Effect of Pasteurization of Bottled Unfiltered Beer to Beer Volatile Substances

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Abstract

Pasteurization is a heat-treatment process that has been used since the 19th century to extend the shelf-life of food and beverages by eliminating most pathogens and spoiling microorganisms that may harm the quality and safeness of the product. It is a commonly used method in the food industry ever since the market became more demanding and more awareness regarding the influence of diet on personal health has been raised. However, in the case of beer, a number of contradictory reports has emerged concerning the influence of pasteurization in its flavor attributes. Some of them mention pasteurization enhances beer staling and causes a more prominent loss of fresh flavors, whereas others report this heat-treatment process favors the stability of beer organoleptic properties in addition to preventing its spoilage. Therefore, this study was performed in order to evaluate the impact of pasteurization on the sensory quality of beer, as well as on its more influent aroma-active compounds. For this, triangle and preference tests were performed using tunnel and flash pasteurized 12% lagers and 20% imperial lagers to assess which pasteurization method produced better results regarding sensory quality maintenance. In addition, IPA samples were used to thoroughly evaluate and compare the sensory profile of pasteurized and non-pasteurized bottled aged beers, which were furtherly submitted to analysis of hop-derived compounds, to check the influence of pasteurization on these flavoring agents through storage time (up to 4 months). Furthermore, sets of pasteurized and non-pasteurized 12% lager and 20% imperial lager matured samples were used to perform analysis with regard to their carbonyl profile, for testing the effect of pasteurization on the formation of staling indicators. Tunnel pasteurization proved to be a more efficient method to ensure a long-term quality product. Use of pasteurization appears to have a positive effect on perseverance of overall flavor attributes and, in contrast, dismissing this treatment caused spoilage notes on the tested beers within a period of 4 months. Hop-derived and carbonyl compounds analysis on their respective beer types did not exhibit any significant consequence of pasteurization usage, apart from slight evidences of a higher degree of oxidation for pasteurized samples in comparison to non-pasteurized ones, although further studies with beers stored for longer periods are needed, in both cases, to reach more accurate conclusions.

Keywords: Beer, Pasteurization, Spoilage, Carbonyls, Staling, Hop oils

1. Introduction

Beer is one of the oldest beverages to ever been produced by humans. The first written reports about its production are dated back from the fifth millennium B.C. coming from the ancient region of Babylonia, although it is believed that beer existed far before these written records.(1)(2) As centuries of development and introduction of new techniques went by, the brewing industry has become a huge global business whether through big multinational companies or smaller producers ranging from regional

breweries to local brewpubs.(3) The widespread increasing business results on a more demanding and competitive market, in which the search for quality and affordable products is constant.(4) Therefore, quality maintenance and reproducibility are significant distinguishing factors and a main concern for the leading exporting beer brands and are mostly related to storage conditions as beer aging appears to be the main issue affecting the quality of the product.(5) The chemical composition of beer is changed during its storage, which can alter the sensory properties such as aroma, color and taste. Unlike some wines, beer aging is usually considered to have a negative impact for its sensory quality as a variety of new different flavors may arise, depending on the beer type and storage conditions.(5) The shelf-life of food products is an important feature for both manufacturers and consumers. Its evaluation is mostly aimed at assuring food safety, although it additionally concerns quality aspects including physical, chemical, and sensorial properties. Therefore, shelf-life studies can provide important information to ensure a high-quality product during its storage period.(6) The shelf-life of beer is especially related to its microbiological, colloidal, foam, color and flavor stabilities. In the past, the appearance and growth of beer spoiling microorganisms was treated initially as the primary trouble causing phenomena. However, the main concern of investigation works is currently focused on the factors affecting beer aroma and taste, being these considered as the most important parameters regarding the quality of the product, since technology development applied to brewing industry allowed assuring control over microbiological issues.(5) Consumers expect the flavor of a certain beer brand to be constant every time. Nonetheless, if not consumed fresh, beer aging may be responsible for noticeable flavor changes or even flavor loss. Although several efforts have been made through numerous studies and experiences, beer aging phenomena remains difficult to control since multiple chemical changes and reactions may occur throughout storage time leading to beer staling and spoilage.(5) Flavor deterioration is caused mainly due to contact of beer with oxygen and can happen as rapidly as higher it is the oxygen content of bottled beer. However, further studies proved that beer staling can still occur at very low oxygen levels, suggesting that beer staling phenomena is in part anon-oxidative process.(7) Another aspect to take into account which also plays a big role concerning beer aging characteristics are storage temperatures, as they directly affect the chemical reactions that occur within the bottle.(5) Nonetheless, the reaction rate does not increase constantly with temperature, as it depends on the activation energy of each reaction, which differs according to the reaction type.(5) Deterioration phenomena is the result of both formation and degradations reactions, which lead to the development and dissipation of certain flavors. In order to have noticeable effects, the formation of new compounds must reach concentrations above their respective flavor threshold, while in contrast, degradation of compounds must reach concentrations below their flavor threshold to cause losses of initial fresh beer flavors.(5) Nevertheless, interactions that may occur amongst different molecules, whether they are initially present in beer or not, can also have an effect on the resultant aroma and taste by suppressing or enhancing the impact of certain flavors, which frequently happens amidst volatile compounds.(8) Volatile compounds represent a big part of the composition of every beer. The introduction of gas chromatography techniques allowed a more detailed outlook on the changes in beer volatiles during storage, which were assumed to have a significant relation with the formation of staling compounds.(9)(10) More recently, development of new techniques such as aroma extraction dilution analysis (AEDA) permitted an improvement in order to detect volatiles in food products and also to evaluate their relevance for odor perception.(11) This method allowed the identification of several staling compounds in beer.(12) From the beginning, carbonyl compounds have drawn the most interest amongst volatile substances possibly responsible for beer staling, as they were

previously known to cause flavor changes in other food products such as milk, oils, vegetables and butter.(5) The majority of carbonyl compounds in beer have their origin on raw materials used for its production or emerge through chemical reactions, particularly in the pre and post fermentation stages of the production process. More precisely, carbonyls are mostly produced in heat-enhanced reactions that occur during kilning of malt, mashing, wort processing and pasteurization of the final product, although most of the ones formed during pre-fermentation stages are reduced by yeasts during fermentation, generating the correspondent alcohols and acetate esters.(13) On Table 1 are presented some the most relevant carbonyl aging markers in beer and the respective type of aging reaction involved in their formation during storage. Oxidation of unsaturated fatty acids and hop-derived compounds also play a big part on sensory changes that occur in beer through time. Hops have a significant degree of importance regarding beer flavor stability, preservation and organoleptic properties.(14) Originally, hops were introduced in beer production process to inhibit the growth of microorganisms that could spoil beer.(15) Nowadays, hops are also added to impart bitterness and pleasant aromas to beer.(16) Hop essential oils are prone to many changes in their composition and are relatively easily degradable, which can cause significant flavor loss.(17) Beer has been recognized for a long time as a safe beverage with a remarkable microbiological stability. The reason is that beer is an unfavorable medium for many microorganisms due to the presence of ethanol, hop bitter compounds, the high content of carbon dioxide, the low pH and the presence of only traces of nutritive substances such as glucose and amino acids.(18) However, in spite of these unfavorable features, a few microorganisms still manage to grow in beer and cause an increase of turbidity and unpleasant sensory changes. This phenomena is called beer spoilage.(19)

Table 1- Carbonyl aging markers in beer and the type of reaction involved in their formation during storage.(5)

Carbonyl aging marker	Aging reaction
Acetaldehyde	Oxidation of ethanol
(*E*)-2-Nonenal	Aldol condensation
3-Methylpropanal	Strecker degradation of amino acids
3-Methylbutanal	Strecker degradation of amino acids
4-Methylbutanal	Strecker degradation of amino acids
2,4,5-Trimethyl-1,3-dioxolane	Aldehyde acetalization
Diacetyl	Oxidation of excretion products from yeast metabolism
2,3-Pentanedione	Oxidation of excretion products from yeast metabolism
5-Hydroxymethyl Furfural	Maillard reaction
Furfural	Maillard reaction
Furfuryl Ethyl Ether	Etherification of ethanol and Maillard compounds
Isoamyl Acetate	Hydrolysis of esters produced by yeasts
Benzaldehyde	Strecker degradation of amino acids

The reported beer spoilage microorganisms include mainly Gram-positive lactic acid bacteria belonging to the genera *Lactobacillus* and *Pediococcus*, although some Gram-negative species and wild yeasts can also be responsible for beer spoilage.(18) Hop compounds, namely α-acids, have demonstrated to protect beer from microbial infection, however, it was found that these compounds only inhibit growth of Gram-positive bacteria and not of Gram-negative species, despite lactic acid bacteria have reportedly developed resistance to hop-derived anti-bacterial mechanisms.(20)(21) Therefore, a more effective technique to prevent the growth of beer spoilage microorganisms must be applied, as it is the case of pasteurization. Beer is pasteurized after fermentation in order to keep it microbiologically stable. Even though it is a commonly used method in most breweries, a number of them prefer not to pasteurize their beer. Those which do not pasteurize their beer, usually do not filter it as well, selling an unfiltered product.(22) Therefore, research on pasteurized unfiltered beer remains important, as the influence of pasteurization on the chemical storage stability is not very well described, and only few studies have been carried out on this subject. Additionally, it is still not clear if the effects of pasteurizing beer are mostly positive or negative, since rather contradictory results have been obtained.(22) Because of the resulting changes in flavor stability in some cases, pasteurization has been pointed as a generator of beer staling. Although the discussion that remains has to do with the spoilage of unpasteurized beer after long periods of storage and the even more prominent negative effects of dismissing pasteurization due to the lack of heat treatment necessary for inactivation of enzymes and other deteriorating microorganisms.(23) There are two types of pasteurization process used in beer production: tunnel pasteurization and flash pasteurization. The present study was conducted in the Laboratory of Brewing and Malting of the Department of Biotechnology of University of Chemistry and Technology Prague, in association with Brevnov Monastery Brewery of St. Adalbert from March to July 2019. The main goal was to investigate the effect of pasteurization in unfiltered beer, regarding its chemical stability and sensory properties, after storage periods of 0, 2 and 4 months. For this purpose, samples of Czech typical light lager (12% ABV - alcohol by volume) and imperial lager (20 % ABV), as well as samples of Monastery IPA (Indian Pale Ale), were used, all produced at the brewery. This investigation work was split into four different branches. Samples of the two types of unfiltered lager beer were submitted to both tunnel and flash pasteurization and triangle and preference test trials were performed after each of the mentioned storage time intervals, in order to detect if there were significant sensorial changes caused by each type of pasteurization method and also to evaluate if these changes were more easily distinguishable and have more prominent effects in higher alcoholic beers. In parallel, another group of bottled 12% lager and 20% imperial lager samples were stored for periods of 0, 2 and 4 months, while a number of each was previously tunnel pasteurized, whereas others were not. These samples were taken into the laboratory at the end of each storage period where determination and comparison of carbonyl compounds concentration was performed, to figure out the effect of pasteurization and storage time in these aging markers. Regarding IPA samples, the procedure included the addition of a solution containing essential hop oils to some bottles and similar to what was done with lagers, only a number of those bottles was tunnel pasteurized before being stored for 0, 2 and 4 months. After each storage period, a deep sensory analysis was performed to evaluate the effects of pasteurization on beer essential hop oils and the resultant flavors and also to verify if sensory profile changes were more prominent in samples with higher concentration of essential oils. Each sensory analysis was followed by isolation and purification of essential oils extract from all IPA samples at the laboratory and further analytical methods were used to determine the content of essential oils compounds.

2. Materials and Methods

Pasteurization of beer and storage conditions - The pasteurization of beer took place at Brevnov Monastery Brewery of St. Adalbert on the 4th of March 2019. The flash pasteurizer was used to sterilize 3 kegs containing 15 L of 20% imperial lager and 3 kegs containing 15 L of 12% light lager. All these kegs were pasteurized at 40 PU's. The maximum temperature at which beer remained in the stand was 71-72°C and the residence time was approximately 45 seconds. Pressure was regulated to 10 bar, as this is an important parameter regarding beer carbonation. All these parameters, including flow-rate, were previously set-up and monitorized during the process. Tunnel pasteurization was applied to 4 sets of 12 bottles of 0.75 L. Each different set of bottles corresponded to 12% light lager, 20% imperial lager, Monastery IPA and Monastery IPA with addition of hop essential oils solution. All these bottles were pasteurized in a water bath at 38 PU's and the maximum temperature reached inside the pasteurizer was 62°C. The entire process of pasteurization (heating, pasteurization, cooling) lasted about 50 minutes. In addition to the above-mentioned tunnel pasteurized beers, an equal number of bottles for each type of beer was also used for storage, in the unpasteurized version (for comparison). All bottled beers were stored in a room at 21℃ for periods of 0, 2 and 4 months. After these storage periods, 2 bottles of each different set of pasteurized and unpasteurized beers were taken into the Laboratory of Brewing and Malting of the Department of Biotechnology of University of Chemistry and Technology Prague for further chemical analysis.

Triangle tests - Triangle test was used in order to find differences between tunnel pasteurized and flash pasteurized beer, using coded samples of 12% light lager and 20% imperial lager. The aim of this test was also to verify if the differences were more easily detected in higher alcoholic beers or not. A triangle test was performed after periods of 0, 2 and 4 months of pasteurized beer storage and the results were compared, in order to check if taste differences between tunnel pasteurized and flash pasteurized beer were more effortlessly detected after longer periods of storage.(24)

Preference tests - When statically significant differences were noticed by the panel of assessors in triangle tests, they were followed by preference tests where assessors had the chance to try again both different samples correctly identified during the triangle test and chose which one they think it has the best palate.

Sensory tests - For the purpose of this work, a sensory analysis was done to create the sensory profile of all Monastery IPA samples, with and without the addition of essential oils solution (unpasteurized samples and samples pasteurized before storage periods of 0, 2 and 4 months). The assessors either evaluated the intensity of a certain attribute within a defined scale or gave an overall appreciation. All results were posteriorly compared, with the aim of verifying the differences concerning the sensorial profile of pasteurized and unpasteurized beer, the effect of time on the sensory properties of the ale and if the sensory differences were more prominent when increasing the concentration of essential oils.(25)

Carbonyl aging markers analysis in lagers - The headspace solid-phase micro-extraction method in combination with gas chromatography and mass spectrometry (HS-SPME GC-MS) was used to determine carbonyl compounds. Sample processing included kieselguhr (LABICOM s.r.o, Czech Republic) filtration, derivatization method with PFBOA and addition of an internal standard. Derivatization solutions were initially

prepared by dissolving 0.150 g of PFBOA (≥ 99%, Sigma-Aldrich, Switzerland) in demineralized water in a 25 mL flask. The solution was stored in glass vials at 4°C. A solution of internal standard was achieved by measuring 30 μL of 3-fluorobenzaldehyde (≥ 97%, Sigma-Aldrich, Switzerland) in a 50 mL flask with addition of ethanol until the volume line. 4 mL of this solution were then pipetted into another 50 mL flask and ethanol was added again until the volume line. This solution was stored in glass vials at -18°C. A mixed solution of carbonyl compounds standards was prepared in a 200 mL flask half filled with ethanol to which were added 400 µL of 2-methylpropanal (99%, Sigma-Aldrich, Switzerland); 400 µL of 2-methylbutanal (97%, Fluka, Switzerland); 400 µL of 3-methylpropanal (97%, Sigma-Aldrich, Switzerland); 200 µL of heptanal (97%, Merck, Germany); 200 µL of octanal (98%, Merck, Germany); 1200 µL of furfural (98%, Alfa Aesar, Germany); 300 µL of benzaldehyde (98%, Alfa Aesar, Germany); 300 µL of 2,3-pentanedione (97%, Sigma-Aldrich, Switzerland); 300 µL of diacetyl (97%, Sigma-Aldrich, Switzerland); 20 µL of (E)-2-nonenal (97%, Sigma-Aldrich, Switzerland) and completed with ethanol until the 200 mL volume line. Three different volumes of this solution (0.02, 0.1 and 0.2 mL) were transferred to three different 100 mL flasks to which ethanol was added until the volume line and three replicates were prepared for each concentration. The thus prepared solutions with diluted carbonyl standards were stored at 4°C. In addition to mixed carbonyl standard solutions, another sample was prepared with standard commercial beer for calibration purposes. For the preparation of this sample, a bottle of Gambrinus Originál 10° (4.3% ABV) was used. Approximately 150 mL of cold beer was degassed by shaking on a shaker for 5 minutes at a frequency of 175 min^{-1}. To prepare the samples for monitoring free carbonyl compounds 2.5 g of NaCl (p.a., Lach-Ner, s.r.o., Czech Republic), 10 mL of shake beer (free from carbon dioxide) and 100 μL of 3-fluorobenzaldehyde were introduced in a dark glass vial. Same approach was made for the remaining calibration solutions preparation, to which were added 100 μL of the respective mixed standard solution in addition to the previously mentioned components. In total, 13 calibration solutions were prepared, as follows: 4 containing solely degassed commercial beer (Beer 1, Beer 2, Beer 3 and Beer 4), one of them to be used as a “blank” for GC-MS apparatus calibration (Beer 1), 3 to which were added a low concentration mixed carbonyl standards solution (Low 1 , Low 2 and Low 3), 3 to which were added a medium concentration mixed carbonyl standards solution (Medium 1, Medium 2 and Medium 3) and 3 to which were added a high concentration mixed carbonyl standards solution (High 1, High 2 and High 3). After closure, all calibration solutions contents were mixed on a Vortex mixer for about 1 minute. Derivatization reagent solutions were prepared in dark glass vials with 3 g of NaCl, 10 mL of demineralized water and 20 µL of PFBOA solution. The contents of the vials were then mixed on a Vortex mixer for about 1 minute. The amount of derivatization reagent solutions prepared was equal to the number of prepared calibration solutions, so each can singularly undergo through carbonyl derivatization reaction process. In the end, calibration lines were designed for each analyzed carbonyl, relating peak area in function of their respective concentration measured in all 3 different calibration solutions. The obtained equations were used for determination of carbonyl content in 12% light lager and 20% imperial lager pasteurized and unpasteurized samples. The processing of these samples was similar to the preparation of calibration solutions (2.5 g of NaCl, 10 mL of filtered beer and 100 μL of internal standard). Two replicates for each sample were prepared, achieving a total of 24 samples (12% Lager NP 1, 12% Lager NP 2, 12% Lager P 1, 12% Lager P 2, 20% IP NP 1, 20% IP NP 2, 20% IP P 1, 20% IP P 2 for each storage period – 0, 2 and 4 months). Same number of derivatization solutions was prepared for these measurements. The individual carbonyl content of these samples was also quantified using manual integration tool and peak areas were normalized with the previously selected internal standard area

(belonging to Beer 3 sample). An average between the values obtained for the two replicates of each sample was also performed. By substituting the resulting average peak areas on the y parameter of the respective calibration equations determined for each carbonyl compound, their concentrations can be obtained in every beer sample.

Essential hop oils analysis in Monastery IPA's - Unfiltered Monastery IPA pasteurized and non-pasteurized samples were shifted to the cold chamber within the facilities of University of Chemistry and Technology Prague after 0, 2 and 4 months of storage at the brewery. Each sample was kieselguhr (LABICOM s.r.o, Czech Republic) filtrated before isolation and purification process of hop essential oils, prior to GC-MS analysis for identification and quantification. Internal standard method was also used for homogenization of the results. Isolation of hop essential oils compounds from beer samples comprised three different parts: steam distillation, solid-phase extraction and evaporation. The measured volume for each filtrated sample was added to the boiling flask together with a pipetted amount of silicone antifoam (Sigma-Aldrich, Switzerland), where it was steam-distilled for approximately 1.5 hours. The distillates were collected in three different receivers. In the first receiver, 5 mL of denatured ethanol was prior added, in the second 50 mL and in the third one 10 mL, to prevent volatiles evaporation. The second receiver was placed in a beaker filled with ice and the third one in a thermos filled with ice and sodium chloride. The distillates of a volume of approximately 350 mL for every sample were collected from all three receivers into 500 mL Erlenmeyer flasks and transferred into 600 mL plastic bottles. Secondly, the obtained distillates from the beer matrices were extracted using solid-phase extraction. A SI-1 polar column (Agilent Technologies, USA) was used for pre-cleaning the distillates and a C18 single-use column (Agilent Technologies, USA) was used to trap the essential oils in the sorbent, as essential oils compounds are long-chained non-polar molecules. The columns were prior conditioned, respectively with 5 mL of n-hexane (VWR Chemicals, France) and 5 mL of distilled water for SI-1 and 5 mL of ethanol absolute and 5 mL of distilled water for C18. The columns were connected together, with SI-1 on top, and then with the distillate bottles through rubber tubes. Eventually, this step lasted 3-4 hours. The next task was to isolate the entrapped essential oils fractions in the sorbent of the C18 column. This was achieved by eluting the columns with 6 mL of n-hexane under vacuum. In the end, 100 µL of borneol (0.751 g/L, Merck, Germany) were added as internal standard to all samples. The eluates were then transferred to 100 mL Falcon tubes and dried overnight with anhydrous sodium sulfate (Penta s.r.o., Czech Republic). The next day, the eluates were filtered from the anhydrous salt in PTFE disc filters with n-hexane, followed by evaporation under vacuum in the rotary evaporator to remove solvent content. The remaining fraction was then transferred to a vial and added up to 1.5 mL with GC-grade n-hexane, ready for GC-MS analysis.

3. Results and Discussion

Triangle and preference tests performed for tunnel or flash pasteurized 12% lagers and 20% imperial lagers stored for periods of 0, 2 and 4 months, demonstrated that tunnel pasteurized lagers are preferred over flash pasteurized ones after a period of 4 months of storage. Results demonstrated no significant flavor differences between both tested pasteurization methods application after 0 and 2 months of beer storage. Only after 4 months of storage, significant palate differences were noticed, however only for 20% imperial lagers, suggesting that aging effects were more prominent for higher

alcoholic beers, and preference tests unanimously selected tunnel pasteurization as the method that preserved better these lagers sensory properties. Flash pasteurized lagers denoted higher oxidation degree after 4 months of storage, which was assumed to be due to higher oxygen contact and exposure to higher temperatures during flash pasteurization, in comparison to tunnel pasteurized samples.

Carbonyl aging markers analysis in lagers did not allow to reach major conclusions since the third set of analyzed samples, which included pasteurized and non-pasteurized 12% lagers and 20% imperial lagers stored for 4 months after pasteurization, produced illogical results. Notwithstanding, when comparing samples stored for 0 and 2 months, the aging effect could be confirmed, particularly for 20% imperial lagers. Although no regular variation could be observed for 12% lagers, since an increase on concentration was verified for some carbonyl compounds while for some others it decreased, the mean results show that 2 months of storage had a negative impact on these lager samples. Furfural concentrations always displayed higher values for non-pasteurized lagers in comparison to their respective pasteurized version and furfural levels were far superior in 20% imperial lagers than in 12% lagers after 2 months of storage, confirming the premise that furfural is present in higher concentrations in high alcoholic beers. Moreover, vicinal diketones and (*E*)-2-nonenal concentration was also far superior in imperial lagers. Regarding the effect of pasteurization process in these unfiltered lagers, only a slight improvement for 12% lager samples can be mentioned, as non-pasteurized version produced a carbonyl profile slightly more indicative of a higher staling degree.

Sensory analysis of Monastery IPA's resulted in a verification of positive effects of pasteurization regarding flavor and aroma perseverance as, regardless of the storage time the tested samples were subjected to, pasteurized beers scored better results on overall beer ratings in comparison to their non-pasteurized versions. Furthermore, the worst scores attributed to non-pasteurized IPA's were given to samples stored for 4 months, the longest storage period tested, and assessors made reference to their acidic taste and unpleasant aroma, including some remarks from specialized members who recognized both of these samples as oxidized spoiled products, possibly due to microbial growth, namely lactic acid bacteria, resultant from the lack of heat treatment. Thus, it was concluded that the shelf-life of these unpasteurized products is inferior to 4 months. Pasteurized samples also exhibited a more regular sensory profile through storage time regarding all 8 different flavor attributes assesses: overall intensity of flavor, intensity of hop, citrus, floral, fruity, spicy, resin and aromas and intensity of bitterness.

Essential hop oils analysis in Monastery IPA's revealed that overall, β-myrcene, D-limonene, linalool and humulene epoxide I are the components that play a bigger role on the sensory profile perceived for these studied Monastery IPA samples. Moreover, linalool concentration increased over storage time in every tested sample, which was assumed to be due to yeast metabolism mechanisms, such as release of linalool from linalool glycosides or isomerization of nerol and geraniol to linalool. Oxidation traces were evaluated by following humulene epoxide I levels though storage time on each sample. Humulene epoxide I concentration increase was more accentuated on Monastery IPA samples to which were added essential oils, which is related to higher availability of oxidizable substrate. Moreover, humulene epoxide I levels suggested that pasteurization induces oxidation reactions in these ales, as it was found in relatively higher concentrations in pasteurized samples, in comparison to their correspondent non-pasteurized version.

4. Conclusions and Future Prospects

Regarding tunnel or flash pasteurization of two types of Czech unfiltered lager beers (12% light lager and 20% imperial lager), results obtained from triangle and preference tests allow to suggest that tunnel method is better suited for preserving the organoleptic properties and flavor quality of lager beers in a long-term. The preference found for tunnel pasteurized beers over flash pasteurized ones is possibly due to higher oxygen contact and subjection to higher temperatures during flash pasteurization, which cause further damage on the sensory profile of beer. However, statistically significant results regarding flavor differences between tunnel and flash pasteurized lagers were only noticed on samples stored for 4 months. Thus, tunnel pasteurization is a more appropriate method for lagers to be exported or beforehand require longer storage periods before consumption, while flash pasteurization is more suitable for lagers about to be shifted from the brewery to a local market selling point or to any other commercialization route which merely implies a storage period equal or inferior to 2 months, since flash method has less associated expenses and apparently equally affects the flavor properties of lagers, in comparison to tunnel method, within this period. However, it would be relevant to collect samples and preform triangle trials within shorter storage periods, in order to have a more precise notion on what is the aging period required to detect significant flavor differences between flash and tunnel pasteurized lagers, since only periods of 2 and 4 months were tested. Furthermore, these trials should be attended by a higher number of assessors than those who attended the trials performed for this experimental work, so a higher accuracy level of the results can be achieved.

Pasteurization of unfiltered IPA samples produced a positive effect on flavor stability and consistency over time. In contrast, absence of pasteurization in tested IPA's resulted in a more irregular sensory profile over time and spoilage notes perceived after 4 months of aging, meaning that the shelf-life of non-pasteurized samples is inferior to 4 months due to product quality issues. Furthermore, assessments performed suggested that pasteurization preserves better the overall quality of the product, along with some specific flavor attributes such as bitterness and intensity of hop aroma, in any stage of maturation process. Addition of essential hop oils did not produce significant differences, apart from a higher perception of certain flavor attributes. In order to complement these results, it would be interesting to perform a microbiological test to identify which microorganisms may be possibly responsible for IPA samples spoilage after 4 months of storage. Although it was assumed beer spoilage is most likely due to lactic acid bacteria growth, as acidic notes were remarked during sensory tests, it could also be relevant to identify and quantify all microorganisms present in spoiled beer, so a microbiological profile of these analyzed IPA samples can be developed and used for future comparative analysis.

Analysis of carbonyl aging markers performed in unfiltered 12% light lagers and 20% imperial lagers did not allow to reach major conclusions about the effect of pasteurization on beer staling. Especially because the third set of analyzed bottles, which contained samples stored for 4 months, generated absurd results which were not taken into account. It is only mentionable that a higher concentration of staling carbonyls was detected in samples aged for 2 months, in comparison to their respective versions stored solely for a period of days prior to analysis, and that staling effect seemed to be more prominent in higher alcoholic beers and slightly more evident in pasteurized samples. Therefore, these analyses should be repeated before further conclusions can be reached. It would have also been positive to test these lagers after longer storage periods in order to

investigate about the aging time from which carbonyls concentration stabilizes and aging reactions cease.

Essential hop oils analysis of unfiltered IPA samples revealed that pasteurization enhances oxidation of hop-derived aroma-active compounds, although in not such a superior rate in comparison to non-pasteurized samples. Moreover, β-myrcene, D-limonene, linalool and humulene epoxide I were identified as the components most responsible for the aromas perceived in the sensory analysis of these samples, with particular relevance to linalool, to which increasing concentrations through time were assumed to be related with yeast metabolism. Notwithstanding, more studies aimed at determining the effect of pasteurization on hop-derived compounds in unfiltered beer should be carried out, since it was not possible to perform a comparative analysis due to lack of published reports on the topic.

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