Thesis Booklet of PhD Dissertation

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Production of organic einkorn beer and analysis of healthcare potential compounds

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The Doctoral School

Name of the Doctoral School: Doctoral School of Food Science

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Approving signature of the supervisor and of the Doctoral School:

The PhD candidate fulfilled all requirements of the Szent István University, observations were considered, suggested modifications and corrections made during the defense workshop were applied in the corrected thesis. The thesis meets the standards of the Doctoral school and it can be subject of a public defense.

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Approval of the Doctoral School Approval of Supervisor

1 Background of the Work and its Objectives

Beer is one of the oldest drinks of mankind. Its popularity is due mostly to the fact that it contains clean, healthy, valuable components, and there are many different types. Purity is guaranteed by natural ingredients such as hops, malt, yeast and water. In addition to a moderate consumption, beer is a healthy drink, rich in vitamins and minerals. Interestingly, 1 liter of beer contains less carbohydrates and calories than the same amount of apple juice. However, no preservatives are added, because of its organic and inorganic acids, it is said to be a medium acidity food. Generally, the ratio of alcohol to water is physiologically beneficial. Of the most important beer components, the ethanol is the most effective for the human body. During the fermentation in addition to carbon dioxide ethanol is also formed in beers, the rate will generally vary from 3-8% (abv). Alcohol is oxidized to acetaldehyde in several stages and then converted into acetate. Acetate is easily converted into the brain into small amounts of neurotransmitter to acetylcholine through acetyl CoA, which has a brain-stimulating effect. Acetate that metabolizes larger amounts during the process will later have a neurotransmitter blocking effect.

Biochemical processes that are naturally occurring in cells typically produce free radicals during oxidation processes, some of which play an important role in maintaining the normal functioning of the body. Free radicals are reactive oxygen, nitrogen, sulfur or carbon-based molecules or molecules that have unbounded electrons and are therefore extremely aggressive and short-lived since they react chemically with other compounds for electron capture very quickly. The appearance of free radicals induces oxidative stress. Oxidative stress is called a condition where the balance of prooxidants and antioxidants is shifted to the benefit of the foregoing. Maintaining antioxidant - prooxidant balance is an important means of maintaining health. Free

radicals can be induced by external and internal factors, biotic and abiotic stress effects.

For the proper functioning of the living organism, mineral elements are indispensable. Minerals are added to the beer from basic and auxiliary materials used in brewing.

Vitamins are a variety of organic chemical compounds, which the human body cannot produce in sufficient quantities, but in small quantities are indispensable for its smooth functioning.

During my PhD research, I laid my focus on three pillars: mapping of einkorn as a potential brewing raw material and on einkorn malt production; producing beer from malted and unmalted einkorn; and on the examination of potential healthcare compounds from einkorn and other cereals, from brewing technology and comparing einkorn beer with different beers. In the first topic, I mapped the einkorn from the point of brewing. I have determined its physical and chemical parameters. In addition, I optimized the malting process of einkorn using a response surface optimization statistical method. Finally, I examined the potentially health-protective components of einkorn and other potential domestic breeds wheat and their malts. In the second topic, I first made wort under laboratory conditions from malted and unmalted einkorn, and then adapted to pilot scale brewing by the upscaling of the selected recipe. After successful brewing, I used the ready recipe in industrial conditions. In the third case I have investigated and compared the mineral and antioxidant content of Organic Einkorn beer and beers that can be found on domestic markets to each other and examined their vitamin content.

To address the above mentioned the following milestone targets were set:

1. Examination of applicability of einkorn wheat for brewing process according to the brewing process.
2. Development of a malting process by means of a micromalting equipment, qualification of finished malt. In addition, I set out to set up a statistical model to optimize the malting process of the einkorn.
3. Mapping the brewing operations, which show a deviation from the traditional brewing practices in the case of malted and unmalted use of einkorn. I intend to carry out the experiment series with a scale increase, and finally to develop a technological process that can be implemented at industrial level.
4. Quantification of health-protective compounds in einkorn and other cereals, and their malts.
5. I aimed to develop the amount of health-protective components known from international literature in the technological process of brewing, and then quantitative analysis of components with potentially health-protective effects.

2 Methods and Materials

During the ALKOBEER project taking place in the Department of Brewing and Distilling I had choose as raw material for my doctoral research the MV einkorn wheat, because it had a proper parameters from perspective of brewing. I have worked with einkorn grown by Körös-Maros Biofarm Ltd. originated from crops 2009, 2010 and 2011. In the beginning of my research I have worked with hulled einkorn, but at the end I have returned to the unhulled einkorn. Subsequent results already present the qualification of this subordinate. My doctoral thesis included the mapping of antioxidant properties of organic einkorn wheat. For comparison, I also examined a number of winter and optional winter varieties breed by the Agricultural Research Institute of the Hungarian Academy of Sciences, and their malts. For the malting process, I used the micromalting equipment produced by the Schmidt-Seeger German company, situated at the Szent István University, Faculty of Food Science, Department of Brewing and Distilling. For laboratory mashing experiments, I used a 1-CUBE brand mashing bath. The pilot experiments were also carried out on the 50-liter pilot plant brewery at the Department, where I could model all the steps of industrial brewing, such as infusion and decoction mashing, beer filtration, hop boiling, whirlpool process, wort cooling, fermentation and aging.

I studied the einkorn and its malt based on brewing process standards (EBC, MEBAK). During the mechanical tests, I determined the grading, the thousand kernel weight, the hectolitre mass, the floury-glasses grains ratio. During the physiological examination I determined the germination energy by BRF method. During the physical examination I examined the moisture content and the gelatinization temperature off einkorn starch. During the qualification of malt, I did standard Congress mashing, from which I determined the extract content and the extract difference. For the

determination of the enzyme composition of malt, the mashing method of Hartong-Kretschmer was used. After determining the protein content and dissolved nitrogen, I also examined the Kolbach index of malt. I determined the amylose-amylopectin ratio of the einkorn starch and the temperature optimum of β-amylase enzyme. From worth I examined the free α-amino nitrogen (SZAN) content and with HPLC I have determined the carbohydrate content.

In order to determine the antioxidant capacity from different plant materials, I had to adapt a multi-step method of extraction:

1. In the first step, the grain / malt sample was milled in a mill size of less than 0.5 mm, and then the meal was immediately used. Of this, 0.5 g of crude was closed in a centrifuge tube and then 20 ml of methanol: water (50:50, v / v, pH 2) was added and shaken at room temperature for one hour. After centrifugation at 2500g for 10 minutes the resulting supernatant was collected. The remaining sediment was measured with 20 ml of acetone: water (70:30 v / v) and repeated the shaking and centrifugation. The resulting supernatant was poured onto the supernatant obtained in the previous step. The resulting mixture was the sample of the first phase of sample preparation.

2. In the second step, 20 ml of methanol and 2 ml of concentrated sulfuric acid were applied to the remaining sediment and placed in a shaking water bath at 85 ° C for 20 hours. Then it was centrifuged (10 minutes, 2500 g) and the supernatant was removed. After duplicate distilled water rinsing, the supernatant was supplemented with distilled water to 50 ml. The resulting mixture formed the sample of the second phase.

Four methods were used to determine the antioxidant capacity:

- **DPPH** (1,1-difenil-2-pikrilhidrazil) assay
- **TEAC** (Trolox Equivalent Antioxidant Capacity) assay

- **FRAP** (Ferric Reducing Ability of Plasma) assay
- **TPC** (Total Polyphenol Content) assay

ICP-AES technique was used for the determination of mineral content. For the determination of water and fat-soluble vitamins, I used an HPLC method using an UV-PDA detector.

The processing of the data obtained during the various measurements is mathematical in accordance with its nature. All statistical analyzes were performed at 5% significance level ($p = 0.05$). In the beer analysis, as there was no absolute control sample, the main component analysis (PCA) was used for the analyzes. To optimize malting, I used the statistical surface-optimization response method (RSM). Statistical analyzes were carried out using Statistica 10 (StatSoft, Tulsa, USA) software.

3 Summary of the results

One of the main components of healthy nutrition is raw material from organic farming. The amount of intake of undesirable chemicals in our body can be reduced by the use of bio foods that are taken daily. To conserve the health of our body exposed to increased external effects, it is not enough to consume only non-chemical foods, but also foods that have a health-protective effect.

Organic beer is increasingly spread in the world, but is still available in small quantities. In addition to a number of brewing raw materials experiments, einkorn wheat (Triticum monococcum L.) is still relegated to the background, and little scientific research dealing with einkorn wheat beer industry usability.

Mapping einkorn wheat as possible raw material for brewing. Optimizing malt production. Determination of its antioxidant capacity.

The main guideline of my research was to develop the technology of Organic Einkorn beer. To make the beer from einkorn I supported the applicability of einkorn in brewing industry with scientific research. I have found that the nutritional composition is similar to barley, but its size is smaller than that. The einkorn is loosely surrounded by chaff, the ratio of which is 30% to the core. During the study, I determined the starch amylose-amylopectin ratios (15/85) of einkorn, and determined the gelatinization temperature of einkorn starch (83 °C) and the optimum temperature (65 °C) of the starch-degrading β-amylase enzyme. Due to the high gelatinization temperature of einkorn starch, it was necessary to modify the mashing technology.

I have made malting trials with einkorn on a micromalting equipment. Examining the nutritional components of einkorn malt I determined the most successful malting process. By varying the malting parameters and on the results of malt nutritional characteristics, I determined the formulas describing the optimum malting parameters of einkorn using a response surface statistical method. The malting of einkorn is optimal, if it is steeped and germinated on 22°C and germination takes 5 days, and the moisture content at the end of germination is 45%. This method results an einkorn malt with acceptable parameters (table **Hiba! A hivatkozási forrás nem található.**.) for brewing.

Four different methods (TPC, FRAP, DPPH, TEAC) were used to determine antioxidant capacity of einkorn, malting barley and other potential brewing raw material grains and then I compared to each other. In order to detect as many antioxidants as possible, I had to adapt the extraction method described by Pérez-Jiménez et al. (2006), which includes two extraction steps, an aqueous and an acidic extraction. During the study, I came to the conclusion

that in the second stage of the extraction, about two-thirds of all the components of the total antioxidant capacity were recovered during the second, acidic extraction step. In addition, it found that the antioxidant capacity of einkorn was in all case lower compared to barley. During malting, I could see an increase in antioxidant capacity. The einkorn malt had superior results in comparison with other wheat in case of DPPH and TEAC determination method.

It can be concluded that organic barley had the highest antioxidant capacity in each of the four different antioxidant capacity determination methods. Among the wheat samples the einkorn had the highest antioxidant capacity, except for the FRAP method, where a wheat sample had a higher iron-reducing capacity.

Production of wort and beer from malted and unmalted einkorn

Unfortunately, my successful malting technology could not be utilized anymore, because in order to make beer at the beginning in a small brewery, I would have had to produce small amounts of malt, which is impossible in Hungary.

From this consideration, I decided to use unmalted einkorn, but due to the high gelatinization temperature of the einkorn starch the use of decoction mashing was inevitable. In the composition of the raw materials, I was striving for more than 50% of the einkorn portion. During the experiment I used the dehulled einkorn for higher extract content, and the hulled einkorn for better filterability of the wort. Since the laboratory trials doesn't cover all the industrial brewing steps (lautering, hop boiling, whirlpooling, fermentation, aging), I have transposed the best laboratory mashing to pilot scale. The beer thus obtained contains 51% of hulled and dehulled einkorn, resulting a beer ready for the market.

Based on the antioxidant capacity of the raw material, I determined the effect of the brewing steps of antioxidant capacity. Most polyphenols were in the first wort, but this diminished greatly, probably due to the considerable dilution. During brewing, the antioxidant capacity showed a greater fluctuation in the FRAP and TPC methods, but in DPPH and TEAC, this cannot be stated.

Investigating potential health-protective components

I compared the antioxidant capacity of the finished product brewed on an industrial scale with other beers from the Hungarian market. They consist beers of a variety of ingredients, different alcoholic content and different amounts of hops.

Regarding the results of the TPC method, it can be stated that the Dreher has detected the lowest values for nonalcoholic beer. Dreher Bak, Ksiazece and Bernard were higher than the average, which is explained by the malt composition, higher extract content, and the addition of other substances. In the category of light beers, Gniewos and Budweiser, which have higher extract content, one of the highlights has Organic Einkorn Beer. Particularly interesting is its antioxidant content so high that it exceeds Wells Bombardie's brown beers. In the FRAP method, I came to similar conclusions as above. Organic Einkorn beer showed outstanding results among beers with clear, average extracts. In the DPPH method, we have outstanding values for Fuller's IPA, which contains extra amounts of hops and I found Ksiazece beer with dried rose hips. It can be stated that this method is best suited for compounds found in hops.

I observed that beers with the lowest antioxidant capacity were non-alcoholic beers, the highest being the beers prepared with higher amount of colored malt or higher amounts of hops or additives rich in antioxidant compounds. In the TEAC method, no protruding values were measured. However, it cannot be ignored that one of the highest antioxidant capacity was measured at Organic Einkorn beer. Overall, I can state that Organic Einkorn beer had the same or greater antioxidant capacity as light beers thanks to the high antioxidant capacity of the raw material.

The statistical component analysis (PCA-BiPlot) has confirmed the literature that from the point of view of antioxidant capacity different beers can be grouped into separate groups based on the ingredients and the technologies. Based on the above mentioned statistical analysis, I can state that Dreher Bak and Bernard samples have outstanding free radical elimination properties, whereas Gösser Zitrone, Hoegaarden, Carlsberg and Dreher non-alcoholic beers have shown modest values. Corona Extra, Zywiec Premium, Tyskie and Dreher Classic beers also showed modest values. The other beers were relatively close to each other in the reduced factor space, so there was no significant difference between them. The relationship between the different methods used is that while the DPPH, FRAP TPC method correlates well with each other (small angle of the vectors representing them), the TEAC method is significantly separated from these. Taking into account the PCA analysis, The Organic Einkorn beer does not show much difference compared to light beers in terms of antioxidant capacity, but there is a significant difference between other wheat beers.

During the mineral content test I examined the mineral content of the water used to brew beer to exclude the effect of the brewing water on the

finished product. The mineral content of the mineral water has proved that the used brewing water may have little effect on the composition of the final mineral. When examining the mineral content of einkorn seed and husk, I have found that the largest amount of copper and sodium is removed during dehulling, however, taking into account the 30% of the core / husk is not significant. Compared with the literature on barley, it contained more than ten times the amount of calcium, iron, manganese and sodium, but the content of potassium, magnesium and phosphorus was also several times the barley.

Since I was able to produce beer according to market expectations and to produce a ready-to-use product under scale, I examined their mineral content and compared it with the literature. The results show that the potassium content of the beer made on pilot plant is highest, followed by a small difference between the potassium concentration of the industrial beer and the average value of the domestic beers, which almost half of the potassium content of the beer is made on pilot plant. The phosphorus content of the beer made on pilot plant is almost identical with the industrial beer, which is about two-thirds of the average value of domestic beers. Calcium content of the einkorn is more than ten times of barley. During the brewing experiments, we encountered lower calcium content in the industrial samples, due to the water treatment technology. In the case of magnesium, both samples exceeded the literature average values.

In order to prove the impact of einkorn beer on health, I examined the vitamin content of the finished product and compared it with other beers found on the Hungarian market. The vitamins tested are originating from malt, in this case the einkorn and yeast activity. I studied a number of vitamins concentrations in different beers, but I found detectable values only for thiamin and nicotinamide. During the experiment, the IPA type of beer had the highest amount of vitamin due to the higher amount of raw material. The content of

nicotinamide in Organic Einkorn beer is considerably higher than the other beers tested, while the content of thiamine remains only in the case of beers made from special malt.

With my results, I have demonstrated the applicability of einkorn wheat for the brewing process and thanks to the developed brewing technology the Organic Einkorn beer enriches the Hungarian beer palette with an organic beer, being the first one on the market.

4 New scientific results

1. During my research, I have mapped a new grain from a brewing point of view, through its properties new type of beer can be produced.
2. Since the einkorn wheat was an unknown raw material for the brewing industry, I developed its malting methodology.
3. Developing a mashing process for brewing applicability of einkorn.
4. I determined the antioxidant content of einkorn wheat, compared it with other wheat suitable for brewing purposes and examined the antioxidant capacity of the malt products
5. I examined the potentially health-protective components of einkorn beer and compared them with other beers. I observed the effect of the technological steps of beer making on the amount of antioxidants.

5 Conclusions and proposals

Based on my research results, einkorn wheat (Triticum monococcum) is suitable for brewing purposes. I have proved that with appropriate malting technology good quality einkorn wheat malt can be done. During the

investigation of the raw material it has been proved that for achieving high extract content wort with using of einkorn, decoction mashing process has to be used because of high gelatinization temperature of einkorn starch. Furthermore, the same β-amylase enzyme temperature optimum of einkorn and barley made possible to use them together. I have proved that if malted einkorn is not available, unmalted einkorn can be used in more than 50% mixed with barley malt with the help of appropriate industrial enzymes. Beer from einkorn can be easily made in industrial environment by upscaling with respecting the appropriate mashing procedure.

When examining potential health-protective components, I determined the mineral content of einkorn. I compared with the literature data and I came to the conclusion that with using einkorn in brewing greater amount of minerals can be transferred to the finished product. This was supported by laboratory and pilot experiments.

I examined the antioxidant capacity of einkorn and its malt using four methods (TPC, FRAP, TEAC, DPPH), and I have compared to other wheat breaded for brewing purposes and to brewing barley, and with its malts. The results showed that the malting barley resulted the highest values, but compared with the wheat samples, the einkorn was on the first place.

At the same time, the antioxidant capacity continued to increase during malting. I determined the effect of brewing on antioxidant capacity and found that antioxidant capacity showed greater fluctuations in FRAP and TPC methods, but in DPPH and TEAC, this cannot be stated. Compared to other beer's antioxidant capacity, I found that Organic Einkorn beer had the same or greater antioxidant capacity than light beers due to its raw material high antioxidant capacity.

In order to prove the health protection potential of einkorn beer, I examined the vitamin content of the finished product and compared it with other beers found on the Hungarian market. During the experiment, the IPA type beer had the highest amount of vitamin due to the higher amount of raw material. The content of nicotinamide in Organic Einkorn beer is considerably higher than the other beers tested, while the content of thiamine remains lower only in the case of beers made from special malt.

In order to attribute higher healthcare effect for Organic Einkorn beer, further research is required, it is also recommended to make research with low-alcohol and alcohol-free einkorn beer.

6 Publications related to the thesis

Impact Factor publications

Attila-Levente Fogarasi, Beáta Hegyesné-Vecseri, Stefanovits-Bányai Éva, Szilárd Kun (2015): A comparative assessment of antioxidant properties, total phenolic content of einkorn, wheat, barley and their malts. FOOD CHEMISTRY 167: pp. 1-6. IF: 4,529

Attila Szöllősi, Quang Duc Nguyen, Attila Gábor Kovács, **Attila-Levente Fogarasi**, Szilárd Kun, Beáta Hegyesné-Vecseri (2016): Production of low or non-alcoholic beer in microbial fuel cell. FOOD AND BIOPRODUCTS PROCESSING 98: pp.196-200. IF: 1,97

Non-Impact Factor publications

Fogarasi Attila Levente, Hegyesné Dr. Vecseri Beáta, Kiss Zsuzsanna, Dr. Kun Szilárd **(2011**): Sör, ami bio és organikus. Az alakor a sörkészítésben. Biokultúra újság, 2011/3.

Fogarasi Attila Levente, Hegyesné Dr. Vecseri Beáta, Kiss Zsuzsanna, Dr. Kun Szilárd **(2014**): Az alakorból készült első magyar biosör gyártástechnológiájának kidolgozása Biokultúra újság, 25:(3) pp. 26-30.

Symposium publications

Hungarian Symposium publication (Summary)

Fogarasi Attila-Levente, Kiss Zsuzsanna, Kun Szilárd, Hegyesné Vecseri Beáta: Alakor búza sör – a magyar egészségvédő bio sör. Táplálkozástudományi kutatások. Innováció - Táplálkozás - Egészség - Marketing. Konferencia, Kaposvári Egyetem, Kaposvár, 2012.

International Conference (summary)

Attila-Levente Fogarasi, Zsuzsanna Kiss, Beáta Vecseri Hegyes: Examination of the Applicability of Einkorn (Triticum monococcum L.) for Beer Production and Determination it's Healthcare potential. 8th International Conference of PhD Students, University of Miskolc, Hungary, 05-11 August, 2012

Attila-Levente Fogarasi, Beáta Vecseri Hegyes, Zsuzsanna Kiss, Szilárd Kun: Einkorn (Triticum monococcum L.) in Organic Beer Production; Malting Organic Einkorn. Third International Young Scientists Symposium for the Brewing, Distilling and Malting Sectors, Nottingham, United Kingdom, 23-25 October, 2012.

Attila-Levente Fogarasi, Beáta Vecseri Hegyes, Zsuzsanna Kiss, Szilárd Kun: Examination of organic einkorn wheat (Triticum monococcum L.) and organic einkorn wheat beer healthcare potential. Food Science Conference 2013 – With research for the success of Darányi Program, Budapest, Hungary