

Original article

# The Influence of Protein Content on Beer Quality and Colloidal Stability<sup>1</sup>

Ariola Devolli <sup>a,\*</sup>, Frederik Dara <sup>b</sup>, Merita Stafasani <sup>a</sup>, Edlira Shahinasi <sup>a</sup> & Mariola Kodra <sup>a</sup>

<sup>a</sup> Department of Chemistry, Faculty of Biotechnology and Food, Agricultural University of Tirana, Albania

<sup>b</sup> Department of Applied Mathematics, Faculty of Natural Sciences, University of Tirana, Albania

## Abstract

Protein content and beer composition depend on the raw materials and enzymatic reactions used in brewery technology. In order to improve the colloidal stability of beer, it is necessary to remove both protein and polyphenolic complexes or prevent their formation. This study aims to determine the protein content in all production stages and to evaluate its influence on beer quality and colloidal stability.

Analyzes of total protein content were performed by Kjeldahl and spectrophotometric methods.

Statistically significant change was observed in the protein content of the final product, which was less than that in wort ( $P < 0.05$ ). Turbidity, colour, extract, alcohol, bitterness, foam and pH were also measured.

Haze forcing tests (incubation at 20<sup>o</sup>C, 40<sup>o</sup>C and 60<sup>o</sup> C) were conducted to evaluate the colloidal stability of the final product. Results of tests showed that the highest level of product stability was ensured after treatment of beer with both silica gel and polyvinylpolypyrrolidone (PVPP).

**Keywords:** Beer quality, Colloidal stability, Protein content.

**Received:** 31 August 2018 \* **Accepted:** 23 November 2018 \* **DOI:** <https://doi.org/10.29329/ijjaar.2018.174.12>

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## \* Corresponding author:

Ariola Devolli, Department of Chemistry, Faculty of Biotechnology and Food, Agricultural University of Tirana, Albania.  
Email: [adevolli@ubt.edu.al](mailto:adevolli@ubt.edu.al); [arioladevolli@gmail.com](mailto:arioladevolli@gmail.com)

<sup>1</sup> A part of this study was presented at the International Agricultural, Biological and Life Science Conference, Edirne, Turkey, September 2-5, 2018.

## **INTRODUCTION**

Beer consists of various ingredients such as proteins, carbohydrates, polyphenols, fatty acids, nucleic acids, amino acids etc. These ingredients can precipitate and haze is formed. Malted barley contains 70–85% total carbohydrates, 10.5–11% proteins, 2–4% inorganic matter, 1.5– 2% fat and 1– 2% other substances (Kunze, 2007).

Proteins and macromolecules from raw materials undergo several changes during brewing. Throughout mashing proteins are solubilized and transferred into the produced wort; in wort boiling proteins are glycosylated and coagulated and during fermentation and maturation process, proteins aggregate as well, because of low pH (Steiner et al., 2011).

Mashing is a biochemical process step of brewing that completes the enzymatic degradation started during malting. In order to provide good quality of beer production, part of the insoluble native protein must be converted into soluble protein during malting and mashing (Jones et al., 2005).

Beer proteins may be defined as a more or less heterogeneous mixture of molecules containing the same core of a peptide structure, originating from only one distinct protein present in the brewing materials (Steiner et al., 2011).

Several classes of phenolic compounds have been found in beer, including simple phenols, benzoic acid derivatives, cinnamic acids, coumarins (Dvorakova et al., 2007; Nardini 2004), chalcones, flavanones, flavones, flavan3-ols, proanthocyanidins (Callemien et al., 2008b), alpha acids, iso-alpha acids and other miscellaneous compounds (Gerhäuser, 2005).

The final polyphenols content of beer depends largely on brewing practice and raw materials. In beer, flavonols could be responsible for bitterness but do not participate in beer haze formation (Callemien et al., 2010).

Beer haze consists of several components: the most common organic parts are proteins (40–75%), polyphenols (in combination with proteins) and to a smaller proportion carbohydrates (2– 15%) (Steiner et al., 2010).

Foam occurs on dispensing the beer as a result of the formation of CO<sub>2</sub> bubbles released by the reduction in pressure. Beer foam is stabilized by the interaction between certain beer proteins and isomerized hop  $\alpha$ -acids, but destabilized by lipids (Van Nierop et al., 2004).

Proteins play a major role in beer stability; hence, they are, beside polyphenols, part of colloidal haze. There exist two forms of haze; cold break (chill haze) and age-related haze (Steiner et al., 2011a).

The formation of this, so-called cold haze, represents a reversible process and it disappears at +20°C. While low-molecular flavonols in the reaction with tannins do not influence haze, irreversible haze is formed in the reaction with oxidized tannins (Marković et al., 2003).

Cold break haze forms at 0°C and dissolves at higher temperatures. If cold break haze does not dissolve, age-related haze develops, which is non-reversible. Chill haze is formed when polypeptides and polyphenols are bound non-covalently. Permanent haze forms in the same manner initially, but covalent bonds soon form and insoluble complexes are created which will not dissolve when heated (Siebert et al., 1996).

Particles which do not originate from organic sources such as barley, hops, yeast and water are, in this context, called inorganic matter. Inorganic components are often dirt particles, which are present due to poor cleaning and filter aids. These substances are comprised of dust particles, remains of labels, filtration aids, etc. Filter and stabilization aids can appear in beer as haze, if these particles pass the filters and the trap-filters (Smythe et al., 2002).

The most common reason for non-biological haze formation in beer during storage is aggregation of haze-forming polyphenols with haze-forming proteins (Bamforth, 1999).

Consumers expect lager and filtered beer to be 'bright' or haze free, a quality resulting from colloidal stability. A beer is considered 'bright' if no haze forms when chilled to 4°C or below (Briggs et al., 2004; Lewis et al., 2002).

The technological differences between the production methods of different beers strongly affect the significance and influence of individual haze-forming precursors (Dienstbier et al., 2011).

Colloidal instability due to interactions between polyphenols and proteins limits the shelf life of beer. A lag phase is usually observed in lager beers before chill-haze development (Collin et al., 2013).

The time needed to form critical amounts of tanning polyphenols leading to visible chill-haze particles corresponds to the lag phase. At different batches, the longer the lag phases, the better the colloidal stability (Leemans et al., 2003).

Colloids in beer contribute to beer haze and premature shelf-life of the product. Methods to minimize, and in some cases, eliminate, the formation of colloidal particles such as proteins and polyphenols have been developed since beer production began, and those methods are continuously improving today (Heverley et al., internet source).

For beer quality parameters evaluation several storage temperature regimes were elected: +10±1 °C, +20±1 °C, +30±1 °C temperature. Data from scientific literature indicate that if quality of food product could be without changes during 20 weeks storage at +20±1°C and during 10 weeks storage at +30±1 °C, as a result the speed of reaction become redouble. During beer storage at temperature +40±1 °C, the speed of reactions increases 2.5–3.0 times (Steele, 2004).

This paper aimed to determine the protein content and its influence on beer quality during production stages. Our study provides information about colloidal stability and beer shelf life. In order to reduce haze formation there were applied various techniques of removing colloidal particles.

### **Materials and methods**

This work was carried out during 2015-2016 in an Albanian brewery. The experiment and laboratory tests were conducted in brewery laboratory according to beer standard methods issued by the European Brewery Convention. Subject of experiment was lager beer. Beer analyses were performed during all production stages.

#### ***Sample Preparation***

Beer samples were tempered at 20°C and degassed prior to testing, as bubbles will cause fault results. There was used magnetic stirrer until all gas has been released.

#### ***Analytical methods***

Analytical methods for determination of extract, alcohol, bitterness, color, pH, foam, haze and shelf life of beer are described in Analytica EBC by European Brewery Convention (2008, 1998).

Beer pH was measured with pH-meter. Before pH measurement, the CO<sub>2</sub> was removed from the beer samples by shaking at room temperature (18–20°C).

Total acidity measurement was conducted with titration method expressed as mg acetic acid/100 ml beer.

The determination of alcohol content was performed by measuring the density of distillate from the degassed beer sample, which distillate is assumed to contain all the alcohol in the sample and nothing else except water. The alcohol content of beer is expressed in percentage (% v/v).

#### ***Color determination***

Color of the degassed beer was measured at 430 nm by a UV–vis spectrophotometer according to the EBC method (European Brewery Convention, 1998). Color was expressed in EBC units and calculated according to the formula:

$$C = A_{430} \cdot f \cdot 25$$

where C gives the color (EBC), f is the dilution factor and A<sub>430</sub> is the absorbance at 430 nm.

#### ***Determination of turbidity***

Determination of haze and beer turbidity was conducted by nephelometric method. There was used Nephelometer Models 800 and 800P with measurement range (0-19.99) – (0-199.9) NTU and precision (+/- 2% or 0.05 NTU). The amount of turbidity is expressed in EBC units. The instrument was

standardized according to European Brewery Convention (2008) with formazin suspension in EBC units (EBC u.). The amount of turbidity was measured in NTU units and was expressed in EBC unit.

#### ***Determination of bitterness***

Bitterness was measured using a spectrophotometer (UV–vis) at 275 nm. A 10 ml sample of degassed beer, 1 ml of 3 M HCl and 20 ml of iso-octane were pipetted into a test tube and intensively mixed for 5 min. After 10 min of incubation, the phase of iso-octane was carefully decanted in test cuvettes and covered. After 30 min of resting in the dark, samples were measured at 275 nm in 10 mm quartz cuvettes. Pure iso-octane was used as a blank. Beer bitterness was calculated according to the formula:

$$B = 50 \cdot A_{275}$$

where B gives beer bitterness (IBU) and  $A_{275}$  is the absorbance at 275 nm (European Brewery Convention, 1998).

#### ***Determination of total polyphenols***

Total polyphenol content in the beer was measured using a spectrophotometer UV–vis. A 10 ml degassed beer sample and 8 ml of CMC/EDTA reagent (Carboxymethyl cellulose) were transferred into a volumetric 25 ml flask and thoroughly mixed. A 0.5 ml aliquot of ferric reagent (3.5% ammonium iron citrate) was added to the sample and homogenized. Afterwards, 0.5 ml of ammonia reagent (ammonia–water, 1:2) was added and mixed again. Finally, the volume was topped up to 25 ml with distilled water, homogenized and incubated for 10 min. The solution was measured at 600 nm. Total polyphenols were calculated according to the formula:

$$P = A_{600} \cdot 820$$

where P gives the total polyphenol concentration (mg/L) and  $A_{600}$  is the absorbance at 600 nm (European Brewery Convention, 1998).

#### ***Determination of protein content***

The standard method for determining protein content of beer is the Kjeldahl method. Nitrogenous compounds in the beer were digested with hot sulphuric acid in the presence of selenium–copper sulphate catalyst, to give ammonium sulphate. The digest was made alkaline with sodium hydroxide solution and released ammonia is distilled into an excess of boric acid solution. The ammonia was titrated with standard acid solution (European Brewery Convention, 2007).

Calculation: 
$$\%N = \frac{[V(1)-V(BI)] \cdot F \cdot c \cdot f \cdot M(N)}{m \cdot 1000} \cdot 100\%$$

$$\%P = \%N \cdot PF$$

where V(1) - consumption of titrant, sample (ml); V(BI) - average consumption of titrant, blank (ml), F - molar reaction factor (1 = HCl, 2 = H<sub>2</sub>SO<sub>4</sub>); c - concentration of titrant (mol /L); f - factor of titrant; M(N) - molecular weight of N (14,007 (g/mol); m - sample weight (g); 1000 - conversion factor (ml in L); PF - protein factor (6.25); % N - % of weight of N; %P - % of weight of protein content.

Protein content was determinate also by spectrophotometric methods. In this method a diluted beer sample was measured spectrophotometrically to determine the protein content (% , w/w) in finished beer. Absorbance of the sample is measured at 215 nm and 225 nm. From these values (plus the total polyphenol content for stabilized beer samples), the protein content can be determined (Manual, 2017).

### ***Haze forcing tests***

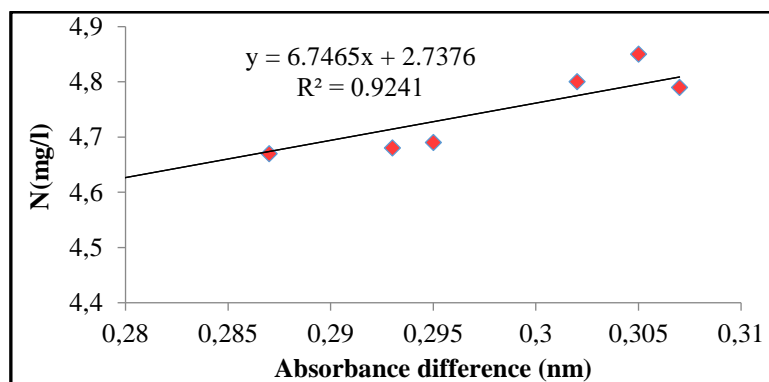
Accelerated aging of beer samples in bottles was performed by storage at an elevated temperature according to MEBAK Analytica (2002). The colloidal stability of the final product was evaluated after samples incubation at 20°C, 40°C and 60°C.

### ***Statistical analysis***

Beer analyses were conducted with 3 replications. Data are expressed as mean ± standard deviation. The statistical analysis of data was carried out with one-way analysis of variance (ANOVA) using the SPSS software (significance level at 0.05). Graphs were performed by using Graph Pad Prism 6 software.

### **Results and discussion**

This research was focused on the influence of protein content on beer quality and its shelf life. The impact of the malt quality on the chemical composition of beer was evaluated. In the experimental part of this work we analyzed different malt types used for wort and beer production. Table 1 shows data analysis of malt. Total protein content in malt was within the permitted levels of lager beer production.



**Figure 1.** Calibration curve of total nitrogen content

**Table 1.** Measured values of the analyzed malt samples

Parameters	Min	Max	Mean	St.dev	C.I.
Moisture content (%)	4.97	6.18	5.533	0.609	0.689
Fine extract (%)	77.34	81.3	79.38	1.983	2.244
Coarse dry extract (%)	74.87	79.25	77.337	2.242	2.537
Extract difference	1.61	2.47	2.043	0.43	0.487
Wort color (EBC)	3.6	4.3	3.9	0.361	0.408
Friability (%)	80.42	83.67	81.95	1.633	1.848
Total nitrogen (N %)	1.71	1.79	1.747	0.04	0.046
Total Protein (%)	10.7	11.2	10.917	0.257	0.29
Soluble Protein (%)	4.85	5.2	4.983	0.189	0.214

Total nitrogen values were obtained from the sum of all nitrogenous compounds present and were determined by the Kjeldahl and spectrophotometric method. Figure 1 shows calibration curve prepared for total nitrogen determination. Absorbance is given as difference of  $\lambda_{215}-\lambda_{225}$ .

Proteins influence the whole brewing process not only in the form of enzymes but also in combination with other substances such as polyphenols. As enzymes, they degrade starch,  $\beta$ -glucans, and proteins. In protein-protein linkages, they stabilize foams and are responsible for mouth feel and flavor stability, and in combination with polyphenols, they are thought to form haze (Steiner et al., 2009).

Depending on beer type, up to 80% of beer polyphenols are said to derive from malt, with the remainder originating from hops (Callemien et al, 2008a). Table 2 presents wort data analysis.

**Table 2.** Measured values of the analyzed wort samples

Statistic results	Extract (°P)	pH	Colour (EBC)	Bitterness (IBU)	Total acidity (mg acetic acid/100 ml beer)	Polyphenols (mg/l)
<b>Min</b>	10.60	5.08	6.10	26.50	0.90	139.40
<b>Max</b>	11.10	5.24	13.25	35.00	1.50	221.00
<b>Mean</b>	10.81	5.19	7.65	29.39	1.08	165.56
<b>Stdev</b>	0.13	0.05	2.08	2.08	0.17	23.08
<b>C.I.</b>	0.06	0.02	0.99	0.99	0.08	10.97

During the brewing process, there are three possibilities to discard the (unwanted) proteinic particles. The first opportunity is given during wort boiling, where proteins coagulate and can be removed in the “whirlpool”. The second, during fermentation, where the pH decreases and proteinic particles can be separated by sedimentation. The third step is during maturation of beer. During maturation, proteins adhere on the yeast and can be discarded (Wiesen et al., 2011).

Measurement of protein content expresses as total nitrogen content in mg/L was performed to all beer production stages. Results of protein content obtained from each production stages are presented in Table 3.

**Table 3.** Total nitrogen content in different beer production stages

Process	Malt	Wort in brew house	Wort after whirlpool	Beer maturation	Beer after filtration	Bottled beer	Beer after 12 months
N (mg/l)	1765 ± 35.66	164 ± 9.86	88.33 ± 2.85	63.33 ± 9.62	38.33 ± 3.64	37.33 ± 3.64	20.66 ± 3.64

From the data obtained (Table 3) it can be seen a significant reduction of total nitrogen content from malt to brew house as well as in other studies. Protein content was further reduced due to maturation and filtration processes.

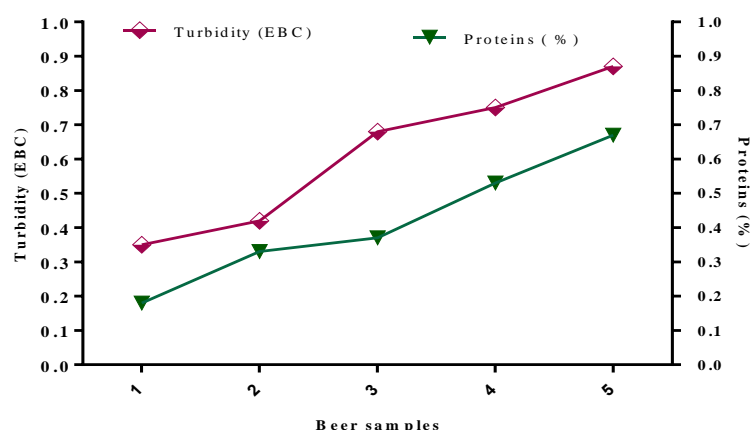
Based on paired samples test was observed statistically significant change in the protein contents of wort and final product which was less than that in wort (Table 4).



**Table 4.** Paired Samples Test for total nitrogen content

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% C.I of the Difference				
					Lower	Upper			
Pair 1	Wort - Bottled beer	51.0000	5.29150	3.05505	37.85518	64.14482	16.694	2	0.004

Figure 2 shows the influence of protein content on beer turbidity (haze formation). There were tested five beer samples with different protein content.



**Figure 2.** The influence of protein content on beer turbidity

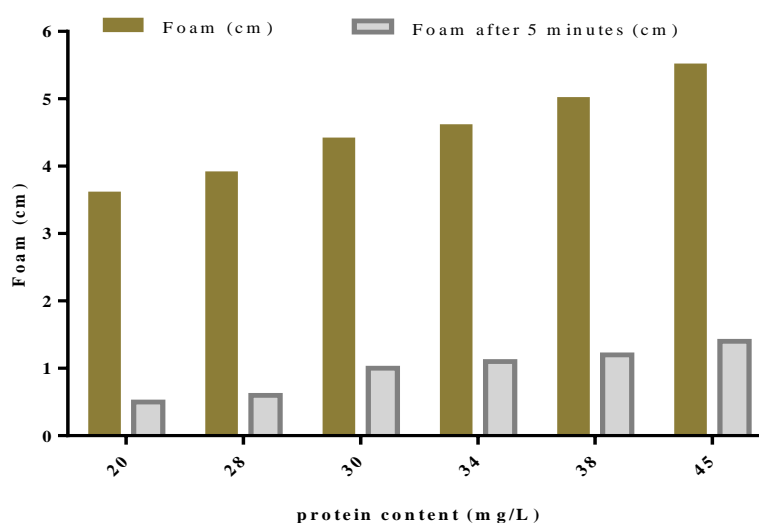
It was noticed reduction of turbidity on beer samples with low protein content (Figure 2). Sample 5 presented high level of protein content and hence higher beer turbidity and lower colloidal stability.

Proteins influence two main quality aspects in the final beer: foam stability and haze formation (Evans et al., 2002).

Most beer drinkers are inclined to prefer beer displaying stable foam and prefer seeing foam adhering to the side of the glass (cling, lacing) (Roza et al., 2006).

The production of foam represents a huge increase in surface area, which is counter to the force of surface tension (Bamforth, 2011).

Beer samples with different protein content were tested for formation and retention of head foam (Figure 3).



**Figure 3.** The influence of protein content on beer foam

Foam stability (head retention) was measured by drainage methods based on a simple glass apparatus. Measurement of foam height was performed before and after five minutes. It was obviously that higher foam head and good stability was achieved in beers with high level of protein content.

Beer haze is primarily formed through complexation of protein and polyphenolic beer ingredients. The problem of reducing susceptibility of beer haze formation can be done either by lowering protein and/or polyphenol levels, or by minimizing the molecular size of protein/polyphenols (Marković et al., 2003).

To preserve beer colloidal stability, brewers usually remove haze-active materials (Siebert, 1999).

To get rid of haze-active proteins, precipitation and adsorption to bentonite or silica gel are very effective, but unfortunately in some cases, such procedures also remove foam proteins (Leiper et al., 2003).

Unfiltered beer samples (100 ml) were treated with different silica gel amounts as absorption agent. Beer parameters before filtration were: 0.55% protein content, color =8.35 EBC, polyphenols= 180.56 mg/L and turbidity= 11.45 EBC. Table 5 shows results of beer analysis after treatment.

**Table 5.** Results of beer analysis after treatment with silica gel

Silicagel quantity (g/100ml beer)	filtration time (min)	Turbidity (EBC)	Filtrate volume (ml)	Colour (EBC)	Polyphenols (mg/L)	Protein content (%)
3	12	1.1343	88	9.95	158.26	0.55
7	24	1.3597	88.3	10.15	157.44	0.5
12	31	1.7052	84	10.725	155.68	0.33
15	34	2.8567	80	10.925	145.04	0.2

Based on results (Table 5) we can say that addition of silica gel amount and extension of filtration time decrease the protein content.

Although conditioning-maturation, clarification, and stabilization plays an important role in reducing yeast and haze loading materials, a final beer filtration is needed in order to achieve colloidal and microbiological stability (Goldammer, 2008). The most commonly used stabilizers for removing proteins is amorphous silica gel and kieselguhr (diatomaceous earth). Polyvinylpolypyrrolidone or PVPP is typically used for removing haze-active polyphenols (Devolli et al., 2015).

Beer filtration was performed using three types of kieselguhr: DIF-rough-size kieselguhr, CBR-middle-size kieselguhr and CBL3 -fine-size kieselguhr. There was prepared the filter cake and a mixture of kieselguhrs for dosage. Three different experiments were conducted to unfiltered beer (100 ml) with parameters: 0.65% protein content, 180.5 mg/L polyphenols, colour 10.85 EBC and turbidity 10.65 EBC (Devolli et al., 2015).

**Table 6.** Results of beer analysis after the filtration with kieselguhr

Experiments	Filtration time (min)	Filtrate volume (ml)	Turbidity (EBC)	Colour (EBC)	Poliphenols (mg/l)	Protein (%)
1	18	78	0.432	8.125	168.1	0.48
2	25	86	0.375	7.87	178.25	0.5
3	15	80	0.735	11.35	180.5	0.62
No	Filter cake		Filtration dosage			
Exp 1	2g CBR + 2g DIF		2g CBR + 2g CBL <sub>3</sub>			
Exp 2	2g CBR + 2g DIF		4g CBL <sub>3</sub>			
Esp 3	2g CBR + 2g DIF		4g CBR			

From the data of Table 6 it can be seen that lowest turbidity, polyphenols and protein content was found in the sample of experiment 1.

Maintaining beer quality during production stages, distributions and shelf storage remains an extensive challenge. While several attributes are used to establish overall beer quality, two aspects in particular have received considerable attention: colloidal and flavor stability. The establishment of colloidal stability in beer renders a beer 'bright', or hazes free (Aron et al., 2010).

Elimination of turbidity, reduction of protein and polyphenol levels, is obtained by applying low temperature (beer cooling) before the final step of filtration and the usage of insoluble absorbent PVPP (Polyvinylpolypyrrolidone).

Unfiltered beer (200 ml: polyphenols 185.5 mg/L, turbidity 9.56 EBC, color 11.35 EBC and protein content 0.6%) was treated with different PVPP amounts. Results of those tests are shown in Table 7.

**Table 7.** Results of beer analysis after the treatment with PVPP

PVPP quantity (g/100ml beer)	Filtration time (min)	Turbidity (EBC)	Filtrate volume (ml)	Colour (EBC)	Polyphenols (mg/l)	Protein content (%)
3	35.23	1.72	196.22	8.87	130.25	0.52
7	42.3	1.95	192.65	11.55	111.25	0.45
12	44	4.95	187.36	11.78	98.25.6	0.31
15	48.57	6.47	183623	12.35	80.7	0.17

It was found that treatment of beer with PVPP decrease the polyphenols, turbidity and protein content even using a small quantity (3 gr PVPP). The experiments have proved that selective removal of polyphenoles via PVPP is the best way to prevent the formation of turbidity and to keep the taste unchanged. In this conditions beer remains fresh and clear for a long time. These results agreed with other studies (Devolli et al., 2015).

Experiments of beer treatments with bentonite (silica gel) and filter aids (kieselguhr and PVPP) were conducted at laboratory conditions.

Haze formation is an important problem in beer production, as it affects the quality of the end product (Lingzhen Ye et al., 2015).

Haze is customarily divided into “chill haze”, which develops when beer is chilled to 0°C, but returns into solution when the beer is warmed to 20°C, and “permanent haze”, which is present in beer at all practical temperatures (Clark et al., 2007).

In bright beers, the formation of permanent haze is a serious quality problem that places limitations on the storage life of the product (Robinson et al., 2004).

The main objective of the brewery was to achieve good flavor and beer haze stability. To obtain a good quality and a long shelf life, it was necessary to keep under control beer turbidity (haze formation). Beer samples treated with absorption agents and pre-filtered with kieselguhr and PVPP at brewery scale were subjected to haze forcing tests.

There were performed three different haze forcing tests as follow: first test: 20 ±1°C for 2 days and 1 day in 0°C; second test: 40 ±1°C for 2 days and 1 day in 0°C and third test: 60±1°C for 2 days and 1 day in 0°C.

**Table 8.** Beer turbidity measured before and after haze forcing tests

	First test	Second test	Third test
Turbidity before (EBC)	0.54±0.029	0.533±0.017	0.535±0.015
Turbidity after (EBC)	0.76±0.017	0.92±0.06	2.08±0.85

Table 8 shows beer turbidity measured (haze formation expressed as turbidity level) before and after each forcing test. A good colloidal stability of beer samples was achieved after the first and second tests (turbidity 0.76- 0.92 EBC), while the third test decrease beer colloidal stability (turbidity 2.08 EBC).

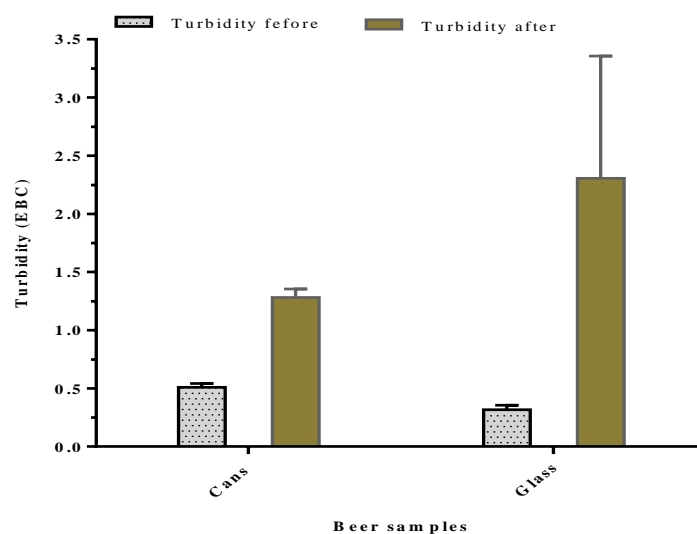
**Table 9.** Measured values of beer samples after haze forcing tests

Statistics	Polyphenols (EBC)	Turbidity before (EBC)	Turbidity after (EBC)	pH	Acidity (mg acetic acid /100 ml beer)	Colour before (EBC)	Colour after (EBC)	Bitterness (EBC)	Alcohol (%v/v)
<b>cans (0.5 l)</b>									
min	194.60	0.47	1.18	4.20	2.10	7.50	9.80	18.70	4.40
max	204.60	0.55	1.36	4.31	2.40	8.75	10.37	21.75	5.25
Mean	199.22	0.51	1.28	4.25	2.20	7.96	10.05	20.00	4.84
Stdev	3.94	0.03	0.07	0.05	0.12	0.48	0.21	1.35	0.34
C.I.	3.45	0.03	0.06	0.04	0.11	0.42	0.18	1.18	0.30
<b>glass (0.5 l)</b>									
min	118.08	0.26	0.98	4.08	1.80	6.87	9.78	19.83	4.45
max	179.30	0.35	3.10	4.34	2.30	8.45	10.85	23.50	5.15
Mean	155.86	0.32	2.31	4.20	2.07	7.61	10.17	22.12	4.76
Stdev	31.42	0.04	1.05	0.10	0.20	0.63	0.44	1.51	0.26
C.I.	27.54	0.03	0.92	0.09	0.17	0.55	0.39	1.32	0.23

During storage, oxidizing and reducing reactions take place in beer samples under the influence of several physical and chemical factors including temperature, light and dissolved oxygen. Oxidizing reactions change the chemical composition of the sample and bring about sample aging, which influences colloidal and sensorial stability. The colloidal system is displaced away from the equilibrium, some compounds aggregate and haze develops (Bamforth et al., 1999).

The colloidal stability was analyzed in bottled beer (glass and cans). Haze measurement expressed as turbidity level and other chemical beer analysis were performed after application of haze forcing tests (Table 9).

Beer chemical parameters had not significant difference before and after haze forcing tests, except turbidity. Turbidity provides the consumer's first visual impression of beer quality. Consumers expect a filtered beer to be a clear, bright, non-hazy product that remains so during its shelf life. Hazy products are often regarded as defective and perhaps even potentially harmful. Therefore, controlling haze formation is an important problem in beer production.



**Figure 4.** The Impact of haze forcing test on beer turbidity

The initial turbidity of all beer samples was less than 0.5 EBC. As it can be seen from Figure 4 turbidity level was increased in both beer samples. Beer bottled in glass had higher level of turbidity than one in cans (2.3 and 1.36 EBC, respectively) due to light and temperature effects.

### Conclusions

Proteins play an important role for mouth feel, foam stability and shelf life of final product. These aspects are important for brewers, since consumer judge beer also according to these quality attributes.

Analytical investigation showed a significant reduction of protein content through brewing processes.

Protein content was an important factor in beer stability and haze formation. Based on experimental results it was noticed that beer samples with high protein content increase the level of turbidity and tend to form haze.

This research was focused on protein content influence on beer quality, haze formation and colloidal stability. Special emphasis was placed on protein content analyses. Filtration trials have showed that the material used to filter the beer (kieselguhr and PVPP) were able to remove haze protein, thus improving the colloidal stability of beer. The experiments have proved that selective removal of polyphenols via both silica gel and PVPP was the best way to prevent the formation of turbidity, to keep the taste unchanged and therefore a long shelf life of beer.

It was detected, that during haze forcing tests at  $+40\pm 1$  °C and  $+60\pm 1$  °C, significant changes of turbidity was observed, which mainly could be explained with polyphenolic compounds and protein content.

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