THESES OF THE DOCTORAL DISSERTATION

Investigation of the mineral substance content during the operations of beer production

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1. Background and aims of the work

It has been known for a long time that beers contain mineral substances. However, detection and determination of the precise amount of macro- and microelements have been possible only for the past few decades, as the analytical methods have evoluted. Most of these substances appear in very small amount (in the order of μg/ml), thus detection of them by classical chemical analytical methods is difficult or not possible at all.

Analysis of the minerals found in beer is important for two reasons. The first – the most important viewpoint – is their physiological effect on the consumer. The macro- and microelements of beer get into the body and influence its functioning. In Hungary the average beer consumption per capita is about 75 liters per year, which in case of a beer drinker can reach several 100 liters. The mineral content of beer may possess great importance when consumed in such an amount, which in case of suitable ion composition can be physiologically beneficial for the body, and can contribute to the loss of minerals. But the question has to be examined from a food safety point of view: unhealthy substances may get into beer due to the increasing environmental pollution. So it is understandable that it is an essential consideration to know the exact amount of minerals in beer.

The other viewpoint is the effect of mineral substances on the technology of beer production. Mineral substances that derive from brewing water and dissolve from the raw materials influence the efficiency of mashing; may activate or hinder the enzymatic degradations. The macro- and microelement content of the wort and yeast affect the course of fermentation, change the composition and proportion of fermentation by-products. The mineral content of final product affects the taste of beer, and influences its shelf-life.

In the past few years, several technological problems have emerged in the brewing industry that showed connection with the concentration of mineral substances. Reports in the literature regarding the effect of macro- and microelements on the brewing technology are rather conflicting and incomplete. All these have drawn my attention to survey the change of minerals in the course of the operations of beer production, and if necessary to determine the optimal macro- and microelement concentrations.

Main goals of the research work:

- Elaboration of a measurement method to determine the mineral content of beer. Determination and statistical analysis of the mineral content of different beer types.
- Following the changing of concentration of the minerals (calcium and zinc) and its effect on the technology in the course of beer production.
- Investigating the factors affecting the stability of beers.

2. Materials and methods

2.1 I determined the mineral content of beers by Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES).

2.2 Role of calcium and zinc during the operations of beer production

2.2.1 Investigation of the role of calcium in brewing

- I measured the activity of α-amylase by the Phadebas method, while I followed the degradation of starch by determining the reducing sugars (Schoorl and Somogyi-Nelson methods) and by photometric iodine test. The mashing was modelled in a laboratory mashing equipment.
- Fermentation was performed in laboratory in fermentation vessels recommended by the European Brewery Convention (EBC). The alcohol and extract content were measured by semiautomatic beer analyser.

2.2.2 Investigation of the role of zinc in brewing

- I examined utilization of zinc with decoction mashing in a pilot plant. I determined the concentration of zinc by ICP-AES method.
- I used VARION type cation-exchange resin was used for the separation of organic and inorganic zinc.
- Fermentation was performed in a pilot plant. The alcohol and extract content were measured by semiautomatic beer analyser; amino acids were determined by HPLC, while the aroma compounds were analysed by gas chromatography. I measured yeast growth gravimetrically.

2.3 Investigation of technological steps affecting the stability of the final product

2.3.1 Role of calcium in the stability of beer

- I have used ICP-AES technique for the determination of the concentration of calcium, while for the measurement of oxalate an enzymatic method (Bohringer test).

2.3.2 Determination of mineral substances desorpting from filtering aids

- Determination of the concentration of minerals desorpting from kiesel guhr and perlit was measured by ICP-AES technique after digestion by destruction.

3. Summary of results

3.1 Determination of the mineral content of beers

- Elaborating a measuring method

I had chosen ICP-AES, a modern spectroscopic technique as measuring method, because this simultaneous multi-element method provides considerable information on the individual concentration of all elements in wort and beer. During the accomplishment of the research project it was aimed to elaborate such a sample preparation procedure which gives reproducible results, that eliminates the differences among the heterogeneous compositions of the analyzed solutions. I compared five sample preparation procedures considering the reliability, labor intensity and time requirement of them. I achieved the best results by applying the digestion with nitric acid and hydrogen peroxide that corresponds with the preparation methods published by Alcazar and co-workers (2002), Wyrzykowska and co-workers (2001), and Matsushige and Deoliveira (1993).

- **Analysis of the mineral content of beer samples**

In the course of determining the mineral content of beers, I examined them by their type, and I classified them in four groups according to their raw material composition.

In the examined beer cadmium, gallium, mercury, nickel and titan were not detected. The beers contained barium, and vanadium in very small concentrations. Arsenic and lead were detected in some of the samples in very small concentration. I had compared my results with the medical limits, and I found that the mineral content of beers did not exceed them in any case.

I had also analyzed the effect of beers on health. I compared the mineral content of beers with the recommended daily allowances. Among the minerals present in beer potassium can be found in the highest concentration (385 mg/l). The average sodium content of the Hungarian beers is 35 mg/l, which makes the beverage low in sodium. Selenium is found in relatively high amount that depends on the habitat of barley, thus it shows considerable fluctuation among beers. The microelements of beers also have significance: iron and copper take part in blood formation, while zinc is building block of enzymes essential for the body.

By analyzing the macro- and microelement content of beers with Principal Component Analysis I had found that the macroelements are located close to each other in the consensus plot, which means that the change of one of the principle components goes together with the change of the other one. This may be explained with that these elements originate mainly frm the malt, and the malt contains them in nearly the same amount.

3.2 Role of calcium and zinc in mashing and fermentation

- **Role of calcium in mashing**

In my research I aimed to determine what the most favorable calcium concentration is for the α-amylase enzyme originating from malt, from the point of view of its effect on the heat stability of the enzyme and the effectiveness of hydrolysis.

I had measured the activity of malt α-amylase in the 13 to 220 mg/l calcium concentration range with the Phadebas method. Next I had performed model mashing procedures at different temperatures (70-80°C) in the 13 to 440 mg/l calcium concentration range. I had examined the change of enzyme activity to which I had deduced from the rate and extent of starch hydrolysis. In addition, I studied the effect of calcium concentration on the free α-amino acid content and colour of wort.

As a result of studying the relation between the α-amylase enzyme activity and the calcium concentration, I determined the optimal and limiting calcium concentrations at different mashing temperatures. I concluded that calcium has no effect on the free α-amino acid content of worts, but the colour of wort becomes lighter with the increase of concentration. There is a linear relationship between the added and the measured calcium concentration in wort, which means that the whole amount of calcium remains in solution, and neither precipitation nor complex-formation takes place.

- **Role of calcium in fermentation**

In the course of my work normal gravity (11.3 m/m %) and higher gravity (14.8 m/m %) worts were fermented with the addition of calcium in different concentrations, and I studied the effect of calcium on the fermentation rate and yeast flocculation.

Analysing the results of the fermentation experiments I concluded that in case of normal gravity worts the addition of 0 to 200 mg/l calcium has no effect on the profile of fermentation. 200 to 600 mg/l concentration had inhibiting effect on fermentation; it slowed down the consumption of substrate. On the fermentation of high gravity worts 150-200 mg/l calcium addition had favourable effect; both the fermentation rate and the final attenuation degree increased. The phenomenon may be explained by the effect of calcium ions on the stress tolerance of yeast. No difference was experienced in the flocculating ability of yeast in either case. The effect on yeast growth was not unambiguous either, results fluctuated and no tendency was found.

- **Investigation of factors affecting zinc concentration and effects on fermentation**

In the following section of my work I brewed worts with barley and maize adjunct, and followed the change in zinc concentration of mash and wort during the steps of the process. In case of the brewing of both kinds of wort strong decrease of zinc concentration was observed. By the end of wort boiling, the amount of zinc decreased to 0.02 mg/l in both worts made with adjunct. I examined the variations in zinc concentration in case of all-malt wort, as well. The result was similar to the one measured by Donhauser and co-workers (1983) and by Narciss (1980). In my investigation I had found that the loss is greater in case of beer made with adjunct, although the amount of zinc in the raw materials was nearly the same. The difference may be explained with the structure of adjunct which is less digested than the structure of malt.

In the next step of my research work I examined the possibilities of zinc addition during fermentation of worts made with adjunct. Since yeast can absorb only the ionic form of zinc during fermentation, I had elaborated a method for the separation of the organic and inorganic form of zinc. I found the VARION KS type of cation-exchange resin the best for the separation of zinc in the ionic form and in complex binding. I examined the separation ability of the column, and I determined the optimal dilution rate of the samples. Based on my results I concluded that the applied cation-exchange resin is capable to bind the ionic form of zinc found in wort. In case the samples are diluted three-fold the complication caused by organic substances does not set in, and due to this dilution even the concentration of zinc does not approach the detection limit, so the amount of zinc in complex binding is possible to determine without digestion.

I had studied the effect of zinc concentration on the fermentation rate; moreover I followed the variation of the amount of amino acids and fermentation by-products.

The fermentation rate was the lowest in case of samples containing 0.02 mg/l zinc; on the other hand even the overdosing of zinc did not decrease the fermentation rate. Concentrations of the free amino nitrogen and valine showed similar tendencies. The maximal diacetyl concentration was measured in the zinc deficient beers. In case of esters the zinc deficiency resulted in lower concentration, while the increase of zinc amount brought on the increase of ester concentration. The lowest yeast growth rate was experienced at the lowest zinc concentration. Increase of the concentration made the yeast growth increase to a certain extent, but it decreased at 80 mg/l zinc concentration.

As result of my work I concluded the optimal zinc concentration value for beers made with adjunct (0.4 mg/l). Overdosing of zinc did not cause technological problems; it had negative effect on only the yeast growth. During my experiments performed on worts made with adjunct I found that the degree of zinc supply of worts affects not only the fermentation rate, but the fermentation by-product composition of the final product. My results agree with the results of Donhauser and co-workers (1983), which drew the same conclusion during fermentation of all-malt worts.

3.3 Investigation of factors affecting the stability of beers

- **Possibilities to avoid gushing**

In this section of my work I studied the variation of amount of calcium and oxalate in the course of beer production, and I studied which technological steps promote crystal formation. Next, I looked for an answer to the question of how can be the calcium oxalate formation promoted before filtration, and what are the possibilities to forecast the precipitations before filling.

As a result of my work I determined those technological steps in the process of beer production where the extent of calcium oxalate precipitation is the highest. I found that the temperature and the calcium concentration had the biggest effect on the crystal formation. At the temperature of -2°C in 30 minutes precipitation takes place, and crystals can be removed during filtration.

- **Investigation of the process of filtration**

I looked for an answer to the question whether during the last step of the technology can the mineral substance composition of beer change, i.e. can macro- and microelements dissolve into the beer. If yes, will they affect the stability and quality of beer? To give a summary of my results, I concluded that in the early stage of filtration minerals dissolve into the beer, but later adsorption is the typical process. It is practical to perform filtration at a lower temperature, and if necessary to decrease the mineral substance content of the filtering aid by washing it with diluted hydrochloric acid.

3.4 New scientific results

- I had determined the mineral content of beers. I classified the analysed beer in four groups on the bases of the applied raw materials. I compared my results with the medical limits and with the requirement to the individual mineral substances. I had concluded that the moderate consumption of beers has favourable physiological effect. The rate of potassium to sodium in beer should be emphasised that was 11 to 1 in case of the analysed beers.

- I had determined the calcium concentrations that are optimal for the α-amylase enzyme during brewery mashing. I followed the rate of starch degradation and the variation of degradation degree at various mashing temperatures and calcium concentrations. As a result of my work I specified the optimal calcium concentration of the mash at four different mashing temperatures that provides the fastest starch degradation and highest degree of degradation. I measured the highest enzyme activity in mashes containing 45-105 mg/l calcium.

  I had found that the calcium has no effect on the α-amino nitrogen content of worts, but the colour of them became lighter as the calcium concentration increased.

- Investigating the effect of calcium on fermentation of normal and high gravity worts, I found that in case of normal gravity wort calcium concentration below 200 mg/l had no effect on the rate of fermentation. However, addition of 150-200 mg/l calcium was beneficial for the fermentation of high gravity worts, which is due to the positive effect of calcium ions on the stress tolerance of yeast.

- Experiments had not been performed so far regarding the zinc content of worts made with adjunct. In my work I examined the zinc content of worts made with barley and maize adjunct in the course of wort production, and I determined the degree of zinc utilization. I had found that in case of wort made with adjunct, the zinc concentration of wort getting to fermentation is less than that of all-malt worts. This is due to the structure of adjunct which is less digested than the structure of malt.

- During fermentation only the ionic form of zinc is available for the brewer's yeast. The trace element analytical methods of today (ICP-AES, AAS, and AFS) can determine only the total zinc content in samples. In order to be possible to determine the ionic zinc and the zinc in complex binding, as well in the wort, I had elaborated an ion-exchange chromatographic method to separate them.

  After the ion-exchange separation I measured the concentration of ionic zinc in wort samples, and I found that only 50-70% of zinc is available for the yeast, thus the fermentation can be performed safely only with the addition of zinc.

- I had determined the optimal zinc concentration for the fermentation of worts made with adjunct. Beside the effect of zinc on the rate of fermentation and on the alcohol content I examined how the various concentrations affect the amount and composition of fermentation by-products, and the yeast growth. I concluded that the optimal zinc concentration is 0.4 mg/l in case of fermentation of worts made with adjunct, but the inhibition was not caused by higher concentration.

- I studied those technological steps during which calcium oxalate is formed in the beer. I had found that precipitation is caused by cooling, thus in case of every technological step that involves the decrease of temperature, the inclination to formation of precipitation increases. In beers calcium oxalate formation takes place at -2°C in 30 minutes that can be removed by filtration from beer, improving its stability.

- Filtering aids contain large amount of mineral substances. In my work I examined that under what circumstances the minerals dissolve into the beer. In course of my industrial experiments I found that both absorption and desorption take place during filtration, and the mineral content of beers do not change significantly. In case the calcium and iron content of the filtering aid is high, it can be decreased by washing with diluted hydrochloric acid.

4. Conclusions

- By setting the optimal calcium concentration, the efficiency of brewery mashing may be increased by 10-12% that will manifest in the extract yield, the energy consumption and the exploitation of capacity. Fermentation of high gravity worts becomes safer by the addition of calcium.
- Zinc content of worts made with adjunct does not assure an undisturbed fermentation process. Thus it is suggested to add 0.4 mg/l zinc upon fermentation. It is practical to add the zinc to the yeast. Experiments performed under industrial circumstances prove that by so doing fermentation rate will increase and the quality will improve.
- It is practical to keep beers at -2°C for 30 minutes before filtration to promote precipitation of calcium oxalate.
- Filtering aids that contain high amount of iron and calcium should be washed with diluted hydrochloric acid in order to decrease concentration of iron and calcium.

5. Publications on the subject of dissertation

Publications in scientific journal

Peer-reviewed articles

Vecseri-Hegyes B., Fodor P., Hoschke Á. (2005): The role of zinc in the brewing process. I. Wort production. Acta Alimentaria. **34. (3).**

Vecseri-Hegyes B., Fodor P., Hoschke Á. (2005): The role of zinc in the brewing process, II. Fermentation. Acta Alimentaria. **34. (4).**

Articles

Beata Vecseri-Hegyes, Gergely Kővári, Renáta Kovács, Balázs Búza, Gabriella Farkas, Ágoston Hoschke (2004) Fermentation of honey enriched beer with different Saccharomyces species. EBC Brewing Science Group Bulletin

Vecseri-Hegyes B. (1992): Macro- and microelement content of beers. (In Hungarian) Söripar, XXXVI.vol. 4.

Vecseri-Hegyes B., Kabai G. (1996): Production of high gravity beers with the use of adjunct (In Hungarian) Söripar, XLIII.vol. 2. 55-58.

Vecseri-Hegyes B. (2002): Beer and health (In Hungarian) Élelmezésipar, 11. 340-342.

Summaries in conference publications (In Hungarian)

Hegyesné Vecseri B. (1990): Macro- and microelement content of beers Lippay János Scientific Meeting, Budapest

Hegyesné Vecseri B., Hoschke Á., Fodor P. (1992): Role of microelements during the fermentation of beer Lippay János Scientific Meeting, Budapest, 1992. 266-267.

Hegyesné Vecseri B., Kabai, G. (1996): Production of high gravity beers with the use of adjunct Vas Károly Scientific Meeting, Budapest

Hegyesné Vecseri B., Kiss Zs., Czaha G., Vízi B. (2000): Role of calcium in the process of beer production Lippay János – Vas Károly Scientific Meeting, Budapest

In a foreign language

Hegyes-Vecseri B., Hoschke Á., .Fodor P. (1993): The role of microelements in beer fermentation. Abstract in Acta Alimentaria **22**. 248

Hegyes-Vecseri B., Kabai G. (1997): High-gravity Brewing with unmalted grains. Abstract in Acta Alimentaria 26. 318-319.