

# Effect of high hydrostatic pressure on quality parameters of lager beer

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**Abstract:** Unpasteurized lager beer samples from a commercial brewery were treated either by high hydrostatic pressure (HHP; 200, 250, 300, 350 MPa for 3 and 5 min at 20 °C) or by conventional heat pasteurization (60 °C for 15 min). The main attributes of the beer, such as ethanol content, extract and pH, were not affected by either treatment; however HHP and heat pasteurization affected colour, chill haze, protein sensitivity and bitterness. Change in bitterness was higher in conventional heat pasteurization, but pressures up to 300 MPa had no significant effect on bitterness. Although more studies should be carried out to investigate the effects of HHP treatment on different types of lagers and ales, our results revealed that HHP could be successfully used to process beer, even at temperatures well below those required for heat pasteurization, without affecting some of the quality attributes.

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**Keywords:** high hydrostatic pressure; quality parameters; lager beer

## INTRODUCTION

Foods having high quality and fresh-like attributes are in demand, and consequently less extreme treatments and/or fewer or no additives are desired.<sup>1</sup> This growing demand for safe and 'fresh' products and if possible products with longer shelf life, is influencing the development of so-called non-thermal food processing techniques.<sup>2</sup> Among these, high hydrostatic pressure (HHP) is gaining in popularity with food processors, not only because of its food preservation capability but also its potential to achieve interesting functional effects. The application of HHP ranging from 100 to 1000 MPa allows the preservation of foods without altering quality to the same extent as thermal treatment having a comparable preservation effect.<sup>3</sup> Therefore, in many cases, HHP could replace more conventional methods such as chemical additives and/or high temperature treatments.<sup>4</sup> These advantages have generated international research and development activity and, within a short time, produced several commercial liquid HHP products in the market, namely HHP-treated orange juice is available in France<sup>5</sup> and non-bitter grapefruit juice and mandarin juice are available in Japan.<sup>6</sup>

Among the alcoholic beverages, the first trials with HHP were made by Hara *et al*<sup>7</sup> on rice wine and HHP-treated Japanese unrefined rice wine (nigori-sake) appeared on the market.<sup>6</sup> Different types of wines containing yeast and lactic acid bacteria were also successfully stabilized by HHP at a range of 350–600 MPa.<sup>8,9</sup> However, fewer studies were

reported about the use of HHP on beer, most probably because of the complex nature of beer as some of the constituents are derived from the raw materials and others are the result of chemical and biochemical transformation of the raw materials during malting, mashing, boiling, fermentation and conditioning.<sup>10</sup> Among these, the effect of increasing pressure (300, 500 and 700 MPa for 5 min) on the brewing process and the beer was studied by Fischer *et al*,<sup>11</sup> and Castellari *et al*<sup>4</sup> studied the effect of HHP (600 MPa for 5 min) on two unfiltered pale ales and a mild ale in comparison with traditional heat pasteurization. The parameters reported were measured either at a single pressure or single time value in both of the above studies, creating a difficulty in demonstrating the exact impact of HHP treatment on beer samples studied. In order to overcome this limitation different pressure and time parameters were selected in the present study.

Since brewers are very concerned whether the finishing techniques they use are the best in terms of product quality, studies detailing the effects of HHP on beer deserve to be investigated more deeply. Therefore the objectives of this study were to determine the impact of different HHP treatment pressure and time combinations on some important quality parameters of filtered lager beer and to compare with conventional heat pasteurization. In this respect, bitterness, ethanol, density, extract (real, apparent, original), fermentation degree, pH, protein sensitivity, colour, haze after chilling (HAC) or chill haze were analyzed. The

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pressurization effect was studied below 400 MPa since (1) alterations in food texture, aroma and colour may occur at elevated pressures and (2) capital costs amount to a substantial share of overall costs of HHP-processed foods, and equipment costs decrease if the machinery is designed for 'mild' pressure application.

## MATERIALS AND METHODS

### Beer

Lager beer samples were obtained from a commercial brewery (Anadolu Efes Biracılık ve Malt Sanayii AS, Ankara, Turkey). Homogenous samples of filtered bright beer were taken directly from the storage tanks, using 10-ml sterile cryovials (Sterilin, London, UK) with silicone seals for pressurization and 100-ml sterile glass bottles with screw caps for heat pasteurization studies and minimum headspace when filled. Before filling the vials and bottles with beer, carbon dioxide was pumped through a nozzle into the vials and bottles from a supply near the storage tanks in order to reduce the negative impact of oxygen on beer. Each of the analyses was by standard methods of the European Brewery Convention (EBC).<sup>12</sup> The samples were stored at 4 °C and processed (HHP and heat pasteurization) within 12 h.

### High hydrostatic pressure treatment

A HHP unit capable of operating up to 600 MPa between 10 and 90 °C with water and glycol mixture as pressure transmitting medium was used. The fluid was heated prior to pressurization to the desired temperature (20 °C) by an electrical heating system surrounding the unit. The rate of pressure increase was about 300 MPa min<sup>-1</sup> and pressure release time was less than 1 min. For pressurization, vials were separately placed inside the cylindrical vessel of the HHP equipment (only one vial was pressurized in each pressurization cycle), the chamber was closed and the samples were treated at 200, 250, 300 or 350 MPa for 3 or 5 min at 20 °C. Pressurization time reported in this study did not include the pressure increase and release times. Reported temperature is the actual process temperature during hold time at reported pressure levels. The pressure level, time and temperature were all recorded during the pressurization cycle. Controls were not pressurized. Heat pasteurization was performed by immersing samples in glass bottles in a water-bath set to 62 °C for 60 °C treatment for 15 min (one at a time) and then the samples were cooled with water at 20 °C. This temperature and time relationship was determined earlier. Bottles were continuously stirred during pasteurization to improve heat transfer. Untreated samples were used as controls. All samples were stored in the dark and analyzed within 24 h. Experiments were repeated and the average of six replicates (three vials/bottles × two replicates) was used to analyze the results.

### Chemical and physical analyses

Bitterness, ethanol content, density, extract (real, apparent, original), fermentation degree, pH, protein sensitivity, colour, haze after chilling (HAC) or chill haze were measured. The measurement of the bitterness was done by spectrophotometry (UV-1601 Shimadzu, Tokyo, Japan) of an iso-octane extract of beer. The extinction value of the iso-octane layer in a 1-cm cell was measured at 275 nm, using pure iso-octane in the reference cell.

Density, the real, apparent and original extract, fermentation degree and ethanol content were measured by an Anton Paar Beer Analyzer (Vienna, Austria). Degassed beer was filtered through a folded paper-filter and to fill a cylindrical cuvette, which was then placed into the beer analyzer.

The pH was measured by a pH meter (WTW 537; London, UK). Colour was measured by spectrophotometry at a wavelength of 430 nm. The absorbance value was multiplied by a factor of 25 to yield European Brewery Convention (EBC) units of colour.<sup>13</sup>

Protein sensitivity and chill haze were determined with the Tannometer (Pfeuffer, Munich, Germany) and results were expressed in EBC units. Gallic tannin at a concentration of 0.1 g dry matter l<sup>-1</sup> was used to precipitate sensitive proteins. The sample was filled in a fluorescence cuvette with optical layer thickness of 2 cm. The integrated magnetic agitators ensured that the reagent and the sample were well mixed in the cuvette. Transmitted light was measured at a wavelength of 510 nm. In order to measure chill haze, a 4-ml beer sample was pipetted into a clean cuvette, 0.24 ml of ethanol (975 g kg<sup>-1</sup>) was added to decrease the solubility of the protein-tannin complex and finally the beer was chilled to -8 °C before the results were recorded.

### Statistical analyses of the data

Results of this study were submitted to a one-way ANOVA. Significant differences between means were tested using a Duncan's multiple range test with a probability level of  $p < 0.05$ . Statistical treatments were carried out with SPSS (Chicago, IL, USA) 10.0 for Windows.

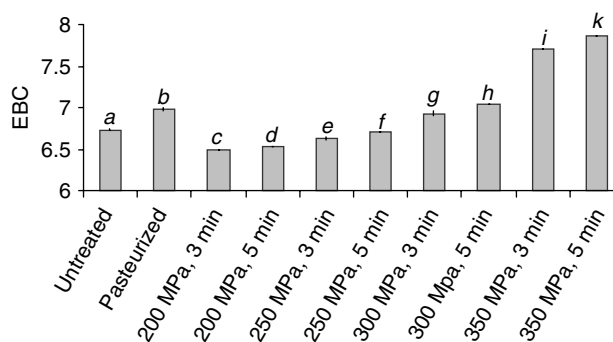
## RESULTS AND DISCUSSION

The comparisons of treated and untreated lager beer samples revealed that ethanol content, extract (real, apparent, and original), fermentation degree, density and pH were not significantly affected ( $p < 0.05$ ) either by HHP treatment at the pressure range studied or by heat pasteurization (Table 1). HHP (600 MPa for 5 min) with no significant affect on ethanol content, extract and pH of pale ale beer has already been reported.<sup>4</sup> Although HHP-treated beers were different in type (lager and pale ale) in previous studies, our results indicated that much lower pressures (<350 MPa) could be used for beer stabilization without significantly affecting primary beer parameters, including ethanol content, extract and pH.

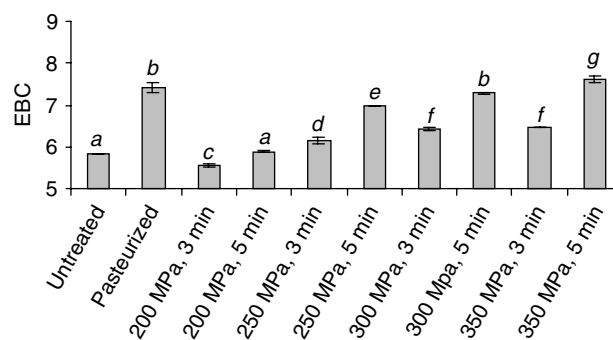
**Table 1.** Effect of HHP treatment on ethanol content, extract, density, fermentation degree and microorganisms in lager beer<sup>a</sup>

Units	Untreated	HHP treatment at												
		Heat pasteurization (60 °C, 15 min)				200 MPa			300 MPa			350 MPa		
		50.3 <sup>a</sup>	1.00579 <sup>a</sup>	3.82 <sup>a</sup>	2.03 <sup>a</sup>	3 min	5 min	3 min	5 min	3 min	5 min	3 min	5 min	
Ethanol	50.3 <sup>a</sup>	50.3 <sup>a</sup>	50.3 <sup>a</sup>	50.3 <sup>a</sup>	50.3 <sup>a</sup>	50.3 <sup>a</sup>	50.3 <sup>a</sup>	50.3 <sup>a</sup>	50.3 <sup>a</sup>	50.3 <sup>a</sup>	50.3 <sup>a</sup>	50.3 <sup>a</sup>	50.3 <sup>a</sup>	
Density	1.00579 <sup>a</sup>	1.00578 <sup>a</sup>	1.00578 <sup>a</sup>	1.00579 <sup>a</sup>	1.00580 <sup>a</sup>	1.00579 <sup>a</sup>	1.00578 <sup>a</sup>	1.00578 <sup>a</sup>	1.00578 <sup>a</sup>	1.00578 <sup>a</sup>	1.00577 <sup>a</sup>	1.00577 <sup>a</sup>	1.00577 <sup>a</sup>	
Real extract	3.82 <sup>a</sup>	3.82 <sup>a</sup>	3.82 <sup>a</sup>	3.82 <sup>a</sup>	3.82 <sup>a</sup>	3.81 <sup>a</sup>	3.81 <sup>a</sup>	3.80 <sup>a</sup>	3.80 <sup>a</sup>	3.81 <sup>a</sup>	3.81 <sup>a</sup>	3.81 <sup>a</sup>	3.82 <sup>a</sup>	
Apparent extract	2.03 <sup>a</sup>	2.03 <sup>a</sup>	2.03 <sup>a</sup>	2.03 <sup>a</sup>	2.02 <sup>a</sup>	2.03 <sup>a</sup>	2.02 <sup>a</sup>	2.03 <sup>a</sup>	2.03 <sup>a</sup>	2.02 <sup>a</sup>	2.02 <sup>a</sup>	2.02 <sup>a</sup>	2.02 <sup>a</sup>	
Original extract	11.49 <sup>a</sup>	11.49 <sup>a</sup>	11.48 <sup>a</sup>	11.49 <sup>a</sup>	11.48 <sup>a</sup>	11.48 <sup>a</sup>	11.48 <sup>a</sup>	11.48 <sup>a</sup>	11.48 <sup>a</sup>	11.48 <sup>a</sup>	11.49 <sup>a</sup>	11.49 <sup>a</sup>	11.48 <sup>a</sup>	
Fermentation degree	82.31 <sup>a</sup>	82.31 <sup>a</sup>	82.31 <sup>a</sup>	82.30 <sup>a</sup>	82.31 <sup>a</sup>	82.32 <sup>a</sup>	82.31 <sup>a</sup>	82.31 <sup>a</sup>	82.31 <sup>a</sup>	82.31 <sup>a</sup>	82.31 <sup>a</sup>	82.31 <sup>a</sup>	82.31 <sup>a</sup>	
pH (20 °C)	4.37 <sup>ab</sup>	4.37 <sup>a</sup>	4.37 <sup>ab</sup>	4.37 <sup>ab</sup>	4.38 <sup>ab</sup>	4.37 <sup>a</sup>	4.37 <sup>a</sup>	4.37 <sup>a</sup>	4.37 <sup>a</sup>	4.37 <sup>a</sup>	4.37 <sup>a</sup>	4.37 <sup>a</sup>	4.38 <sup>b</sup>	

<sup>a</sup> Different letters following the numbers on the same line indicate differences between means,  $p < 0.05$ . Data are the means of six replicates.



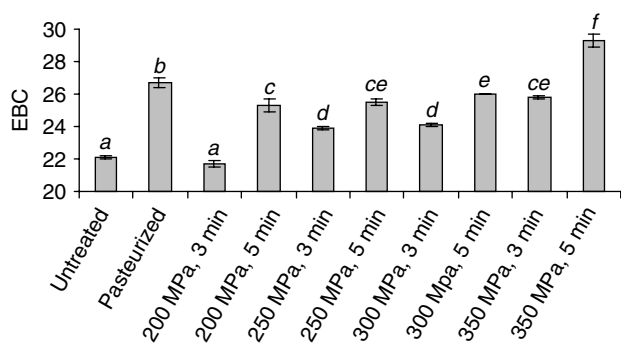
**Figure 1.** Effect of HHP on colour of lager beer. Different letters indicate differences between means at  $p < 0.05$ . Data are the means of six replicates.



**Figure 2.** Effect of HHP on protein sensitivity of lager beer. Different letters indicate differences between means at  $p < 0.05$ . Data are the means of six replicates.

The effect of HHP on colour of the beer samples is given in Fig 1. The EBC value increased as the magnitude of pressure and pressurization time increased significantly ( $p < 0.05$ ). Fischer *et al*<sup>11</sup> reported that HHP treatment of beer had no significant effect on colour. It has also been reported that heat pasteurization and HHP (600 MPa for 5 min) had no influence on the lightness, but all the other colour indices were higher in heat processed pale ale.<sup>4</sup> Basically, the kind of beer and differences in treatment conditions seem to be the causes of the different results reported in literature.

As the pressure and pressurization time increased, the protein sensitivity and chill haze of the samples increased slightly compared with untreated samples (Figs 2 and 3). Since HHP causes partial protein denaturation by rearrangement and/or destruction of the tertiary and quaternary structure, lower pressures and pressurization times (ie 200 MPa for 3 min) had less effect than the other pressure–time combinations when compared with controls (Fig 2). Probably at low pressure–time combinations the proteins rearrange themselves through denaturation, removing available binding sites for phenolic compounds (tannins). However, as the pressure and pressurization time increase, new binding sites may be formed owing to destruction of protein structure so that protein sensitivity increases. It has been reported that permanent and chill haze turbidity increase as pressure



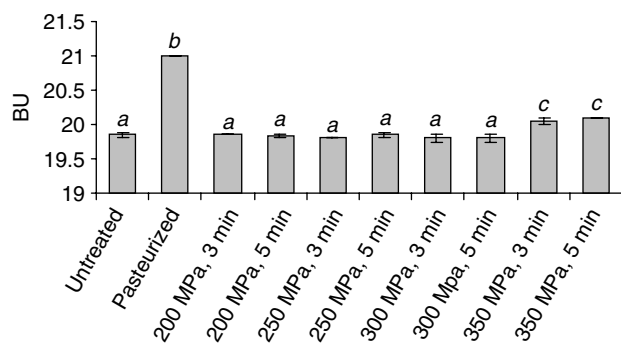
**Figure 3.** Effect of HHP on chill haze of lager beer. Different letters indicate differences between means at  $p < 0.05$ . Data are the means of six replicates.

increases.<sup>4,11</sup> Beer, if chilled, often produces a 'chill haze' which normally disappears when it is warmed; however cyclic warming and delayed cooling cause a permanent haze, which will not disappear on warming.<sup>14,15</sup> Chill hazes create a more serious problem with lager beers, which are served at lower temperatures, than ales.<sup>10</sup> Prolonged cold storage at  $-1$  or  $-2$  °C followed by filtration is used to remove the haze-forming material in the brewery. Since pressure treatment increased the chill haze values of lager beer, application of HHP at 20 °C could be an alternative to low temperature storage for removal of unwanted haze.

The behaviour of HHP samples must be evaluated by taking into account that every beer contains a considerable amount of haze-active proteins. Denaturation of these proteins exposes additional hydrophobic binding sites and this leads to strong protein-phenolics binding developing haze.<sup>4,16</sup> The different degree and modality of protein denaturation may influence the interaction between colloidal substances and could be the basis of observed effects on beer protein sensitivity and chill haze.

Bitterness in beer arises when the  $\alpha$ -acids in hops are isomerized during boiling with wort. The formation of iso- $\alpha$ -acids or isohumulones as a result of heat treatment at elevated temperatures has been previously reported.<sup>17</sup> Our results revealed that the bitterness units (BU), which are an index for isohumulone content in beer, of the samples were increased after heat treatment (60 °C, 15 min) whereas a decrease was expected as isohumulones decrease due to oxidation during heat pasteurization. This contradiction might be explained by the presence of some components which have no direct relationship with isohumulones ie bitterness units (BU).

Although HHP-treated samples resulted in bitterness units (up to 300 MPa) similar to controls (Fig 4), this is not yet sufficient to conclude that lager beer bitterness does not change significantly during HHP treatment. The influence of high pressure on the hop constituents, the solution and possibly on the quality of the soluble materials and isomerization could cause substantial changes during processing which necessitates further in-depth research. Although the BU



**Figure 4.** Effect of HHP on bitterness of lager beer. Different letters indicate differences between means at  $p < 0.05$ . Data are the means of six replicates.

value obtained from the spectrophotometric method is a good representation of the sensory bitterness of beer,<sup>14</sup> the selectivity of this method is not as high as HPLC analysis. For further investigation HPLC analysis could be performed to determine whether bitterness *per se* changes during the treatment.

## CONCLUSIONS

The main attributes of lager beer such as ethanol content, extract and pH were not affected by either conventional heat pasteurization or HHP. HHP and heat pasteurization affected colour, chill haze, protein sensitivity and bitterness. However, the change in bitterness was greater in conventional heat pasteurization, but pressures up to 300 MPa had no significant affect on bitterness. Our results revealed that HHP could be successfully used to produce stable lager beer even at temperatures well below those required for heat pasteurization. The results presented here could be seen as a basis for possible operational use of HHP technology in the brewery.

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