet radiation lamps, e) beers stored in darkness, f) forced aging beers

Storage at room temperature resulted in a decrease in the count of identifiable particles in the range of 0-0.05 mm (Fig. 5c). However, the malted laboratory beer exhibited an increase in the particle content, suggesting potential initial microbiological contamination in this particular variant. The chosen storage temperature provides optimal conditions for the growth of various microorganisms. Additionally, the laboratory beers did not undergo pasteurization, further diminishing their microbiological resistance. Although particles larger than 0.1 mm were detected in all the beer variants, their presence was minimal.

Ultraviolet radiation had a significant impact on the increase in the numer of particles with an equivalent diameter of 0-0.05 mm in commercial beers, as well as larger particles in all the investigated samples (Fig. 5d). The UN COM variant displayed particular sensitivity to these changes, with a 10-fold increase in the smallest particles and over a 67-fold increase in particles ranging from 0.05 to 0.1 mm compared to the control sample. This variant exhibited the highest number of large particles (108).

Storage in darkness (Fig. 5e), similar to ultraviolet radiation, strongly influenced the particle count and haze formation. Among the smallest particles, the

malted commercial beer demonstrated the highest sensitivity, with the observation of 590 particles. Furthermore, a 5-fold increase in this particle type was observed in the UN COM sample, and a 2-fold increase in the laboratory beer with the unmalted adjuncts. The count of particles ranging from 0.05 to 0.1 mm was also higher compared to the control sample. Although particles with an equivalent diameter larger than 0.1 mm were detected in all the variants, their presence was minimal.

Forced aging is employed to determine the shelf life of beer. This method serves to determine the beer's stability. The particle size analysis (Fig. 5f) revealed that in most cases, this process resulted in an elevation of identifiable particles. Nontheless, an exception was observed in the malted laboratory beer, where the aging process led to a reduction in the particle content compared to the samples not subjected to the aging process.

The storage conditions influenced the formation of haze in both the malt and adjunct-containing beers. It is worth noting that storage at a constant temperature had a lesser impact on the count of identifiable particles compared to light exposure. The temperature ranges used during storage contribute to a decrease in the number of detectable particles, except for the malted laboratory beer stored at 21 ± 2°C, where the warm temperature may have had facilitated the growth of microorganisms, resulting in increased haziness. Special attention should be given to ultraviolet radiation, which had a significant influence on haze formation, color, and particle counts. Light exposure leads to the degradation of specific compounds present in beer, such as vitamin B2 [29] and isohumulones [13]. Therefore, the packaging materials commonly used for beer, such as cans or bottles made of green or brown glass, act as light barriers [13]. To fully understand the impact of UV radiation on haze formation, a comprehensive chemical analysis should be conducted to determine the relationship between degradation of the chemical compounds in beer under UV influence and their susceptibility to haze formation. In beers containing unmalted adjuncts, the particle content in the control sample was lower compared to the malted variants. This indicates that the addition of unmalted adjuncts effectively reduces the content of polyphenols, nitrogen compounds, or β-glucan, which are involved in haze formation. For the malted beers, a decrease in the particle count was observed after storage at low temperatures. This phenomenon is attributed to the formation of cold haze resulting from interactions between polyphenols and proteins, leading to sedimentation. All the samples for analysis were taken from the middle portion of the solution, disregarding the sedimented particles. Storing beer alternately at high and low temperatures during the force aging process also significantly influenced the formation of particles, particularly in the size range of 0.05-0.1 mm in the commercial malt beer. It can be presumed that due to the fluctuating temperatures, more substantial and permanent haze formations began to develop in this beer, surpassing the colloidal haze.

The Shadow Sizing method provides a means to measure and mathematically analyze solutions or materials containing particles with regular or irregular shapes [11]. However, the camera utilized for Shadow Sizing analysis lacked the required sensitivity to identify colloidal particles ranging from 0.1 to 1 μm in size. To investigate changes in colloidal stability, application of the Shadow Sizing method with a higher-resolution camera would be necessary.

3. Conclusion

The results presented in the study show the potential for turbidity formation depending on the raw material used, storage methods or the scale of production. The findings provide a basis for determining which factors in the storage process should be avoided in order for the beer to retain its physical stability for as long as possible. Unmalted additives are becoming increasingly popular in brewing processing. This study shows how they affect the turbidity potential compared to beers without them. This is important from the production technology point of view and to introduce appropriate measures to minimize the formation of turbidity, e.g. through the addition of enzymes and the selection of raw materials.

References

[1] Aguiar D., Pereira A.C., Marques J.C.: The Influence of Transport and Storage Conditions on Beer Stability – a Systematic Review. Food and Bioprocess Technology, 2022, 15, 1477. https://doi.org/10.1007/s11947-022-02790-8

[2] Bilinski C. A., Russell I., Stewart G.G., 1987, Applicability of yeast extracellular proteinases in brewing: physiological and biochemical aspects. Applied and Environmental Microbiology, 53(3), 495–499.

[3] Buiatti S., Bertoli S., Passaghe P.,2018) Infuence of glutenfree adjuncts on beer colloidal stability. European Food Research and Technology 244(5):903–912. https://doi.org/10.1007/s00217-017-3010-3
[4] Cimini A., Moresi M.: Beer Clarification Using Ceramic Tubular Membranes. Food and Bioprocess Technology, 2014, 7, 2694–2710. https://doi.org/10.1007/s11947-014-1338-2
[5] Cimini A., Moresi M.: Combined enzymatic and crossflow microfiltration proces to assure the colloidal stability of beer. LWT Food Science and Technology, 2018, 90, 132-137, https://doi.org/10.1016/j.lwt.2017.12.008
[6] Ciont C., Epuran A., Kerezsi A.D., Coldea T.E., Mudura E., Pasqualone A., Zhao H., Suharoschi R., Vriesekoop F, Pop O.L.: , Beer Safety: New Challenges and Future Trends within Craft and Large-Scale Production. Foods, 2022, 11, 2693. https://doi.org/10.3390/foods11172693
[7] Ciosek A.: Zmętnienia w piwie – przyczyny powstawania i ich identyfikacja. Przemysł Fermentacyjny i Owocowo-Warzywny, 2022, 3/2022, 4-7. DOI 10.15199/64.2022.3.2
[8] Delcour J.A., Hennebert M. M. E., Vancraenenbroeck R., Moerman M.: Unmalted cereal products for beer brewing. Part I. The use of high percentages of extruded or regular corn starch and sorghum. Journal of The Institute of Brewing, 1989, 95(4), 271-276. https://doi.org/10.1002/j.2050-0416.1989.tb04631.x
[9] Figueroa, J. D. C., Martínez, B. F., & Ríos, E.: Effect of sorghumendosperm type on the quality of adjuncts for the brewing industry. Journal of American Society of Brewing Chemists, 1995, 53(1), 5–9. https://doi.org/10.1094/ASBCJ-53-0005
[10] Glatthar, J., Heinisch, J. J., Senn, T.: Unmalted triticalecultivars as brewing adjuncts: effects of enzyme activities andcomposition on beer wort quality. Journal of the Science of Food and Agriculture, 2005, 85(4), 647–654. https://doi.org/10.1002/jsfa.1941
[11] Jakubowski M., Antonowicz A., Janowicz M., Streczyńska M., Piepiórka-Stepuk J., Poreda A.: An assessment of the potential of shadow sizing analysis and Particle Image Velocimetry (PIV) to characterise hot trub morphology. Journal of Food Engineering, 2016, 173 (3), 34-41. https://doi.org/10.1016/j.jfoodeng.2015.10.033
[12] Kahle E.M., Zarnkow M., Jacob F., 2020, Beer Turbidity Part 2: A Review of Raman Spectroscopy and Possible Future Use for Beer Turbidity Analysis. Journal of the American Society of Brewing Chemists, 79(2), 115-137. https://doi.org/10.1080/03610470.2020.1800345
[13] Keukeleire D.D., 2000, Fundamentals of beer and hop chemistry, Quim. Nova 23(1). https://doi.org/10.1590/S0100-40422000000100019
[14] Kordialik-Bogacka E., 2005, Surowce a stabilność piany piwa, Kierunek Spożywczy 3/2005, 33-35
[15] Kordialik-Bogacka E., Bogdan P.: Wpływ surowców niesłodowanych na proces produkcji brzeczki i piwa. Przemysł Fermentacyjny i Owocowo-Warzywny, 2017, 61 (09).
[16] Kunz T., Woest H., Lee E.J., Müller C., Methner F.J.: Improvement of the Oxidative Wort and Beer Stability by Increased Unmalted Barley Proportion. BrewingScience, 2011, 64(7), 75-82.
[17] Leiper K.A., Miedl M.: Beer a quality perspective ed. Bamforth C.W. Academic Press is an imprint in Elsevier, 2009.
[18] Lopez M., Edens L.: Efective prevention of chill-haze in beer using an acid proline-specifc endoprotease from Aspergillus niger. Journal of Agricultural and Food Chemistry, 2005, 53(20), 7944–7949. https://doi.org/10.1021/jf0506535
[19] MEBAK - Wort, Beer, Beer-Based Beverages, 1st Edition, p. 185, 2012.
[20] Morris T. M.: Particle size analysis of beer solids using a coulter counter. Journal of The Institute of Brewing, 1984, 90(3), 162-166. https://doi.org/10.1002/j.2050-0416.1984.tb04257.x
[21] Munroe J. H.: Aging and Finishing, Handbook of Brewing Second Edition ed. Priest F.G., Stewart G.G., Taylor & Francis, Boca Raton, 2006.
[22] Palmer G.H.: Dodatki niesłodowane w piwowarstwie i gorzelnictwie, Materiały III Szkoły Technologii Fermentacji, Kraków-Zakopane, 1998.
[23] Paynter, B., Young, K.: Managing grain protein in malting barley. Agriculture Western Australia South Perth, 1996, 2 (37).
[24] PN-A-79098:1995 – Piwo.
[25] PN-A-79093-9: 2000 – Piwo – Metody badań – Sprawdzanie napełnienia butelek i puszek, oznaczanie klarowności, oznaczanie trwałości metodą forsowania.
[26] Poreda A., Zdaniewicz M., Sterczyńska M., Jakubowski M., Puchalski C.: Effects of wort clarifying by using carrageenan on diatomaceous earth dosage for beer filtration. Czech Journal of Food Sciences, 2015, 33(4). DOI: 10.17221/92/2015-CJFS
[27] Psota V., Skulilova Z., Hartmann J.: The efect of the barley variety, location and year crop on the haze of congress wort. Czech Journal of Food Sciences, 2009, 27(3):158–164. https://doi.org/10.17221/156/ 2008-CJFS
[28] Rehmanji M., Gopal C., Mola A.: Nowoczesna metoda stabilizacji piwa – Polyclar Brewbrite. Przemysł Fermentacyjny i Owocowo-Warzywny, 2002, 46, 03.
[29] Sikorska E., Górecki T., Khmelinskii I.V., Sikorski M., Keukeleire D.D.: Monitoring beer during storage by fluorescence spectroscopy. Food Chemistry, 2006, 96(4), 632-639. https://doi.org/10.1016/j.foodchem.2005.02.045

[30] Steiner E., Auer A., Becker T., Gastl M.: Comparison of beer quality attributes between beers brewed with 100% barley malt and 100% barley raw material. Journal of the Science of Food and Agriculture, 2012, 92(4):803–813. https://doi.org/10.1002/jsfa.4651
[31] Steiner E., Becker T., Gastl M.: Turbidity and haze formation in beer – insights and overview. Journal of The Institute of Brewing, 2012, 116(4), 360-368, https://doi.org/10.1002/j.2050-0416.2010.tb00787.x
[32] Sterczyńska M., Stachnik M., Poreda A., Pużyńska K., Piepiórka-Stepuk J., Fiutak G., Jakubowski M.: Iconic composition of beer worts produced with selected unmalted grains. LWT, 2021, 137 (2). https://doi.org/10.1016/j.lwt.2020.110348
[33] Vaughan A., O'Sullivan T., Sinderen D.: Enhancing the Microbiological Stability of Malt and Beer — A Review. Journal of The Institute of Brewing, 2012, 111(4). https://doi.org/10.1002/j.2050-0416.2005.tb00221.x
[34] Yorke J., Cook D., Ford R.: Brewing with Unmalted Cereal Adjuncts: Sensory and Analytical Impacts on Beer Quality. Beverages, 2021, 7(1):4. https://doi.org/10.3390/beverages7010004
[35] Żyła K., Kujawski M.: Stosowanie preparatów enzymatycznych w wybranych gałęziach przemysłu spożywczego. Biotechnologia, 1999, Kraków, 2 (45), 38–49.