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Effects of Wort Clarifying by using Carrageenan on Diatomaceous Earth Dosage for Beer Filtration

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Abstract

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Diatomaceous earth makes a great contribution to the amount of brewing waste. In this work, attempts were made to reduce the dosage of diatomaceous earth used during beer filtration, via the application of carrageenan at the stage of wort boiling. Carrageenan was added to the boiling wort (50 mg/l) and, after fermentation, the beer was filtered using different doses of diatomaceous earth (0.05–0.40 g/l). The filtration performance of beers obtained from carrageenan-pretreated wort was compared with the filtration of reference beer. It was found that beer produced from wort treated with carrageenan, had a significantly lower haze before filtration compared to the reference beer (other quality parameters showed no significant differences). The reference beer had a higher haze after filtration with 0.40 g/l of diatomaceous earth than the carrageenan pre-clarified beer after filtration with 0.05 g/l. It was concluded that the use of preliminary clarification of wort with carrageenan allows for a significant reduction of the diatomaceous earth application while maintaining high clarity of beer.

Keywords: fermentation; wort boiling; beer production; haze

The clarity of beer has been an important factor influencing consumer preferences. The occurrence of haze in beer is often associated with low quality, so during the wort and beer production the removal of haze forming compounds is of high importance. In the brewing industry, the elimination of excess proteins and tannins (commonly associated with the haze formation) is known as colloidal stabilisation (Siebert 1999).

The most popular way to achieve the clarity of beer is the beer filtration, with the use of diatomaceous earth (DE). This auxiliary material operates via two mechanisms (Michen *et al.* 2011). One of them is the creation of hydrogen bonds between the carboxyl groups of proteins and the hydroxyl groups of DE. The second one is the absorption of the protein molecules within the diatomaceous earth pores that are of the size of 4–8 μm. The characteristic surface area of DE is 3–100 m^2/g (Tsai *et al.* 2006). The dosage of the diatomaceous earth required for the beer filtration depends on the raw materials quality, technological process, application of other stabilising agents, and the required shelf life of the beer. Usually, it is applied in the amount of 70–200 g/hl of the beer filtered (Debourg 1996; Braun *et al.* 2011).

Despite being very effective in the beer clarification, diatomaceous earth is considered quite problematic in terms of the health and environmental issues. The exposure to DE dust causes lung cancer and non-malignant respiratory disease (Checkoway *et al.* 1993). The remaining DE slurry after filtration makes a large contribution to the brewery solids wastes, as the initial weight of diatomaceous earth used is almost tripled after this process (Olajire 2012). Although there are ways to regenerate DE (Johnson 1997) in order to reuse it in various applications,

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the regeneration process is energy consuming and requires the use of chemicals. These factors make DE regeneration environmentally challenging (Tsai *et al.* 2004a).

Consequently, the best way to reduce the negative environmental impact of diatomaceous earth is to reduce its use, which can only be done when appropriate beer clarity is maintained. One of the options is to start colloidal stabilisation in the early stages of production, namely during wort boiling (Leather *et al.* 1995). At this stage carrageenan can be added to remove some of the haze forming compounds before the wort is transferred to the fermentation equipment, followed by filtration. Carrageenan is a family of linear polysaccharides, comprising repeating units of disaccharide (1,3) linked β-D-galactose-4-sulphate and (1,4) linked 3,6 anhydro-α-D-galactose (Dale *et al.* 1995). It is distributed and dissolved in the wort during boiling, to enhance the precipitation of cold trub during subsequent cooling, making its removal easier (Rehmanji *et al.* 2005). So far, research regarding the application of carrageenan in the brewing industry has been focused mainly on the technological aspects, process optimisation and wort quality (Leather *et al.* 1995; Dale *et al.* 1996a,b). However, there are no references available that would join the two aspects: the carrageenan mediated clarification of wort and reduction of diatomaceous earth requirements at the stage of the beer filtration. Since the application of DE creates some environmental problems, it is worth to show an alternative approach that allows for a reduction of the DE dosage in the brewing industry.

The presented study fulfills this gap, aiming at assessing the possibility of decreasing the dosage of diatomaceous earth through the application of carrageenan during wort boiling. The work was carried out on laboratory scale and the promising results achieved encourage performing scale-up experiments.

MATERIAL AND METHODS

Raw materials and auxiliary materials. Malted barley Pilsner Malt (Weyermann, Germany) was used to produce the laboratory wort. Carrageenan GPI CARRACLEAR XS (74-GPICARRXS/25; Promar, Łomianki, Poland) was used to clarify the wort prior to fermentation.

Execution of experiments. A portion (50.5 g) of malt was milled (laboratory miller WZ-1; ZBPP, Bydgoszcz, Poland) for 3 s and used to produce the laboratory wort according to the Congress procedure (EBC Analytica 2004) . After the mash clarification, the resulting wort was boiled (without hopping) for 60 min with or without the addition of carrageenan. Carrageenan was added to obtain the concentration of 50 mg/l (this dosage was chosen based on trials with the application of 50, 100, and 200 mg/l) (Poreda *et al.* 2014). It was added 10 min before the end of boiling in the form of water solution (5 ml) containing an appropriate amount of the polysaccharide (to the reference sample, 5 ml of pure water was added). The wort was cooled at ambient temperature until it reached 20°C. Due to the evaporation of water during boiling, the resulting wort extract content was 10%.

Fermentation (200 ml of wort) was performed with the use of yeast *Saccharomyces pastorianus* W34/70 (Fermentis Division of Lesaffre, Wołczyn, Poland). The yeast suspension was prepared prior to pitching by suspending 20 g of dried yeast in 1 l of physiological solution of NaCl (7 g/l). It was added to the cooled wort so as to obtain 1 g of yeast/l of wort. Fermentation took 7 days and was run in a refrigerated cabinet (14°C, Q-Cell 240; Poll-Lab., Wilkowice, Poland).

To assess the requirement for diatomaceous earth, beer samples (30 ml) were collected after the yeast biomass sedimentation, and mixed with various amounts of diatomaceous earth for 20 min (magnetic stirrer ES24, 100 rpm). The doses of diatomaceous earth applied were: 5, 20, and 40 g calculated per 100 l of beer. Next, the beer was run through a paper filter and the haze of the beer was measured.

Analytical procedures. Alcohol and real extract: the resulting beer (50 g) was distilled and the densities of the distillate and of the remaining water solution of the extract were measured with the use of pycnometer. On the basis of the density, alcohol content and real extract content were calculated. Colour and haze in the beer were determined spectrophotometrically (Beckman DU 650; Beckman Instruments, Fullerton, USA) by measuring the absorbance of light at 430 and 560 nm respectively, and the results were expressed in EBC units. The pH of the beer was measured with a pH meter CP-501 (Elmetron, Zabrze, Poland).

Statistical analysis. The data (3 replications) were analysed by one-way analysis of variance (ANOVA) to evaluate the significance of the main effect of carrageenan application on the parameters analysed. Significant differences between the means were verified by Duncan test ($P < 0.05$) with the use of Statistica v. 10 (StatSoft Polska, Kraków, Poland).

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RESULTS AND DISCUSSION

Figure 1 shows the haze of unfiltered beer, obtained from the reference wort and the wort treated with carrageenan. Turbidity of the beer produced with the addition of carrageenan was by a factor of almost six lower (ca. 12 EBC units) compared with the reference (ca. 70 EBC units). Such a significant difference can be explained by the fact that the haze forming proteins precipitate more intensively through the use of carrageenan. The reaction between the carrageenan and the polypeptides is mediated by electrostatic interactions (Dale *et al.* 1996b), but the necessary condition for precipitation and flocculation of the resulting conglomerates is to cool the wort (Dale *et al.* 1995).

The unfiltered beer clarity is generally obtained only by lagering (storage of the beer at temperature just above freezing). The main purpose of this process is the clarification of beer, which later improves the filtration performance and increases its colloidal stability. During lagering, the yeast cells and other haze forming particles settle and the number of yeast cells remaining in the suspension prior to filtration is very low (Debourg 1997).

In order to determine the potential impact of carrageenan mediated clarification of wort on the requirements for diatomaceous earth dosage during the beer filtration, the beer was filtered with different amounts of DE: 5, 20, and 40 g (calculated per hl).

The filtration of the reference beer with 40 g/hl of DE decreased the turbidity by ca. 50% (from 70 to

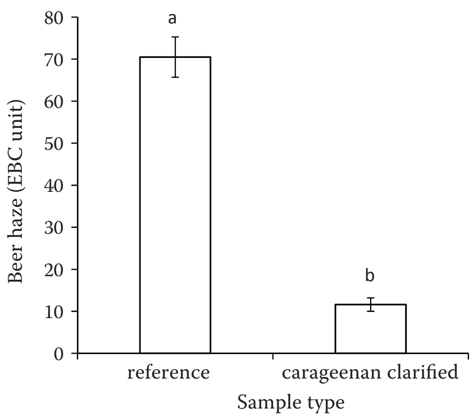

Figure 1. Haze of non-filtered beers: the "reference" prepared from wort without the carrageenan treatment and the "carrageenan clarified" prepared from wort treated with carrageenan

Values are means ± SD (n = 3), the letters indicate non homogenous groups

34 EBC units), while the carrageenan pre-clarified beer (at the same dose) was characterised by the haze reduction by about 90% (Figure 2), from 11 to ca. 1 EBC unit).

When 40 g/hl of DE was used, the turbidity of the beer pre-clarified with carrageenan, remained at a level of 1 EBC unit, which in the analytics EBC is named "almost crystal" (or almost brilliant). At the same dose, the reference beer haze was 34 EBC units. In commercial conditions, diatomaceous earth beer filtration is aimed at achieving haze of less than 1 EBC unit (Martinovic *et al.* 2006), with a dosage usually in the range of 70–200 g/hl (Debourg 1997). The results shown in Figure 2 revealed that the reference beer filtered with DE in a dose of 40 g/hl was more hazy than the pre-clarified beer filtered with only 5 g/hl of DE. Therefore we propose that the demand for diatomaceous earth in the filtration of beer pre-clarified with carrageenan may be many times lower. The use of carrageenan allowed for a significantly higher clarity of beer with a lower than usual, consumption of diatomaceous earth for the beer filtration, i.e. a very low dose (40 g/hl).

The results obtained on the laboratory scale are usually not easily transposed directly onto the technical scale. However, the significant effect noted in model studies (Figure 2), gives reasonable hope for positive results in scaling-up experiments. This is of particular importance because of the nature of the environmental problems, created at the beer filtration stage by the production of waste, especially the diatomite (Olajire 2012).

An important issue when introducing new solutions to the existing production conditions is to ensure that the impact on the fermentation process is as small as possible. Carrageenan does not interact with the low molecular weight components of brewers' wort, making their nutritional value appropriate for the yeast cells (Dale *et al.* 1996b). To estimate the efficiency of fermentation, brewers measure the differences between the extract contents in the wort and beer (before and after the process). In this study, the initial content of extract in wort was 10.0%, whereas the extract of beer after fermentation was 3.1% (reference beer) and about 3.5% (pre-clarified beer) (Figure 3). The difference was significant, but the lower attenuation level (final extract divided by the initial extract) did not influence the alcohol content. It was equal in both samples, 4% v/v, which is typical of light beers of 10% initial extract content. The fermentation of wort of the same original grav-

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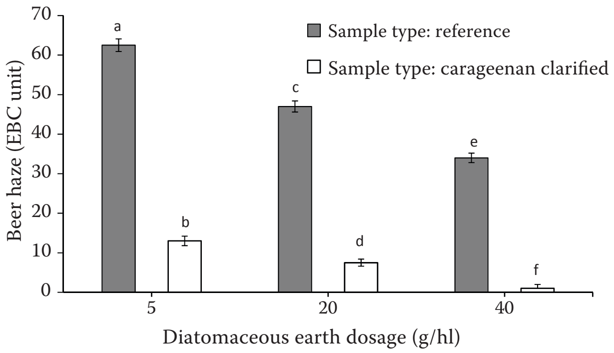

Figure 2. Haze of beers filtered with various doses of diatomaceous earth

Values are means ± SD (n = 3), the letters indicate homogenous groups

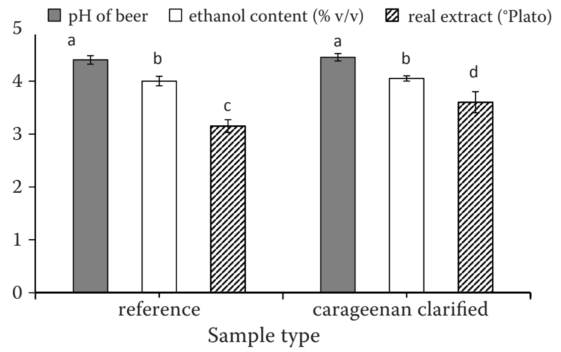

Figure 3. Real extract, ethanol content, and pH of the beers: the reference beer and the beer clarified with carrageenan

Values are means ± SD (n = 3), the letters indicate homogenous groups

ity produced beer of a higher extract content in the preclarified trials, while ethanol content was the same as in the reference beer. A number of authors have been cited in a review publication (Kühbeck *et al.* 2006) supporting the thesis that turbid worts may contain lipids, such as unsaturated long-chain fatty acids and ergosterol, which have a significant influence on the yeast growth. Consequently, the more turbid wort (reference trial) could provide a better environment for the yeast growth, which results in more extract being used during the process. The higher final extract of beer is actually beneficial, as it contributes to the mouthful effect, making the taste of beer more pleasant.

It has been also revealed that the carrageenan clarification has no significant effect on the pH of beer (Figure 3). During the fermentation, the pH of the wort essentially decreases from about 5.3–5.6 to 4.5–4.6 due to the uptake of phosphates, ammonium, and potassium ions by the yeast cells and due to deamination, which releases acids (Coote & Kirsop 1976). These processes have a significant impact on the quality of beer. Furthermore, the pH value of around 4.5 improves the conditions for the precipitation of protein-tannin trub, thereby improving clarity. In addition, at low pH the beer maturation is faster and a better taste bouquet and greater microbiological stability are achieved. In both cases (Figure 3), the pH of the young beer was within the range of 4.4–4.5. No effect of carrageenan used in the amount of up to 6 g/l on the pH of wort and beer was demonstrated by Leather *et al.* (1995) either. It is worth noting that the carrageenan gel formation depends on the pH of the solution, wherein a noticeable degradation of the clarification performance is found at pH lower than 4.5 (Leather *et al.* 1995). In the case of brewers' wort, the pH at the beginning of fermentation is generally significantly higher than this (O'Rourke 2002).

The filtration process tends to affect the beer colour: beers after filtration are typically somewhat lighter then before. Figure 4 shows the colour of beers before and after filtration. The reference beer, was slightly darker before filtration than the pre-clarified beer (Figure 4). The difference was about 2.5 °ECB and was probably due to the removal of some protein-like compounds from the wort clarified with carrageenan. Due to the filtration, a decrease of colour was noted only in the reference beer – the pre-clarified beer carrageenan colour did not change. It seems that some of the protein-like colour forming compounds were removed from the reference beer during filtration, whereas in the pre-clarified beer they were removed earlier in the process, when carrageenan precipitation took place.

To comparison of the colour in the samples after filtration revealed no difference between the reference and carrageenan clarified beers. It can therefore be concluded that the addition of carrageenan does not affect the colour of the final product.

To provide a rough estimation of the economical aspect of the carrageenan mediated decrease of DE dosage, we have made the following calculations. The assumptions made: carrageenan cost ca. 3760–7520 EURO/1000 kg; cost of DE, ca. 600 to 800 EURO/1000 kg (average prices taken from the internet pages http://diatomaceousearthonline.com.au/ and http://www.21food.com/). All calculations are made per 1000 hl of beer. In the production conducted according to the traditional technology

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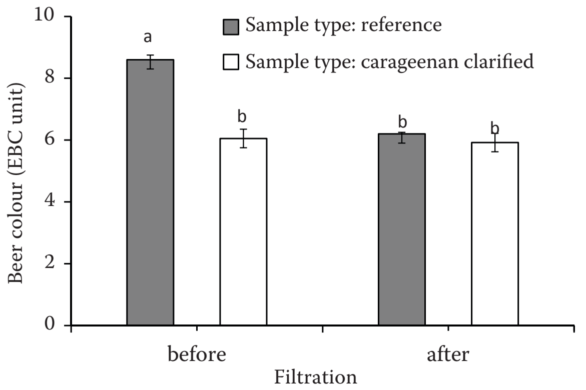

Figure 4. Colour of the beer before and after diatomaceous earth filtration: the samples produced with and without the addition of carrageenan to perform clarification

Values are means ± SD (n = 3), the letters indicate homogenous groups

(DE only), the application of 70 g of DE per hl (a low dosage) generates a cost of ca. 66.5 EURO. Application of 50 mg of carrageenan/l and application of a decreased dose of DE (e.g. 10 g/hl – double the amount used in this study), generate a cost of 25 plus 9.5 EURO (34.5 EURO). So the rough savings calculated per 1000 hectoliters of beer round up to ca. 32 EURO (66.5–34.5). Simple multiplication gives us a saving of ca. 32 000 EURO in a brewery producing 1 mill hl/year. The presented calculation is an underestimated option – original DE dosage may be much higher (220 g/hl, not 70), which will make the savings even more pronounced.

SUMMARY AND CONCLUSIONS

The addition of carrageenan to wort during boiling, aimed at clarifing the beer at the early stage of production does not cause significant differences in the quality of the resulting wort and beer. The concentration of carrageenan that ensures a good clarity of beer is 50 mg/l. The most important finding of this paper is a great reduction of the amount of diatomaceous earth use during the beer filtration, achieved by the application of carrageenan.

The most important conclusions are:

(1) The use of carrageenan allows for a significant reduction in the dosage of diatomaceous earth for the filtration of beer.

(2) The beer made from the wort fined with carrageenan is characterised by a significantly lower haze than the beer produced from the reference wort.

(3) The basic physico-chemical parameters (colour, pH, ethanol concentration) of the beer pre-clarified with carrageenan are not significantly different as compared with those of the reference beer.

(4) Rough estimation of the costs reveals that the application of carrageenan and the relevant savings in diatomaceous earth use may bring a substantial economical gain.

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