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A Pilot Study of Clarifying (Fining) Agents and Their Effects on Beer Physicochemical Parameters

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Abstract

The role of science and technology in enhancing beer quality is crucial amid growing market demands. This pilot study assessed the clarity and physicochemical stability of laboratory beers treated post fermentation with three clarifying (fining) agents: two chitosan-based and one collagen-based (fish bladder/isinglass). The beers were brewed with Polish barley malt and hops (alpha acids 7.5% and 14.5%). The measured parameters included pH, colour, turbidity, viscosity, surface tension, and foam volume. Within this small-scale, low-power dataset, both the collagen- and chitosan-based agents improved clarity, with the collagen agent showing the lowest turbidity in this sample. The clarifying agents also influenced the colour and surface tension, while the pH was largely unchanged. The foam volume increased with fining. Shelf-life checks suggested improved stability in clarified beers, with no clear differences between agents under these conditions. These findings are preliminary. The results should be interpreted cautiously due to the limited number of replicates. Larger scale studies with adequate replication are required before translating these observations into brewing practice. Chitosan's effectiveness as a clarifying agent aligns with its high charge density and ability to coagulate suspended particles. This study underscores the importance of selecting appropriate clarifying agents to optimize beer clarity and stability while maintaining essential physicochemical properties. These findings contribute to the brewing industry's efforts to meet consumer expectations for high-quality, stable beer products.

Keywords: beer; chitosan; fish bladder; clarifying agents; surface tension

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1. Introduction

Given the growing significance of the role of science and technology in the beer production process, it becomes crucial to understand and optimize various aspects of this process to meet the high market expectations regarding beer quality [1]. Beer quality, defined by its physicochemical and sensory characteristics, is now more valued than ever, especially in the context of strong competition in the food product market [2]. The shelf-life of pasteurized beer should not be less than 30 days. A correctly conducted production process allows for beer with colloidal stability lasting up to about 6 weeks. Interestingly, by adjusting the process parameters to the quality of the raw material, the shelf-life can be

extended to 3–4 months, while colloidal stabilization and aseptic filling can yield a product with a shelf-life of 6–12 months [3].

One of the main determinants of beer shelf-life is its clarity, which can be maintained for an extended period through appropriate clarification methods [4]. Colloidal instability in beer, a significant aspect of brewing science, involves various forms of particulate matter affecting beer clarity. These colloids can generally be classified based on their origin and the conditions leading to their formation [5]. Firstly, temperature-induced colloids appear when beer is exposed to extreme temperatures. At low temperatures, as in the case of "ice beer" technology, intentional freezing helps remove colloidal materials [6]. On the other hand, high temperatures can cause precipitation, especially in non-alcoholic beers, due to reactions between clarifying agents and stabilizers [7].

Another category consists of colloids resulting from the insolubility of additives in beer. These colloids may originate from reactions between various additives during processes such as pasteurization or from inherent materials in the beer that can become problematic under certain conditions, such as during agitation in transport [5].

Additionally, beer haze, a common issue in brewing, arises from various materials such as starch, oxalic acid, and protein–polyphenol complexes. This haze can be exacerbated by external factors, such as lubricants covering can lids or the presence of dead bacteria, which contribute to the visible cloudiness in beer [8].

Finally, a more subtle form of colloid known as "invisible haze" or "pseudo-haze" occurs due to extremely small particles that significantly scatter light. These particles often originate from unmodified regions of the barley kernel, reduced starch, or polysaccharides released from yeast cells. Each of these categories highlights the complex nature of colloidal stability in beer and the intricate interactions brewers must manage to ensure the desired clarity and quality of their product [8].

The composition of beer, particularly the polyphenol content and the degree of protein degradation, significantly impacts the intensity of ageing processes [7]. Equally important are carbonyl compounds formed from the oxidation of higher fatty acids and the content of heavy metals such as iron and copper, which act as catalysts in oxidation processes [9]. In the context of cold breaks, carbohydrates, including β-glucans, play a significant role. Various silica gels and sols are used to remove the protein fraction that causes beer haze [10].

Currently, an increasing number of studies focus on the use of chitosan in the clarification process [11]. Chitosan, a natural polymer of partially deacetylated chitin, shows the ability to adsorb heavy metals and a significant portion of polyphenols from beer. Despite its potential benefits, the use of chitosan in brewing is associated with certain limitations, mainly due to its stable and rigid crystalline structure and limited solubility in water [12,13].

The sector of so-called white biotechnology makes a significant contribution to the development of the brewing industry by offering a range of commercial enzyme preparations. For instance, the Ondea® Pro (Novonesis Group, Bagsværd, Denmark) preparation allows for the production of beer exclusively from unmalted barley [14]. The synergistic action of endogenous barley enzymes and exogenous microbiological enzymes can result in the efficient breakdown of starch and proteins, leading to improved clarity [15,16]. In the future, the brewing process may also rely on cross-flow microfiltration (CFMF), although this process still faces certain limitations, such as moderate permeation flow rates [9].

In the global context, leaders in brewing technology must meet the challenges posed by increasing consumer awareness regarding beer quality and rising expectations for sustainable and environmentally friendly production methods. Consequently, research into innovative clarification methods, such as the use of chitosan, is gaining particular importance, both from a scientific standpoint and for practical application in the brewing industry.

The aim of this study was to perform a physicochemical evaluation of beers and determine their stability when subjected to different stabilization conditions after clarification using three different preparations.

2. Materials and Methods

The materials for this study were purchased from the suppliers of brewing raw materials. Standardized methods according to the Analytica EBC (European Brewery Convention) and research apparatus were used for the physicochemical determinations.

2.1. Materials

The research material consisted of laboratory beers using a minimum of 32 L of water, subjected to different types of clarification. The raw materials used for this study included the following:

2.1.1. Viking Malt Pilsner Barley Malt (Viking Malt Oy, Malt House in Strzegom, Poland)

Pilsner barley malt produced in a kiln, suitable as a base for all types of beer. This 2-row brewing barley is of Polish origin.

2.1.2. Magnat T-90 Hops (PolishHops, Karczmiska, Poland)

Magnat hops, a super-bitter variety bred in Poland (derived from Magnum). Alpha acids: 14.0%. It is used in wort hopping to produce beers with a pronounced bitterness. Depending on the harvest, alpha acid levels can reach up to 14.5%. It is recommended for beers such as Pilsner, India Pale Ale, American Ale, Blonde Ale, Pale Ale, Nut Brown Ale, Dark Ale, Bright Ale, Hefeweizen, and Bitter. Leading aroma notes: lemon balm, woody, resinous, spicy.

2.1.3. Puławski T-90 Hops (PolishHops, Karczmiska, Poland)

Puławski hops were released in 2012. This bitter variety, grown in Poland, is distinguished by a fruity–floral, slightly spicy aroma. Alpha acid level is around 7.5%. It can be used for both top and bottom fermentation beers, as well as for dry hopping during secondary fermentation. It is recommended for beers such as American Ale, IPA, Lager, Pils, American Pale Ale, American India Pale Ale, American Wheat, Bitter, Wheat, Red Ale, Altbier, and Rauchbier. Leading aroma notes: earthy, grassy, fruity–floral, herbal.

2.1.4. Yeast

Fermentis Safale S-04 yeast (Lesaffre, Marcq-en-Barœul, France) was used for fermenting the wort. This English yeast strain is chosen for its high fermentation speed and ability to form a very compact sediment at the end of fermentation, which facilitates beer clarification.

2.1.5. Commercial Beer of a Polish Brand

Pale, pasteurized beer containing water, barley malt, and Polish hops. The alcohol content declared by the producer was 5.2% ABV, and the extract was 11.3% by weight.

Three clarifying agents, commonly used among home brewers, were employed to clarify the beer. They were used following the instructions of the producer. The characteristics of these substances are as follows:

2.1.6. Chitozan 1_(Sweden)

This clarifying agent is a two-component system containing kieselsol (colloidal silica) and chitosan, designed for rapid post fermentation clarification. It is considered a strong and neutral fining agent that does not alter the final taste of the beer. In this study, Chitozan

1 was applied after fermentation using the full two-step protocol. First, kieselsol was added at a dose of 1.25 mL per litre of degassed beer, stirred gently for uniform dispersion, and left to react for 1 h. Then, chitosan was added at a dose of 5 mL per litre, previously dissolved in a small amount of water, and stirred gently for 30 s to minimize oxygen incorporation. Following the addition of both components, the beer was stored undisturbed at 4 ± 1 °C. Clarification was visually confirmed within 24 h, and the clarified beer was decanted from the sediment for further analysis.

2.1.7. Collagen (Poland)

This clarifying agent, based on collagen extracted from dried fish swim bladders, is suitable for use in all types of beer. In the acidic environment of beer, the collagen molecules acquire a positive electrostatic charge, enabling them to bind negatively charged suspended particles such as yeast cells, proteins, and lipids. This interaction promotes the rapid formation of sediment, which settles at the bottom of the tank during maturation. The resulting sediment is compact and cohesive, making it difficult to resuspend, and thus facilitates clean decanting. During secondary fermentation in the bottle, it further contributes to sediment stability by encouraging tight packing at the bottom.

In this study, the isinglass-based agent was prepared by dissolving 2.5 g of powdered collagen in 500 mL of cold distilled water ($\leq$10 °C) with gentle stirring. The solution was allowed to hydrate at 10–14 °C for 30 to 60 min. Once fully dissolved, it was added post fermentation to beer cooled to $\leq$4 °C, at a dosage of 0.5 mL per litre. After gentle mixing, the beer was stored undisturbed at 4 ± 1 °C for a minimum of 24 h, allowing sedimentation to occur. Clarification was visually confirmed, and the clarified beer was then carefully decanted for further analysis.

2.1.8. Chitozan 2 (Poland)

This clarifying agent is a two-component formulation intended primarily for the clarification of wine, musts, and fermentation mashes. Although not originally designed for beer, it was evaluated in this study for its suitability in post fermentation beer clarification. The product consists of colloidal silica (kieselsol) and chitosan, applied sequentially. First, the kieselsol component was added at a dose of 1.25 mL per litre of degassed beer and stirred gently to ensure uniform dispersion. After a 1 h reaction period, the chitosan component was added at a dose of 5 mL per litre, previously dissolved in a small volume of water. The mixture was stirred gently for approximately 30 s to minimize oxygen incorporation. Following the addition of both components, the samples were stored upright at 4 ± 1 °C, undisturbed. Visual clarification was observed within 24 to 48 h, typically requiring a slightly longer settling time than the beer-specific agent (Chitozan 1).

2.2. Physicochemical Analyses and Rheological Measurements

Selected alcoholic beverages were subjected to examinations of various physicochemical parameters, such as pH, colour, turbidity, extract and alcohol content, viscosity, surface tension, and foam volume. The methodologies for each analysis are presented below. Analyses were performed with no modification to the original procedure.

2.2.1. Foam Volume Measurement

The foam volume measurement was conducted by pouring the contents of each beer package (can or bottle) along the wall into a 1000 cm^3 graduated cylinder [17]. The volume of the resulting foam was read from the scale marked on the cylinder.

2.2.2. pH Measurement

The pH measurement of degassed beer samples was performed using a HandyLab 100 pH metre (Si Analytics, Mainz, Germany) by immersing the electrode into the beer samples at a temperature of 19 °C. Analysis followed EBC Analytica methodology: 9.35—pH of Beer (formerly published as IOB Method 9.42) [18].

2.2.3. Colour Measurement

The colour of the produced beers was determined using a Ray Leigh UV-1800 spectrophotometer (Beijing Rayleigh Analytical Instrument Corporation (BRAIC), Beijing, China) by measuring the absorbance of the beers (Abs) at a wavelength of 430 nm. Before each measurement, the samples were degassed and filtered twice (with diatomaceous earth and then through a syringe filter). Analysis followed EBC Analytica methodology: 9.6—Colour of Beer: Spectrophotometric Method (IM). The absorbance results were converted to EBC units using the following equation [19]:

$$EBC = Abs_{430} \times 25. \quad (1)$$

2.2.4. Beer Clarity Measurement

Initially, the turbidity values of the selected beers were measured using a TB 300 IR turbidimeter (Lovibond, Amesbury, UK). The obtained values were then converted to the EBC (unit of turbidity) scale by dividing the results by 4. The beer samples were degassed prior to measurement. Measurements followed EBC Analytica methodology: 4.23—Method to measure Congress Mash wort turbidity [20].

2.2.5. Surface Tension Measurement

The measurement began by degassing each sample on a magnetic stirrer for 12 h. The surface tension of each beer was determined using a tensiometer (Figure 1), applying the du Nouy ring method [21]. The apparatus used consisted of an analytical balance, a platinum du Nouy ring, a force actuator for ring detachment, and a computer. The measurement was fully automated, utilizing an electric drive with programmable ring emergence speed. The du Nouy method involves precisely measuring the force required to detach the ring from the liquid surface. The Zuidema–Waters correction method was applied for accuracy.

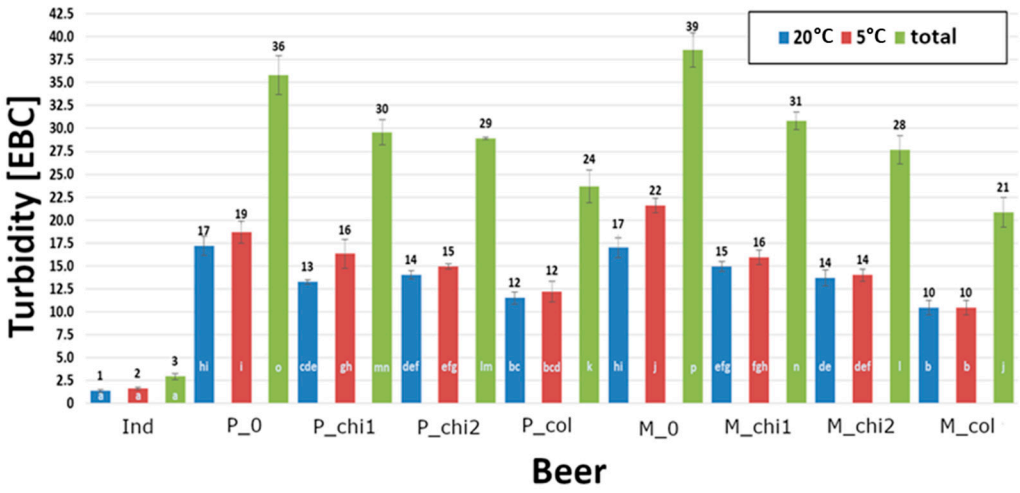

Figure 1. Turbidity of produced beers (n = 3, α = 0.05; homogenous groups of results within the given parameter are indicated by letters).

2.2.6. Rheological Properties Measurement

Rheological measurements were performed using a HAAKE Viscotester iQ Rheometer (Thermo Scientific, Waltham, MA, USA). Before starting the measurements, the samples were degassed on a mechanical shaker for 18 h at ambient temperature. During the last half hour of degassing, the samples were stirred using a glass rod. The geometry used was a double-gap concentric cylinder system. For the analysis, a 2.6 cm^3 sample was taken. Measurements were performed in triplicate, each time using a fresh sample. The measurements were conducted under controlled rate (CR) conditions. Test settings were selected based on preliminary studies and the measurement ranges of the geometry. Correct settings were those where three consecutive measurements showed a difference of less than 7% [22].

A rheological test was conducted, in which the flow curve included the following stages:

1. Heating Stage: shear stress τ = 0.000 Pa; time t = 600.00 s; set temperature T = 20.00 ± 0.50 °C
2. Measurement Stage: shear rate = 1400.0 1/s to 2500.0 1/s in a logarithmic distribution; Time t = 300.00 s; number of measurement points collected = 300; temperature as set in the previous step.

These stages allowed for the precise determination of the rheological properties of the beer samples, ensuring reliable and reproducible results.

2.3. Determination of Shelf-Life Using the Forced Ageing Method

The determination was carried out using a combination of methods described in EBC Analytical 9.30 (Prediction of Shelf-Life of Beer) [23]. Bottles of beer from the analyzed experimental variants were alternately stored at temperatures of 0 °C and 40 °C (so-called thermal day). The clarity of the beers was measured after each thermal day. The procedure was stopped once the beer reached a turbidity increase of 2 EBC units.

The predicted shelf-life of the beer (T), in days, was calculated using the following formula:

$$T = f \times a \tag{2}$$

where a—number of thermal days, f—conversion factor, calculated from the formula:

$$f = \frac{T_{20}}{A_{40}} \tag{3}$$

where T_{20}—shelf-life of the beer, in days, at 20 °C; A_{40}—number of thermal days at 40 °C.

2.4. Statistical Analysis

Each experimental variant was performed in three repetitions due to the material limitation (standard scale of congress wort production) and the preliminary nature of the study. This approach allowed the management of a large number of variants while ensuring internal consistency in the measurements. All procedures were conducted under controlled conditions with standardized instruments, and the variation among replicates remained within acceptable ranges. The obtained results were grouped, and their mean values and standard deviations were calculated. The values were compared and subjected to statistical interpretation. The significance of the influence of the studied variables was determined using a one-way analysis of variance (ANOVA). The significance of differences between the means, in both cases, was verified using Duncan's test ($p < 0.05$). Statistical analysis was conducted using Statistica 13.1 software from StatSoft (Tulsa, OK, USA).

2.5. Experimental Characterization

Laboratory studies were conducted using a beer production apparatus with a Speidel Braumeister (Ofterdingen, Germany) mash–boiling kettle with a capacity of 50 dm^3. Iodine tests were performed for each experimental variant to verify the correctness of the mashing process.

Mashing for each batch was carried out with guidelines similar to the production of congress wort [22] to provide optimal conditions for enzyme activity. The temperature range included the following:

- 46 °C for proteolytic enzymes and β-glucanases.
- 68 °C for β-amylase activity.
- 70–75 °C for optimal α-amylase activity.
- 78 °C for the completion of mashing and filtration of the sweet wort.

The worts were boiled for 60 min. During this time, hops were added twice, according to the specific recipe, at the beginning of the boil and 15 min before the end of the process.

Each batch was produced using 32 dm^3 of water (including 5 dm^3 used for sparging). Two types of laboratory beers were produced. In none of the experimental variants were enzymes used; only clarifying agents were employed. The beers were not subjected to pasteurization. However, selected physicochemical properties were compared with a pasteurized beer available on the Polish market. The experimental variants are summarized in Table 1.

Table 1. Symbols and description of experimental variants.

Variant Symbol	Description
Ind	Industrial beer: water, barley malt, hops
P_0 (without clarifying agent)	Laboratory beer with water (32 dm^3 including sparging), 5 kg barley malt, 60 g Puławski hops
P_chi2 (with chitosan)	
P_chi1 (with chitosan)	
P_col (with collagen)	
M_0 (without clarifying agent)	Laboratory beer with water (32 dm^3 including sparging), 5 kg barley malt, 60 g Magnat hops
M_chi2 (with chitosan)	
M_chi1 (with chitosan)	
M_col (with collagen)	

3. Results

3.1. Turbidity and Clarity of Beers

Figure 1 presents the turbidity values of the experimental beers produced with different hop varieties and clarifying agents, in comparison to a commercial industrial beer. Turbidity was measured at two temperatures: 20 °C (room temperature) and after cooling to 5 °C. Across both temperatures, the industrial beer consistently exhibited the lowest turbidity values (EBC), serving as a benchmark for clarity. All experimental beer variants displayed higher turbidity than the industrial reference at both temperature points. However, beers treated with clarifying agents showed significantly lower turbidity than the unclarified samples, particularly after cooling. Among the clarifying agents tested, the collagen-based agent resulted in the greatest clarity, yielding the lowest EBC values. In contrast, the sample treated with chitosan 2 exhibited the least effective clarification, regardless of hop variety or alpha acid content. A comparison of total turbidity (defined as the sum of values at both temperatures) further supports these observations. Beers

produced without any clarifying agent, irrespective of hop variety, showed the highest total turbidity—36 EBC and 39 EBC. The lowest total turbidity values were recorded in the collagen-treated samples: 10 EBC for beers brewed with Magnat hops and 12 EBC for those with Puławski hops.

3.2. Colour

Figure 2 presents the results of the colour intensity of the analyzed beer variants. The beer produced in the laboratory exhibited a significantly higher colour intensity compared with the industrial beer, regardless of the type of hops or clarifying agent used.

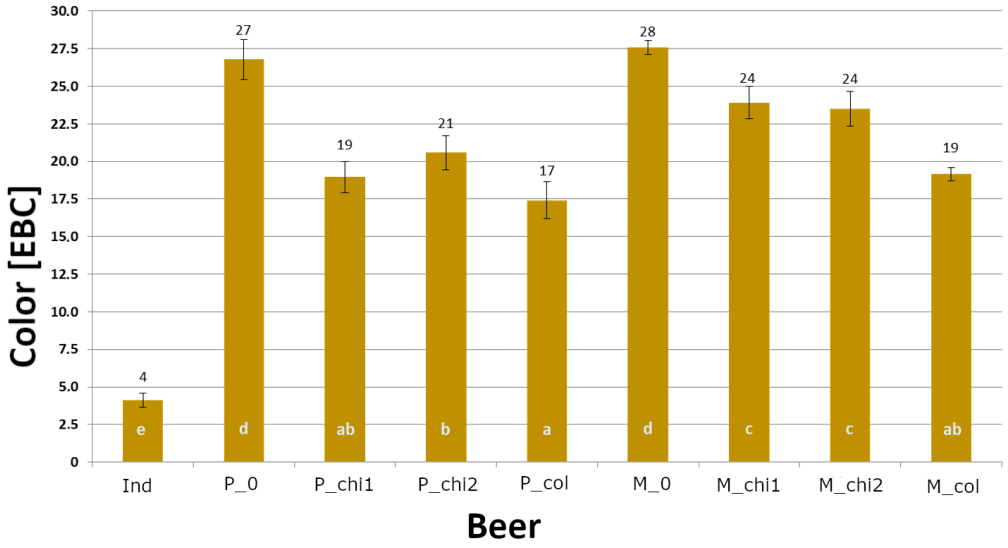

Figure 2. Colour intensity of produced beers (n = 3, α = 0.05; homogenous groups of results within the given parameter are indicated by letters).

The use of clarifying agents significantly reduced the colour intensity of the beers. The greatest difference in colour (less intense colour compared with the unclarified sample) was mainly observed in samples clarified with the agent without chitosan. For the beer with Puławski hops, the colour intensity was reduced from 27 EBC to 17 EBC. For the beer hopped with Magnat, the colour intensity was reduced from 28 EBC to 19 EBC.

3.3. pH

The beers produced in the laboratory with Polish hop varieties exhibited higher pH levels compared with the industrial beer. However, all samples remained within acceptable ranges (Figure 3). All beers had pH values between 4.2 and 4.5. The clarifying agents with chitosan 1 (P_chi1 and M_chi1) resulted in pH values statistically similar to the industrial beer (pH = 4.2). Conversely, beers clarified with chitosan 2 had slightly higher pH values (4.4–4.5) and were not statistically different from most other treatments.

It is important to note that tap water, suitable for drinking but untreated, was used in the production of the laboratory beers.

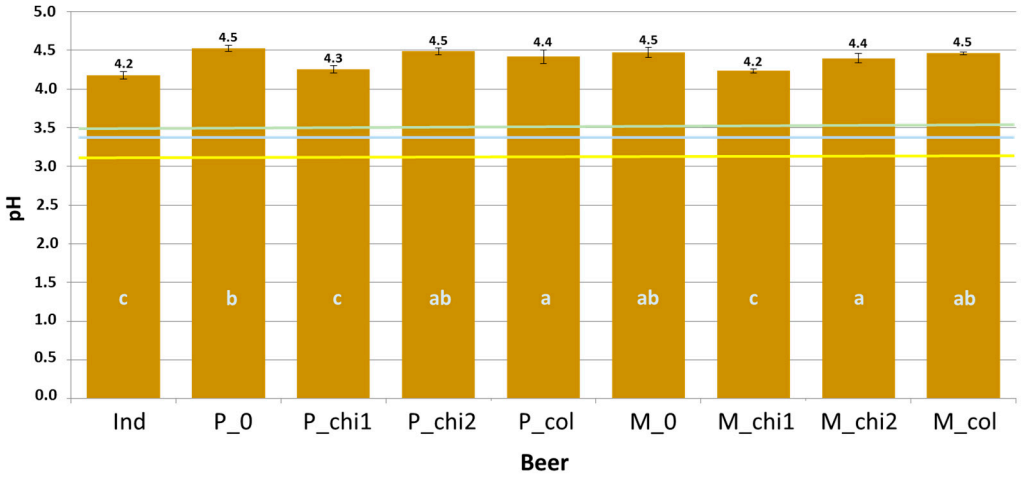

Figure 3. pH levels of produced beers (n = 3, α = 0.05; homogenous groups of results within the given parameter are indicated by letters).

3.4. Surface Tension

The industrial beer exhibited a lower surface tension compared with the laboratory beers (Figure 4). All of the obtained results were significantly lower than the surface tension of distilled water, to which the measuring device was calibrated.

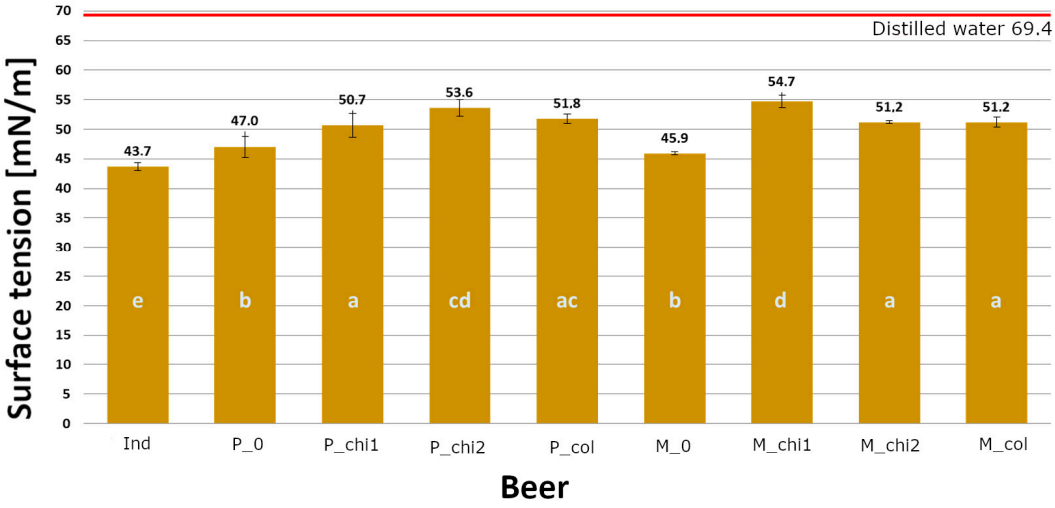

Figure 4. Surface tension of produced beers (n = 3, α = 0.05; homogenous groups of results within the given parameter are indicated by letters).

Surface tension is a physical phenomenon occurring at the interface of a liquid with a solid, gas, or another liquid, manifesting as an elastic, tense membrane. This phenomenon is related to the presence of unbalanced intermolecular attraction forces at the surface and is equal to the force per unit length acting perpendicular to the surface of the liquid.

The addition of clarifying agents increased surface tension values from 47.0 mN/m in the unclarified Pulawski-hopped beer (P_0) and 45.9 mN/m in the unclarified Magnat-hopped beer (M_0) to values exceeding 50 mN/m in all clarified variants.

3.5. Foam Volume

The use of clarifying agents (regardless of composition) contributed to an increase in foam volume in the laboratory beer samples (Figure 5). The laboratory beers had lower foam volumes compared with the industrial beer (620.0 cm^3), with 453.3 cm^3 in the unclarified Pulawski-hopped variant (P_0) and 436.7 cm^3 in the unclarified Magnat-

hopped variant (M_0). After clarification with the collagen-based agent, foam volume increased to 551.7 cm^3 in P_col and 578.3 cm^3 in M_col. The chitosan-based preparations also contributed to an increase in foam volume, although to a lesser extent.

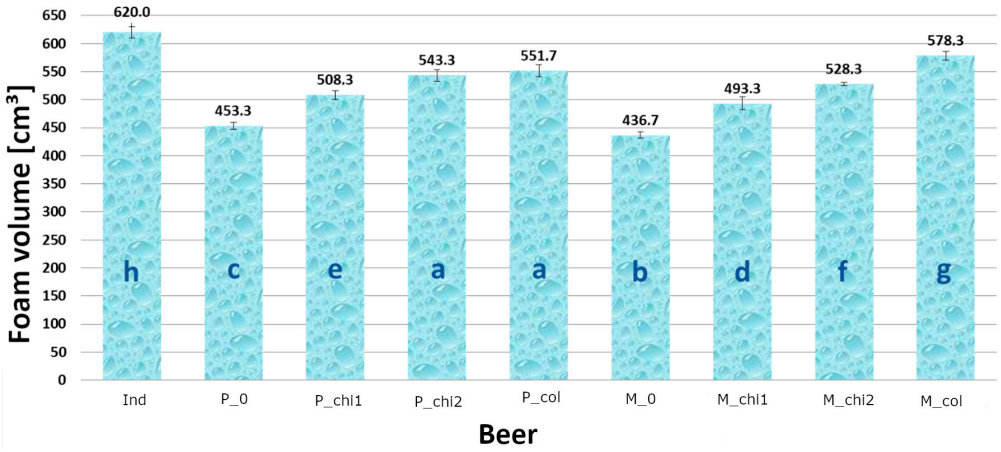

Figure 5. Foam volume of analyzed beer variants (n = 3, α = 0.05; homogenous groups of results within the given parameter are indicated by letters).

3.6. Beer Shelf-Life

The addition of clarifying agents (regardless of composition) significantly affected the shelf-life results of the beer (Figure 6). In unclarified beers, the shelf-life was shorter by an average of 3.5–4.5 days. The composition of the clarifying agents (with or without chitosan) did not significantly affect the extension of the shelf-life of the produced alcoholic product—the difference between treatments was up to 2 days.

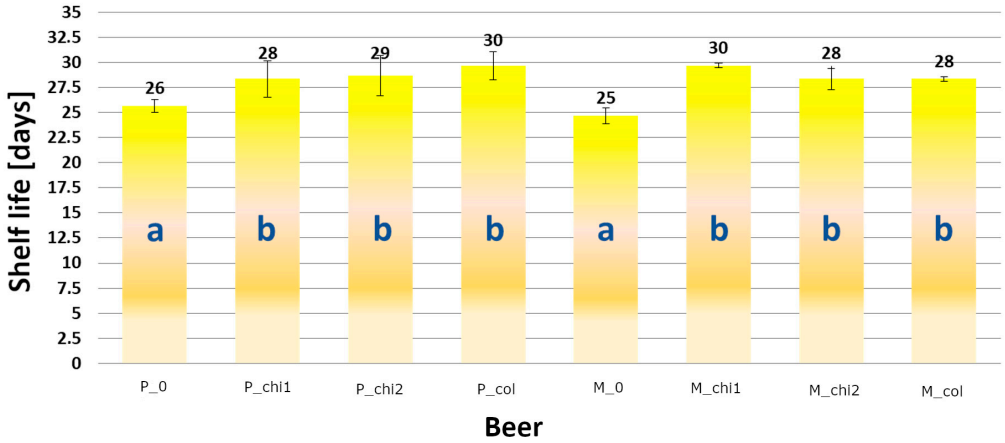

Figure 6. Shelf-life of produced beers (n = 3, α = 0.05; homogenous groups of results within the given parameter are indicated by letters).

3.7. Viscosity

Figure 7 presents the viscosity values of the analyzed beer variants. The laboratory beers exhibited a lower viscosity compared with the industrial beer. The highest dynamic viscosity was observed for the beer clarified with natural collagen (isinglass). Conversely, the lowest viscosity was measured in the variants clarified with chitosan-based preparations and in the laboratory beers that were not clarified (regardless of the hop variety used).

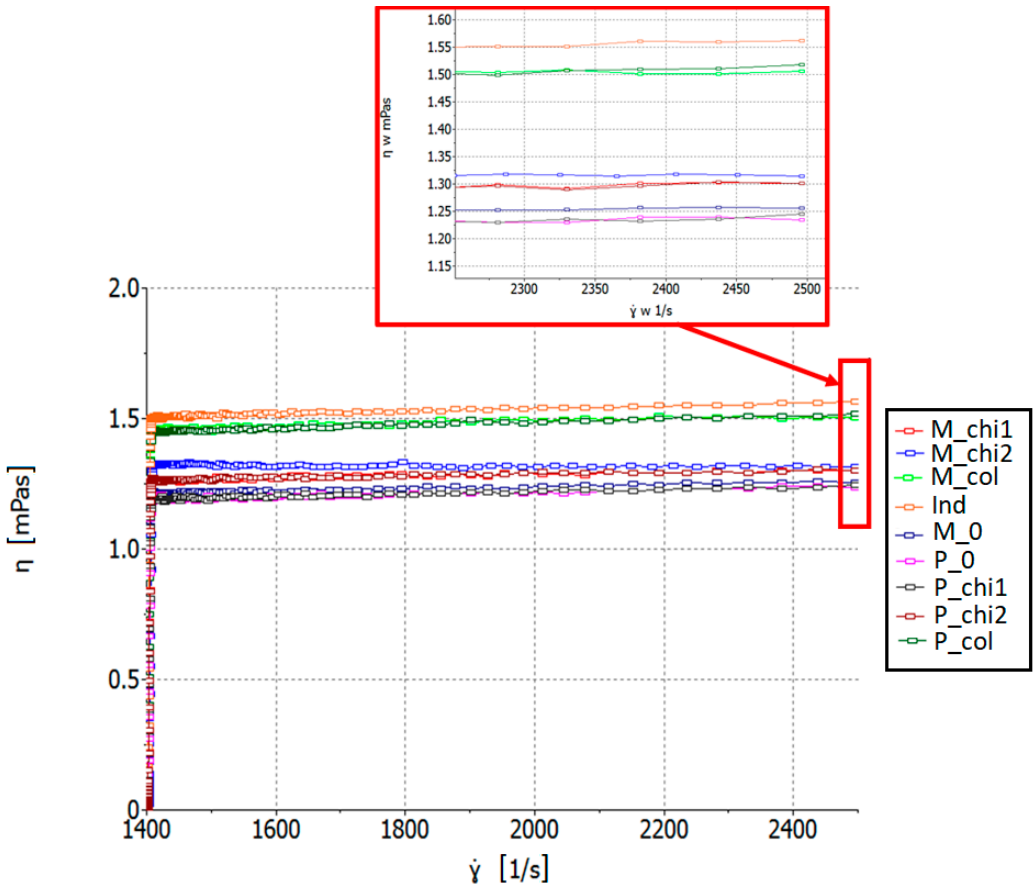

Figure 7. Viscosity of analyzed beer variants.

4. Discussion

Clarity is a key quality parameter in beer, strongly influencing consumer perception and market appeal [24]. This study confirms the importance of clarity in preserving the perceived quality of beer over time, with pasteurized beer generally maintaining clarity and sensory acceptability for 3 to 4 months, depending on processing conditions and raw material quality. These findings align with prior research indicating that consumers generally prefer clear beers, particularly in popular styles such as lager and pilsner [10,25].

The recent literature highlights growing concerns with conventional beer clarification methods such as diatomaceous earth filtration, cold crashing, or centrifugation, which may result in operational inefficiencies, high losses, or undesirable changes in foam stability and flavour [26,27]. Our findings align with the brewing industry's efforts to identify sustainable, non-thermal, and low-impact solutions such as chitosan and collagen. When applied strategically, these agents can improve clarity while preserving sensory quality and reducing equipment demands, especially in small- and medium-sized breweries [28].

The present study demonstrates that both tested clarifying agents—fish-derived collagen (isinglass) and chitosan—effectively improve the visual clarity of beer. Fish collagen exhibited slightly greater effectiveness in turbidity reduction than chitosan, particularly after cold storage. Clarification significantly reduced colour intensity and increased surface tension, while the changes in pH were minimal and not statistically significant. For instance, while fish collagen led to a slight decrease in beer pH, the values remained well within acceptable brewing limits. These findings underscore the suitability of both agents for commercial use, as they enhance clarity without negatively impacting the key physicochemical properties. This balance positions both collagen and chitosan as practical options for brewers aiming to improve haze stability while preserving beer character.

Chitosan, owing to its strong flocculating capacity and unique physicochemical properties, has emerged as a viable alternative to traditional clarifying agents. It effectively coagulates anionic suspended particles such as proteins and pectins, thereby reducing turbidity. In addition to beer, chitosan has been successfully applied in the clarification of various juices—including apple, grape, lemon, orange, passion fruit, and acai—and green tea [29–32]. In those applications, its effectiveness was influenced by concentration and juice pH. For example, the clarification of passion fruit juice at pH 6 resulted in a significantly greater turbidity reduction. Chitosan has been approved for use in wine and grape must clarification by the International Organization of Vine and Wine [13].

In the context of brewing, the present study reinforces the efficacy of chitosan, particularly when used in post fermentation clarification protocols. At a concentration of 5 mL/L, chitosan significantly improved beer clarity, which is consistent with findings by Gassara et al. [12], who reported a high effectiveness at similar dosages. The mechanism of action is linked to chitosan's high charge density, which enhances its ability to form flocs with suspended solids. Compared with traditional agents such as bentonite or gelatin, chitosan has in some cases demonstrated superior performance, such as in apple juice clarification [33,34]. However, the cost of chitosan remains a relevant consideration when evaluating its large-scale applicability in breweries.

Beyond clarifying agents, multiple intrinsic beer properties influence haze formation. The pH level plays a critical role in modulating the charge of proteins and polyphenols, affecting their aggregation potential. Lower pH values tend to reduce free polyphenols, which are involved in haze formation through interactions with proteins [10,35]. Carbohydrates such as α- and β-glucans, pentosans, and various oligosaccharides contribute to colloidal instability [36]. Metal ions (Fe, Cu, Zn, Ca, and K) can accumulate haze particles and also catalyze the oxidative polymerization of polyphenols, converting reversible haze into permanent haze [10]. Additionally, oxygen ingress promotes free radical formation, which accelerates flavour degradation and leads to stale aroma development [9]. These factors emphasize the complex interplay between raw materials, processing, and storage in determining final beer clarity.

Regarding colour, a marked decrease in EBC colour values was observed after clarification, especially in samples treated with fish collagen. Colour values decreased from 27 and 28 EBC to 17 and 19 EBC, suggesting that isinglass not only improves clarity but also impacts beer colour by possibly removing pigments or colloids. This contrasts with observations by Cimini and Moresi [10,29], where microfiltration led to only a minor colour shift (from 7.3 to 6.9 EBC-U). The greater impact observed in this study may stem from higher initial turbidity or differences in the pigment-binding properties of the clarifying agents.

The pH of the beers remained stable after clarification, with values averaging around 4.5. This is consistent with the findings of Cimini et al. [29], indicating that neither isinglass nor chitosan significantly alter the acidity profile of beer. However, differences were observed in the foam characteristics. In this study, foam volume increased slightly after clarification, suggesting that the removal of haze-forming proteins may enhance foam formation by reducing surfactant-like particles that destabilize foam. Conversely, Cimini et al. [29] noted reduced foam retention in microfiltered beer. These differing outcomes highlight the importance of clarifier selection and processing context.

The viscosity analysis revealed further distinctions between clarifying agents. The beers clarified with chitosan exhibited a lower viscosity (1.20–1.30 mPa·s), while those treated with fish collagen (without chitosan) had a higher viscosity (1.50–1.60 mPa·s). These values were in line with the previous literature, including microfiltered beer reported by Sadosky et al. [36], which showed a viscosity of 1.4 mPa·s. The increased viscosity in the

collagen-only variants may be attributed to residual polysaccharide or protein interactions not fully removed by the clarifier.

Finally, while this study focused on collagen and chitosan, alternative agents such as avian collagen and pea protein extracts have shown comparable clarifying potential. Walker et al. [5] demonstrated that these agents performed similarly to fish collagen in terms of clarity and the preservation of beer quality. As consumer demand grows for non-animal and sustainable brewing inputs, further industry trials are warranted to evaluate the cost-effectiveness, sensory impact, and regulatory acceptance of these alternatives. The emerging literature increasingly acknowledges the limitations of conventional clarification techniques—such as filtration with diatomaceous earth, centrifugation, or thermal treatments—which can compromise product quality or increase operational burdens. For instance, Bertuzzi et al. [37] demonstrated that while filtration improves clarity, it may also reduce the phenolic content of and associated antioxidant activity in beer, affecting both the nutritional value and sensory complexity. Similarly, Ghosh et al. [38] emphasized that aggressive processing steps can alter foam retention and body, key parameters for consumer acceptance. Moreover, Gobbi et al. [31] highlight a growing demand among craft brewers for functional, sustainable solutions that enhance visual and nutritional qualities without resorting to resource-intensive methods. Against this backdrop, the present study contributes by validating collagen and chitosan as effective, low-impact clarifying agents, compatible with evolving quality standards and consumer expectations in both the large-scale and artisanal brewing sectors [39–43].

These findings collectively support the use of collagen and chitosan as effective tools in beer clarification, offering substantial improvements in visual clarity while maintaining other physicochemical and sensory parameters. Nevertheless, a larger scale study with more repetition is still needed to ensure the greater statistical power and reliability of the results. As the brewing industry continues to evolve, integrating consumer-driven preferences for clear, stable, and ethically produced beers, such agents may play an increasingly central role in modern brewing practice.

5. Conclusions

This pilot study evaluated collagen- and chitosan-based clarifying (fining) agents and their effects on beer clarity, stability, and physicochemical properties using laboratory brews produced with Polish hop varieties. The key takeaways—each qualified for low statistical power—are as follows:

1. The effectiveness of clarifying agents
   In this pilot dataset, the collagen-based fining agent achieved the lowest turbidity (EBC) across hop variants and temperatures, indicating stronger visual clarity improvements under our conditions.
   Pilot caveat: The result reflects a small n and laboratory scale; the effect size and rank order require confirmation with more replicates, beer styles, and process settings.
2. The role of hop variety
   Across the two hop lots (alpha acids 7.5% and 14.5%), we did not detect clear differences in clarification effectiveness or core physicochemical parameters relative to agent choice.
   Pilot caveat: The absence of detected differences may reflect limited statistical power; broader hop chemistries (oil profiles, polyphenols) and more replicates could reveal effects.
3. Shelf-life and physical stability
   Clarification was associated with improved short-term stability (e.g., lower turbidity, altered surface tension). We did not observe a consistent advantage of one agent over

another for longer term stability in this study.
Pilot caveat: The stability findings are time- and condition-limited and based on a small sample; longer, replicated storage trials are needed.

4. The effect on viscosity
Chitosan-treated beers tended toward a lower viscosity than collagen-treated beers and the industrial reference, which could aid operations (pumping/filtration), while a higher viscosity can benefit sediment compaction.
Pilot caveat: The viscosity trends are preliminary and method-specific; confirmation with more batches and process scales is required.

The overall conclusion is as follows:

Both collagen- and chitosan-based finings appear effective for clarity and stability in this preliminary, low-power study, with collagen showing the strongest turbidity reduction in our conditions and chitosan showing potentially favourable viscosity trends. These results are not definitive and should be validated in larger scale, well replicated brewery trials before informing industry practice.

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