CONVENTIONAL AND ALTERNATIVE PRINCIPLES FOR STABILIZATION OF PROTEIN AND POLYPHENOL FRACTIONS IN BEER

Romeo S. Marković, Olgica S. Grujić and Jelena D. Pejin

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. In experimental part of this work the shelf life of unstabilized beer is being compared with beer stabilized with various standard products, such as PVPP and silica gel. Furthermore, the trials have been made to prove the functionality of a new product consisting of carrageenan and cross-linked PVPP. The method used to determine shelf life was haze forcing test (0/60°C). Extract, alcohol, bitterness, foam, haze, color and pH were also monitored. The test results showed expectedly that combined treatment of beer ensures the highest level of product stability. Through selective stripping of polyphenols and protein fractions it is possible to improve shelf life of beer to a significant extent.

KEY WORDS: beer, PVPP, stabilization, protein, polyphenol, haze forcing test, carrageenan, kettle

INTRODUCTION

During the past several years it has been observed an abrupt change in the habits of beer consumers. Beer is nowadays mainly sold through the commercial and distributive channels, which ensure supply to the market. Beer selling through commercial channels, as well as the intensified competition on the market, have set new standards to beer quality when its shelf life is concerned. Once talking about beer quality, haze surely represents one of most considered quality parameters. Presently, breweries deliver beer with shelf life from 6 to 12 months and, not rarely, guarantee its stability over the whole 18 months.
Like breweries, the suppliers had also to adjust themselves to new requirements for using appropriate means for this purpose. What effect these stabilization agents will have on the colloidal stability of beer and what are the resulting effects, it can be judged on the basis of experiment and experience. Here, thermal stabilization technology will not be considered.

To explain the problem it should be mentioned that proteins, polyphenols, carbohydrates and metal ions are primarily responsible for forming beer haze. Phenolic compounds come to beer from malt and hops and their content in beer depends on the technological process involved. Depending on structure and molecular size they influence color, taste and foam. Tannins are formed by polymerization of simple phenols. Their reaction with high-molecular nitrogen compounds yields tannin-protein complexes. Most important polyphenols involved are anthocyanogens, catechins and flavones (1).

![Fig. 1. Compounds influencing formation of haze of various types (2)](image)

Hydrogen bridges between molecules of polyphenols and polypeptides are formed by hydroxyl groups of polyphenols and oxygen atom of the peptide group. If the temperature is in the range from +5 to – 2°C, the reaction of phenol and protein compounds produces haze (Fig. 1). The formation of this, so-called cold haze, represents a reversible process and it disappears at +20°C. While low-molecular flavanols in the reaction with tannins do not influence haze, irreversible haze is formed in the reaction with oxidized tannins (3).

In order to improve the colloidal stability of beer it is necessary to remove both protein and polyphenolic fractions. In the present-day technology this is carried out with hydro- and xerogels, polyvinylpolypirrolidone (PVPP), as well as with combined agents. Besides, in a number of countries it is allowed to use enzymes, ascorbic acid, and other preparations. Whether the agents applied produce desired effects it is usually established using the haze forcing test (0/40/0°C or 0/60/0°C). Although being reliable, this method is also connected with analytical errors, it is time-consuming, and does not allow a timely intervention, i.e. the adjustment of stability parameters in the course of beer filtration. Beer haze is measured using process and laboratory photometers with two absorption angles (25° and 90°). It should be noticed that neither filtration nor stabilization are conceived as measures for correcting mistakes made in the production process. Hence, from the very beginning, it is necessary to perform stringent control of the quality of all techno-
logical factors that influence beer quality, and especially of those that influence stability 
of its sensory parameters (2):

- Nature of stabilization agent
- Amount of agent added
- Combination of more agents
- Stage of adding the stabilization agent
- Time of agent adding
- Contact time

To make more legible documentation, these parameters can be followed using special 
forms, as presented in Table 1 (4).

<table>
<thead>
<tr>
<th>Sample for analysis</th>
<th>Type of analysis</th>
<th>Frequency</th>
<th>Limit value</th>
<th>Test/Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVPP</td>
<td>Swelling volume</td>
<td>Each batch</td>
<td>&lt; 60 ml</td>
<td>Acc. to test method and specification</td>
</tr>
<tr>
<td>PVPP suspension from dosing vessel</td>
<td>Concentration</td>
<td>After each regeneration</td>
<td>not limited</td>
<td>Not later than the 5th regeneration</td>
</tr>
<tr>
<td>PVPP suspension from dosing vessel</td>
<td>Ash</td>
<td>Once a year</td>
<td>&lt; 0.4 %</td>
<td>Acc. to test method and specification</td>
</tr>
<tr>
<td>PVPP suspension from dosing vessel</td>
<td>Control of regeneration</td>
<td>Once a year</td>
<td>Absorption capacity the same as with single-use PVPP</td>
<td></td>
</tr>
</tbody>
</table>

The stabilization agents are added together with kieselguhr, i.e. at the stage of precoat filtration. These agents can improve quality of filtration and at the same time reduce the amount of kieselguhr. However, one must not forget that their addition increases the load of filtration surface (in kg/m²), irrespective of filter type (5). Often, stabilization is made with silica gels. Silica gels represent a group of highly porous adsorption means composed of colloidal silica. Silica gels serve for the removal of protein fractions and beer haze, i.e. for achieving optimal physico-chemical stability.

Silica gel can be added at the different stages of production process. The moment and amount depend on the product quality, technology applied, and technical facilities of the brewery. In Fig. 2 is sketched one of the possibilities of using silica gel. Silica gel can also be added in the course of pumping green beer from the fermentation tank to maturation tank, to the buffer tank, after beer maturation prior to kieselguhr filtration. Most often, silica gel is added together with kieselguhr or with single-use PVPP.

When adding silica gel between the fermentation and maturation tank it is practiced to add 1/3 of the total amount via special dosage pump, the remaining 2/3 being added as usual at filtration. We distinguish essentially two types of silica gels – hydrogels with total water content <60% and xerogels with <10%. Depending on beer characteristics and desired shelf life, the recommended amounts are 40-100 g/hl for hydrogel and 30-70 g/hl for xerogel (6).
Use of enzymes in the process of beer production is not allowed in all countries. For this purpose it is possible to use, for example, the enzyme obtained from the skin of papaya fruit (*Carica papaya*), whose enzymatic activity is due to two peptide hydrolases, papain and chymopapain. The enzymes do not remove haze fractions but reduce them to products that do not influence haze formation. They cleave high-molecular proteinaceous fraction to medium-molecular proteins (molar mass $<10^3$), peptides and amino acids. The addition of 2-4 ml/hl of enzyme preparation directly to maturation tank it is possible to achieve a high degree of enzymatic degradation (6).

Beer stabilization can also be achieved with the aid of ascorbic acid; however, its usage is not allowed in some countries. Ascorbic acid improves colloidal stability of beer but does not solve the problem of excessive exposure to air in the course of packaging. A dose of 2-8 g/hl is added directly to beer, and in no case before or during filtration. Iron from the kieselguhr has an adverse effect on the reduction capacity of ascorbic acid (6).

In addition to beer, PVPP is presently used for stabilization of wines and juices. There are two groups of PVPPs: for single and multiple use (recoverable PVPPs). In both cases unhindered adsorption of PVPP is achieved by its soaking in water before use. Beer stabilization with PVPP is carried out immediately after filtration and the amounts vary from 10 to 50 g/hl (2).
adsorption of polyphenols on insoluble stabilization agent (2). The amount of PVPP used for beer stabilization is limited by its physical properties. PVPP can be compressed, which, at a higher dosage can bring about an uncontrolled increase in pressure during filtration and filter clogging. For adding PVPP to a beer stream it is necessary to prepare first its 10% solution in warm water. Depending on the filter type, the precoat values vary between 175 and 225 g/m². The allowed surface load of the filter must not be exceeded. With the single stabilization, the sum of all amounts used must not exceed the amount prescribed by the producer of the filtration equipment. The water-absorption capacity of PVPP is 6 l/kg. While maximal load of filtration elements can be easily calculated on the basis of the nominal parameters, the actual dosage of PVPP depends on the quality of filtration raw material and technological procedure. A usual dose is in the range of 20-50 g/hl (5).

In addition to single-use PVPP large breweries have also systems for beer stabilization using recoverable PVPP, and stabilization process is carried out on a separate filter with horizontal sieves or filter candles. Stabilization process is stopped at the moment when the filtration cake on circular horizontal sieves fills all the free space between the sieves. Regeneration is carried out with hot alkali (NaOH 1.2-1.6%, 65°<T<85°C), which removes the polyphenols adsorbed on PVPP. Hot water (50°<t<70°C) is used to remove the excess of alkali from the stabilization cake, while cold, acidic washing (0.5-1% phosphoric acid) lowers pH value to below 7. Final rinsing with clean cold carbonized water removes the mineral residues. After completing regeneration PVPP is removed from the sieves by rotating the filter element, whereby it falls down to the filter bottom. Then, it is pumped to a receiver, to be dosed in the next stabilization operation (5).

In previous sections we have seen that a balanced adsorption of proteins and polyphenols is more desirable than either removal of polyphenols or proteins alone for enhancing colloidal stability of beer. Therefore, the industry has developed various products which can be used in different production stages for beer stabilization. The addition of a new composition of a selected carrageenan and micronized PVPP improves the wort clarity and gives brighter wort colors. The use of this new product (Figs. 4 and 5) increases yield and throughput through the remainder of the downstream brewing process. It also allows minimizing of trub which is comprised of spent hops, precipitated proteins and other insoluble materials including tannoids.

This experiment should show that the inadequate use of stabilization aids, lack of industrial equipment just as reliable and a fast determination method for prediction of shelf life result in negative influence on beer stability and fast creation of beer haze.

Fig. 4. κ-carrageenan

Fig. 5. Cross-linked polyvinylpyrrolidone
The experiment and laboratory tests were carried out in an Austrian brewery. The stabilisation and filtration aids were obtained at this site. The new product “Brewbrite” was supplied from ISP and added (15 g/hl) during the last 10 minutes of the kettle boil, hence shortly prior to second (aroma) hopping. Subject of experiment was in all cases a “Lager” type beer with parameters shown in Table 2.

Filtered beer loses its clarity during maturing, and especially at low temperatures when it becomes opaque, i.e. when a precipitate is formed. To determine beer quality in the shortest time use is made of the accelerated haze forcing test, described in the MEBAK methods of analysis (7). Result can be obtained already after several days upon beer packaging, and is expressed in “warm days”.

Analytical methods for extract, alcohol, bitterness, color, pH, foam, haze and shelf life of beer are described in MEBAK (7).

There is a whole range of the so-called global methods for the determination of polyphenolic compounds based on the characteristics of particular compounds for the determination. For this purpose use is made of EBC Method for the determination of total polyphenols. The analysis is based on the reaction of Fe$^{2+}$ ions, forming in alkaline solution the colored complexes that can be measured spectrophotometrically (7). Beer bitterness was determined via iso-alpha-acids that are extracted with iso-octane. The method is also described in MEBAK (7).

Swelling volume was determined on the basis of the known amount of PVPP that is soaked in water and measured after 24 h. To 10 g of PVPP in a vessel of 250 ml, 50 ml of water were added and stirred for several minutes. The suspension was then transferred to a measuring cylinder and filled with water to 100 ml. Direct volume reading is carried out after 24 h.

Ash was determined after soaking PVPP in 2 ml of sulfuric acid (1:1) in a weighed vessel. The vessel with PVPP was placed on a heater and incinerated mildly to the disappearance of white smokes. After that it was heated at $T = 800^\circ$C ± 25°C and, upon cooling, weighed to calculate percentage with respect to the blank (4).

Control of PVPP regeneration was carried out by the method of Harris and Rocketts described in MEBAK (7). Concentrations of anthocyanogens and tannins of the PVPP from the dosage vessel are compared after regeneration with those of pure, unused PVPP.

Tannins were determined using PVP (polyvinylpyrrolydone), which precipitates tannins from beer. PVP binds through hydrogen bonds to tannins, forming thus insoluble complexes, i.e. producing the beer haze. With PVP addition, beer haze increases to the moment when all the tannins reacted out with PVP. Excessive addition of PVP yields haze disappearance. The amount of added PVP at which maximal haze was achieved is proportional to the amount of tannins. Use was made of the Tannometer of the firm Pfeuffer GmbH, Kitzingen, Germany. Beer haze was read in EBC units, whereas content of tannins is expressed in mg/l PVP.
RESULTS AND DISCUSSION

In the experimental part of this work we examined the change of shelf life from the totally unstabilized beer to the beer stabilized with different doses of PVPP, as well as using combination of silica gel and new agents for beer stabilization after the treatment with PVPP.

In Fig. 6 is illustrated the effect of different doses of PVPP on stability, i.e. shelf life of beer. The haze value of 2 EBC units is a limiting value for “acceptability” on the market.

![Fig. 6. Effect of different doses of single-use PVPP on beer stability](image)

With export beer, it is expected that the haze will not exceed 2 EBC in the course of 12 months. While the value for nonstabilized beer is only two weeks, with a PVPP dose of 20 g/hl shelf life shows a strong increase. To achieve a shelf life over 6 months it is necessary to use 40-50 g/hl of single-use PVPP, which in practice often leads to accelerated filter clogging. Recoverable PVPP has smaller swelling volume, so that higher dosages are possible. The effect of the above agents on beer stability is shown in Table 2.

<table>
<thead>
<tr>
<th>Beer analysis</th>
<th>Blank unstab. beer</th>
<th>50 g/hl PVPP irreco.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract (% mas)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Alcohol (% vol)</td>
<td>4.3</td>
<td>4.3</td>
</tr>
<tr>
<td>Bitterness (BE)</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>Color (EBC)</td>
<td>8.0</td>
<td>7.5</td>
</tr>
<tr>
<td>pH</td>
<td>4.2</td>
<td>4.2</td>
</tr>
<tr>
<td>Foam (s) accord. to NIBEM</td>
<td>105</td>
<td>112</td>
</tr>
<tr>
<td>Initial haze (EBC) in bottle</td>
<td>0.72</td>
<td>0.64</td>
</tr>
<tr>
<td>Haze forcing test (0/60°C) (warm days)</td>
<td>0.4</td>
<td>9.5</td>
</tr>
<tr>
<td>Haze forcing test 1 week (0/60°C) (EBC)</td>
<td>13</td>
<td>1.1</td>
</tr>
</tbody>
</table>
In the test shown in Fig. 7, stability was monitored by taking multiple samples of filtered beer after each 60 minutes of filtration. Shelf life was determined by the haze forcing test (0/60°C). The highest degree of colloidal stability showed the beer that was stabilized using the combination of silica gel (xerogel) and irrecoverable PVPP. The beer that was not stabilized (blank) only in the samples from the first hour of filtration showed a stability of 5 warm days, whereas soon after that the value decreased toward zero. A linear decrease of colloidal stability was observed with the beer that was stabilized with silica gel only. In contrast to this, the beers stabilized only with PVPP lost their stability after first hours of filtration, and then the level of colloidal stability remained constant.

![Fig. 7. Comparison of the effect of silica gel (xerogel) and PVPP (irrecover.) and filtration duration on colloidal stability of beer](image-url)

Similar experiments carried out in the laboratory of the Chair of Beer Technology II in Weihenstephan, served to examine the efficiency of stabilization using the haze forcing test based on the change of concentration of particular groups of compounds such as anthocyanogens, total polyphenols, and total proteins before and after filtration, i.e. stabilization. For this purpose HPLC (High Pressure Liquid Chromatography) was used to determine polyphenol compounds relevant to haze formation, such as (+)-catechin, (+)-epicatechin, prodelphidine B₃ and procyanidine B₃. The method made possible the determination of a large number of still unidentified but for haze formation relevant compounds. To measure the haze produced by protein fractions use was made of ELISA (Enzym Linked Immuno Sorbent Assay) test. By introducing the obtained values into the corresponding formulas, beer stability can be established in a more precise way than by conventional methods of analysis (1).

When using 15g/hl Polyclar Brewbrite the wort fermentation process was completed after 128 hours, while the fermentation without this additive lasted 144 hours. In the haze forcing test (0°/60°/0°C) the finished beer showed the difference in shelf life of two days. The unstabilized beer appeared to be stable only two days, whereas the physicoche-
mical stability of the beer after the addition of 15 g/hl Polyclar Brewbrite lasted four days. Also, the amount of remaining tannins in the treated beer was insignificant, whereas the content of compounds measured by the method PVP K-90 in untreated beer was 39 mg/l (8). Total polyphenols were reduced by 18% compared to untreated beer.

CONCLUSION

To achieve colloidal stability of beer it is necessary to remove protein-polyphenolic complexes or prevent their formation. The stabilization process must not have adverse effects on sensorial characteristics of beer such as appearance, odor, taste, and foam. The combined treatment with silica gel and PVPP has become a standard procedure that results in a product of very high quality. On the basis of the tests described it can be concluded that for obtaining optimal results, a dynamic regime of dosage should be conceived. This regime would assume timely adjustment of the agent dose in proportion to the adsorption capacity, which is subject to change in the course of filtration. In addition to qualified technological-technical staff in charge of process control, efficient stabilization requires also the adequate processing equipment. The main equipment parts are the PVPP precoated filters with regeneration station, dosage vessels and valves, dosage pumps, clarifying filters, haze measuring device, as well as a modern control system. The analytical problem stems from the fact that it is very difficult to predict what will be the stabilization effect after using a chosen agent. To solve this problem it is necessary to develop such analytical methods that will provide results at the necessary speed and precision that they can be used to adjust the process of beer stabilization before it reached the consumer.

REFERENCES

СТАНДАРДНЕ И АЛТЕРНАТИВНЕ МОГУЋНОСТИ ЗА СТАБИЛИЗАЦИЈУ ПРОТЕИНСКИХ И ПОЛИФЕНОЛНИХ ФРАКЦИЈА У ПИВУ

Ромео С. Марковић, Олгица С. Грујић и Јелена Д. Пејин

Мутноћа пива настаје првенствено као последица настајања комплекса између протеинских и полифенолних састојака пива. Проблем смањења склоности настајања мутноће се може решити било смањењем садржаја протеина и/или полифенола, било смањењем величине молекула протеина/полифенола. У експерименталном делу овога рада разматрана је промена дуготрајности пива између потпуно нестабилизованог пива, пива стабилизованог ПВПП-ом при различitim количинама и након употребе ПВПП-а у комбинацији са силика гелом. Рок трајања одређен је методом форсир теста (0/60°C). Надаље, учињени су покушаји да се потврди функционалност једног новог производа који се састоји од карагинана и умреженог ПВПП. Убрзани тест (0/60°C) је примењен као метода за одређивање трајности пива. Поред тога праћене су промене основних параметара у пиву као што су екстракт, алкохол, горчица, пена, мутноћа, боја и pH вредност. Комбиновани третман је, као што је и очекивано, омогућио највиши ниво стабилности продукта. Селективним одстрањивањем дела полифенолних и протеинских фракција, тј. успостављањем равнотеже ових фактора, могуће је значајно побољшати трајност пива.

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