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ASLT Method for Beer Accelerated Shelf-Life Determination

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***Abstract*—**The aim of current research was to investigate ASLT method suitability for accelerated beer shelf-life determination. The research was accomplished on popular Latvian beer: light filtrated and unfiltered pasteurized beer with alcohol content 5.2%; dark filtrated pasteurized beer with alcohol content 4.2% with shelf-life five months. Bottled in dark glass bottles beer samples were storage during 20 weeks at several temperature regimes: +10±1 °C, +20±1 °C, +30±1 °C, +40±1 °C. Samples quality parameters as physically-chemical and microbiological was tested every two weeks using standard methods. It is possible to determine beer shelf-life rapidly during storage at +30±1 °C for filtered pasteurized light beer by 2.5 times, unfiltered pasteurized light beer by 1.4 times and for filtered pasteurized dark beer by 1.7 times. During preset experiments it was proved, that it is possible to determine beer shelf-life rapidly using ASLT method if beer storage temperature could be increased by +10±1 °C.

***Keywords*—**Beer, shelf-life, ASLT method.

I. Introduction

ETHANOL is one of the most commonly used recreational drugs worldwide and it is often ingested as a component of beer. When beer is consumed, ethanol is absorbed from the gastrointestinal tract by diffusion and is swiftly distributed in the blood before entering tissues. Ethanol is metabolized to acetaldehyde mainly in the stomach and liver. Acetaldehyde is highly toxic and binds cellular constituents generating harmful acetaldehyde adducts [1].

Fermented beer is a complex mixture of fluids, cells, aggregates and macromolecules. Fermentation of beer is done by yeast cells, converting starch derived maltose into alcohol and CO_2. The grain also delivers proteins, which in part are required for foam formation. Next to grains, also hop is used, whose compounds render the bitter taste and preservative functionality. One of these functional ingredients is a type of polyphenols like tannins. They are anti-oxidants, which protect aromas and taste compounds, like hop bitter acids (humulones), from oxidation [2].

Iso-α-acids, the main bitterness substances in beer, are particularly sensitive to degradation during storage, which results in a decrease in sensory bitterness. The iso-α-acids comprise six major components: the *trans*- and *cis*-isomers of isocohumulone, isohumulone and isoadhumulone. The *trans*-isomers are much more sensitive to degradation than the *cis*-isomers. The concentration ratio *trans/cis* isomer was proposed as a good marker for the flavour deterioration of beer [3].

Turbidity is basically cloudiness in a liquid, which in beer is caused by the presence of un-dissolved particles such as yeast, proteins or diatomaceous earth. These particles scatter the incident light and consequently lead to a milky or even opaque appearance of the liquid [4].

The integrity of an official beer sample is vitally important when authenticity and adulteration allegations have been made. The percentage alcoholcontent by volume (% ABV) and original gravity (OG) are highly indicative parameters and can be used to identify a beer's authenticity. OG is defined as the specific gravity (SG) of the wort or liquor from which the beer is made [5]. Beer strength varies by local custom. British ale tends to average 4.4% ABV. Belgian beers tend to average 8% ABV. The strength of the typical global pale lager is 5% ABV. The yeast used for brewing beer normally cannot get the strength much beyond 12% ABV; however, in the 1980s the Swiss brewery Hurlimann developed a yeast strain which could get as high as 14% for their Samichlaus beer. Since then, breweries have experimented with using champagne yeasts, continually pushing up the strength. Samuel Adams reached 20% ABV with Millennium. The strongest beer sold in Britain was Dogfish Head's World Wide Stout, a 21% ABV stout which was available from UK Safeways in 2003. In Japan
in 2005, the Hakusekikan Beer Restaurant sold an eisbock, strengthened through freezing, (believed to be) 28% ABV. The beer that is considered to be the strongest yet made is Hair of the Dog's Dave – a 29% ABV barley wine made in 1994. For a comprehensive list of the strength of all beers check out the link below [6]. However, Latvians ale tends to average from 4.0 to 6.8% ABV.

One of the main concerns of brewers is to preserve the organoleptic stability of beer during ageing. A key factor influencing beer ageing and its stability is pH. From a sensory standpoint, if the pH of fresh beer decreases below 4.0, sharp, acid, bitter, and drying effects increase rapidly in intensity, with a markedly enhanced metallic after-palate for pH values below 3.7. On the other hand, above 4.0, palate effects relate to increased mouth-coating, with higher scores for biscuity and toasted characters, and even soapy and caustic notes if the

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pH rises above 4.4 [7].

The anaerobic bacteria are a very important group of beer-spoiling microorganisms. Among them, the highest risks are associated with the genus *Pectinatus*, but the genus *Megasphaera* is also often detected in breweries. It is supposed that these microorganisms are able to contaminate beer thanks to their ability to form or to be a part of microbial biofilms present on the surface of pipelines, floors, machinery etc. [8].

Beer is an inherently unstable product and can undergo significant changes if not stored correctly. The % ABV can decrease as the beer goes ''off'' by microbial conversion of ethanol to acetic acid. Potential losses of alcohol from sample portions may also occur due to the migration of ethanol through container walls, or if the container is not properly sealed. The same is true of draught and pasteurized beers. However, there is potential for the % ABV to increase by further fermentation if not stored correctly. This is particularly relevant to live ''real ales''. ''Real ale'' is a living fresh beer that undergoes a natural second fermentation in the cask. Like any natural product, the beer will age and go off, and therefore must be drunk within a strict timescale. It requires care in handling on its way to the selling point, and care there to bring it to perfection. Yeast is still present in the cask, but has to settle out, so it is not poured into the glass. ''Real Ale'' must be distinguished from a brewery conditioned or ''keg beer'', the aim of which is to produce a product with a long shelf life which is ready to drink as soon as it leaves the brewery. The conditioning is completed in the brewery; the beer is chilled and filtered to remove all the yeast, and pasteurized to make a sterile product [9].

Nowadays large competition takes place between beer makers. Therefore, increasing of beer assortment, as a result of beer prescriptions and technologies variegating, it is necessary new product quickly supply to consumers. For that reason is necessary to determine beer shelf-life in short time period. Hence beer accelerated shelf-life determination method is necessary to apply.

Accelerated shelf-life testing (ASLT) involves storing the products under a controlled set of storage conditions designed to accelerate the rate of deterioration of the product. The rate of deterioration can then be related to that occurring in normal ambient conditions, and the results can be used to devise models to predict shelf life in different storage conditions. Although the storage conditions used in ASLT need to increase the rate of deterioration, the deteriorative changes must be the same as those occurring under normal conditions. The accelerated tests assume that the deteriorative processes will fit a kinetic model [10].

The major objective of current research was to investigate ASLT method suitability for accelerated beer shelf-life determination.

II. Materials and Methods

A. Materials

The research was accomplished on in Latvia produced beer. Beer samples were: light filtrated pasteurized and light unfiltered pasteurized beer with alcohol content 5.2%; dark filtrated pasteurized beer with alcohol content 4.2%. The shelf-life of tested beer samples within storage at traditional conditions at temperature +20±1 °C was 5 mouths.

B. Imitation of Beer Storage Conditions

Bottled in dark glass bottles beer samples were storage within 20 weeks at several temperature regimes: +10±1 °C, +20±1 °C, +30±1 °C, +40±1 °C. Samples quality parameters as physically-chemical and microbiological was tested every two weeks.

C. Bitterness Substances

The iso-α-acids occur in beer in concentrations varying between 15 and 100 mg/l and their sensory detection threshold are around 5 mg/l. About 80% of the beer bitter taste is caused by the iso-α-acids but there are large differences in the bitterness of the individual compounds. It is known that a mixture of cis- and trans-isohumulones tastes bitterer in beer than trans-isohumulones alone. Furthermore, the cis component is significantly bitterer than the trans counterpart and the isohumulones are more bitter than the isocohumulones [11].

Beer bitterness substances content changes during beer storage at several temperatures regimes were measured by spectrophotometer *Shimadzu UV-1601PC* at 275 nm [11]. Beer bitterness was expressed in unites as BU.

D. Beer Turbidity

Beer turbidity was measured using *Hazemeter VOS ROTA 90/25* and acquired results were expressed in EBC (Formazine Nephelometric Unit).

Scattered light caused by haze particles is measured at 90º and 25º angles. A red light source is used, operating at a light wavelength of 650±30 nm [12].

E. Alcohol, Dry Matter Content, Color and pH

For the alcohol and dry matter content, color and pH value measurement *Alcolyzer Beer ME DMA 4500M* equipment was used [13].

F. Microbiological Parameters

During beer storage at previously selected conditions microbiological parameters were tested using standard methods: *Lactobacillus spp.* was measured accordingly with ISO 9332:2003, *Enterobacteriaceae spp.* accordingly with LVS ISO 21528-2:2007, *Escherichia coli spp.* accordingly with LVS ISO 7251:2006 and yeasts accordingly with ISO 21257-2:2008.

G. Mathematical Data Processing

Data are expressed as mean ± standard deviation; for the mathematical data processing p-value at 0.05 (One Way analysis of variance, ANOVA), was used to determine the significant differences. In case of establishing statistically significant differences, homogeneous groups were determined by Tukey's multiple comparison test the level of confidence α=0.05. The statistical analyses were performed using

Microsoft Excel 2007. Experiments were carried out in fivefold.

III. Results and Discussion

Accelerated shelf-life testing (ASLT), in the usual context meaning a cheap, fast method of estimating the shelf life of a new product, is a bit of a will-o'-the-wisp. Developing a sound, scientific, accelerated test takes a great deal of time and resources and probably won't stand by itself as a wise expenditure. Developing some accelerated methods as part of the shelf-life testing during development of a new product can be justified much more easily. Shelf-life testing (SLT) as a new product is developed encompasses a wide range of tests. Label the whole process accelerated shelf life data accumulation (ADA). This means accumulating data from the earliest samples through to the final product in the final packaging. All along, the goal is to have representative product under test that is far enough ahead of production product that any problems can be identified in time to take systematic corrective action. That is opposed to suddenly getting inundated with returned product and product complaints because the behavior of the product during storage and distribution was not adequately tested [15].

For beer quality parameters evaluation several storage temperature regimes were elected: +10±1 °C, +20±1 °C, +30±1 °C and +40±1 °C temperature. Data from scientific literature indicate [14], that if quality of food product could be without changes during 20 weeks storage at +20±1 °C and during 10 weeks storage at +30±1 °C, as a result the speed of reaction become redouble. Therefore, it is possible to forecast, that quality of beer could be stable during beer storage at temperature +10±1 °C and +20±1 °C, it is, in traditional conditions. However, during beer storage at temperature +30±1 °C, the speed of reactions become redoubles. During beer storage at temperature +40±1 °C, the speed of reactions increases 2.5–3.0 times.

A. Dry Matter

Gorinstein S. (1998) proved that the dry matter of red wine and beer are the most effective beverages: they exercise beneficial lipidemic and antioxidant effects by reducing total cholesterol (TC), triglycerides, and lipid peroxides and elevating high-density lipoprotein (HDL-C)/TC ratio [16]. Therefore it was necessary to control mentioned parameter changes in several types of beer during storage at several temperature regimes. In the preset experiments was detected, that dry matter content of light filtrated pasteurized beer before bottling was 11.83±0.05%. During beer samples storage for 20 weeks at several temperature regimes, changes of dry matter content was not significant (p=0.05).

Acquired results demonstrate, that during light filtered pasteurized beer storage, the dry matter content changes do not to exceed 12.0±0.1% (Fig. 1), what is maximally allowed.

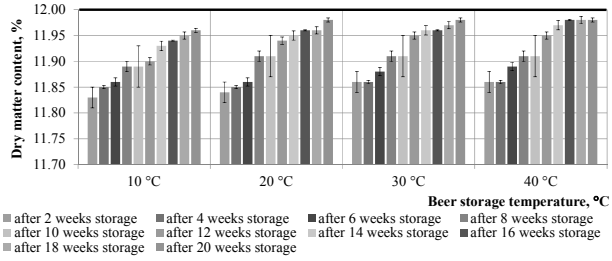

Fig. 1 Dry matter changes of light filtered pasteurized beer during storage at several temperature regimes

Dry matter content of non-storage unfiltered pasteurized and dark filtered pasteurized beer was 11.86±0.30% and 11.79±0.03% respectively; the maximally allowed could be 11.9±0.3% and 12.1±0.5%. Within experiments it was found, that during beer storage at several temperature regimes for 20 weeks, changes of beer dry matter was not significant (p=0.11) and don't exceed allowable limit.

B. Alcohol

Alcohol content of fresh non-bottled light filtered and unfiltered pasteurized beer was 5.13±0.25% (allowed amount 5.20±0.25%), dark filtered pasteurized beer was 4.5±0.4% (allowed amount 4.2±0.4%). Within present research it was proved, that elevated storage temperature conditions not significantly (p=0.13) influence alcohol content changes in analyzed beer samples within 20 weeks storage.

C. Color

Apart from storage temperature affects the aging characteristics of beer, by affecting the many chemical reactions involved. The reaction rate increase for a certain temperature increase depends on the reaction activation energy. This activation energy differs with the reaction type, which means that the rates of different reactions do not equally increase with increasing temperature. Consequently, beer storage at different temperatures does not generate the same relative level increase of staling compounds. Cardboard flavor shows different time courses during lager beer storage at 20 and 30 °C. In the early phase of beer aging, this result in a sensory pattern with relatively more cardboard character when beer is stored at 30 °C compared to 20 °C. This agrees with the lager beer aged at 25 °C tends to develop a predominantly caramel character whereas, at 30 or 37 °C, more cardboard notes are dominant [17]. Therefore, during beer storage main sensory properties what could be changeable could be a color.

The color intensity of non-bottled light filtered pasteurized beer was 10.3±0.1 EBC. During beer storage at several temperature regimes during 20 weeks (Fig. 2) found significant color changes, especially if beer was storage at +30±1 °C and +40±1 °C (p=0.0001).

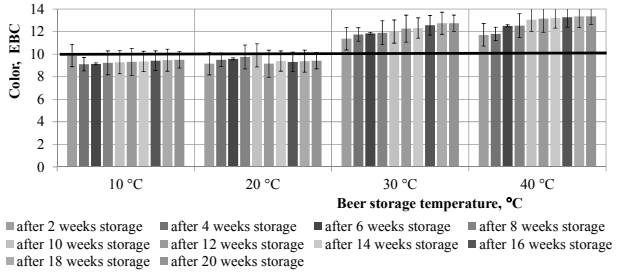

Fig. 2 Color changes of light filtered pasteurized beer during storage at several temperature regimes

For the light filtered pasteurized beer allowed color value could be 8±2 EBC. Experimentally it was found, that during beer storage at temperature +30±1 °C and +40±1 °C the color value increase after two weeks storage already, what mainly could be described with possible oxidation processes beginning during beer storage at mentioned temperature regimes.

The color of light fresh un-bottled unfiltered pasteurized beer was 13.0±0.1 EBC (allowed value – 12±2 EBC). Experimentally was ascertained, that during beer storage color value exceed allowed parameters during storage at +30±1 °C after 12 weeks and at +40±1 °C – after 10 weeks (Fig. 3), what mainly indicate spoilage.

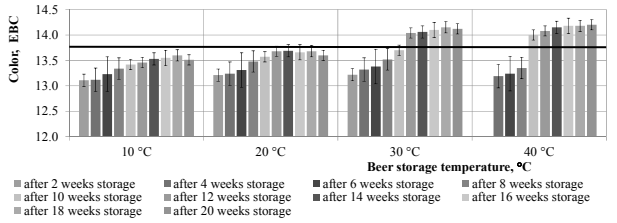

Fig. 3 Color changes of light unfiltered pasteurized beer during storage at several temperature regimes

The color of light fresh un-bottled unfiltered pasteurized beer was 88.9±1.0 EBC (allowed value – 85±5 EBC).

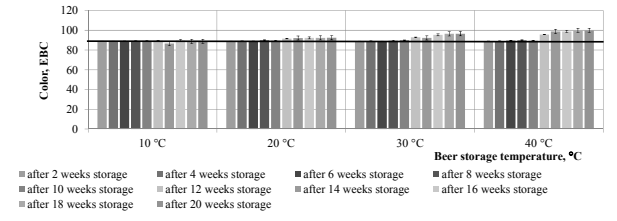

Fig. 4 Color changes of dark filtered pasteurized beer during storage at several temperature regimes

It was detected, that during beer storage at +30±1 °C and +40±1 °C, significant (p=0.001) color changes was established after 12 weeks storage (Fig. 4), what mainly could be explained with polyphenolic compounds oxidation and with elevated oxygen amount in battle, what is the main reason of beer to becoming out-of-date.

D.pH and Turbidity

Oxygen and pH are two of the main factors likely to influence the astringency perception. Oxidation enhances the polymerization degree of polyphenols, and consequently increases their astringent properties. Concerning pH, it is accepted that astringency is intensified at low pH, at least in model solutions and wine, although pH 4.4 is the optimum for beer colloidal instability [18]. From the other hand, the pH trend was found to have a close relationship to the consumption of amino acids (nitrogen-source) in the broth [19]. pH value of fresh non-bottled light filtered- and unfiltered-pasteurized beer was 4.46±0.20 and 4.60±0.20 respectively (allowed amount 4.4±0.1), and pH value of dark filtered-pasteurized beer was 4.34±0.2 (allowed amount 4.4±0.1%). Within present research it was proofed, that elevated storage temperature conditions not significantly (p=0.1254) influence pH value changes in analyzed beer samples within 20 weeks storage.

Turbidity of fresh non-bottled light filtered- and unfiltered-pasteurized beer was 0.5±0.1 EBC (allowed amount <1 EBC), dark filtered-pasteurized beer was 0.6±0.1 (allowed amount <1 EBC). Within present research it was proofed, that elevated storage temperature conditions not significantly (p=0.06) influence turbidity changes in analyzed beer samples within 20 weeks storage.

E. Bitterness Substances

The main hop resins are bitter acids as a source of bitterness of beer. They are divided into two groups, humulones (α-acids) and lupulones (β-acids). Both types of these bitter acids have several homologs, such as normal-, co-, and ad-homologs. Minor hop acids including post-, pre- and adpre-homologues can be found in hops in addition to main types of bitter acids. During the brewing process, humulones are transformed into isohumulones (iso-α-acids), which are responsible for the specific bitter taste and the stability of beer foam. The foam is one of the first qualitative signs of beer quality recognized by consumers. The foam stability can be affected by other compounds, such as proteins, metal ions, lipids and amino acids [20].

Bitterness substances content in light filtrated pasteurised beer samples before bottling was 15.1±1 BU (allowed amount 15±1 BU). During beer storage at temperature +30±1 °C product spoilage properties appear after 8 weeks storage, however during beer keeping at +40±1 °C – after 6 weeks storage (Fig. 5).

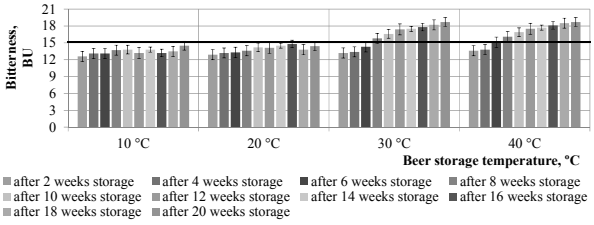

Fig. 5 Bitterness changes of light filtered pasteurized beer during storage at several temperature regimes

Bitterness substances content in light un-filtrated pasteurised beer samples before bottling was 20±1 BU (allowed amount 20±2 BU). Experimentally it was ascertained, that during beer storage at +10±1 °C a content of bitterness substances in beer samples exceed allowed amount after 20 weeks storage, it means, that beer quality was acceptable during validity (Fig. 6).

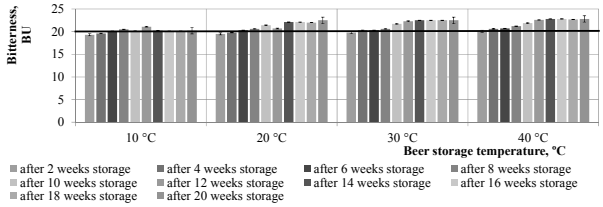

Fig. 6 Bitterness changes of light un-filtered pasteurized beer during storage at several temperature regimes

For all, that during beer storage at +20±1 °C, a content of bitterness substances in beer samples exceed allowed amount after 16 weeks storage, however during beer storage at +30±1 °C and +40±1 °C – after 14 weeks storage (Fig. 6), what mainly indicate beer stalling, what mainly could be explained with possible isohumulones oxidative splitting and α-acids polymerization.

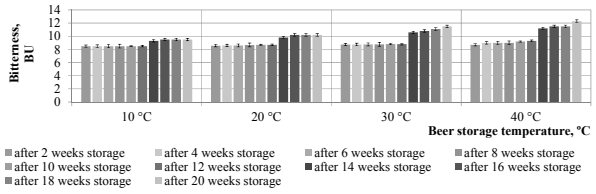

Fig. 7 Bitterness changes of dark filtered pasteurized beer during storage at several temperature regimes

Similar results (Fig. 7) were acquired during investigation of dark filtered pasteurized beer storage at several temperature regimes. The content of bitterness substances of un-bottled dark filtered pasteurized beer was 10.2±1.0 BU (allowed amount 10±2 BU). It was ascertained, that amount of analyzed substances exceed allowed amount after 20 weeks storage of beer at +40±1 °C.

F. Microbiological Parameters

Beer has been recognized for hundreds of years as a safe beverage. It is hard to spoil and has a remarkable microbiological stability. However, in spite of these unfavorable features, a few microorganisms still manage to grow in beer. These, so-called beer spoilage microorganisms, can cause an increase of turbidity and unpleasant sensory changes of beer. Needless to say that these changes can affect negatively not only the quality of final product, but also, the financial gain of the brewing companies. A number of microorganisms have been reported to be beer spoilage microorganisms, among which both Gram-positive and Gram-negative bacteria, as well as so-called wild yeasts. Gram-positive beer spoilage bacteria include lactic acid bacteria belonging to the genera *Lactobacillus* and *Pediococcus*. They are recognized as the most hazardous bacteria for breweries since these organisms are responsible for approximately 70% of the microbial beer-spoilage incidents. The second group of beer spoilage bacteria is Gram-negative bacteria of the genera *Pectinatus* and *Megasphaera*. The roles of these strictly anaerobic bacteria in beer spoilage have increased since the improved technology in modern breweries has resulted in significant reduction of oxygen content in the final products. Wild yeasts do cause less serious spoilage problem than bacteria but are considered a serious nuisance to brewers because of the difficulty to discriminate them from brewing yeasts [21].

In the present experiments it was proved, that during three samples of beer storage at chosen temperature conditions all tested samples was microbiologically stabile, it means, that neither population of lactic acid bacteria, nor acetic acid bacteria, nor *Escherichia coli* and yeasts was not detected.

G.Summary of the Research

Results of acquired experiments give a possibility to affirm, that ASLT method is suitable for accelerated beer shelf-life determination. Main tested quality parameters of filtered and un-filtered pasteurized dark and light beers could be color and bitterness substances.

If color and bitterness substances content of light filtrated pasteurized bottled beer exceed allowed amount during beer storage at +30±1 °C after 8 weeks, as a result shelf-life of beer could be 20 weeks during storage at +20±1 °C. therefore, for beer producers using ASLT method it is possible to determine real beer shelf-life 2.5 times fast.

If color and bitterness substances content of light un-filtrated pasteurized bottled beer exceed allowed amount during beer storage at +30±1 °C after 14 weeks, as a result shelf-life of beer could be 20 weeks during storage at +20±1 °C. therefore, for beer producers using ASLT method it is possible to determine real beer shelf-life 1.4 times fast.

If color and bitterness substances content of dark filtrated pasteurized bottled beer exceed allowed amount during beer storage at +30±1 °C after 12 weeks, as a result shelf-life of beer could be 20 weeks during storage at +20±1 °C. therefore, for beer producers using ASLT method it is possible to determine real beer shelf-life 1.7 times fast.

IV. Conclusions

Within preset experiments it was established that dry matter and alcohol content, pH value, turbidity and microbiological parameters changes of analyzed beer samples was not relevant if beer was storage for 20 weeks at +20±1 °C, +30±1 °C and +40 °C.

Increase of beer storage temperature by +10±1 °C and by +20±1 °C during 20 weeks storage negatively influence beer color and bitterness substances content, what indicate beer spoilage.

It is possible to determine beer shelf-life rapidly during

storage at +30±1 °C for filtered pasteurized light beer by 2.5 times, for unfiltered pasteurized light beer by 1.4 times and for filtered pasteurized dark beer by 1.7 times.

During preset experiments it was proved, that it is possible to determine beer shelf-life rapidly using ASLT method if storage temperature could be increased by +10±1 °C.

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