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STUDY OF THE INFLUENCE OF A HIGHER HOPPING DEGREE OF WORT ON THE FUNCTIONAL PROPERTIES OF ALE BEER

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Abstract

Experiments have been conducted to determine the effect of a higher hopping degree of wort (120 mg/l and 200 mg/l bitter α-acids) on the antioxidant capacity of ale beer. In one of the experimental variants, dry hopping with aromatic hop pellets (3 g/l) was applied. The higher hopping degree of wort did not lead to disturbance in the fermentation process. For beers containing 200 mg/l α-acids, the most significant was the increase in flavonoids (20.6-23.8%) compared to the variant with 120 mg/l α-acids. The polyphenols in highly hopped beer were increased by 6.6-7.1% compared to moderately hopped beer. The content of anthocyanins was not significantly affected by the addition of more hops. Antioxidant activity, expressed as equivalent vitamin C mmol/l, showed an increase of 4-5.2% for highly hopped beers compared to the control variant; when calculated relative to vitamin E, the increase was 5.8-7.5%. All beers were light, with excellent clarity and enhanced hop aroma.

Key words*: ale beer, antioxidant activity, hops.*

INTRODUCTION

The most common beer in the world is the so-called lager (Helweg, 2013), produced by bottom fermentation with brewing yeast *Saccharomyces pastorianus*. The second main type of beer is ale, which is obtained by top fermentation at 15-24°C with *S. cerevisiae*. The yeasts form a foam on the surface of the fermenting wort; they have increased alcohol tolerance, so they produce beers with higher alcohol content (Eckhardt, 2008). The number of generations is significantly higher. Elevated temperature facilitates the reduction of diacetyl; due to the accelerated fermentation process, the final product has a relatively low pH (4.1-4.3) (Eßlinger, 2009). Ale beer is characterized by an increased content of esters, higher density, fruity taste and enhanced aroma compared to the lager.

Top fermentation is a much older method than the bottom one (Eßlinger, 2009). Until the 16th century, ale was the main type of beer in Europe (Pavsler & Buiatti, 2009). Although ale is currently less popular than lager (Standage, 2020), there are many variants of this type of beer. They differ significantly in their color, aroma, taste, alcohol content, esters, etc.

Hops (*Humulus lupulus*) are the most expensive (Walters, 2004) and very important raw materials for brewing. In addition to their bitter and aromatic properties, they also act as preservatives of beer (Olsovska et al., 2016). Hops secondary metabolites include hop resins, essential oils and polyphenols (Almaguer et al., 2014). They have many biological activities: antimicrobial, antioxidant and anticancer properties (Bocquet et al., 2018; Van Cleemput et al., 2009), sedative and anti-inflammatory potential (Knez Hrnčič et al., 2019), etc.

The hop resins are classified into two main groups: soft and hard resins (Taniguchi et al., 2014). Alpha acids (humulones) are the most important fraction of hop resins (Skomra & Koziara-Ciupa, 2020); they give a bitter taste to the beer and contribute to the foam stabilization (Schönberger & Kostelecky, 2011; Kunimune & Shellhammer, 2008). Beta acids (lupulones) are usually found in smaller amounts than α-acids (Almaguer et al., 2014) but also affect bitterness (Haseleu et al., 2009).

Hop oil is one of the most complex essential oils in plants (King & Dickinson, 2003). It contains 3 main fractions. The first fraction includes mono- and sesquiterpenes, as well as aliphatic hydrocarbons. The second fraction consists of oxygenated compounds (terpene and sesquiterpene alcohols), and the last fraction of

sulphur-containing compounds (Knez Hrnčič et al., 2019).
According to Biendl (2009), the polyphenols in hops are divided into 4 groups. The first group includes flavonols; the second - flavanols (catechins), which form oligomers known as proanthocyanidins (Almaguer et al., 2014). The third group consists of phenolic carboxylic acids, and the last one of other phenolic compounds (prenylflavonoids, stilbenoids). Aromatic hop varieties contain more polyphenols than hops with a high content of bitter acids (Kammhuber, 2005). Hops contribute to the phenolic content in beer - about 30% (70% comes from barley malt) (Callemien & Collin, 2010).
Beer is known for its health benefits in moderate consumption (it reduces the risk of cardiovascular diseases, contains vitamins and antioxidants, etc.) (Denke, 2000). The aim of this work is to study the influence of a higher hopping degree of wort on the functional properties of ale beer.

MATERIALS AND METHODS

Wort was prepared with a Bender & Hobein mash apparatus; boiling and hopping were performed on a Kjeldahl apparatus. The wort consisted of 100% barley malt. It was hopped according to the following scheme: I batch - 50% bitter hop pellets Galena, α-acids 13.6%, added 10 minutes after the start of boiling; II batch - 35% aromatic hop pellets Perle, α-acids 7.9%, added 30 minutes after the start of boiling; III batch - 15% aromatic hop pellets Hallertau Mittelfrueh, α-acids 3.3%, added 10 minutes before the end of boiling. The control version of wort contained 120 mg/l bitter α-acids and the samples 200 mg/l α-acids. The wort was analyzed according to methods of European Brewing Convention (EBC) (Analytica EBC, 1998).
Experiments were performed according to the methodology of EBC, in fermentation tubes with a volume of 0.5 l, at 15°C. Brewing yeast strain Bry-97 West Coast Ale Yeast was used; the inoculation amount of cells was 18-19.10^6/ml. At 72-th hour of fermentation, 3 g/l of Hallertau Mittelfrueh hop pellets were added to one of the 200 mg/l α-acids samples, and remained in the fermenting wort until the end of the process. During fermentation, the following parameters were monitored: development of the reproductive process; rate and degree of fermentation; pH change. After the main fermentation (7 days), the beer matured for 14 days at 4°C. The young and aged beers were tested according to EBC methods (Analytica EBC, 1998; Analytica Microbiologyca EBC, 2001) and radical capture ability according to a modified method of Marinova & Batchvarov (2011).

RESULTS AND DISCUSSIONS

Physico-chemical parameters of wort are summarized in Table 1.

Table 1. Physico-chemical parameters of hopped wort containing 120 mg/l α-acids and 200 mg/l α-acids

Parameters	120 mg/l α-acids (control variant)	200 mg/l α-acids
Extract, %	10.88	10.91
pH	5.84	5.75
Color, EBC units	18	20.6
Isohumulones, mg/l	31.7	70.7
Bitterness, bitter units	38.0	72.1
α-amino nitrogen, mg/l	192	188
Polyphenols, mg/l	324.0	355.6
Flavonoids, mg/l	27.1	31.5

The extracts of the two variants of wort were similar, and the higher hopping degree led to a slight decrease in pH. In the case of polyphenols and flavonoids, there was an increase (by 10% and 16%, respectively) for the highly hopped wort. The content of isohumulones, as expected, increased in the wort with a higher degree of hopping (the bitterness increased by about 90%).
Key parameters were monitored during the main fermentation (Tables 2, 3, 4).

Table 2. Fermentation with wort containing 120 mg/l α-acids

Hour	120 mg/l α-acids (control variant)			
	Number of cells, ml.10^6	Extract, %	pH	Budding cells, %
0	19	10.88	5.84	-
24	74	5.12	4.45	33
48	64	2.93	4.47	22
72	43	2.81	4.51	15
96	32	2.74	4.52	9
End	15	2.65	4.55	3

Table 3. Fermentation with wort containing 200 mg/l α-acids

Hour	200 mg/l α-acids			
	Number of cells, ml.10^6	Extract, %	pH	Budding cells, %
0	18	10.91	5.75	-
24	73	5.09	4.40	27
48	65	3.02	4.48	23
72	47	2.85	4.50	16
96	34	2.78	4.51	10
End	16	2.68	4.58	3

Table 4. Fermentation with wort containing 200 mg/l α-acids with 3 g/l dry hopping

Hour	200 mg/l α-acids with 3 g/l dry hopping			
	Number of cells, ml.10^6	Extract, %	pH	Budding cells, %
0	18	10.91	5.75	-
24	73	5.03	4.35	24
48	62	3.00	4.42	20
72	46	2.92	4.44	15
96	35	2.86	4.46	9
End	17	2.71	4.56	2

The development of the strain proceeded in the same way for all variants, with the maximum number of cells being recorded at 24-th hour. The percentage of budding yeasts was the highest in the control variant - about 1/3 of the total number of cells; for highly hopped versions, budding cells were about 1/4 of the total. Over the next 24 hours, the amount of yeasts in the fermenting wort began to decline gradually; their number was 62-65.10^6 cells/ml, and the budding ones were about 1/5 of them. From this moment the formation of cell sediment began. During the following days, the gradual precipitation of the yeasts continued, and at the end of the fermentation, the unprecipitated cells were 15-17.10^6 cells/ml.

The higher hopping degree of wort did not lead to disturbances in the development and reproduction of yeasts.

The initial wort extract was 10.88% for the control and 10.91% for highly hopped versions. At the beginning of the process, due to the rapid reproduction of yeasts, there was a sharp decline; at 24-th hour the residual extract was about 1/2 of the initial (for all variants). At 48-th hour, the residual extract was 26.9% for the control; for the variants with a higher degree of hopping the values were 27.5-27.7%. From this point on, the depletion of the extract was delayed (due to the beginning of cell lysis and precipitation). In the following days, the trend was maintained, and at the end of the process, the residual extract was similar for all variants.

The initial pH of the control wort was 5.84; the higher degree of hopping led to a slight decrease - 5.75. The sharpest drop in pH was reported at 24-th hour (due to the rapid reproduction of yeasts at the beginning of the process). From this point on, a slow alkalization of the fermenting wort was observed, associated with the cell's lysis. In the following days, the alkalization continued, and at the end of the process the pH of the young beer was 4.55-4.58.

Based on the parameters monitored during the process, no deviations in the normal course of fermentation were detected.

After the main fermentation, physic-chemical (Table 5) and microbiological analyses (Table 6) were performed.

Table 5. Physico-chemical parameters of young beer

Parameters	120 mg/l α-acids (control variant)	200 mg/l α-acids	200 mg/l α-acids with 3 g/l dry hopping
Initial extract, %	10.46	10.55	10.61
Apparent extract, %	2.65	2.67	2.71
Real extract, %	4.13	4.16	4.21
Alcohol, %	3.24	3.27	3.28
pH	4.55	4.58	4.56
α-amino nitrogen - residual, mg/l - assimilated, %	 64.8 66.25	 75.2 60	 72.6 61.38

The apparent extract at the end of the fermentation varied within a narrow range, suggesting that the fermentation of the initial extract was similar for all variants. The values for 120 mg/l α-acids and 200 mg/l α-acids were almost identical, which suggests that the fermentation of the initial extract was not significantly affected by the higher degree of hopping. The highest value was for the dry-hopped variant (the most significant part of the initial extract remained unfermented). The same trend was observed for the real extract.

The alcohol content and pH of the young beer were similar for all three experimental variants, which suggested that these two parameters were not significantly affected by the degree of hopping.

Moderate hopping (120 mg/l) contributed to better assimilation of α-amino nitrogen by cells. The percentage of assimilated α-amino nitrogen was the highest for the control variant (over 66%). For highly hopped variants, the values were 60-61%.

Table 6. Microbiological analyses of the brewing yeast strain at the end of main fermentation

Parameters	120 mg/l α-acids (control variant)	200 mg/l α-acids	200 mg/l α-acids with 3 g/l dry hopping
AP-test (fermentation activity)	2.74	2.70	2.69
Biomass increase, times fold	1.81	1.80	1.76
Dead cells, %	11	12	13

The results of the fermentation activity test (AP test) varied within a narrow range and proved the good fermentation ability of the yeast strain. The control value was slightly higher than the highly hopped variants.

The increase in biomass amount was 1.8 times fold for beers with 120 and 200 mg/l α-acids; the value was slightly lower for the dry hopped variant. The brewing yeast strain showed good viability, with a dead cell content of 11-13% at the end of the main fermentation.

Microscopic observation showed that the culture was homogeneous. The yeast cells were large in size, round and with clear protoplasm (except for dead cells, whose protoplasm was granular and darker in color).

After maturation, physico-chemical analyses of the finished beer were performed (Table 7).

All beer variants were light, with excellent clarity and enhanced hop aroma (especially for the ale with dry hopping).

Table 7. Physico-chemical parameters of aged beer

Parameters	120 mg/l α-acids (control variant)	200 mg/l α-acids	200 mg/l α-acids with 3 g/l dry hopping
Initial extract, %	10.72	10.77	10.76
Apparent extract, %	2.56	2.61	2.65
Real extract, %	4.11	4.16	4.19
Alcohol, %	3.38	3.39	3.37
Apparent degree of fermentation, %	76.11	75.76	75.39
Real degree of fermentation, %	61.67	61.39	61.08
pH	4.75	4.80	4.80
Color, EBC units	9.2	8.5	9.8
Isohumulones, mg/l	23.3	35.7	36
Bitterness, bitter units	25.5	36.4	36.6
Usage of bitter substances, %	21.25	18.18	18.3
α-amino nitrogen - residual, mg/l - assimilated, %	 62.5 67.45	 73.1 61.12	 70.8 62.34
Clarity	0.7	0.6	0.7
Polyphenols, mg/l	280.5	298.9	300.4
Flavonoids, mg/l	21.4	25.8	26.5
Anthocyanins, mg/l	85.2	85.8	86.0

The apparent extract showed a slight decrease after maturation. Like the young beer, after maturation, the apparent extract remained the lowest for the control variant, which suggested the most complete fermentation of the initial extract. This was confirmed by the value of the apparent degree of fermentation - 76.11%, the highest for all experimental variants. For the two highly hopped variants, the values of the apparent extract were slightly higher, but they also had a sufficiently high apparent degree of fermentation - over 75%.

The alcohol content of the finished beer increased slightly compared to the young beer, varying within narrow limits. The production of alcohol from brewing yeasts was not significantly affected by the higher hopping degree of wort.

The pH increased slightly after maturation, as the values for beers with 200 mg/l α-acids were identical - 4.80. The control value was slightly lower.

The amount of assimilated α-amino nitrogen increased slightly after maturation, remaining the highest for control variant - over 67% (two thirds of the initial amount of α-amino nitrogen in the wort was absorbed by the yeasts). For beers with a higher hopping degree, the assimilated α-amino nitrogen was 61-62%.

The bitterness of the beer containing 120 mg/l α-acids was 25.5 bitter units, while for the 200 mg/l α-acids the values were expectedly higher. Dry hopping contributed to enhancing the hop aroma but did not significantly affect the bitterness of the beer. The usage of bitter substances was higher for the control (over 21%). A higher hopping degree of wort resulted in higher losses of bitter substances, which was associated with precipitation losses.

Polyphenols are considered to be the main natural antioxidants in brewing (Whittle et al., 1999). Their antioxidant activity is extremely important in terms of human health (Kocyigit & Selek, 2016), so it is important to monitor their content in food and beverages. In our study, the polyphenol content of the control variant ale (120 mg/l α-acids) was 280.5 mg/l. For the highly hopped variants, a slight increase was reported (about 1.07 times fold).

Flavonoids are a large group of natural polyphenolic compounds having a benzo-γ-pyrone structure (Kumar & Pandey, 2013).

They are considered to have health-promoting properties due to their high antioxidant capacity *in vivo* and *in vitro* systems (Cook & Samman, 1996; Rice-Evans et al., 1995).
The amount of flavonoids in the control variant ale was over 21 mg/l. Like polyphenols, beers with a higher hopping degree showed an increase (about 1.2 times fold).
Another important parameter related to the total antioxidant capacity of beer is the content of anthocyanins. They are water-soluble pigments belonging to the phenolic group, known for their antioxidant properties (Khoo et al., 2017).
The amount of anthocyanins for the control variant ale was 85.2 mg/l. For the other experimental variants, the values showed an insignificant increase. The higher hopping degree of wort did not have a significant effect on the content of anthocyanins in beer.
The antioxidant activity of beers (Table 8) was expressed as equivalent of two important antioxidant vitamins, C and E (Wassmann et al., 2004).

Table 8. Antioxidant activity of aged beer

Parameters	120 mg/l α-acids (control variant)	200 mg/l α-acids	200 mg/l α-acids with 3 g/l dry hopping
Antioxidant activity - equ. vit. E mmol/l - equ. vit. C mmol/l	 531.31 1380.53	 562.24 1436.34	 571.08 1452.29

Antioxidant activity, expressed as equivalent vitamin C mmol/l, was 1380.53 for the control variant. For the beers with 200 mg/l α-acids, a slight increase was reported (1.04-1.05 times fold). The increase in calculation relative to vitamin E was similar (1.06-1.08 times fold).
Table 9 shows the increase in the content of polyphenols, flavanoids, anthocyanins and antioxidant activity in beers with a higher hopping degree than the control variant.

Table 9. Increase in the main parameters related to the total antioxidant capacity of beer, for the variants with a higher hopping degree compared to the control variant, %

Parameters	200 mg/l α-acids	200 mg/l α-acids with 3 g/l dry hopping
Polyphenols	6.6	7.1
Flavonoids	20.6	23.8
Anthocyanins	0.7	0.9
Antioxidant activity - equ. vit. E mmol/l - equ. vit. C mmol/l	 5.8 4	 7.5 5.2

There was an increase (to varying degrees) in the parameters related to the total antioxidant capacity of beers with a higher hopping degree than the control variant. The most significant increase was observed for flavonoids - 20.6% for 200 mg/l α-acids and 23.8% for 200 mg/l α-acids with 3 g/l dry hopping. The content of polyphenols was increased by 6.6-7.1%, and for anthocyanins the increase was insignificant (less than 1%). Antioxidant activity, expressed as equ. vit. C mmol/l, increased by 4% and 5.2%, respectively, compared to the control. When calculated relative to vitamin E, the increase was 5.8-7.5%.

CONCLUSIONS

The higher hopping degree of wort did not lead to disturbances in the development and reproduction of yeasts. No deviations in the normal parameters of the fermentation process were found.
For beers containing 200 mg/l α-acids, the most significant was the increase of flavonoids (20.6-23.8%) compared to the 120 mg/l α-acids variant.
Polyphenols in highly hopped beers were increased by 6.6-7.1% compared to moderate hopped beer.
The content of anthocyanins in beer was not significantly affected by the addition of more hops.
The antioxidant activity showed an increase of 4-5.2% in samples with a higher hopping degree compared to the control variant. When calculated relative to vitamin E, the increase was 5.8-7.5%.
Based on the data it can be concluded that a higher hopping degree contributes to improved functional properties of ale beer.

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