

Theses of doctoral (PhD) dissertation

**EFFECTS OF DIFFERENT MATERIALS OF
PLANT ORIGIN ON THE QUALITY
PARAMETERS OF BEER**

Written by: Loránd Alexa

Supervisor: Dr. Nikolett Czipa, associate professor



UNIVERSITY OF DEBRECEN
Kerpely Kálmán Doctoral School

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1. Background and objectives of the doctoral dissertation

Beer is an extremely popular alcoholic beverage worldwide, its consumption is significant even in such countries that do not have a long history and tradition of the craft of brewing (ALCÁZAR et al., 2002). Based on the data collected in 2019, the Czech Republic is at the top of the European list of annual beer consumption with an intake of 141 L/capita. Although Hungary occupied the 19th place at the same list, its 64 L/capita/year consumption is also considerable (STATISTA, 2021.06.08.). The changing consumption patterns causes structural changes in numerous countries. The most important goal of the Craft Beer Campaign of the 1980's was to reinvent the standard, featureless beer market developed after the prohibition in the USA, which has also reached Hungary three decades later, due to the amendment of the tax laws in 2012, that enabled citizens to perform brewery with a volume lower than 1000 litres (MAGYAR KÖZTÁRSASÁG, 2011). Craft beers may be appealing to consumers who would prefer complex, novel taste instead of the common mainstream beverages (JAEGGER et al., 2020). The most important criteria relating to craft beers is that they must be prepared by traditional technologies in a facility with low production capacity (ELZINGA et al., 2015). The development of new tastes and properties is an easier task for small facilities, since they can freely experiment with new primary ingredients, flavourings, technologies due to their low production volume (DONADINI – PORRETTA, 2017).

The application and combination of different ingredients and technologies might modify the quality and nutritional parameters of these beverages. The aim of my study was to find correlations between the quality parameters and the composition of beers prepared by the application of different raw materials, therefore, to explore the concentrations of different compounds with health benefits (polyphenols, flavonoids, vitamins, minerals) in beers containing additional materials of plant origin. For this purpose, I have carried out the analysis of commercial beers. Most of the samples were traditional pale and dark beers and wheat beers, but I have also measured the parameters of some ale beers, flavoured beers and mixed beer beverages. The measurements were carried out in the Institute of Food Science, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen. The analysis included the determination of total phenolic content (TPC), flavonoid content, colour, total acidity and mineral content.

Then, I have prepared my own samples by using a micro-brewery system available in the Institute. After creating a few base recipes, I have investigated the effect of different products of plant origin on the quality parameters of the samples. I have mostly prepared pale ale beers with additional plant-origin products, such as fruit juices and chocolate, which were added to the beer at different steps of the production in different concentrations. Total phenolic content, flavonoid content, vitamin C content, colour, total acidity, bitterness and mineral content of the above-mentioned samples were determined. After the measurements, the statistical analysis of the created data was also carried out to determine whether there are statistically verified differences between the parameters of the samples or not, and whether these samples could be differentiated based on the test results or not.

2. Material and methods

Production and analysis of the samples were carried out in the Institute of Food Science, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen. Commercial beers were collected from supermarkets in Debrecen, and included 30 pale beers, 10 dark beers, 11 pale wheat beers, 2 dark wheat beers, 8 ale beers (IPA, APA, stout), 8 flavoured beers and 5 mixed beer beverages. Malts, hops, yeasts and other materials and equipment required for the brewing were purchased in a Budapest-based brewing specialty shop. The additional materials were mostly collected in local markets right before the brewing process. The applied materials and the technological parameters of the base recipes are shown in Table 1; and the flowchart of the production of these samples are demonstrated by Figure 1.

Table 1.: Description of the base recipes

Raw materials and parameters		Flavoured beers	Oat beers	Stout beers
Malt 1.	Type	Pale ale	Oat	Pale ale
	Weight (kg)	4	5	1
Malt 2.	Type	Wheat	-	Maris Otter
	Weight (kg)	0.5	-	3
Malt 3.	Type	Caramel	-	Cara-crystal
	Weight (kg)	0.2	-	0.6
Malt 4.	Type	-	-	Chocolate
	Weight (kg)	-	-	0.25
Surrogate 1.	Type	-	-	Oat flakes
	Weight (kg)	-	-	0.8
Surrogate 2.	Type	-	-	Roasted barley
	Weight (kg)	-	-	0.25
Mashing water (L)		15	18	15
Protease step	Temperature (°C)	50	50	52
	Time (min)	20	30	30
α-amylase step	Temperature (°C)	67	65	69
	Time (min)	75	60	120
β-amylase step	Temperature (°C)	-	75	74
	Time (min)	-	15	20
Leaching water (L)		11	10	10
Hop boiling (min)		60	60	80
Hop 1.	Type	Citra	Magnum	Fuggles
	Alpha-acid (%)	13	14.5	5.2
	Weight (g)	40	10	55
	Time (min)	60	60	35
Hop 2.	Type	Citra	Cascade	-
	Alpha-acid (%)	13	6.8	-
	Weight (g)	10	20	-
	Time (min)	10	30	-
Hop 3.	Type	-	Saaz	-
	Alpha-acid (%)	-	3.6	-
	Weight (g)	-	10	-
	Time (min)	-	5	-
Yeast type		Fermentis S-05	Fermentis S-04	Fermentis S-04
Fermentation	Temperature (°C)	20	18	18
	Time (days)	14	14	14

Source: Own editing

The application of alternative primary ingredients, surrogates and flavourings was carried out as follows:

- Flavoured beers: Fruits were collected on the day of the brewing process from local markets, their juice was extracted by single pressing. The addition of the juices happened in three different ways.
Type 1.: Juices were added before the fermentation process, with the volume of 7.5% of the total fermentation volume (1725 ml in 23 L). Henceforward: Fruity ales.
Type 2.: Fermentation was carried out without containing any fruit juice. Juices were added to the samples before bottling with different volumes: proportion of the juice was 2.5% (12.5 ml), 5% (25 ml) or 7.5% (37.5 ml) of the bottling volume (500 ml). Henceforward: Simple fruity ales.
Type 3.: Combining the two above-mentioned methods, I have added extra quantities of fruit juices to the fruity ale samples with the previously described proportions (2.5; 5 and 7.5%), therefore the samples prepared by fermentation with the juices was enriched again before bottling. Henceforward: Double fruity ales.
- Oat beers: Table 1. contains the materials used for the production of the sample containing oat malt only. Besides a control sample, which contained only barley malt instead of oat malt, I have prepared two more samples with this recipe. In these cases, rice or corn flakes were added for the whole mashing process, substituting 2.5 kg of the oat malt.
- Stout beers: Four different flavoured stouts were prepared: milk chocolate, dark chocolate, white chocolate and coffee stout. 200 g of the flavouring product was added to each batch for the last 10 minutes of the hop boiling process. Before the addition, the chocolates and the coffee were crushed. Coffee beans were just soaked in the hot wort in a mashing bag.

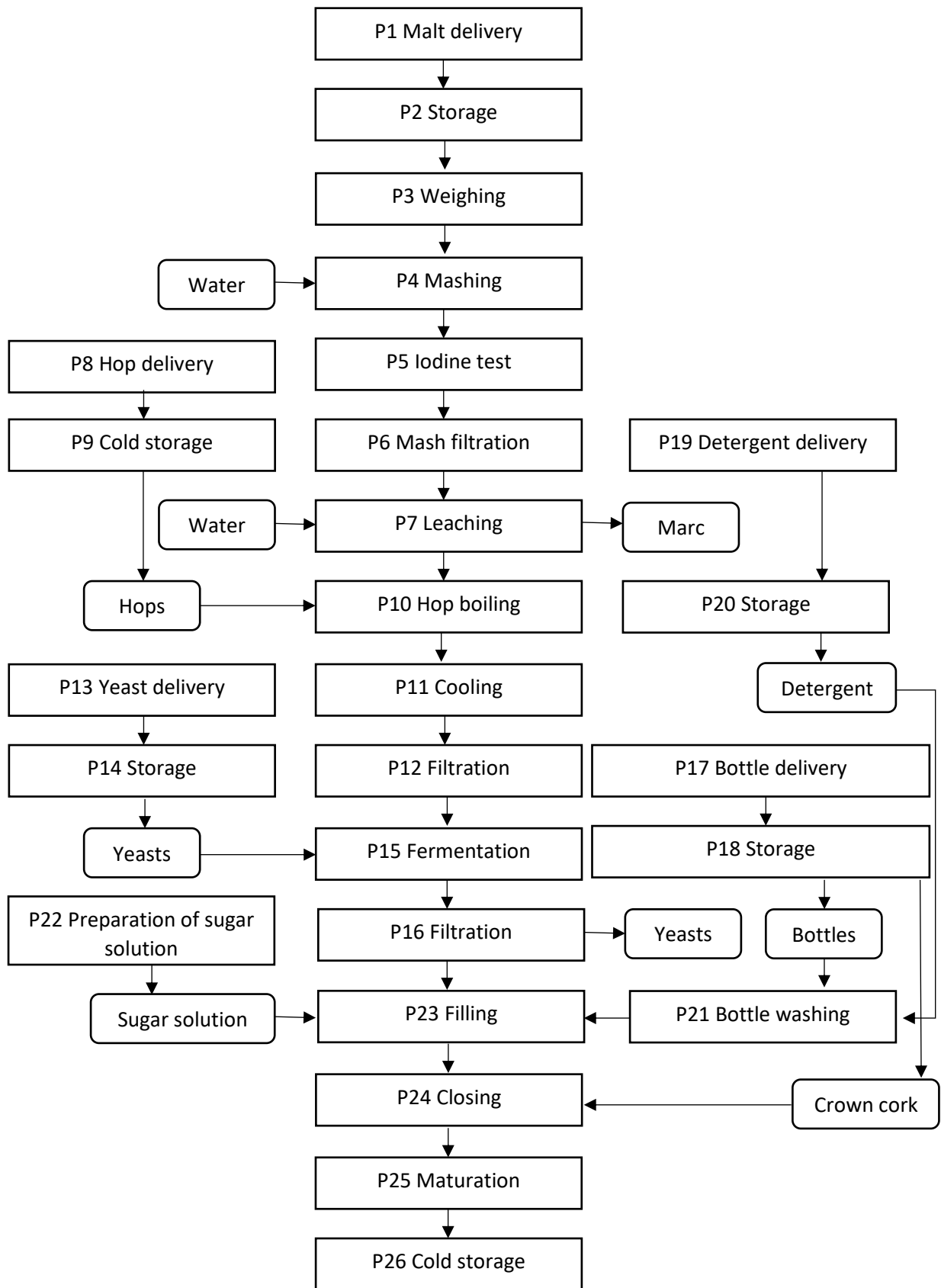


Figure 1.: The brewing process

Source: Own editing

2.1. Analytical methods

Before the analysis, solid samples must be ground and homogenized. Beer samples were degassed by using an ultrasonic water bath (Bandelin Sonorex Digital DT 255H, Germany), then filtered through folded filter paper (Munktell Ahlstrom, grade: 292, Helsinki, Finland).

2.1.1. Determination of Total Polyphenol Content (TPC)

The principle of the method is that phosphotungstic and phosphomolybdic acid found in Folin-Ciocalteu reagent oxidize phenolic compounds, resulting in a blue-coloured solution. Colour intensity is proportionate to the concentration of phenolic compounds, therefore the absorbance of the mixtures is measured by spectrophotometer (Evolution 300 LC, Thermo Electron Corporation, England) at a wavelength of 760 nm, against the mixture of methanol and distilled water (80:20).

To prepare the calibration solutions, a gallic acid stock solution is used. Applied chemicals: 3,4,5-trihydroxybenzoic acid (Alfa Aesar GmbH & Co. KG, Karlsruhe, Germany), sodium carbonate (Sigma-Aldrich Chemie GmbH, Germany), methanol (Scharlab S.L., Spain), Folin-Ciocalteu reagent (VWR International S.A.S., France). Results were expressed in mg GAE/100 g (or ml) (mg gallic acid equivalent/100 g) (SINGLETON et al., 1999).

2.1.2. Determination of flavonoid content

The determination of flavonoid content was also carried out by a spectrophotometric method. Absorbance of the rose-coloured complex created during the analysis was measured at a wavelength of 510 nm by spectrophotometer (Evolution 300 LC, Thermo Electron Corporation, England) against a blank solution. To prepare the calibration solutions, a catechin stock solution was used. Applied chemicals: catechin (Cayman Chemical Company, USA), aluminium chloride (Scharlab S.L., Spain), sodium nitrite (Scharlau Chemie S.A., Spain), sodium hydroxide (Sigma-Aldrich Chemie GmbH, Germany), methanol (Scharlab S.L., Spain). Results were expressed in mg CE/100 g (or ml) (mg catechin equivalent/100 g) (ZHISHEN et al., 1999).

2.1.3. Determination of vitamin C content

The principle of the metaphosphoric acid method is that potassium iodate releases iodine from potassium iodide, which reacts with ascorbic acid.

After all the ascorbic acid is consumed, the released iodine infiltrates the helix of starch, resulting in a blue colour change. The volume of the potassium iodate consumed during the titration is proportionate to the ascorbic acid content. Applied chemicals: metaphosphoric acid (Thermo Fischer GmbH, Germany), potassium iodide (Sigma-Aldrich Chemie GmbH, Germany), hydrochloric acid (VWR International S.A.S., France), starch indicator (VWR International S.A.S., France), potassium iodate (Sigma-Aldrich Chemie GmbH, Germany). Results were expressed in mg/100 g or mg/100 ml (CZIPA, 2014).

2.1.4. Determination of beer's colour

To determine the colour of beer samples, the previously degassed samples were poured into cuvettes, and the absorbance was measured at a wavelength of 430 nm by spectrophotometer (Evolution 300 LC, Thermo Electron Corporation, England), against distilled water. Results were expressed in EBC ($EBC = A_{430} * 19,7$) (CZIPA, 2014).

2.1.5. Determination of acid content of fruit juices

20 ml of the juice was poured into an erlenmeyer flask, which was diluted to 150 ml with distilled water, then taken to a water bath at 85-95 °C for 30 min. After the sample had cooled down to room temperature, it was filtered through cotton wool into a 250 ml volumetric flask, then volumed up with distilled water. Taking 25 ml of the solution and diluting it to 100 ml, it was titrated by 0.1 M NaOH solution in the presence of phenolphthalein indicator until the pink colour appeared. Results were expressed in % (CZIPA, 2014).

2.1.6. Determination of beer's total acidity

Beer samples were degassed and diluted with boiled distilled water, then they were titrated by sodium hydroxide (Sigma-Aldrich Chemie GmbH, Germany) in the presence of phenolphthalein indicator (Scharlab S.L., Spain). Results were expressed in %lactic acid (AOAC 950.07, 1995).

2.1.7. Determination of beer's bitterness

During the determination of total isomerized alpha acid content, bitter acids are extracted from the sample by an organic solvent in an acidic medium.

After the extraction and the separation of the two phases, absorbance of the solvent phase is measured at a wavelength of 275 nm by spectrophotometer (Evolution 300 LC, Thermo Electron Corporation, England), against the solvent. Applied chemicals: 2,2,4-trimethyl pentane (VWR International S.A.S., France), hydrochloric acid (VWR International S.A.S, France). Results were expressed in mg/l (AOAC 965.21, 1995).

2.1.8. Determination of mineral content

Sample preparation was carried out based on the method of KOVÁCS et al. (1996). Digestion of the samples was carried out by the addition of nitric acid (69%, VWR International LTD., Radnor, USA) and hydrogen peroxide (30%, VWR International LTD., Radnor, USA), at the temperature of 60 and 120 °C. Samples were diluted by ultrapure distilled water (Millipore S.A.S., Molsheim, France) and filtered (qualitative filter paper, grade: 388, Sartorius Stedim Biotech S.A., Gottingen, Germany), then the mineral content was determined by ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer) (Thermo Scientific iCAP 6300, Cambridge, UK). Operating parameters for the determination were the followings: Power rating – 1350 W; nebulizer gas flow rate – 1 dm³/min; cooling gas flow rate – 12 dm³/min; auxiliary gas flow rate – 1 dm³/min; sample input speed – 1 cm³/min; stabilisation time – 3 sec. The emission wavelengths (nm) were as follows: Na – 589.5; K – 766.4; Ca – 317.9; Mg – 279.5; P – 185.9; S – 182.0; B – 208.8; Ba – 233.5; Cu – 324.7; Fe – 259.9; Mn – 259.3; Sr – 407.7; Zn – 213.8.

2.2. Statistical analysis

The analytical measurements were carried out in triplicate. For the statistical analysis, SPSS (version 13, SPSS Inc. Chicago, Illinois, USA) was applied, thus minimum and maximum values, average values and standard deviations were determined. To compare the samples within one group, One-Way ANOVA was applied, for which a homogeneity test was carried out first at the level of 5% of significance. Tukey test was performed in case of homogeneous variables, while heterogeneous variables were investigated by Dunnett's T3 test. To classify samples into the previously defined groups, linear discriminant analysis (LDA) was performed. In case of my own flavoured beer samples, Pearson's correlation analysis was carried out to find linear correlations between the analysed parameters.

3. Results

Based on the results of the commercial beers, dark beers contain phenolic compounds and flavonoids in higher concentrations than pale beers. Flavonoid content of the dark beers (20.3 ± 5.3 mg CE/100 ml) were twice as high as the pale beers'. Besides those, the difference between flavoured beers and mixed beer beverages is also worth mentioning. Considerably higher concentrations of polyphenols and flavonoids were measured in most of the flavoured beers than the mixed beer beverages.

Mineral content of the commercial beers was not outstanding compared to the homemade beverages, but out of those, dark samples had high calcium (49.0 mg/l), magnesium (82.8 mg/l) and phosphorus content (245 mg/l) compared to the other sample groups. Dark wheat beers were abundant in calcium (51.5 mg/l) and sulphur (61.5 mg/l), but phosphorus (233 mg/l) and sulphur content (57.1 mg/l) of pale wheat beers was also relatively high.

Commercial beers were categorized into sample groups of pale beer, dark beer, pale wheat beer, dark wheat beer, ale, flavoured beer and mixed beer beverage by linear discriminant analysis (LDA). The listed sample groups were applied as grouping variables, while independent variables included polyphenol and flavonoid content, colour, acidity, copper, manganese, phosphorus and strontium content of the samples. Six discriminant functions were determined. In the first dimension, pale beers were separated from every other group except for two samples, while mixed beer beverages were separated from every other sample in the second dimension. In the third dimension, dark beers and pale wheat beers separated from every other sample, ale samples were separated from every other sample in the fourth dimension, while means were quite close to each other in the sixth dimension. Graphic representation of the results is illustrated by Figure 2. Based on the cross validation, 93.3% of pale beers, 80% of dark beers, 81.6% of pale wheat beers, 50% of dark wheat beers, 75% of flavoured beers and 80% of mixed beer beverages were correctly classified. Overall, 85.1% of the samples was correctly categorized.

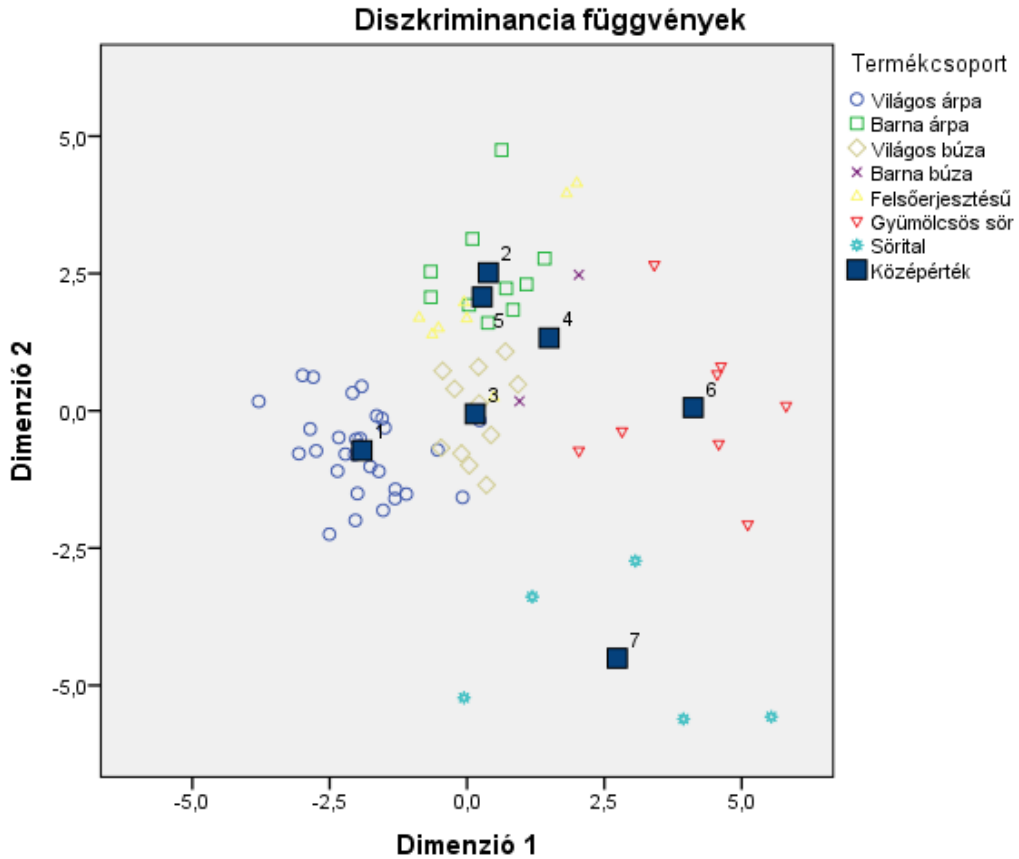


Figure 2.: The result of the linear discriminant analysis
Source: Own editing

In case of the homemade flavoured beers, polyphenol and flavonoid content was influenced by the additional fruit species and the proportion and method of the addition too. Phenolic content was increased effectively in case of the addition after fermentation, proportionate to the quantity of the juice added. Samples fermented with the juices showed lower phenolic contents than the control sample's in every case, which could be statistically verified, except for apricot ($p = 1.00$) and nectarine ($p = 0.365$) ale. On the contrary, samples fermented with the juices showed higher flavonoid content than the control sample in every case. Fermentation with cherry juices resulted in twice as high, and fermentation with sour cherry juice resulted in 150% higher flavonoid content than the control sample's. Furthermore, every fruity ale showed significantly higher flavonoid content compared to the control sample's, except for nectarine ale ($p = 0.213$).

Application of the juices were efficient with different methods of addition. Enrichment before fermentation increased flavonoid content efficiently, however the polyphenol content of these samples was lower than the control sample's. Nevertheless, addition before bottling caused an increase in the phenolic content and flavonoid content proportionate to the quantity of the juice used, furthermore, flavonoid content of every sample exceeded the flavonoid content of the control sample, except for the 5% simple watermelon and apricot ale.

Vitamin C content of the strawberry juice was 2.5 times higher than the concentration measured in the sample with the second highest ascorbic acid content, which has manifested in the end products too. Although the ascorbic acid content of strawberry ale did not exceed the concentration measured in the control sample, the addition of strawberry juice before bottling increased vitamin C content significantly, proportionate to the quantity of the juice added. The highest ascorbic acid content (32.3 mg/100 ml) was measured in the 7.5% simple strawberry ale, which was almost twice as high as the control sample's vitamin C content, and was significantly higher than the concentration measured in any other sample.

The addition of fruit juices with high acidity definitely increases the acid content of the end product. Out of the prepared samples, beers containing sour cherry juice had the highest titratable acidity, which was significantly higher than the acid content measured in the control sample ($p < 0.000$). 7.5% double sour cherry ale showed the highest acidity (0.138 ± 0.001 %lactic acid), which was twice as high as the control sample's acid content. It could also be stated that the increase in the acidity of the samples was proportionate to the quantity of the juice added in almost every case.

Mineral contents of the samples containing fruit juices were various. Potassium content of every sample containing juices was higher than the control sample's potassium concentration, and the potassium content of the fruity ale samples was higher than the concentration measured in the simple fruity ales, but lower than the double fruity ales in almost every case. Typically, the potassium content increased proportionate to the quantity of the juice used.

Calcium content of the samples also increased compared to the control sample in most of the cases of post-fermentation or double addition, however fermentation with the fruit juices resulted in lower calcium content in case of the cherry ale, and no significant changes could be observed in case of plum, watermelon, melon, apricot and nectarine ale samples either. Similar observations can be made in case of magnesium content, which was increased by the addition of grape juice the most efficiently.

Phosphorus content of the simple fruity ales was higher than the concentration measured in the double fruity ales in many cases. For instance, the difference between the phosphorus contents of simple and double cherry ales was statistically verified in every case with p values of 0.004; 0.001 and 0.013, furthermore, in case of every watermelon ($p < 0.000$), peach ($p < 0.000$), nectarine ($p < 0.000$) and apricot ($p_1 = 0.001$; $p_2 = 0.003$; $p_3 = 0.003$) ale too.

Regarding to the sulphur content, concentrations measured in the simple fruity ales were considerably higher than in the double fruity ales. For instance, the difference between simple and double apricot ales were over 100%, which could be statistically verified ($p < 0.000$) too. The difference was also significant in case of cherry, sour cherry, watermelon, peach, nectarine and grape ales.

The opposite could be observed in case of the boron contents, the concentrations measured in the double fruity ales were mostly higher than the contents measured in the simple fruity ales. The difference was more than double in case of peach and nectarine ales, but it could also be statistically verified in case of the samples prepared by the application of apricot and grape juice ($p < 0.000$). In most cases, boron concentration increased proportionate to the quantity of juice added.

The highest copper contents were measured in samples prepared by the addition of grape juice, which verifies the high copper content of grapes in relation to the plant protection technologies. Both grape ale compared to the control sample and each double grape ale sample compared to each other showed significant difference in their copper contents ($p < 0.000$). However, this outstanding copper content did not have any negative effect on the fermentation process, since the highest copper content was only 10% of the concentration which would inhibit the metabolism of yeast cells.

Less significant increase of copper content was caused by the addition of juices in case of simple and double strawberry, simple and double plum, double sour cherry, double melon, double apricot and double nectarine ales.

Manganese content of the beers was corresponding to the manganese content of the juices. The highest manganese concentrations were measured in strawberry, apricot and nectarine ale samples, but no obvious conclusions could be drawn from the results given by further addition of the juices.

Regarding to the strontium content, grape, apricot, nectarine, peach and plum ales showed higher concentration than the control sample, which could be statistically verified ($p < 0.000$). However, the further addition of these juices did not increase strontium content significantly, furthermore, concentrations measured in simple fruity ales was higher than double fruity ales' strontium contents.

Zinc content of the samples was increased significantly by the fermentation with fruit juices only in case of plum ale, but this difference could not be verified statistically either ($p = 0.060$). The background of this is the fact that the additional fruit juices increased the concentration of fermentable carbohydrates in the wort, which also increased the zinc demand of the yeast cells, since it is essential to numerous metabolic processes. For instance, zinc is the activator of alcohol dehydrogenase.

Pearson's correlation analysis was carried out on the results of homemade flavoured beers, which included every analysed parameter except for barium content. Results of the analysis are shown by table 2. During the evaluation, I have only considered those cases, in which Pearson's coefficient was higher than 0.7 whether in positive or negative range, at the significance level of 0.01. Therefore, only the correlations between five minerals can be found in the table. There is a moderately strong positive correlation between sodium and sulphur ($r=0.718$), and between sodium and phosphorus ($r=0.754$). Calcium showed moderately strong positive correlation with sulphur ($r=0.738$) and phosphorus ($r=0.786$), and it was in strong positive correlation with magnesium content ($r=0.884$). Magnesium also showed a strong positive correlation with phosphorus ($r=0.878$) and sulphur ($r=0.896$).

Table 2.: Results of Pearson’s correlation analysis

	Na	Ca	Mg	P	S
Na				0.754	0.718
Ca			0.884	0.786	0.738
Mg		0.884		0.878	0.896
P	0.754	0.786	0.878		
S	0.718	0.738	0.896		

Source: Own editing

The application of oat malt as a primary ingredient, or the application of corn and rice flakes as surrogates decreased polyphenol, flavonoid, potassium, magnesium and phosphorus content of the beer significantly in every case, which was statistically verified in numerous cases compared to the control sample. On the other hand, the usage of oat malt increased sodium content of the samples by 26%, doubled their sulphur content, and it increased manganese content by 150%. The substitution of 50% of the oat malt by rice or corn flakes decreased sulphur content, but manganese concentrations of these samples were also less than half of the concentrations measured in the pure oat beer. These surrogates only increased zinc content of the beer. The addition of corn flakes increased zinc content by 26%, while rice flakes increased that by 10%.

Stout beers contained significantly higher concentration of polyphenols than pale beers, which was increased further by the addition of the flavouring products in every case. Polyphenol content of the control stout was only 60% of the concentration shown by coffee stout, but dark chocolate has also increased polyphenol content of the control sample by 30%. The above-mentioned flavouring products caused 2-4 times higher flavonoid content than the control sample’s. Furthermore, samples containing chocolates or coffee showed higher bitterness, sodium, potassium, magnesium and phosphorus content than the control sample, thus the addition of these products increase the concentration of antioxidants and important macro elements too. The results are in total compliance with those observations that suggest the increase of antioxidant compounds by the increase of cocoa solid content of chocolates, and that arabica coffee is one of our most antioxidant-rich luxury item.

Table 3. summarizes the order of the average results of the sample groups regarding to the determined parameters. Based on that, pale beers did not show outstanding results of any analysed parameter, while dark beers had the highest average concentration of flavonoids, the second highest calcium, magnesium and phosphorus content, and the third highest polyphenol and copper content.

Table 3.: Order of sample groups based on the average results of the analysed parameters

<i>Para- meter</i>	<i>Beer type</i>									
	Commercial							Homemade		
	<i>Pale beer</i>	<i>Dark beer</i>	<i>Pale wheat</i>	<i>Dark wheat</i>	<i>Ale</i>	<i>Flav. beer</i>	<i>Mixed beer</i>	<i>Fruity ale</i>	<i>Oat ale</i>	<i>Choc. stout</i>
<i>TPC</i>	6	3	8	9	5	4	10	2	7	1
<i>Flav.</i>	7	1	6	2	9	5	8	4	10	3
<i>Acid</i>	10	7	8	9	6	1	3	4	5	2
<i>Na</i>	5	8	9	10	6	2	7	3	1	4
<i>K</i>	9	7	6	8	2	3	10	1	5	4
<i>Ca</i>	5	2	4	1	7	3	10	8	6	9
<i>Mg</i>	6	2	5	3	1	8	10	7	9	4
<i>P</i>	4	2	3	7	1	5	10	6	9	8
<i>S</i>	6	4	3	2	7	8	10	5	1	9
<i>B</i>	9	8	10	7	5	1	6	2	3	4
<i>Ba</i>	8	6	9	7	5	2	1	4	3	10
<i>Cu</i>	7	3	9	5	4	8	10	6	2	1
<i>Mn</i>	9	8	5	4	6	3	10	7	1	2
<i>Sr</i>	7	6	10	9	8	5	1	2	4	3
<i>Zn</i>	7	6	8	9	5	4	10	2	1	3

Source: Own editing

Pale wheat beers showed the third highest phosphorus and sulphur content, while dark wheat beers had the highest average calcium content, the second highest flavonoid and sulphur content, and the third highest magnesium content.

Consequently, dark beers usually show higher flavonoid content than pale beers due to the heat treatment applied during the production of caramel and roasted malts, which raw materials are necessary to produce dark beers.

Commercial ale beers had the highest average magnesium and phosphorus content, and the second highest potassium concentration.

Significant differences could be observed regarding to the analysed parameters between commercial flavoured beers and mixed beer beverages, which could be explained by the ingredients of these beverages. The highest titratable acidity and boron content, the second highest sodium and barium content, and the third highest potassium, calcium and manganese contents were measured in the flavoured beers. Mixed beer beverages showed the highest barium and strontium concentration, the third highest titratable acidity. On the other hand, these samples were poor in every other compound determined, compared to the other sample groups.

Homemade flavoured beers showed the highest potassium content of all sample groups, and they also had the second highest polyphenol, boron, strontium and zinc content, and the third highest sodium content. Therefore, these samples were rich in antioxidants and minerals too.

Beers prepared by alternative primary ingredients (oats) and surrogates (corn and rice flakes) contained the highest amounts of sodium, sulphur, manganese and zinc, the second highest copper content, and the third highest amount of boron and barium, which is in compliance with the fact that oats are considered to be rich in minerals.

Homemade chocolate and coffee stouts showed the highest polyphenol and copper content, the second highest acid and manganese content, and the third highest flavonoid, strontium and zinc content.

Based on the average results of the sample groups, it could also be declared that there is no univocal correlation between polyphenol and flavonoid content of the samples. It is possible in spite of the fact that flavonoids are a group of polyphenols. Polyphenol content of beers mostly come from the malts and hops, however, most of these compounds are phenolic acids and hydroxycinnamic acid derivatives, which constitute as non-flavonoid phenols. Although hops are rich in xanthohumol and other prenylated flavonoids, the applied quantity of hop products is negligible compared to the malt. It also should be noted that the type, quantity and application of malts and hops could be various, which could cause significant differences in the concentrations of polyphenols and flavonoids in the end products.

4. New scientific results of the dissertation

1. Samples were categorized into the groups of pale beers, dark beers, pale wheat beers, dark wheat beers, ale beers (IPA, APA, stout), flavoured beers and mixed beer beverages, then they were classified by linear discriminant analysis. Polyphenol and flavonoid content, colour, copper, manganese, phosphorus and strontium content of the samples were applied as independent variables. Total result of the cross validation was 85.1%.
2. I did not find correlation between polyphenol and flavonoid content of the samples. Based on the average results of the sample groups, some samples' polyphenol and flavonoid contents were both high. For instance, dark beers showed the highest flavonoid content (20.3 mg CE/100 ml) and the third highest polyphenol content (39.5 mg GAE/100 ml). On the other hand, there were groups with high polyphenol and low flavonoid content, for instance, ale beers had a polyphenol content of 35.4 mg GAE/100 ml, but their average flavonoid content was only 5.66 mg CE/100 ml. On the contrary, dark wheat beers showed the second highest flavonoid content (15.9 mg CE/100 ml), but only the second lowest polyphenol content (25.2 mg GAE/100 ml). It could be explained by the observations that polyphenol content of the beer mostly comes from the malt and hops, which compounds are mostly phenolic acids and hydroxycinnamic acid derivatives, which constitute as non-flavonoid phenols. Although hops are rich in xanthohumol and other prenylated flavonoids (YAMAGUCHI et al., 2009), the applied quantity of hop products is negligible compared to the malt. It also should be noted that the type, quantity and application of malts and hops could be various, which could cause significant differences in the concentrations of polyphenols and flavonoids in the end products.

3. Polyphenol content of the homemade flavoured beers decreased in case of the fermentation with the substitution of 7.5% of the wort by fruit juices, however, the addition of the juices after fermentation (before bottling) increased the concentration of phenolic compounds proportionate to the volume of the juice added. In case of cherry juice, the polyphenol concentration of cherry ale was only 85.1% of the control sample's polyphenol content. However, the addition of 37.5 ml juice before bottling (7.5%) increased fruity ale's polyphenol content by 23.1%, which was higher than the concentration measured in the control sample. This result is in compliance with the observation which suggests that the volume of alcohol created during fermentation can decrease polyphenol content of the wort or beer.
4. The presence of the fruit juices' mineral content in the end products depends on the mineral demand of the yeasts. Some macro elements, such as potassium are present in the wort in such high concentration which covers the yeasts needs, so the minerals added by the juices are not taken up by the yeasts, therefore it increases the mineral content of the end product proportionate to the quantity of the juice added. In case of potassium, the sour cherry juice added before fermentation was expected to increase the original, 381 mg/l potassium content to 520 mg/l approximately. Based on the measurements, potassium content of the sour cherry ale was 549 mg/l. Considering this concentration, the addition of 37.5 ml sour cherry juice before fermentation (7.5% double sour cherry ale) was expected to increase its potassium content to 713 mg/l, while the actual result was 690 mg/l. These differences are negligible, the increase in the potassium content was proportionate to the potassium concentration of the juice. On the other hand, 1725 ml sour cherry juice added before fermentation should have increased zinc content of the control sample from 186 µg/l to 207 µg/l, but I have measured a zinc concentration of 101 µg/l in the sour cherry ale. The juice added to produce 7.5% double sour cherry ale was expected to increase zinc content from 101 µg/l to 134 µg/l, but the result of the measurement was only 111 µg/l. The expected zinc concentration of 7.5% simple sour cherry ale was 220 µg/l, I have only measured 191 µg/l instead. These results can be explained by the excessive demand for zinc of the yeast cells because of the increased concentration of fermentable carbohydrates in the wort, since zinc is taken up in high quantities by the yeasts to activate alcohol dehydrogenase.

5. The exclusive application of oat malt as primary ingredient increased sodium content of the control sample by 26%, doubled its sulphur content, and increased its manganese content by 150%. Substituting 50% of the oat malt with corn or rice flakes decreased sulphur content, which were similar to the concentrations measured in the control sample. In case of manganese, concentrations measured in the samples containing the above-mentioned surrogates was about half of the pure oat beer's manganese content, however it was still 9% higher than the control sample's in case of the addition of corn flakes, and 30% higher in case of the addition of rice flakes. These surrogates increased only the zinc content compared to the pure oat beer. The addition of rice flakes increased zinc content by 10%, while this value was 26% in case of the corn flakes.

5. Practical results

1. Classifying commercial beers into product groups by linear discriminant analysis based on their polyphenol and flavonoid content, colour, copper, manganese, phosphorus and strontium content was correct in 85.1% of the cases. Therefore, by expanding the independent variables, this method can be suitable for the identification of unknown samples too.
2. Concentration of antioxidant compounds of beers was effectively increased by the addition of fruit juices. However, it was mostly successful if they were added after fermentation, since the concentration of these compounds decrease during the fermentation, depending on the wort's alcohol content too. The addition of sour cherry juice before fermentation with a proportion of 7.5% of the total volume decreased the control sample's polyphenol content to 85.1%, but the addition of the juice with the same proportion before bottling resulted in an increase of 23.1% of polyphenol content.
3. The addition of fruit juices rich in vitamin C can increase ascorbic acid content of beers significantly, which is beneficial from the aspect of product development. Ascorbic acid content of strawberry juice was outstanding compared to every other juice. The addition of strawberry juice before fermentation, with a proportion of 7.5% of the bottled volume increased beer's vitamin C content by 66%.

4. Most of the analysed fruit juices increased potassium, calcium, magnesium and phosphorus content of the samples significantly, since the wort already contained the necessary amount of these elements for the yeasts. In case of some micro elements, the contents of the fruit juices do not appear in the end products. The additional fruit juice increases fermentable carbohydrate content of the wort, and since the yeasts need zinc to activate alcohol dehydrogenase, zinc content of these samples decreased significantly. In case of the addition of sour cherry juice before fermentation, zinc content was less than half of the expected concentration based on the zinc content of the wort and the juice.
5. The application of oat malt as a primary ingredient, or unmalted oat as a surrogate increases the concentrations of some micro elements found in the beer, particularly the amount of manganese and zinc. In case of the homemade oat beers, the exclusive application of oat malt increased the beer's manganese and zinc content by 150%. The application of corn and rice flakes as surrogates decreased manganese content of the oat beer, but the addition of rice flakes increased its zinc concentration by 10%, while the addition of corn flakes increased it by 26%. Overall, the production of gluten-free beers by the application of oat malt is beneficial regarding to the mineral content of beer.

6. References

- Alcázar, A. - Pablos, F. - Martín, J. - González, A. G.: 2002. Multivariate characterization of beers according to their mineral content. *Talanta*. 57. (1): 45-52. [https://doi.org/10.1016/S0039-9140\(01\)00670-1](https://doi.org/10.1016/S0039-9140(01)00670-1)
- AOAC.: 1995. Acidity (Total) of beer. Indicator titration method. AOAC Official Method 950.07.
- AOAC.: 1995. Bitterness of beer. Iso-alpha acids method. AOAC Official Method 965.21.
- Czipa N.: 2014. Élelmiszeranalitika gyakorlati jegyzet élelmiszermérnök BSc. III. évfolyam részére. Debreceni Egyetem.
- Donadini, G. – Porretta, S.: 2017. Uncovering patterns of consumers' interest for beer: A case study with craft beers. *Food Research International*. 91: 183-198. <https://doi.org/10.1016/j.foodres.2016.11.043>
- Elzinga, K. G. – Tremblay, C. H. – Tremblay, V. J.: 2015. Craft beer in the United States: History, numbers, and geography. *Journal of Wine Economics*. 10. (3): 242-274. doi:10.1017/jwe.2015.22
- Jaeger, S. R. – Worch, T. – Phelps, T. – Jin, D. – Cardello, V. A.: 2020. Preference segments among declared craft beer drinkers: Perceptual, attitudinal and behavioural responses underlying craft-style vs. traditional-style flavour preferences. *Food Quality and Preference*. 82. <https://doi.org/10.1016/j.foodqual.2020.103884>
- Kovács B. – Győri Z. – Csapó J. – Loch J. – Dániel P.: 1996. A study of plant sample preparation and inductively coupled plasma emission spectrometry parameters. *Communication in Soil Science and Plant Analysis*. 27: 1177-1198. <https://doi.org/10.1080/00103629609369625>
- Magyar Köztársaság.: 2011. 2011. évi CLVI. törvény egyes adótörvények és azzal összefüggő egyéb törvények módosításáról. *Magyar Közlöny*. 140.: 33404-33586.
- Singleton, V. L. – Orthofer, R. – Lamuela-Raventos, M.: 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*. 299: 152-178. [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1)

Yamaguchi, N. – Satoh-Yamaguchi, K. – Ono, M.: 2009. In vitro evaluation of antibacterial, anticollagenase, and antioxidant activities of hop components (*Humulus lupulus*) addressing acne vulgaris. *Phytomedicine*. 16 (4): 369-376.

Zhisen, J. – Mengcheng, T. – Jianming, W.: 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemisrty*. 64 (4): 555-559.

STATISTA: Per capita consumption of beer in selected countries in the European Union (EU) in 2019*

<https://www.statista.com/statistics/625214/per-capita-beer-consumption-european-union-eu/>

Letöltve: 2021.06.08.

7. Publications



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Tel.: 52/410-443, e-mail: publikaciok@lib.unideb.hu

Nyilvántartási szám: DEENK/431/2022.PL
Tárgy: PhD Publikációs Lista

Jelölt: Alexa Loránd
Doktori Iskola: Kerpely Kálmán Doktori Iskola
MTMT azonosító: 10065423

A PhD értekezés alapjául szolgáló közlemények

Idegen nyelvű tudományos közlemények hazai folyóiratban (3)

1. Novák, A., **Alexa, L.**, Kovács, B., Czípa, N.: Comparative study of special honey products and herbhoneyes.
Agrártud. Közl. 74, 117-120, 2018. ISSN: 1587-1282.
DOI: <http://dx.doi.org/10.34101/actaagrar/74/1675>
2. **Alexa, L.**, Kántor, A., Kovács, B., Czípa, N.: Determining the antioxidant compounds of beer.
Agrártud. Közl. 74, 5-10, 2018. ISSN: 1587-1282.
DOI: <http://dx.doi.org/10.34101/actaagrar/74/1657>
3. Czípa, N., Kántor, A., **Alexa, L.**, Kovács, B.: The effect of collecting area on the element content of Hungarian acacia honeys.
Agrártud. Közl. Klsz., 129-138, 2018. ISSN: 1587-1282.
DOI: <http://dx.doi.org/10.34101/actaagrar/150/1709>

Idegen nyelvű tudományos közlemények külföldi folyóiratban (3)

4. Orhotohwo, O. L., Czípa, N., Kovács, B., **Alexa, L.**: Impacts of the use of gluten-free cereals and spices on the quality parameters of beer.
J. Microbiol. Biotech. Food Sci. 11, 1-4, 2021. ISSN: 1338-5178.
DOI: <https://doi.org/10.15414/jmbfs.3838>
5. **Alexa, L.**, Kántor, A., Kovács, B., Czípa, N.: Determination of micro and trace elements of commercial beers.
J. Microbiol. Biotech. Food Sci. 7 (3), 432-436, 2018. EISSN: 1338-5178.
DOI: <http://dx.doi.org/10.15414/jmbfs.2018.7.4.432-436>
6. Czípa, N., **Alexa, L.**, Phillips, C. J. C., Kovács, B.: Macro-element ratios provide improved identification of the botanical origin of mono-floral honeys.
Eur. Food Res. Technol. 244 (8), 1439-1445, 2018. ISSN: 1438-2377.
DOI: <http://dx.doi.org/10.1007/s00217-018-3057-9>
IF: 2.056





Idegen nyelvű konferencia közlemények (1)

7. **Alexa, L.**, Kántor, A., Kovács, B., Papp-Topa, E. A., Czipa, N.: Risk assessment for forest honey's toxic element content.
In: Scientific researches in food production : 3rd meeting of young researchers from V4 countries. Ed.: Béla Kovács, Nikolett Czipa, Ferenc Peles, Éva Bacskainé Bódi, Andrea Kántor, Flóra Mária Szabóné Petróczki, Loránd Alexa, University of Debrecen, Debrecen, 6-11, 2018. ISBN: 9789634900320

Magyar nyelvű absztrakt kiadványok (2)

8. **Alexa, L.**, Mihály, K., Kerekes, G., Nagy, T., Takács, F., Karaffa, E. M.: Antioxidáns hatású vegyületek mennyiségének változása tárolás és preharvest kezelések függvényében három magyarországi meggyfajta eredményei alapján.
In: Magyar Táplálkozástudományi Társaság XLIV. Vándorgyűlése programja és az előadások összefoglalói. Szerk.: Antal Emese, Biró Lajos, Gelencsér Éva, Lugasi Andrea, Rurik Imre, Magyar Táplálkozástudományi Társaság, Budapest, 11, 2019. ISBN: 9786155606090
9. **Alexa, L.**, Kántor, A., Kovács, B., Czipa, N., Papp-Topa, E. A.: Erdei mézek toxikus elemtartalmának kockázatbecslése.
In: Óshonos- és Tájfajták - Ökotermékek : Egészséges táplálkozás : Vidékfejlesztés Minőségi élelmiszerek : Egészséges környezet: Az agrártudományok és a vidékfejlesztés kihívásai a XXI. században. Szerk.: Irinyiné Oláh Katalin, Tóth Csilla, Nyíregyházi Egyetem Műszaki és Agrártudományi Intézet, Nyíregyháza, 80, 2018. ISBN: 9786155545818

Idegen nyelvű absztrakt kiadványok (3)

10. Novák, A., Kovács, B., **Alexa, L.**, Kántor, A., Czipa, N.: Comparative study of acacia honeys and herbhoney's quality parameters.
In: Scientific researches in food production, FBFS, SUA in Nitra - Proceedings of abstracts. Ed.: Miroslava Kačániová, Slovak University of Agriculture in Nitra, Nitra, 33, 2017. ISBN: 9788055217369
11. **Alexa, L.**, Kántor, A., Kovács, B., Czipa, N.: Contribution of beer to cover human's nutritional needs.
In: Scientific researches in food production, FBFS, SUA in Nitra - Proceedings of abstracts. Ed.: Miroslava Kačániová, Slovak University of Agriculture in Nitra, Nitra, 18, 2017. ISBN: 9788055217369
12. **Alexa, L.**, Czipa, N., Kincses, S.: Effect of beer's mineral content on humans' health.
In: The International Conference for Students - Student in Bucovina, Stefan cel Mare University of Suceava, Romania, Bucovina, 29, 2016. ISBN: 20687648





További közlemények

Idegen nyelvű, hazai könyvek (2)

13. Szerk. Kovács, B., Czipa, N., Peles, F., Bódi, É., Kántor, A., Petróczki, F. M., **Alexa, L.**: Scientific researches in food production, University of Debrecen: Proceedings of abstracts. University of Debrecen, Debrecen, 30 p., 2018. ISBN: 9789634900412
14. Szerk. Kovács, B., Czipa, N.; Peles, F., szerk. Bódi, É., Kántor, A., Petróczki, F. M., **Alexa, L.**: Scientific researches in food production - 3rd meeting of young researchers from V4 countries. University of Debrecen, Debrecen, 58 p., 2018. ISBN: 9789634900320

Magyar nyelvű tudományos közlemények hazai folyóiratban (2)

15. Papp-Topa, E. A., **Alexa, L.**, Kántor, A., Kovács, B., Czipa, N.: Piros gyümölcsök minőségi jellemzői friss és liofilizálás utáni állapotban.
Élelmiszervizsgalati Közlemények. 67 (4), 3649-3656, 2021. ISSN: 2676-8704.
DOI: <http://dx.doi.org/10.52091/EVIK-2021/4-2-HUN>
16. Papp-Topa, E. A., **Alexa, L.**, Kántor, A., Kovács, B., Czipa, N.: Száritott bazsalikommal dúsított kenyerek vizsgálata és eredményeinek értékelése.
Élelmiszervizsgalati Közlemények. 67 (4), 3665-3671, 2021. ISSN: 2676-8704.
DOI: <http://dx.doi.org/10.52091/EVIK-2021/4-3-HUN>

Idegen nyelvű tudományos közlemények hazai folyóiratban (2)

17. Kántor, A., **Alexa, L.**, Papp-Topa, E. A., Kovács, B., Czipa, N.: Change of antioxidant compounds of spices during drying.
Agrártud. Közl. 2, 77-81, 2019. ISSN: 1587-1282.
DOI: <http://dx.doi.org/10.34101/actaagrar/2/3682>
18. Kántor, A., **Alexa, L.**, Kovács, B., Czipa, N.: Determination of element contents of commercial and homemade jams.
Agrártud. Közl. 74, 71-75, 2018. ISSN: 1587-1282.
DOI: <http://dx.doi.org/10.34101/actaagrar/74/1667>

Idegen nyelvű tudományos közlemények külföldi folyóiratban (1)

19. Kántor, A., **Alexa, L.**, Kovács, B., Czipa, N.: Determination of nutritional parameters of commercial and homemade jams.
J. Microbiol. Biotech. Food Sci. 7 (3), 407-411, 2018. EISSN: 1338-5178.
DOI: <http://dx.doi.org/10.15414/jmbfs.2017.7.4.407-411>





Idegen nyelvű konferencia közlemények (2)

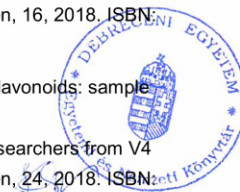
20. Papp-Topa, E. A., Posta, J., **Alexa, L.**, Kántor, A., Czipa, N., Kovács, B.: Analysis of flavonoids: Characterization, sample preparation for GC-MS.
In: Scientific researches in food production : 3rd meeting of young researchers from V4 countries. Ed.: Béla Kovács, Nikolett Czipa, Ferenc Peles, Éva Bacskainé Bódi, Andrea Kántor, Flóra Mária Szabóné Petróczki, Loránd Alexa, University of Debrecen, Debrecen, 43-49, 2018. ISBN: 9789634900320
21. Kántor, A., **Alexa, L.**, Papp-Topa, E. A., Kovács, B., Czipa, N.: Nutritional parameters of different cereal flour's bread.
In: Scientific researches in food production : 3rd meeting of young researchers from V4 countries. Ed.: Béla Kovács, Nikolett Czipa, Ferenc Peles, Éva Bacskainé Bódi, Andrea Kántor, Flóra Mária Szabóné Petróczki, Loránd Alexa, University of Debrecen, Debrecen, 33-37, 2018. ISBN: 9789634900320

Magyar nyelvű absztrakt kiadványok (1)

22. Fischinger, L. Á., Czipa, N., **Alexa, L.**, Kovács, B., Kántor, A.: Beltartalmi paraméterek vizsgálata kereskedelmi forgalomban kapható fokhagymákban és fokhagymakrémekben.
In: Óshonos- és Tájfajták - Ökotermékek : Egészséges táplálkozás : Vidékfejlesztés Minőségi élelmiszerek : Egészséges környezet: Az agrártudományok és a vidékfejlesztés kihívásai a XXI. században. Szerk.: Irinyiné Oláh Katalin, Tóth Csilla, Nyíregyházi Egyetem Műszaki és Agrártudományi Intézet, Nyíregyháza, 93-94, 2018. ISBN: 9786155545818

Idegen nyelvű absztrakt kiadványok (4)

23. Kántor, A., **Alexa, L.**, Papp-Topa, E. A., Kovács, B., Czipa, N.: Determination of nutritional parameters of different cereal flours' bread.
In: Scientific researches in food production : 3rd meeting of young researchers from V4 countries : Proceeding of Abstracts, University of Debrecen, Debrecen, 22, 2018. ISBN: 9789634900412
24. **Alexa, L.**, Kántor, A., Papp-Topa, E. A., Kovács, B., Czipa, N.: Examination of changes in the antioxidant compounds and acidity of the mash of different fruits.
In: Scientific researches in food production : 3rd meeting of young researchers from V4 countries : Proceeding of Abstracts, University of Debrecen, Debrecen, 16, 2018. ISBN: 9789634900412
25. Papp-Topa, E. A., **Alexa, L.**, Kántor, A., Kovács, B.: GC-MS analysis of flavonoids: sample preparation methods and derivatization.
In: Scientific researches in food production : 3rd meeting of young researchers from V4 countries : Proceeding of Abstracts, University of Debrecen, Debrecen, 24, 2018. ISBN: 9789634900412





26. Kántor, A., **Alexa, L.**, Kovács, B., Czipa, N.: Determination of micro elements of commercial and homemade baby food.
In: Scientific researches in food production, FBFS, SUA in Nitra - Proceedings of abstracts.
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