THE PRODUCTION AND THE STUDY OF VITAMIN ENRICHED BEERS

THESIS OF THE PHD DISSERTATION OF

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1. INTRODUCTION

In these days, a healthy life-style is being encouraged all over the world and consequently different kinds of foods and beverages enriched with vitamins or minerals are becoming more and more popular. Adding an antioxidant vitamin is good from the point of view of the consumer's health and also for shelf-life improvement of the food itself due to its antioxidant activity.

The beer contains many vitamins and antioxidants, for examples phenolic acids, melanoidins, xanthohumol, humulons, lupulons and the vitamins derived from the malt or the yeast. There can be found some riboflavin, pantothenic acid, niacin, pyridoxin, pholic acid and biotin int he beer, but there is neither vitamin E nor vitamin C.

On the grounds of the data released by the Hungarian Brewing Society the consumption of the beer is 70 liter/person in a year in Hungary, so the vitamin content of the beer has a serious importance, which can be helpful for the human body. Besides that the addition of the antioxidant vitamins to the beer can influence its flavour stability, other analytical parameters or the sensory characteristics. The vitamins can influence the shelf-life of the beer, because the deteriorate of the beer is caused by oxidative reactions. The vitamin E and C can block or slow down these reactions by their antioxidant effect. However the vitamin addition can influence the fermentation process, the growth of the yeast, so it can change the amount of the compounds arisen during the fermentation.

I found it important to determine the stability of vitamin E and C in other beverages, in wine and in orange juice. These experiments can help to decide which other beverages are suitable for vitamin enrichment.

2. OBJECTIVES

The possibilities of the vitamin enrichment of different beer samples were investigated in this PhD work. The objectives of the study can be defined as follows:

- Development of a fast and reliable analytical method for the determination of vitamin C and E in beer, wine and orange juice.
  - Investigation of an efficient sample preparation treatment.

- Investigation of the stability of vitamin C and E in beer, wine and in orange juice.
  - Determination of the dependence of the stability from the alcohol content
  - Determination of the dependence of the stability from the vitamin concentration
  - Investigation of the synergic effect of vitamin C and E
  - Determination of the effect of other parameters (pH, storage temperature, etc.) influencing the stability

- Investigation of the effect of the vitamin C addition on the flavour stability of beer
  - Determination of the technological stage which is suitable for the vitamin addition
  - Investigation of the effect of the different added vitamin concentration

- Sensory analysis of the vitamin enriched beer samples

- Determination of the analytical parameters of the vitamin enriched beer samples, when the vitamin addition was carried out after cooling the wort.

3. MATERIALS AND METHODS

Reagents and standards

The following regaents and standards were used during the work: destilled water, DL-α-tocopherol, methanol, ethanol, L-(+)-ascorbic acid, potassium-dihydrogen-phosphate, ortho-phosphoric acid, N-tert-butyl-phenylnitrone, wort, packaged beer samples

Instrumentation

The following instruments were applied: pH meter, magnetic stirring plate, centrifuge, HPLC-UV system equipped with C18 (150 x 4.60 mm, 5 μm), and XDB-C8 (150 x 4.60 mm, 5 μm) coloumn, GC-ECD system equipped with CP-Wax 52 C.B coloumn, E-scan spectrometer, beer analyser, spectrophotometer, turbidity meter and foam stability meter.

Tasting

Untrained consumers (n=315) participated in the tasting panels. The samples were rated by consumers in separate booths illuminated by white light. Each consumer tasted 10-11 beer samples at one tasting. Participants scored beer clarity, foam, flavour acceptability and overall acceptability using a nine-point hedonic scale (1=“strong dislike”, 5=“neutral”, 9=“high preference”). Four demographic and behavioural questions were included in the questionnaire.

Samples

Beers with different alcohol content were examined during this work from different technological stages. A white wine and an orange juice sample were also analysed.

Sample preparation

In the case of the stability experiments, when the examination of the stability of vitamin C in beers with different alcohol content was the target, the used vitamin C concentration was 30 mg/L. The storage temperature of the vials was 4 ^{o}C (refrigerator) and they were covered with aluminium foil to prevent the effect of the light.

When the comparison of the stability of vitamin C at different added concentrations was the target of the experiments, 10, 20, 30, 40 and 50 mg/L vitamin C concentration was

examined using three different matrix (beer with medium alcohol content, wine and orange juice) The samples were analysed for five weeks.

In case of examining the effect of vitamin E on the stability of vitamin C, 30 mg/L vitamin C and 4mg/L vitamin E concentration was used. Two different storage temperatures (4 ^{o}C and 20 ^{o}C) were used at this experiment in case of each matrix.

The effect of the pH value on the stability of vitamin C was also investigated. In case of beer, five different pH values were applied. The pH of 5 mL beer sample was adjusted to the required value by addition of phosphoric acid. The concentration of vitamin C was adjusted to 30 mg/L. Two different storage temperatures (4 ^{o}C and 20 ^{o}C) were used.

When the comparison of the stability of vitamin C at different added concentrations at lower pH was the target of the experiments, the pH values were modified by addition of concentrated phosphoric acid. The new pH values were 3.08 for beer, 2.97 for wine, and 3.04 for orange juice. 10 mL acidic sample was prepared from each matrix. The examined vitamin C concentrations were 10, 20, 30, 40, and 50 mg/L. The samples were analysed for five weeks.

In case of the vitamin E stability experiments two different storage temperature was used (4 ^{o}C és 20 ^{o}C). The vials were covered with aluminium foil to prevent the effect of the light.

When the examination of the stability of vitamin E in beers with different alcohol content was the target, the used vitamin E concentration was 4 mg/L, and three different alcohol content was examined.

When the comparison of the stability of vitamin E at different added concentrations was the target of the experiments, 1, 2, 3, 4, and 5 mg/L vitamin E concentrations were prepared for beer with medium alcohol content, wine and from orange juice. The samples were stored in the refrigerator and they were analysed for ten weeks.

When the effect of vitamin C on the stability of vitamin E was examined, 30 mg/L vitamin C and 3 mg/L vitamin E concentration was used. Two different storage temperatures (4 ^{o}C and 20 ^{o}C) were used at this experiment in case of each matrix. The samples were analysed once a week for ten weeks.

When the effect of the vitamin addition on the flavour stability of beer was investigated, three different technological stages were examined.

When the vitamin addition was carried out on package beer samples, 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.

50 mL aliquots from the beer samples were placed into 60 mL screw top brown vials and the vitamin concentrations were adjusted to 1 – 4 mg/L vitamin E and 10 – 40 mg/L vitamin C concentration. Until analysis, prepared samples were stored at $0^{o}C$.

When the vitamin addition was carried out 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. 90 mL aliquots from the beer samples were placed 100 mL screw top vials to obtain the final concentrations, 1 – 4 mg/L vitamin E concentration and 10 – 40 mg/L vitamin C concentration. The prepared samples were covered using aluminium foil and stored at 4 ^{o}C for seven days. Following this conditioning period the samples were centrifuged, the supernatants decanted to a centrifuge tube and frozen. For analysis the samples were placed into water bath at room temperature and when completely thawed , they were placed on ice until the commencement of the lag time analysis.

When the vitamins were added to wort, 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 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. The concentrations of the vitamins were adjusted to 1 – 4 mg/L vitamin E concentration, and 10 – 40 mg/L vitamin C concentration. The prepared samples were covered using aluminium foil and stored at 10 ^{o}C for ten days. Following this time period, the samples were centrifuged, the supernatants decanted to a centrifuge tube and frozen. For analysis, the samples were placed into water bath at room temperature and when completely thawed, they were placed on ice until the commencement of the lag time analysis.

In the case of sensory analysis different beer samples were examined. Three samples had the largest proportion of surrogates: maize grits, barley or high fructose corn syrup substituted for 20-30% of malt. These beers had an original extract content of 10.5 m/m%. Three beer samples had higher original extract (min. 12 m/m%) than the previous beers. The proportion of the maize grits was max. 20% of the amount of malt. The last sample was a brown doppelbock beer brewed from high-coloured malts. It was an extra strong, very malty lager beer. This beer had the highest original extract content (13.3 m/m%) from the examined

beers. Packaged beer samples belonging to the same type of beer were derived from the same batch, so they had the same quality properties (oxygen and carbon dioxide content).

Fifty-two samples were prepared from each type of beer for one tasting. Twenty-five samples did not have any added vitamins, so they were the original, reference beers. The other twenty-seven samples were prepared in the following way. Each unopened, original packaged beer sample was stored in the refrigerator at 10 ± 1 ^{o}C. Immediately before tasting a sample, 50 mL cold original beer sample was poured gently into a vessel, and the vitamin content was adjusted to 0 – 5 mg/l vitamin E and 0 – 50 mg/L vitamin C concentration using all the combinations of the concentrations.

The sensory analysis of the vitamin enriched beer samples was also carried out at lower pH value (3.00 +/- 0.10) because of the decay of vitamin C. The pH value of the beer was modified by adding concentrated phosphoric acid.

When the effect of the vitamin addition on the analytical parameters of beer was investigated, the pH value of the original wort was 5.14. Half the amount of this wort was spiked with phosphoric acid to get lower pH. The lower pH value was 3.02.

For the examination, 500 mL Schott vials were used with a screw top with a hole in the middle. A retaining valve was placed into this hole. The vials contained 450 mL wort (after cooling) and 6.43 g yeast. The vitamin concentrations were adjusted to 0 – 5 mg/L vitamin E and 0 – 50 mg/L vitamin C concentration. The samples prepared in this way were covered with aluminum foil and stored refrigerated (at about 10 ^{o}C) for ten days. After this fermentation time the cups of the Schott bottles were changed to a cup without a hole and the bottles were then stored at 4 ^{o}C for seven days. After this maturation time, 100 mL of each sample was analyzed for alcohol content. The concentration of iso-α-acid, vitamin E and C and the final pH value were determined from 150 mL samples of each. The concentrations of diacetyl and 2,3-pentandione were determined from 200 mL samples of each. After these experiments the remaining portion of each sample was centrifuged at 3100 g, and the centrifuged yeast plug was extracted with 5 mL methanol and the vitamin E content of this solution was determined by HPLC in each case.

4. RESULTS

4.1. Development of an analytical method to analyse the vitamin C and E in beer

The HPLC technique has been chosen to work with UV detection. The main target was on elaboration and mesuring techniques that can be applied easily and quickly during the daily lab routine that provides efficient results. It can be established that there is no significant difference between the analysis of the different matrices, and the usage of the extraction as a sample preparation gives the same result as the direct injection onto to column. Using the optimized parameters both vitamins gave only one peak on the chromatograms. The retention time of vitamin E was 2.0 min, and it was 4.30 min for the vitamin C.

4.2. Determination of the stability of vitamin C

The beers with different alcohol content behaved in a different way and it is agreed by the statistical analysis results. There was a significant decay in the amount of vitamin C during storage in every case, but the gradient of the decay curve depended on the alcohol content.

In case of the beer samples stored at room temperature (20 ^{o}C), the decrease of the pH value doesn't change the stability of vitamin C, however the decay in the amount of vitamin C had a slower tendency. In case of samples stored in the refrigerator (4 ^{o}C), under the pH value of 3.96, the decrease of the vitamin C content of the samples was only 12.4±2.0%. If the pH was 3.96 or higher, there was no significant difference between vitamin C content of the samples stored at different temperatures, the decay of the ascorbic acid concentration was 81.0±6.3% independently from the storage temperature.

The stability of vitamin C using different concentrations was also examined. In case of beer the rate of the decay is the highest at the highest vitamin C concentration

When these vitamins are added at the same time, the addition of Vitamin E influences the concentration of vitamin C. Samples having vitamin E and stored at 4 ^{o}C had slower decay than the samples with same vitamin content stored at 20 ^{o}C, but these samples had still lower gradient than the samples without vitamin E.

The stability of vitamin C in wine and in orange juice was also examined. When wine is the matrix for vitamin addition, the maximum rate of the deterioration can be detected at 30 mg/L vitamin C concentration. In case of orange juice, the reduction of the amount of vitamin C decreased with the increasing ascorbic acid concentration, so the speed of the decay was higher at lower concentrations.

In case of wine and orange juice spiked with phosphoric acid the decay curve shows exponential tendency. The faster decay can be observed at lower concentrations in wine, but in case of orange juice, the decline is the fastest at the highest vitamin C concentration.

4.3. Determination of the stability of vitamin E

There is a significant difference between the stability of vitamin E in beer samples with different alcohol content. The storage temperature had no effect on the behaviour of the α-tocopherol in case of beer with low alcohol content. There was little difference between the vitamin E content of the beer with medium and high alcohol content stored at different temperatures. In these cases, vitamin E was less stable in the samples stored at room temperature than in the others stored in the refrigerator.

The stability of vitamin E applied at different concentrations was examined. A decline was detected in the amount of vitamin E at each concentration, but the tendency of the reduction was different: the gradient of the decline curve grows with the growing amount of vitamin E.

The effect of vitamin C on the stability of vitamin E was also examined. On the ground of these experiments, it can be established, that the amount of vitamin E was independent from the presence of vitamin C

The stability of vitamin E in wine and orange juice was investigated. In case of each sample and at each concentration, a decline was detected in the amount of vitamin E. In case of orange juice, the gradient of the decline curve grows with the growing amount of vitamin E but in case of wine, the gradient is independent from the concentration of vitamin E.

4.4. The effect of the vitamin addition on the flavour stability of the beer

When only vitamin C was added to the wort, 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. If the 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. From the 30 mg/L vitamin C concentration the value of the lag time parameter was higher than 100 min.

When only vitamin E was added to the wort, with 4 mg/L vitamin E concentration, an increase was seen in the value of the measured parameter. 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 presence of vitamin C slowed down the effect of vitamin E. Using the combined vitamin addition at lower pH value, there was a significant improvement from the first examined concentration.

When only vitamin C was added to the sample from the end of the fermentartion, there was no change in the lag time. Using the lower pH value than the original beer, vitamin C addition immediately raised the value of the lag time from the first examined concentration (10 mg/L).

If vitamin E was added separately at this technological stage, there was a growing tendency in the lag time parameter 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. The lower pH was also better from the lag time's point of view than the original pH value in this case.

When the examined vitamins were added to a packaged beer sample, the growth of the lag time was ~ 10% on average and did not depend on the vitamin concentration. An additive effect of vitamin E and C addition was not measurable, and the change of the pH value didn't influence the lag time parameter significantly.

4.5. The effect of the vitamin addition on the sensory characteristics of the beer

The transparency of the samples was also examined by a spectrophotometer and it can be found that the limit concentration of Vitamin E is 3.8 mg/L to get a clear and bright beer.

The original clarity scores ranged from 4.6 to 8.6 on a nine-point hedonic scale. In case of low alcohol content (3.0% and 4.4%) the scores were postured around the median, and when the alcohol content was higher, these scores received almost maximum value.

When phosphoric acid was added to the samples, the presence of phosphoric acid does not influence clarity score. When the clarity score for the original beer was around the median, vitamin addition did not influence significantly the score of this feature. If the clarity

score for the original beer was above the third quartile, samples having 3 mg/L or 5 mg/L vitamin E were considered to be duller, than the original control beer. The only exception was the brown beer, because in this case there was no difference between the clarity scores of the samples. In contrast, ascorbic acid did not influence clarity.

The results of the foam stability measurements showed, that the vitamin addition did not change the foam half-life of the original beer significantly. However, samples with lower pH value had significantly lower foam half-life.

Foam scores for the original samples ranged from 3.8 to 8.6 on a nine-point hedonic scale. It can be stated, that following the vitamin addition there is no regularity in foam scores. Only in case of the brown beer did all samples get the same scores. The reason for the lack of regularity can be this assumption, since the samples they examined later for foam score had less foam.

Mean scores for original flavour acceptability ranged from 5.4 to 8.8 on a nine-point hedonic scale. When vitamin E was the only vitamin added, there were differences between the scores of different matrices. In case of 71.4% of the samples, the addition of vitamin E did not change or improve the original flavour acceptability scores. However, using lower alcohol content the high vitamin E concentration caused flavour deterioration.

In case of ascorbic acid addition, if the original score was around the median, the ascorbic acid addition did not change or improve the flavor acceptability score. If the original score was almost of maximum value, vitamin C addition mildly decreased this score.

When these two vitamins were added to the samples together, 1 mg/L and 3 mg/L vitamin E concentration did not change the flavour acceptability score if 10 mg/l or 30 mg/L ascorbic acid concentration was used. If 5 mg/L vitamin E was added to the samples under the same conditions as previously, the examined score decreased. This effect was not observable only in case of low alcohol content. In case the vitamin E concentration was the constant parameter and the original score was at the median, growing vitamin C concentration decreased the flavour acceptability score. In case the original score was at the third quartile only 50 mg/L vitamin C concentration decreased this score. When the original score reached almost maximum value, the addition of ascorbic acid did not change the flavour acceptability score.

From the point of view of flavour acceptability score, brown beer matrix is very different from the other samples. The original score was around the third quartile but each vitamin enriched samples had higher score than the reference beer.

Overall acceptability scores for original reference beer ranged from 6.4 to 8.6 on a nine-point hedonic scale. When vitamin E was the only vitamin added, and the original overall acceptability score was around the third quartile, two different types of behavior were observable. In case of low alcohol content, this score decreased with the growth of α-tocopherol concentration. If the alcohol content was above 5.2%, all vitamin concentrations resulted in the same overall acceptability score. If the original score was almost the maximum, there was a decline in this score only in case of the 5 mg/L vitamin E addition. In case of ascorbic acid addition, samples having their original score around the third quartile received the same overall acceptability score as the reference beer. However, if the original score was almost the maximum, a decline was observable above 30 mg/L ascorbic acid concentration.

When these two vitamins were added to the samples together, if the beer matrix had low alcohol content and the ascorbic acid concentration was constant, the overall acceptability score did not change with the growth of vitamin E concentration. If the alcohol content of the matrix was higher than 4.7% and vitamin C concentration was constant at a low level, overall acceptability score decreased above 3 mg/L vitamin E concentration. Nevertheless, if the ascorbic acid concentration was 30 mg/L or higher, α-tocopherol addition did not change the overall acceptability score. If the vitamin E concentration was the constant parameter and alcohol content was above 4.7%, vitamin C addition did not change this score, although, in some cases an improvement was observable. If the alcohol content was below this limit, the overall acceptability score decreased or did not change with the growth of vitamin C concentration.

The only exception to the previous statements is brown beer: each examined sample had the same overall acceptability score.

4.6. The effect of the vitamin addition on the analytical parameters of the beer

When vitamin E was added to the samples, the alcohol content increased and it depended on the concentration of vitamin E. When vitamin C was added to the samples, the amount of alcohol was higher than in case of vitamin E addition. The highest alcohol contents were observed when both of the vitamins were added to the samples.

When vitamin E was added to the samples, the concentration of iso-α-acid was higher than the reference sample, and it increased with the increasing concentration of α-tocopherol.

If vitamin C was added to the samples, the concentration of iso-α-acid was lower than in the reference sample, and this depended on the concentration of the ascorbic acid. When these two vitamins were added together to the samples, their effects combined.

The diacetyl and the 2,3-pentandione content of the beer behaved in the same way. The samples having lower pH value always had lower values for these parameters than the samples with the original pH value. When vitamin E was added to the samples, the concentration of diacetyl and 2,3-pentandione was unchanged compared to the reference sample. When only vitamin C was added to the samples, the concentration of these two vicinal diketones was higher than in the case of the reference sample. This increase was dependent on the concentration of vitamin C. When these two vitamins were added together to the samples, their effects also combined.

5. NEW SCIENTIFIC ACHIEVEMENTS

1. A fast and reliable analytical method was developed for the determination of vitamin C and E in beer, wine and orange juice. This method doesn't require any sample preparation steps, so it can be easily applied in a daily laboratory routine.
2. It was established, that the stability of vitamin C and E can be determined in beer, wine and in orange juice. The dependence of the stability on the vitamin concentration, and the effect of the storage temperature and the pH also can be evaluated. It was established, that the quantity of both vitamins decreases during the storage, but the gradient of the decay curve depends on the added vitamin concentration. The stability of vitamin C and E in beer also depends on the alcohol content of the sample: in case of low alcohol content, the vitamins are more stable. It can be found, that the stability of vitamin C can be improved by using lower pH value, or by simultaneous vitamin E addition.
3. It can be concluded, that the vitamin addition has an effect on the shelf-life and the flavour stability of the beer. It was determined, that the most suitable technological stage for the vitamin addition is the wort after cooling. The lag time parameter can be improved using lower pH value in case of vitamin C addition.
4. As a result it can be concluded, the sensory parameters of the beer change due to the vitamin addition. If the production of a bright beer is the purpose, than the concentration of vitamin E must be less, than 4 mg/L. Based on the four examined scores it can be stated that if vitamin E is added, the alcohol content of the beer must be above 4.7% and the proposed vitamin E concentration is 3 mg/L. If the aim of the production is ascorbic acid enriched beer, the suggested vitamin C concentration is maximum 30 mg/L regardless of alcohol content. Adding vitamins is recommended mostly in case of brown beer.
5. The results of the analyses show that vitamin addition has an impact on the examined parameters. Vitamin E addition changed both the alcohol and the iso-α-acid content of the samples. The addition of this vitamin did not change the amount of diacetyl and 2,3-pentandione. In the presence of vitamin C the amount of the vicinal diketones and the iso-α-acids decrease and the alcohol content increases. When vitamin E and C are added together to the samples their effect combines.

6. PUBLICATIONS

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