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Study of health promoting components throughout the malting and brewing process

The Thesis of the Ph.D. dissertation

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Background of the work and its aims

In recent years, the lifestyle of people, especially in western countries, has changed, we are pursuing an accelerated way of life, which also affects our eating habits. Processed foods make up an increasing proportion of the food consumed. As a result of processing, they lose their nutritional value, primarily their biologically active nutrients. Intake of biologically active nutrients such as vitamins and antioxidants are essential for the proper functioning of the body. To know how we can meet our body's need for vitamins and antioxidants, we also need to know how the processing of raw materials during food production affects these components. My dissertation is intended to help answer this question.

It is assumed that on a population level, nutritional requirements for folate cannot be completely covered by a varied diet, as recommended by national health authorities. Dietary intake is below recommendations in numerous countries, including Hungary, owing to low consumption of folate-rich foods, e.g., pulses, citrus fruits, and leafy vegetables (OHRVIK et al. 2011, OTÁP 2014).

Cereals are considered a good source of folate. There are publications dealing with the folate content of different cereals (oat, wheat, barley, rye) but with malting the folate content of cereals can be further increased. Nonetheless there is only one scientific publication investigating the evolution of folate content during malting of barley which was authored by WALKER (2003).

There are numerous scientific publications about the folate content of several types of beers available in commercial trade, in these it has been reported that beer can be a valuable source of folate depending on the beer type, but a considerable deviation is observed between the different products. There is a lack of information about the effect of raw materials and technological steps of brewing on folate content that could be helpful to understand the evolution of folate during the brewing process and to get a deeper insight into what makes the folate content of each product so different.

In addition to folate, the intake of antioxidants is also essential. Nowadays, we are exposed to several external physical and internal psychological stress effects. Stress influences the balance of antioxidants and free radicals in our body, which can lead to various diseases. To maintain this balance, we need to consume foods rich in antioxidants, primarily foods of plant origin. However, it is shown that we do not consume enough.

Supplementation with cereals, malt beverages and alcohol-free beer can be a good alternative to this. In addition to the health effects, antioxidants and phenolic compounds are also very important from the technological point of view in brewing. There are studies in the literature that examine antioxidants and polyphenols during malting or brewing, or in different types of beers, but a comprehensive study that gives a detailed picture of these compounds from the grain to the finished product is not available.

The aim of my dissertation is to form a comprehensive picture about the evolution of folate content, antioxidant activity and phenolic content from the raw materials throughout the malting and brewing process until the final beer. To achieve this, I set the following goals:

Introduce and process the available literature.

Determine the folate content of:
malts produced from different grains; different barley varieties; barley malts produced on pilot and industrial scale.

Investigate the evolution of folate content during:
pilot and industrial scale malting; roasting of malt; an extended protease rest during mashing; laboratory and pilot scale brewing.

Determine the total phenolic composition and antioxidant activity of:
malts produced from different grains; different barley varieties; barley malts; different hop varieties.

Investigate the evolution of total phenolic composition and antioxidant activity during:
pilot and industrial scale malting; roasting of malt; laboratory and pilot scale brewing.

Investigate targeted phenolic compounds, total flavonoid content, total polyphenol content, and DPPH radical scavenging activity:
of congress worts produced with adjuncts and special malts; during infusion and decoction mashing; during high gravity brewing.

Materials and methods

The materials investigated can be divided into two groups. Materials used for the determination of folate content, antioxidant activity and total phenolic composition and materials used to determine targeted phenolic compounds, total flavonoid content, total phenolic content, and DPPH radical scavenging activity.

With the first group of methods malts produced from different grains; malting on pilot and industrial scale; the effect of roasting; the effect of brewing pale and dark beer on laboratory and pilot scale; and different hops were investigated.

With the second group of methods the effect of adjuncts and special malts; comparison of infusion and decoction mashing; and the effect of high gravity brewing were investigated.

As for the analytical methods, the folate content was determined by a microbiological assay suitable for total folate content determination. The antioxidant activity was investigated by five different methods (FRAP, CUPRAC, ABTS, TPC, DPPH).

Both the total and targeted phenolic composition was investigated by HPLC-DAD. For the total phenolic composition the phenolic compounds were identified based on their retention times and spectral characteristics and were quantificated as rutin equivalent. The targeted phenolic composition was determined using standards of the selected phenolic compounds.

The determination of extract content was carried out using an Anton Paar Beer Analyzer System. The moisture content of the barley and malt samples was determined by an AND MX-50 Moisture Analyzer.

Results and discussion

Evolution of folate during malting and brewing

To study the effect of **malting** on folate content, first malts produced from different grains were investigated, then malting was carried out on micro and industrial scale which was followed by a roasting experiment.

Based on my results, it can be stated that the folate content of pale barley malt is outstanding among the malts made from different grains. There is considerable difference between the folate content of spring and winter barleys of different vintages. The folate content of malts produced from winter barley was higher both under micro and industrial scale. Malting increased the folate content of malts of different types and vintages of barleys, which is mainly due to the activity of the embryo and the enzymatic degradation processes during malting. The biggest difference between micro and industrial scale malting was the trend during the malting process, even though the same parameters were used during the processes. Barleys malted at micro scale reached their highest value on the 3rd-5th days of malting, and then their folate content decreased due to kilning. In contrast, barleys malted at industrial scale showed a steadily increasing trend, reaching the highest folate content in the finished malt.

The **roasting** experiment proved that the folate in barley malt was still stable at temperatures around 100 °C, but above that folate began to degrade and is in inverse relationship with temperature and time. At 200 °C folate content dropped to one third in just 5 minutes, proving that special malts that are exposed to higher temperatures are not good sources of folate.

Evaluating the results from the point of view of nutrition, it can be stated that the folate content of barley can be increased up to 10 times by germination, which can be preserved by gentle kilning. When kilning, it is important that the temperature does not exceed 100 °C. In terms of folate content, pale barley malt is the best choice for further processing in the food industry.

To study the effect of **brewing** on folate content worts were produced on laboratory and pilot scale.

During mashing, prolonged protein rest had no effect on folate content. The folate content of the mash increased continuously, which is due to the enzymatic processes. By the end of the first enzyme rest (protease rest), a significant part of the folate was already dissolved. During wort separation, sparging is of paramount importance, which, related to extract content, contained at least the concentration of folate as the first wort. Hop boiling, at which a decrease would have been expected in folate content, did not cause a decrease.

The brewing process is effective in recovering the folate content of malt, so brewed drinks have the potential to contribute considerably to daily folic acid intake. Based on my results, it can be stated that malt beverages or non-alcoholic beers can be produced with a significant folate content if choosing the appropriate raw materials. On the other hand, the metabolism of the yeast greatly influences the development of the folate content, so further studies would be needed on the production of beers with normal alcohol content, which investigate specifically the processes that take place during fermentation, in order to produce beers with higher folate content.

Antioxidant activity and phenolic composition during malting and roasting

Like in case of folate experiments, first the antioxidant activity and phenolic composition of malts produced from different grains were investigated, then the evolution of these compounds was studied during micro and industrial scale **malting** followed by roasting experiments.

From the point of view of antioxidant activity and phenolic content, barley malt is also of outstanding importance among different cereals, however, it is not the pale barley malt, but the special caramel and coloring malt, primarily due to their very high flavanol derivative content.

There was no real correlation found between the antioxidant activity and the phenolic content of barleys. In terms of phenolic content, according to cultivar, spring barleys had a higher concentration, and according to vintage, 2018 barleys had a higher concentration compared to the 2017 crop.

The antioxidant activity and phenolic content of both micro and industrial scale malts reached a peak during germination and then decreased with kilning. As a result of roasting, the antioxidant activity increased in all cases, as did the total phenolic content, which well reflects the results of the preliminary experiment with special malts. The hydroxycinnamic-acid derivative content began to increase intensively around 150 °C and the flavanol derivative content around 175 °C. The flavanol derivative content increased continuously until the longest roasting at the highest temperature, while the other phenolic groups stagnated after the initial growth.

Malts **roasted** at a high temperature are outstanding, which is due to the formation of reductones with antioxidant activity and the structural transformations in the case of phenolic compounds, but this can be negative from the technological point of view, as dark malts contain high concentration of flavanol derivatives which have the ability to form polymers, thereby adversely affecting the colloidal stability of the final product in the case of filtered beers, but may be particularly advantageous for the production of turbid beers. In addition, the antioxidant activity of malts treated at higher temperature is higher, which can improve the oxidative stability of beers.

Antioxidant activity and phenolic composition of hops

In the study 16 different types of hops with different alpha-acid content were involved.

Great differences were found between the different types of hops in terms of both antioxidant activity and phenolic content. No correlations were found between antioxidant activity, phenolic content, and alpha-acid content. It can be stated that the antioxidant activity and phenolic content of hops are high, however, there are significant differences in phenolic composition. Hops can be chosen for brewing keeping in mind the quality requirements of the final product to be produced. If an unfiltered, turbid product is brewed, the right choice is the hop with high flavanol derivative content, if a filtered product is brewed, the right choice is the hop with lower flavanol derivative content.

Antioxidant activity and phenolic composition during the brewing process

For **brewing** experiments a pale and a dark wort were brewed on laboratory scale, furthermore on pilot scale a pale and a dark beer were produced.

During mashing, different degrees of change occurred in pale and dark laboratory and pilot scale samples, however, the antioxidant activity and polyphenol content increased in all cases. The first wort separation had a small negative effect on both antioxidant activity and phenolic content in all cases. It should be emphasized that sparging showed high values in terms of both antioxidant activity and phenolic content related to extract content. Hop boiling caused a decrease in phenolic

content in pale worts, but an increase in dark worts. This may be due to the protective effect of reductones originating from special malts. During fermentation, the flavanol derivative content showed the greatest decrease, which can be positive for the colloidal stability of the final product in case of a filtered beer.
The higher antioxidant activity and phenolic content of the dark malts used is also reflected in the brewhouse process. The antioxidant activity and phenolic composition of the wort and beer can be influenced with the selection of the right amount and type of special malts, considering the desired quality of the final product.

Effect of adjuncts on the antioxidant activity and targeted phenolic compounds of congress worts
In order to study the effect of **adjuncts** on antioxidant activity and phenolic composition of worts, congress worts were produced on a laboratory mashing equipment.
Rice, corn, and wheat used as adjuncts decreased the free phenolic acid content of worts. This is a negative finding from both the nutritional point of view and technology because these compounds can contribute to the longer shelf life of a product and the maintenance of antioxidant/free radical balance in our body. On the other hand, when using these adjuncts, the concentration of procyanidin B3 decreased, which is beneficial for the better colloidal stability of the final product. Barley contributed to a higher phenolic acid content but must be treated carefully from the technological point of view because its flavanol content (catechin, epicatechin, procyanidin B3) was also high. Special malts had a high flavanol content which is in line with the previous results, which clearly showed that this high flavanol content is mainly due to the monomers (catechin, epicatechin) and not to the dimers (procyanidin B3).

Study of the effect of decoction and infusion mashing technology on antioxidant activity and targeted phenolic compounds
In order to study the effect of **infusion and decoction mashing** on antioxidant activity and phenolic content one infusion mash and two decoction mashes were produced on pilot scale.
Among first wort, sparging and sweet wort samples sparging had the highest values of antioxidant activity in most cases, highlighting the importance of this technological step for antioxidant activity and phenolic content. Decoction mashing technology contributed to higher antioxidant activity of worts, but the same cannot be stated for the phenolic content.

Effect of high gravity brewing on antioxidant activity and targeted phenolic compounds
In order to study the effect of **high gravity brewing** two beers were produced from the same raw materials and with the same technology on pilot scale, one was brewed to 9 %m/m original extract content and the other to 14 %m/m original extract content. Before analysis, the 14% beer was diluted to 9% with deoxygenated water.
No significant difference was observed between the antioxidant activity of high gravity brewed and normal beer, on the other hand all the investigated phenolic compounds were significantly higher in the normal beer which was not diluted. High gravity brewing decreased the phenolic content in beer, it can result in worse flavor stability and a less full taste, but the colloid stability of the product may be better.

Conclusion and recommendations

Evaluating the results from the point of view of nutrition, it can be stated that the folate content of barley can be increased up to 10 times by germination, which can be preserved by gentle kilning. When kilning, it is important that the temperature does not exceed 100 °C. In terms of folate content, pale barley malt is the best choice for further processing in the food industry.

The brewing process is effective in recovering the folate content of malt, so brewed drinks have the potential to contribute considerably to daily folate intake. Based on my dissertation, it can be stated that malt beverages or non-alcoholic beers can be produced with a significant folate content if choosing the appropriate raw materials. On the other hand, the metabolism of the yeast greatly influences the development of the folate content, so further studies would be needed on the production of beers with normal alcohol content, which investigate specifically the processes that take place during fermentation, in order to produce beers with higher folate content.

In terms of antioxidant activity and phenolic content, malts roasted at a high temperature are outstanding, which is due to the formation of reductones with antioxidant activity and the structural transformations in the case of phenolic compounds, but this can be negative from the technological point of view, as dark malts contain high concentration of flavanol derivatives which have the ability to form polymers, thereby adversely affecting the colloidal stability of the final product. In addition, the antioxidant activity of malts treated at higher temperature is higher, which can improve the oxidative stability of beers.

It can be stated that the antioxidant activity and phenolic content of hops is high, however, there are significant differences in phenolic composition. Hops can be chosen for brewing keeping in mind the quality requirements of the final product to be produced. If unfiltered, more turbid product is brewed, the right choice is the hop with high flavanol derivative content, if a filtered product is brewed, the right choice is the hop with lower flavanol derivative content.

The higher antioxidant activity and phenolic content of the dark malts used is also reflected in the brewhouse process. The antioxidant activity and phenolic composition of the wort and beer can be influenced with the selection of the right amount and type of special malts, considering the desired quality of the final product.

Rice, corn, and wheat used as adjuncts decrease the free phenolic acid content of worts. This is a negative finding from both the nutritional point of view and technology because these compounds can contribute to the longer shelf life of a product and the maintenance of antioxidant/free radical balance in our body. On the other hand, when using these adjuncts, the concentration of procyanidin B3 decreased, which is beneficial for the better colloidal stability of the final product. Barley can contribute to a higher phenolic acid content but must be treated carefully from the technological point of view because its flavanol content (catechin, epicatechin, procyanidin B3) is also high. On the other hand, there is an advantage in the use of barley compared to other adjuncts; the presence of husk allows easier wort separation.

Decoction mashing technology contributes to higher antioxidant activity of worts, but the same cannot be stated for the phenolic content. No significant difference was observed between the antioxidant activity of high gravity brewed and normal beer, on the other hand all the investigated phenolic compounds were significantly higher in the normal beer which was not diluted. High gravity brewing decreases the phenolic content in beer, it can result in worse flavor stability and a less full taste, but the colloid stability of the product may be better.

The study of both folate content, antioxidant activity and phenolic content during malting and brewing is extremely complex. The type, the sowing and harvesting time of the raw materials, the way of their processing, and proportion of use, the variability of many parameters of the brewing process and the diversity of the final products offers endless research opportunities, not to mention the diversity of the analytes.

New scientific results

1. I have demonstrated that significant part of the folate content of malt is released during the 20 minutes at 52 °C protease rest of the mashing process of brewery wort production. I have proved that a prolonged protease rest (40 minutes at 52°C) does not increase the folate content of mash. Furthermore, I have demonstrated that folate content of the mash increases continuously until the end of the mashing process (20 minutes at 52°C, 45 minutes at 62°C and 15 minutes at 72°C).

2. I have proved that the hop boiling process (one hour at 100 °C) does not result in the decrease of the folate content of wort.

3. I have proved that caramel and coloring malts have outstanding flavanol derivative content. The high flavanol content is mainly due to monomers (catechin and epicatechin) rather than dimers (procyanidin B3).

4. I have demonstrated that antioxidant activity and phenolic content reach a peak during the technological step of germination of the malting process of barley.

5. I have demonstrated that roasting at 100, 125, 150 or 175 °C for 20 minutes and at 200 °C for 10-15 minutes increases the antioxidant activity and total phenolic content of malt.

6. I have demonstrated that the antioxidant activity and phenolic content of mash increase during the mashing process (20 minutes at 52°C, 45 minutes at 62°C and 15 minutes at 72°C). I have proved that decoction mashing technology result in wort with higher antioxidant activity than infusion mashing technology.

7. I proved that the technological step of sparging has an outstanding contribution to the antioxidant activity, phenolic content, and folate content of wort.

8. I have proved that beer produced with high gravity brewing technology contains less phenolic compounds as opposed to beer produced with normal gravity brewing technology.

9. I have proved that flavanol derivative content decreases significantly during the fermentation process (primary fermentation at 16°C for 5 days and secondary fermentation at 3°C for 21 days with ale type brewing yeast).

The publications of the author in the field of studies

Journal articles related to the dissertation with IF:

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