Examination of the Effect of Vitamin E and C Addition on the Beer's ESR Lag Time Parameter

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ABSTRACT

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The electron spin resonance method can be used to analyse the effect of antioxidant vitamins such as vitamin E and C on the flavour stability of beer. The aim of this study was to examine the effect of vitamin addition on the lag time parameter at different beer production stages. The longest lag time values were measured when the vitamins were added to the wort after cooling. Lower pH value helped improve the lag time value when used with ascorbic acid. When the vitamin E concentration was higher than 4 mg/L at original pH, or the vitamin C concentration was higher than 30 mg/L at lower pH, the lag time was higher than 100 min.

Key words: Ascorbic acid, α-tocopherol, beer, ESR, lag time.

INTRODUCTION

The flavour stability of any food is an important part of its quality. Consumers respond to this parameter consciously and unconsciously. When a consumer is faced with packaged beers of different ages, ideally they should not be able to distinguish major differences between them.

The unpleasant flavour of stale beer is thought to arise from free radicals. These radicals are formed during the oxidation of beer. During storage, even trace amounts of transition metals such as iron or copper can catalyze the conversion of molecular oxygen to reactive oxygen species. One of these reactive oxygen species is the hydroxyl radical, which rapidly oxidizes the components of beer to free radicals. These free radicals start a chain reaction in beer, which results in undesirable carbonyl end products[26] and the odour and taste are caused by these different compounds (e.g. aldehydes). Strecker degradation is an important factor in beer ageing.[5,13] Lipid oxidation aldehydes, such as trans-2-nonenal and hexanal, can be determined in malt and sometimes in barley.[21] It has been reported that lower nonenal concentrations in wort result in improved flavour stability of beer.[7,15] Trans-2-nonenal is very flavour active, especially together with the Strecker aldehyde, furfural or 2-ethyl furfural ethyl ester, formed during fermentation. It is known that metal catalysts, for example copper ions, increase the speed of Strecker degradation and fat oxidation.[27]

Many of these staling compounds can be produced during mashing and furthermore both enzymatic activity and oxidation can produce them. It is very difficult to control the levels of enzymes because they are malt dependent.[2,11,14]

All beers have naturally occurring antioxidants that protect flavour from oxidative free radical reactions. Although oxidation is inevitable, it can be minimized by optimizing brewery operations and storage conditions to preserve the antioxidant content in the packaged beer. A beer with higher antioxidant activity can resist oxidation for longer periods, and therefore, has better shelf-life stability.[26]

Most breweries use taste panels to determine the shelf-life of beer, but tasting is not always a practical method for evaluating the effects of several process changes. These organoleptic analyses require weeks to perform and are not ideal due to the subjectivity of the panelists.

Electron spin resonance (ESR) is a powerful method for the determination of the endogenous antioxidant activity of beer. This parameter is expressed as the lag time for OH-radical generation during the forcing test. The lag time value is a total index that refers to the balance of prooxidants and antioxidants in beer.[24] The method can be an effective predictor of a beer's oxidative flavour stability. It can quickly characterize the overall antioxidant status of the beer at each stage of the brewing process. There are differences between measurements at different stages of the brewing process. The OH-radical generation in wort is measurable immediately after the start of the forcing test, so the lag time cannot be observed in this case; whereas the lag time of OH-radical generation for fermenting wort is gradually formed with fermentation time.[23]

ESR is sensitive to the ingress of oxygen and to the presence of certain metals in wort and beer. It also can be used to monitor the quality of yeast used for fermentation. The detection limit of this method is in the μmolar range. ESR can measure the reacted oxygen, a better indicator of oxidative damage than dissolved oxygen content as measured by standard dissolved oxygen meters.[20] It has been demonstrated that a beer's lag time correlates strongly with the data derived from sensory analysis.[22]

It is known, that there are many vitamins and compounds that have antioxidant effects in beer and one of the sources of vitamins is malt. Most of these vitamins and compounds with antioxidant effect are stable and can be found in the bottled beer. Phenolic acids in beer have an

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anticarcinogenic effect and xanthohumol acts as an antioxidant.[6,18] The average vitamin content of beer is shown in Table I.[4,19] It should be noted that filtered beer does not naturally contain ascorbic acid and α-tocopherol.

In nature there are many vitamins with antioxidant effects but they are not found in beer. Vitamin E and vitamin C are widely regarded as important dietary antioxidants.[12] Free radical traps neutralize radicals arising from molecular oxygen, and thus prevent lipid peroxidation.[17]

The L-enantiomer of ascorbic acid is also known as vitamin C. Ascorbic acid and its sodium, potassium and calcium salts are commonly used as antioxidant food additives. However, these compounds cannot protect fats from oxidation and thus for this purpose fat-soluble vitamins are used as antioxidants. The Recommended Dietary Allowance (RDA) of vitamin C is 60 mg per day. To enhance its antioxidant properties it is best taken with other antioxidants, as there is strong evidence for a synergic effect.[16]

Vitamin E is an essential fat-soluble vitamin and it exists in eight forms. Alpha-tocopherol is the most active form of this vitamin in humans and is the best radical trap. The RDA of vitamin E is 10 mg per day for males and 8 mg per day for females.[17]

It is better to take vitamin E with other antioxidants, for example with vitamin C, beta-carotene or selenium. These combinations hold some promise for preventing and possibly easing complications of such disorders as congestive heart failure, alcoholism, cancer, HIV infection, lupus, multiple sclerosis and nail problems.[17]

The pH value of the beer is an important parameter in this study, because the superoxide anion can be protonated to form the perhydroxyl radical, which has a much higher reactivity. The pKa value of this reaction is 4.8. If the beer sample has lower pH value, the quantity of the perhydroxyl radical is higher than at the original pH. This perhydroxyl radical has higher reactivity than the superoxide anion. In addition, the peroxide anion can be protonated to hydrogen peroxide, and hydroxyl radicals can be produced.[9,10]

Another important parameter is the ethanol content of the vitamin E solution. Hydroxyl radicals can non-selectively react with ethanol in beer, because ethanol is a good radical scavenger. The hydroxyethyl radicals can react with oxygen and these reactions result in secondary radicals. Aldehydes and perhydroxyl radicals arise by bimolecular reactions from these secondary radicals.[1] Since the perhydroxyl radicals have lower reactivity than the initial hydroxyl radical, the value of the lag time can change due to the presence of ethanol.

The question arises at to whether these vitamins can help improve the shelf-life of beer with their antioxidant effect. This work tries to answer this question by examining the effect of added vitamin E and C on the ESR lag time parameter of beer at different stages of the brewing process. In addition, the dependence of this effect on the vitamin concentration is described.

MATERIALS AND METHODS

Method of measurement

To study free radical formation, a spin trapping agent (N-tert-butyl-phenylnitrone) was added to the beer at a final concentration of 0.05M. After the addition of this reagent the sample was incubated at 60°C. This accelerated the free radical oxidation process to a rate that was measurable within a one to two hour time period. As free radicals formed, they were trapped by the spin trap and spin adducts began to accumulate. The sample was placed into the spectrometer at specific time intervals and the ESR sign of the spin adducts was measured.

Beer samples

Beer and in process beer samples described in this study were obtained from the same production ale beer, but at different production stages.

Reagents and chemicals

The N-tert-butyl-phenylnitrone (PBN) standard was purchased from CSIR Biosciences (Johannesburg), 4-hydroxy-2,2,6,6-tetramethyl-piperidinyloxy (4-Hydroxy-TEMPO) and L-(+)-ascorbic acid (min. 99.7%) were obtained from FLUKA (Buchs), DL-α-tocopherol (>96%) was purchased from ROTH (Karlsruhe), and HPLC-grade water and absolute ethanol from Merck (Darmstadt).

Equipment

The measurement was carried out using an e-scan spectrometer (Bruker BioSpin GmbH, Karlsruhe) with autosampler equipped with a peristaltic pump, a sample rack and a variable temperature water bath.

Standard solutions

A stock solution of 0.916 g/L of vitamin E was prepared in an absolute alcoholic solution and a stock solution of 9.007 g/L of vitamin C was prepared in an aqueous solution.

Sample handling

Effect of vitamin E and vitamin C addition on packaged beer. The pH value of the packaged beer was 4.31. This beer was divided into two groups and one group was spiked with phosphoric acid to achieve the lower pH of 2.99. The reason for employing these lower pH values was the decay of vitamin C if the pH value is higher than 4. Although the experiment controlled the effect of pH, in practise such a low pH value would give beer an undesirable acidic taste.[3,8]

Aliquots from the stock solutions were placed into 60 mL screw top brown vials (Supelco, Pennsylvania) containing 50 mL beer to obtain the final concentrations (see sample matrix Table II).

Table I. Average values for vitamin content in lager and light beers.[4,19]

Vitamin	Concentration in lager beer	Concentration in light beer[1]
Riboflavin (Vitamin B2)	0.372 mg/L	0.424 mg/L
Niacin (Vitamin B3)	6.452 mg/L	5.552 mg/L
Pyridoxine (Vitamin B6)	0.712 mg/L	0.480 mg/L
Pantothenic acid	0.824 mg/L	0.508 mg/L
Folic acid	84 µg/L	60 µg/L
Biotin (Vitamin B12)	0.28 µg/L	0.16 µg/L
Thiamine (Vitamin B1)	0.084 mg/L	0.128 mg/L

[1] This beer contained fewer calories but had the same alcohol content as the regular beer.

Table II. Added vitamin concentrations and pH values of beer samples belonging to the different groups presented in the figures. The group name refers to the line numbers of the examined concentrations within each experiment (e.g. Group No.1 contains the first examined concentrations).

Sample name	Group name	Concentration of Vitamin E in the sample mg/L	Concentration of Vitamin C in the sample mg/L	pH value
1	Reference beer	0	0	4.31, 4.34 or 5.12[1]
2	No. 1.	1	0	4.31, 4.34 or 5.12[1]
3	No. 2.	2	0	4.31, 4.34 or 5.12[1]
4	No. 3.	3	0	4.31, 4.34 or 5.12[1]
5	No. 4.	4	0	4.31, 4.34 or 5.12[1]
6	No. 1.	0	10	4.31, 4.34 or 5.12[1]
7	No. 2.	0	20	4.31, 4.34 or 5.12[1]
8	No. 3.	0	30	4.31, 4.34 or 5.12[1]
9	No. 4.	0	40	4.31, 4.34 or 5.12[1]
10	No. 1.	0	10	2.99, 2.98 or 3.08[1]
11	No. 2.	0	20	2.99, 2.98 or 3.08[1]
12	No. 3.	0	30	2.99, 2.98 or 3.08[1]
13	No. 4.	0	40	2.99, 2.98 or 3.08[1]
14	No. 1.	1	10	4.31, 4.34 or 5.12[1]
15	No. 2.	2	20	4.31, 4.34 or 5.12[1]
16	No. 3.	3	30	4.31, 4.34 or 5.12[1]
17	No. 4.	4	40	4.31, 4.34 or 5.12[1]
18	No. 1.	1	10	2.99, 2.98 or 3.08[1]
19	No. 2.	2	20	2.99, 2.98 or 3.08[1]
20	No. 3.	3	30	2.99, 2.98 or 3.08[1]
21	No. 4.	4	40	2.99, 2.98 or 3.08[1]

[1] Exact values depend on the examined beer matrix (see Materials and Methods, Sample Handling).

Until analysis, prepared samples were stored at 0°C. Samples were analysed after 24 h storage.

Effect of vitamin E and vitamin C addition at the end of fermentation. The pH value of the end fermented beer was 4.34. This beer was divided into two groups and one group was spiked with phosphoric acid to achieve the lower pH of 2.98.

Aliquots from the stock solutions were placed into 100 mL screw top vials (Schott, Mainz) containing 90 mL end fermented beer to obtain the final concentrations (see sample matrix Table II). The prepared samples were covered using aluminium foil and stored at 4°C for seven days. This storage time and temperature duplicated the plant maturation time (i.e. minimum of a week at less than 4°C). Following this conditioning period the samples were centrifuged, the supernatants decanted to a centrifuge tube and frozen.

The samples were analysed after 24 h storage. For analysis the samples were placed into a water bath at room temperature and when completely thawed, they were placed on ice until the commencement of the lag time analysis.

Effect of vitamin E and vitamin C addition on wort (after cooling). The pH value of the original wort was 5.12. This wort was divided into two groups and one group was spiked with phosphoric acid to achieve the lower pH of 3.08.

Vials, 100 mL screw top with a hole in the middle (Schott, Mainz) and a retaining valve in the hole, were employed with the wort samples. The vials contained 90 mL wort (after cooling) and 1.29 g yeast. Aliquots from the stock solutions were placed into these 100 mL vials to obtain the final concentrations (see sample matrix Table II). The prepared samples were covered using aluminium foil and stored at 10°C for ten days. This storage time and temperature duplicated the plant fermentation time (i.e. ten days at 6–10°C). Following this time period, the samples were centrifuged, the supernatants decanted to a centrifuge tube and frozen. The samples were analysed after 24 h storage. For analysis, the samples were placed into a water bath at room temperature and when completely thawed, they were placed on ice until the commencement of the lag time analysis.

ESR conditions and lag time assay

The method used for lag time assay and ESR analytical conditions were essentially as previously described by Uchida and coworkers.[22,23,25]

Statistical analysis

Comparisons between the lag time values were done using least significant differences (LSD). A 5% significance level was used for the comparisons. The number of parallel experiments was five and the coefficient of variation was determined.

RESULTS AND DISCUSSION

Table II shows the vitamin content of the samples presented in the figures. A coefficient of variation of about 3.5% was obtained for lag time of the same sample.

Vitamin addition to packaged beer

As the results show (Fig. 1), the growth of the lag time was ~ 10% on average and did not depend on the vitamin concentration. Only in the case of vitamin C addition was there an increase of about 20%. At the lower pH value this effect was not observed, suggesting that the addition of acid had not improved the antioxidant effect of ascorbic acid. An additive effect of vitamin E and C addition was not measurable. The lag time was almost the same when vitamin E was the only added vitamin, as compared to

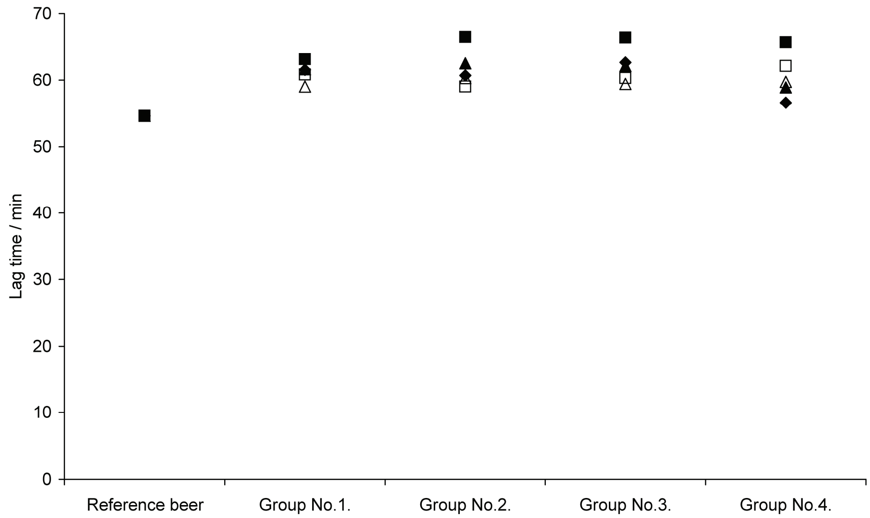

Fig. 1. Lag time values when vitamin E and C were added to packaged beer with the original and lower pH: ♦ only vitamin E added – normal pH; ■ only vitamin C added – normal pH; ▲ only vitamin C added – lower pH value; △ vitamin E and C added – normal pH; □ vitamin E and C added – lower pH value.

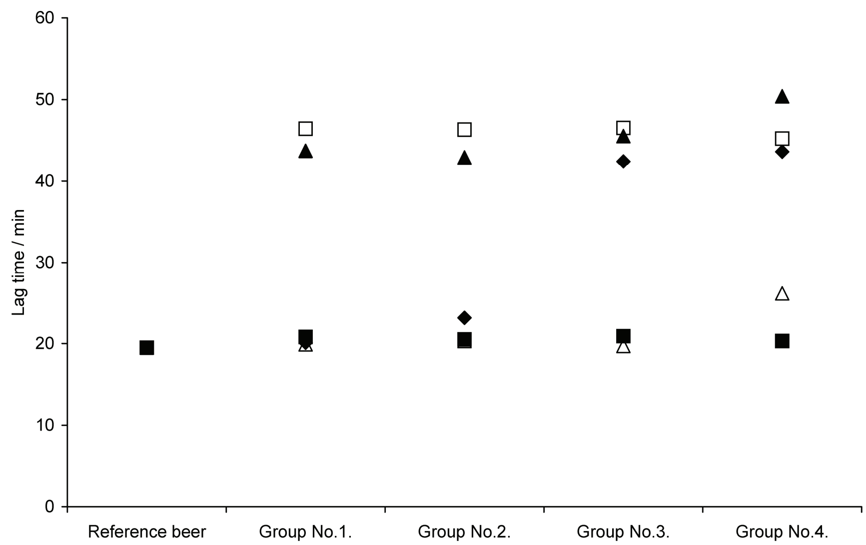

Fig. 2. Lag time values when vitamins were added to beer at the end of fermentation with the original and lower pH: ♦ only vitamin E added – normal pH; ■ only vitamin C added – normal pH; ▲ only vitamin C added – lower pH value; △ vitamin E and C added – normal pH; □ vitamin E and C added – lower pH value.

when these vitamins were added together ($P < 0.05$). Thus, it appears that both vitamins had the same effect on the lag time and did not increase it remarkably ($P < 0.05$).

Vitamin addition at the end of fermentation

It can be seen in Fig. 2, that there were differences between the lag time values of the different samples. When only vitamin C was present, there was no change in the lag time and it was the same as for the reference beer ($P < 0.05$).

When vitamin E was added separately, there was a growing tendency in the lag time parameter and it was dependent on the added vitamin concentration. In the case of 2 mg/L vitamin E concentration, a small increase was found in the lag time parameter compared with the value obtained for the reference beer and with higher concentrations there was a larger increase in lag time ($P < 0.05$). This growing tendency then slowed and from the 3 mg/L vitamin E concentration there was only a mild improvement observable with the parallel growth of the α-tocopherol concentration.

When vitamin E and C were added together, the presence of vitamin C slowed down the effect of vitamin E. With the 4 mg/L vitamin E concentration (concentration of vitamin C 40 mg/L) there was a small increase in the lag time parameter ($P < 0.05$), contrary to the 2 mg/L vitamin E concentration mentioned in the previous case.

The lower pH value had a notable effect on lag time at this processing stage. Using the lower pH value than the original beer, vitamin C addition immediately raised the

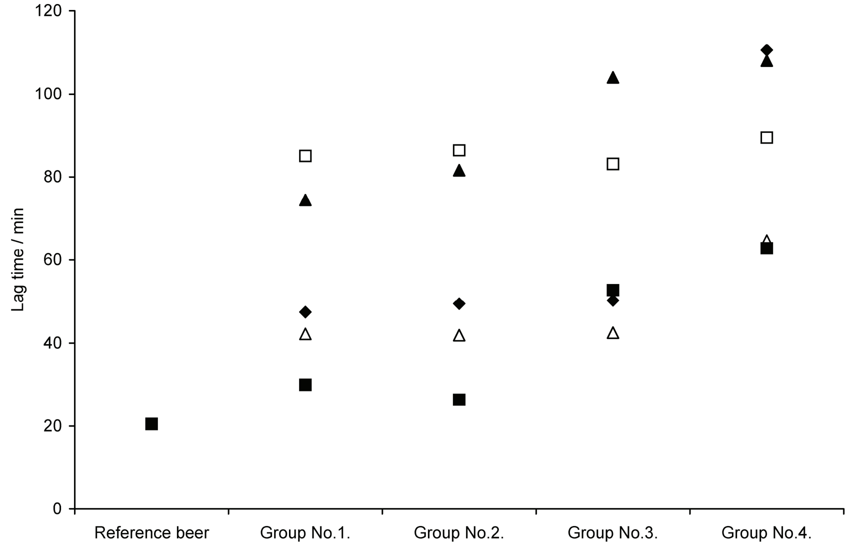

Fig. 3. Lag time values when vitamins were added to wort with the original and lower pH: ♦ only vitamin E added – normal pH; ■ only vitamin C added – normal pH; ▲ only vitamin C added – lower pH value; △ vitamin E and C added – normal pH; □ vitamin E and C added – lower pH value.

value of the lag time from the first examined concentration (10 mg/L) ($P < 0.05$). After this sudden lift, there was a slow increase which depended on the concentration of ascorbic acid present.

When the two vitamins were added together to the same beer, the lower pH was also better from the lag time's point of view than the original pH value. From the first examined concentration (1 mg/L vitamin E and 10 mg/L vitamin C) the lag time had a higher value ($P < 0.05$) and was independent of the vitamin concentration.

Vitamin addition after wort cooling

Fig. 3 shows the effects of vitamin addition on the lag time parameter when the sample matrix was green beer.

When only vitamin C was added, the first two examined concentrations (10 mg/L and 20 mg/L) only caused a small increase in the lag time parameter. When the vitamin C concentration was higher than 30 mg/L, the growth of this parameter was more dynamic and resulted in a medium value ($P < 0.05$).

When only vitamin E was added, in the concentration range of 1 mg/L to 3 mg/L α-tocopherol, a slow increase was observed in the lag time. With 4 mg/L, an increase was seen in the value of the measured parameter ($P < 0.05$). Using this vitamin concentration the lag time was more than 100 min.

When vitamin E and C were added to the same wort together, the individual effects of these vitamins combined was as in the examination of the maturation process. The presence of vitamin C slowed down the effect of vitamin E. At the first three examined concentrations (1, 2, and 3 mg/L vitamin E and 10, 20, and 30 mg/L vitamin C) almost the same effect was noticed as with vitamin E addition alone, but the values of the lag time were lower ($P < 0.05$). A sudden increase appeared at the 4 mg/L vitamin E and 40 mg/L vitamin C concentration, but this increase was lower than in the previous case ($P < 0.05$).

Lower pH value also had a notable effect at this processing stage. When only vitamin C was added to the wort with lower pH, at the first examined concentration (10 mg/L) the lag time parameter had a notable increase, and from this concentration the lag time increased in proportion to higher concentrations ($P < 0.05$). From the 30 mg/L vitamin C concentration the value of the lag time parameter was higher than 100 min.

When the two vitamins were added to the same wort together, the lower pH caused a sudden increase in the lag time parameter at the first examined concentration (1 mg/L vitamin E and 10 mg/L vitamin C concentration) ($P < 0.05$). From this concentration, the growing tendency of the lag time was the same, as was experienced with the combined vitamin addition at the original pH value. The next two vitamin concentrations showed almost the same lag time as with the first concentration ($P < 0.05$) and with the fourth (4 mg/L vitamin E and 40 mg/L vitamin C concentration) there was a little increase in this parameter.

Other parameters influencing the lag time

During these experiments there were three main parameters which influenced the value of lag time. The effect of the pH value of the beer (see Introduction) and beers with lower pH values had lower lag times. In spite of this, when vitamin C was added to the samples with a lower pH, the value of lag time increased. The antioxidant effect of the vitamin C appeared to be higher than the effect of the change in pH.

The ethanol content of the vitamin E stock solution can influence the lag time value (see Introduction). However, the amount of ethanol did not increase this parameter much in these experiments, as the highest increase in alcohol content was only 0.4 percent.

In the activation of oxygen, transition metal ions act as electron donors. The antioxidant activity is based on the capture of reactive oxygen species and free radicals. The capture of metal ions with chelating agents is another possibility. The phosphoric acid used in this study is a good metal chelator[26]. However, its effect on lag time was not observable in these experiments. Each acidic sample had the same phosphoric acid content and treatment, so differences should have been due only to vitamin content.

CONCLUSIONS

The ESR method can be used to analyse the effect of antioxidant vitamins such as vitamin E and vitamin C on the flavour stability of beer. The effect of different vitamin additions at different stages in the beer production process was examined. The combined effects of vitamin E and C in the human body are known and thus the goal was to add these vitamins together to the same beer samples and examine their joint effect. Ascorbic acid decomposes if the pH value is higher than 4, so samples were also prepared at a lower pH.

Comparing the different beer production process stages for vitamin addition, it was established that the best lag time values were measured when the vitamins were added to the wort after cooling. When the vitamin E concentration was higher than 4 mg/L at original pH, or the vitamin C concentration was higher than 30 mg/L at lower pH, the lag time was higher than 100 min. Vitamin addition at the end of fermentation increased the lag time in some cases, but adding vitamins is not recommended for packaged beer.

In the case of original pH, ascorbic acid always had a smaller effect on the value of lag time than vitamin E. When these vitamins were used together, although in the human body they have joint effects, this was not observed in the beer samples examined.

Thus if the growth of the lag time is the target, individual vitamin addition is the correct way to achieve this goal. The best results were obtained when either vitamin E alone was added to the wort at original pH, or when vitamin C alone was added to the wort at a lower pH value.

This topic still requires further examination and experiments should be repeated (in addition to phosphoric acid) with other acids used in the food industry to determine the exact effect of acid and pH. A lower pH which results in acidic tastes and affects the flavour of the beer should be avoided.

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