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RESEARCH OF HOP POLYPHENOLS IMPACT ON MALT HOPPED WORT AROMA FORMATION MODEL EXPERIMENTS

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ABSTRACT

Currently, a lot of research is being done on the flavoring compounds of hops. However, much less attention has been paid to the aroma formation considering the hop polyphenol different groups by various methods at the wort hopping stage. Therefore, the main goal of the research is an impact of hopping conditions on the polyphenolic compounds, when the hop is extracted variously into both wort and water to better understand extraction conditions, mechanisms, and factors as well as aroma formation considering various groups of polyphenols. As shown the hop variety, boiling time, and treatment type affect the hop polyphenols amount extracted into the wort. Aromatics varied upon a hop variety and wort boiling time accompanying a positive softening effect regarding aroma formated from malt wort compounds comparing to hopped aqueous extracts. The research proved the most noticeable pH (7 and above) impact on aroma formation caused by the polyphenol conversion. As also shown the first time, a temperature and acidic pH doubled the rutin amount, as well as the best extraction of the prenylflavanoid isoxanthohumol, was achieved by boiling at an alkaline medium pH. The results obtained indicate that various hydrophilic amino acids containing in the wort can stabilize polyphenols affecting the quality indicators of beer produced from different grain raw materials.

Keywords: hop polyphenol compounds; essential oils; beer aroma; polyphenol stabilizers; terpene hydrocarbon transformation

INTRODUCTION

It has been known that the hops use in brewing is very important from many different angles. The article concentrates on the hop polyphenol′s impact on the beer aroma formation and taste starting from the malt wort obtaining stage.

As researchers say, hop polyphenol′s constitute up to 30% of all raw polyphenols **(Wannenmacher, Gastl and Becker, 2018)**. A wide range of phenolic compounds presenting in beer is extracted from vegetable raw materials (malted and non-malted grain and hop). It is believed that polyphenolic substances mainly provide the colloidal stability and head retention of a beer.

Hop phenolic substances consist of monomeric and oligomeric polyphenol′s, including phenolic acids, coumarins, prenylated chalcones, flavonoids, catechins, and proanthocyanidins **(De Keukeleire et al., 2003; Dostálek, Karabín and Jelínek, 2017; Stevens et al., 1997)**. 20% of the total polyphenols are low molecular weight compounds esterified with sugar-like compounds. Such substances include catechin or proanthocyanidins, phenolic carbon acids and quercetin, kaempferol **(Biendl and Pinzl, 2009)**. Prenylflavanoids and chalcones account for 80% of the high molecular weight compounds of hop polyphenols.

Not all the hop polyphenol′s are present in beer. Thus, the research has indicated that proanthocyanidin B, catechin, epicatechin, quercetin glycoside, kaempferol, isoxanthohumol, caffeine acid are present in hop beer **(Forster, Gar and Gar, 2013)**. Which is caused by biochemical processes occurring during beer fermentation and maturation. Researchers note that 10 – 20% flavan-3-ols quantity in beer decreases after 3 weeks of maturation meanwhile isoxanthohumol as a phenylpropanoid decreases its content by 50% after 1 week of maturation **(Mikyška, Dušek and Slabý, 2019)**.

However, the impact of polyphenol′s on the beer taste has been noted. Taste tones such as astringency, hotness, oiliness, "mouthpiece", stickiness are attributed to polyphenols **(Meilgaard, 2001)**. Scientists note that catechin and epicatechin causing the astringent taste in beer should be contained at levels up to 20 $mg.L^{-1}$ **(Dadic and Belleau, 1973)**.

Simple phenolic acids (ferulic, p-coumaric, vanillic, hydrocinnamic acid, etc.) transformations during the brewing process along with their impact on the finished beer aroma and taste are known and examined **(McMurrough et al., 1996; Meilgaard, 1975; Wackerbauer, Kramer and Siepert, 1982)**.

Polyphenol′s physiologically activate tongue TAS2R bitterness receptors in the mouth and thus the research has indicated the polyphenols overlap causing the beer

bitterness **(McLaughlin, Lederer and Shellhammer, 2008; Roland et al., 2013)**.

Polyphenolic compounds interact with salivary secretion nitrogen-containing compounds to form complexes causing an astringent sensation **(Condelli et al., 2006)**. The polyphenol′s molecular weight affects the astringent taste formation **(El Gharras, 2009)**. When even in minor concentrations certain polyphenols cause a bitter taste **(Soares et al., 2013)**.

The polyphenol′s impact on taste can be not direct only but also indirect due to some polyphenol′s effect (catechin and ferulic acid) on the suppression of the carbonyl compounds formation **(Walters, Heasman and Hughes, 1997)**.

When processed the polyphenol′s resistance to non-enzymatic degradation depends upon numerous factors such as molecular structure, pH, temperature, oxygen, light, processing, interaction conditions, and/or presence of other compounds and components **(Boussetta et al., 2011; Ioannou et al., 2012; Iacobucci and Sweeney, 1983)**. Phenolic acids and flavonol glycosides are reported to be more stable and less susceptible to degradation at higher temperatures **(Teleszko, Nowicka and Wojdyło, 2016; Zorić et al., 2014)**. This can explain the presence of low molecular weight polyphenol′s from hops and significant losses of isoxanthohumulone in beer.

To define exactly the hop polyphenol′s impact on the wort aroma formation, we fix the research goal to examine the hopping effect of both traditionally boiled and dry-hopped on the polyphenol content as wells as the hopped wort and water aroma in model experiments using two different types of hops (bitter Magnum and aromatic Tetnanger).

Scientific Hypothesis

When researching several hypotheses were examined:

a. The wort prolongs boiling with hops causing the release of more polyphenol′s, regardless of the hop type;

b. The liquid medium composition affects extraction and aroma formation bound to hop polyphenolic compounds;

c. The pH medium and different stabilizers affect the aroma formation and polyphenol dissolution.

MATERIAL AND METHODOLOGY

Samples

The hops pellets Hallertaur Magnum and Tettnanger varieties are of the 2019 harvest containing the following amount of oil and α-acids respectively: Magnum (1.6 – 2.6 mL.100g^{-1}, 12.0%) (Figure 1) and Tettnanger (0.5 – 0.9 mL.100g^{-1}, 3.8%) (Figure 2). The precise oil and α-acid content were determined by the producer.

Chemicals

All reagents and standards were of analytical grade. Quercetin, rutin, isoxantogumol, and phenolic acids standards were from Sigma-Aldrich with a purity ≥ of 99%. Potassium dihydrogen phosphate (KH_2PO_4), acetonitrile, acetic acid, orthophosphoric acid (H_3PO_4) were purchased from Galachem (Moscow, Russia).

1-butanol, hydrochloric acid (HCl), iron (II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), methanol, carboxymethylcellulose, ethylenediaminetetraacetic acid (EDTA), ferric ammonium citrate ($(NH_4)_5[Fe(C_6H_4O_7)_2]$), ammonium hydroxide ($NH_4OH$) were purchased from Limited liability company “Reatorg” (Moscow, Russia).

Instruments

This investigation was used centrifuge Armed 80-2 (Russia), photoelectric colorimeter Apel AP-101 (Japan), pH meter Milwaukee (USA), chromatographic equipment "Agilent Technologies 1200" ("Agilent", USA) with diode array detector. HPLC equipment was fitted column Hypersil 5 u C18 250 x 4.6 mm 5 μm (Thermo, USA) with wavelength 270 and 310 nm, and fitted column Luna 5 u C18 (2) 250 x 4.6 mm 5 μm (Phenomenex, USA) with wavelength 290 nm and column Kromasil C18 150 x 4.6 mm 5 μm (Supelco, USA) with wavelength 290 nm.

Animals and Biological Material:

The hops selected for the research represented by T90 pellets of the Hallertaur Magnum and Tettnanger varieties purchased from Joh.Barth & Sohn Company (Freiligratstrasse, 7-9, 90482, Nuremberg, Germany).

Laboratory Methods

Total polyphenol content

The total hop polyphenol content and wort samples were determined according to method 7.14; Analytica-EBC, 2010. Beer sample (10 mL) was mixed with a preparation of carboxymethylcellulose (CMC, 1%) and ethylenediaminetetraacetic acid (EDTA, 0.2%) (8 mL) in a 25 mL volumetric flask; then ferric ammonium citrate (3.5%, 0.5 mL) was added and then followed by ammonium hydroxide solution (33.3%, 0.5 mL) being mixed after each addition. The solution was made with Reverse Osmosis (RO) water up to mark and left at room temperature for 10 min. The absorbance of the solution was taken at 600 nm and multiplied by 820 to give the total polyphenol content in beer (mg.L^{-1}) **(EBC, 2010)**.

Anthocyanogens content of hop, water extracts and wort samples

Anthocyanogens (Method 2.17.2; MEBAK, 2011): Anthocyanogens determination to Harris and Ricketts (photometry at 550 nm) **(MEBAK, 2011)**.

High-performance liquid chromatography (HPLC) analysis

A high-performance liquid chromatography method using "Agilent Technologies 1200" ("Agilent", USA) diode array detector was applied to determine the phenol acids mass concentration. HPLC equipment was fitted Hypersil 5u C18 250 x 4.6 mm 5 μm (Thermo, USA) column with 270 and 310 nm wavelength. The samples and all standards solutions at a volume of 20 μL were injected into a reversed-phase column at 25 °C. The mobile phase was 0.025 mol.L^{-1} potassium dihydrogen phosphate solution (A) (pH 2.5) and acetonitrile solution (B) with the ratio (A:B – 87:13). The eluent flow rate was 1.3 mL.min^{-1}.

A high-performance liquid chromatography method using "Agilent Technologies 1200" ("Agilent", USA) diode array detector was applied to determine the quercetin and rutin mass concentration. HPLC equipment was fitted Luna 5 u C18 (2) 250 x 4.6 mm 5 μm (Phenomenex, USA) column with 290 nm wavelength. The samples and all standards solutions at a volume of 20 μL were injected into a reversed-phase column at 25 °C. The mobile phase was 2% acetic acid solution (A) and acetonitrile solution (B) with the ratio (A:B – 70:30). The eluent flow rate was 1.5 mL.min^{-1}.

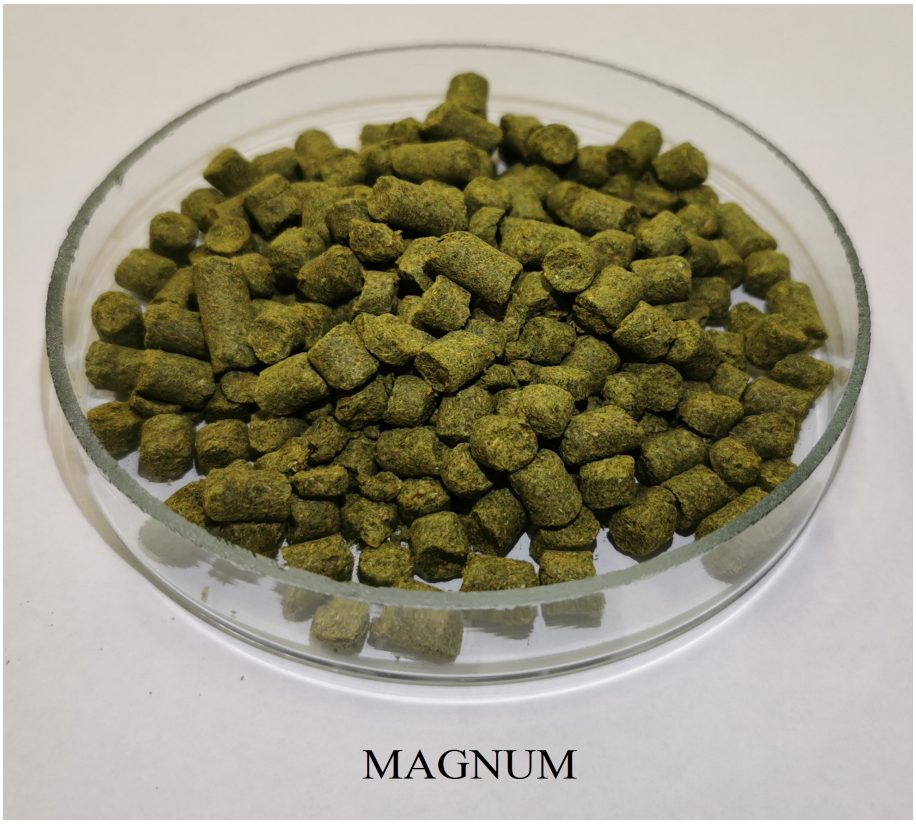

Figure 1 The hop pellets Magnum variety.

Figure 2 The hop pellets Tettnanger variety.

Determination of isoxantogumol concentration in hoped samples

High-performance liquid chromatography (HPLC) analysis

A high-performance liquid chromatography method using "Agilent Technologies 1200" ("Agilent", USA) diode array detector was applied to determine the isoxantogumol mass concentration. HPLC equipment was fitted Kromasil C18 150 x 4.6 mm 5 μm (Supelco, USA) column with 290 nm wavelength. The samples and all standards solutions at a volume of 10 μL were injected into a reversed-phase column at 25 °C. The mobile phase was acetonitrile solution (A), water (B), and orthophosphoric acid solution (C) with the ratio (A:B:C – 40:60:0.1). The eluent flow rate was 1 $mL.min^{-1}$.

Determination of pH samples

Sample pH was determined with a pH meter. All measurements were made in triplicate.

Organoleptic evaluation of hopped samples by descriptors

The organoleptic analysis was done by a professional group of researchers, consisting of 10 people on a 5-point scale according to the characteristic taste descriptors selected. 5 points mean a strong descriptor shade, 4 points – a well-developed descriptor shade, 2 points – a slightly visible descriptor shade, 1 – a subtle descriptor shade. The results obtained were summarized and the average score was recorded.

Description of the Experiment

Sample preparation: Wort production and dry hopping

The wort was produced in a pilot brewery (Bavaria, Germany) of the All-Russian Research Institute of Brewing, Non-alcoholic, and Wine-Making Industry. The original wort density produced from 100% malt was 12 °P. The wort was produced according to the conventional infusion mash procedure with temperature breaks at 52 °C, 63 °C, and 72 °C during 20 – 30 min each.

The wort was hopped with two Hallertaur Magnum and Tettnanger hop varieties to achieve a target of 12 bitterness units (BU). Hop samples were added at 15, 30 and 45 minutes after the wort boiling. The boiling time was 60 min and the post-boiling density was 1.048 (12 °P). Also, hop samples were placed to the whirlpool applying dry-hopping technology. The data is shown in Table 1.

T90 pellet hops at a rate of 4 $g.L^{-1}$ (Hallertaur Magnum and Tettnanger variety) were added to the wort current pumped into the whirlpool at a 35 $m.sec^{-1}$ flow rate. The cold hopping stage lasted 25 minutes;

Model hop water solution preparation

The boiling time for the preparation of model water hopped solutions was 15 minutes at different pH values applying the same hop doses as for the wort. The pH was adjusted by adding a 0.1% citric acid solution.

Number of samples analyzed: The number of one experiment analyzed samples in one replication was 16.

Number of repeated analyses: The analysis was done in triplicate.

Number of experiment replication: The experiment replication was done two times.

Statistical Analysis

Descriptive statistics performed and values expressed as mean ± standard deviation. The Student-Fischer method was used in the studies. The obtained data reliability limit (p <0.05) was considered to assess the various factors that impact the polyphenols content in all studies; the statistical data was processed by the Statistics program (Microsoft Corporation, Redmond, WA, USA, 2006).

RESULTS AND DISCUSSION

Boiling time and liquid medium composition effect studies on extraction and aroma formation bound to hop polyphenolic compounds

It is known that the finished beer organoleptic profile depends upon the hops types used in the technology and forms due to various organic compounds – bitter acids, ether compounds, polyphenolic substances **(Praet, 2016; Van Opstaele et al., 2010)**. Sometimes the sharp shade in the hop compounds taste becomes leveled and changes also due to the presence of organic malt substances (β-glucan, starch dextrins, etc.) in the wort **(Fox, 2018; Langstaff and Lewis, 1993)**. In our terms the boiling time of the wort with hops is of great importance.

Therefore, and at the first research stage, comparative studies of the environmental impact on the various hop polyphenol′s dissolution (polyphenoles (POL) and anthocianogence (ANTH) in malt wort (MW) and aqueous solutions (AS) with pH = 5.2) were presented in Table 2.

Table 2 data indicates that the hop type, hopping procedure (kettle or dry-hopping, boiling time), and all together affects the polyphenolic compounds amount transferred. When comparing the data in Table 2, one can see that the hop variety affects the extraction of polyphenol′s – use of bitter Magnum provides a lower polyphenols release comparing to the finely aromatic Tetnanger which is consistent with the quantitative polyphenolic compounds composition in the original hop varieties **(Callemien and Collin, 2009)**. However, the data indicates that the amount of the polyphenolic compound in the boiled wort hop-free is higher than hopped with Magnum regardless of the boiling time. The polyphenol content is reduced by 33% in the thermal interaction of malt protein substances with polyphenol substances and by 40 – 60% in the thermal interaction of malt protein substances and Magnum hop polyphenol′s as well as by 8 – 40% in the case of Tetnanger. One can note that 15 minutes less prolonged boiling with Tetnanger provides the polyphenol′s dissolution by 78% more than their amount in the boiled wort indicating incomplete protein-polyphenol associates formation due to a short interaction time. In our terms, this may be due to the higher intensity of the association of protein and polyphenolic compounds of malt and hops in the wort **(Mikyška et al., 2002)**. The use of fine-flavored Tetnanger hops provides greater polyphenolic substances extraction both in comparison with Magnum in the hopping dynamics and with the amount in the boiled wort. Cold hopping revealed that the use of the fine-flavored hops provides 4% greater polyphenol′s release comparing to the use of bitter Magnum.

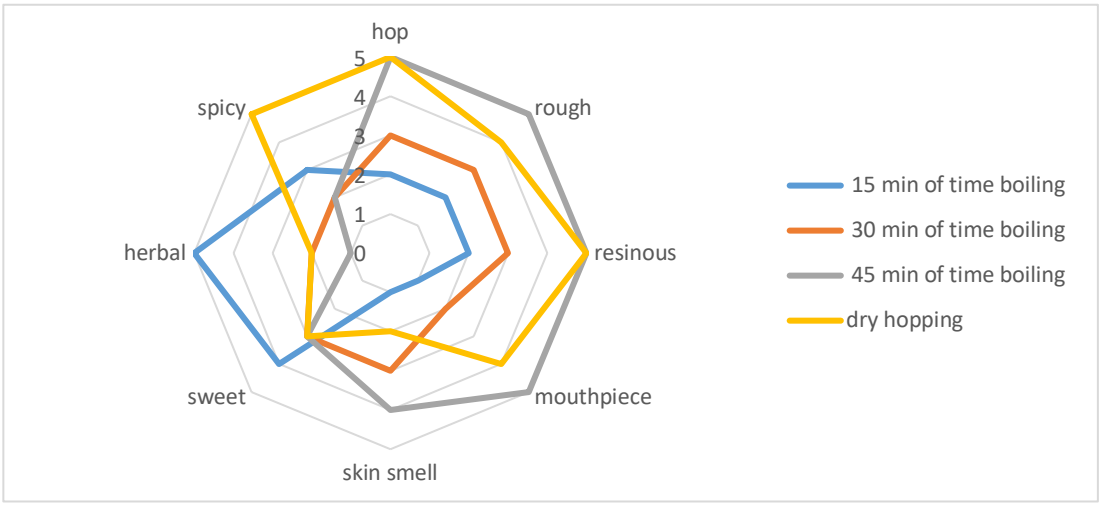

Figure 3 The hopped wort organoleptic profile using Magnum hop variety depending on the application and hopping duration method.

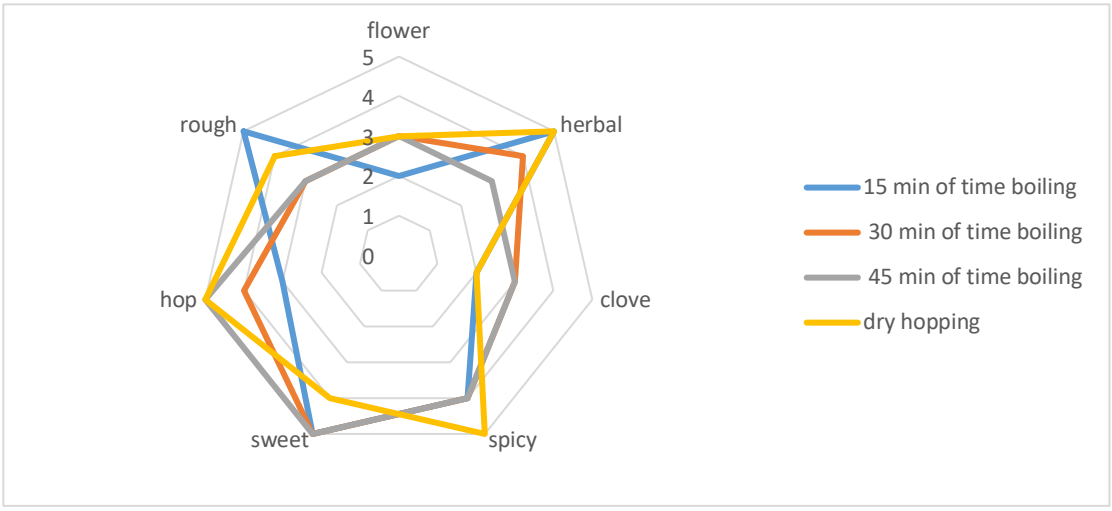

Figure 4 The hoped wort organoleptic profile using Tettnanger hop variety depending on the application and hopping duration method.

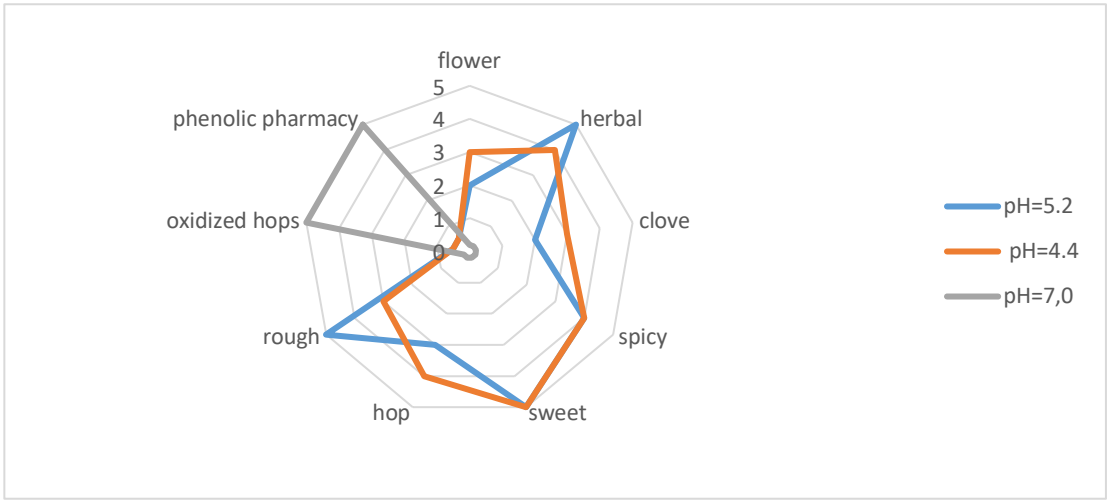

Figure 5 The hoped water organoleptic profile using Tettnanger hop variety depending on the pH medium.

When comparing the intensity of the polyphenol dissolution in wort (MW) and water (AS), the level of polyphenols decreased by 0.5 – 16 times when using Magnum and 2 – 6 times when using Tetnanger relatively to the content in the hopped wort under the same conditions. This indicates that the most complete hop polyphenol′s dissolution requires not only the appropriate pH of the medium (5.2) but also other compounds′ presence allowing their possible release from the hop plant matrix.

As shown in Table 2, anthocyanogens pass into the wort with different intensities; they are labile and their maximum amount is observed when wort hopping with Magnum for 15 minutes and with Tetnanger for 30 minutes. A long boiling time does not provide their presence in the wort and they are more active to the malt wort protein substances responsible for colloidal haze during beer storage. Anthocyanogens amount could be determined in aqueous solutions when using Magnum hops for 15 minutes and anthocyanogens were converted and outside the detection limits in other cases. When Tetnanger hop variety hopping time was 30 minutes the maximum amount of anthocyanogens was observed in aqueous solutions. Dry-hopping conditions did not provide complete anthocyanogens release from hops in wort and aqueous solutions. The polyphenolic substance transition processes were consistent in both research objects as well as during the aqueous solution and wort hopping.

The aroma formation studies were done due to different compounds in hopped wort and aqueous extract. The data is presented in Figure 1 and Figure 2 respectively.

It ought to be remarked that a longer boiling results in deeper organoleptic aromas such as spice, rough, hop, etc. in the case of Magnum bitter hops variety (Figure 3). The herbal flavor is more present in the aroma with minimum heat treatment time while the flower and clove aromas increase when the heat treatment time is longer in the case of Tetnanger aroma hops variety hopping (Figure 4).

It ought to be remarked regarding dry-hopping that noble hop, floral and spicy tones come out first. The wort has a strongly marked hop aroma with a spicy-herbal-floral touch in the case of Tetnanger and resinous-spicy in the case of Magnum.

Researchers note that floral and citrus tones characterize the presence of linalool while floral, citrus and rose-like – geraniol, resinous – myrcene, clove – caryophile which means that they belong to terpene hydrocarbons **(Kishimoto, 2008)**.

Dry-hopping allows enriching the aqueous solution hopped with Magnum by strong hop, resinous, spicy, and mouthpiece shades while with Tetnanger – floral-spicy aromas where the hop is at the sub-tone level. Thus, there is a fundamental difference in the manifestation of different hop varieties aromas specifications: organic compounds of malt wort (starch and non-starch polysaccharides, protein substances, etc. as well as polyphenolic compounds) responsible for secondary subtones. Aqueous solutions obtained after hopping cause more clear secondary specifications for a particular hop variety tone which is explained by a less transfer of bitter hop compounds into aqueous solutions comparing to wort and characterized by the presence of the compounds in the composition such as acids, mineral, and organic compounds, etc. which reduce the external environment polarity coefficient facilitating the transfer of bitter resins into the wort.

Studies of the pH and different stabilizers impact on the aroma formation and polyphenol dissolution in model aqueous solutions

Since polyphenol′s are labile compounds capable of exhibiting not only antioxidant properties but also oxidation and transformation, the pH impact on the organoleptic parameters state and the polyphenol content in aqueous extracts of aromatic hop varieties were researched; furthermore, due to the studies (Table 2), the aromatic hops precisely contains more polyphenolic compounds. The data contains in Table 3 and Figure 5.

Studies done with malt wort polyphenol′s have indicated that lowering the pH changed the chemical structure of some malt polyphenols imparting them more light absorption at 275 nm and potentially more bitterness **(Jurić et al., 2015)** unlike hop polyphenol′s as Table data 4 shows. The medium pH is of great importance for the polyphenol′s and related substances extraction since the hop's aroma is formed due to the hops ether compounds – terpenes, terpenoids, oxygenated sesquiterpenes, sulfur compounds, and glycosylated species **(Almaguer et al., 2014; Dresel, Dunkel and Hoffman, 2015)** – Figure 5.

pH values up to 5.2 cause an increase in the release of polyphenolic compounds. More alkaline extraction conditions cause a conversion of polyphenol′s and anthocyanogens negatively affecting the extracts aroma. Accordingly, the spatial polyphenols configuration with associated ester compounds should be in a more acidic environment.

What is since polyphenol′s and anthocyanidins color particularly are pH-dependent **(Tsao, 2010**) being chemically stable in acidic solutions which are confirmed by experiments results (Table 3). Besides, it is well known that anthocyanidins are unstable and decompose at high temperatures and neutral pH **(Dangles and Fenger, 2018)**. When the medium reacts alkaline at pH 7 or higher, a reduction reaction occurs in the medium. Besides, the composition OH groups responsible for the polyphenol′s stability are oxidized and the polyphenol′s lose their stability and due to the detachment of the carbohydrate residue as well.

On the other hand, it is known that flavanols and proanthocyanidins can react to associating with other polyphenol′s and forming coloration up to brown **(Shoji, 2007)**.

Figure 5 indicates that polyphenol′s critically affect aroma at a pH close to neutral when the aroma shades specifications of the ester compounds disappear and only the phenolic-pharmaceutical aroma is visualized inherent in the hop polyphenols group of hop polyphenols.

We have researched the possible use of amino acids and monosaccharides in a model solution to stabilize various polyphenol′s as well as to examine the various compounds′ impact on changes in the polyphenol structure and state.

Table 1 The studied samples list depending on the hops introduction model.

Sample No	Technological stage of hop varietes introduction			
	15 min after beginning	wort boiling 30 min after beginning	45 min after beginning	into the current of the wort pumped into the whirlpool
		Magnum		
1w/1wt*	+	-	-	-
2w/2wt	-	+	-	-
3w/3wt	-	-	+	-
4w/4wt	-	-	-	+
		Tettnanger		
5w/5wt	+	-	-	-
6w/6wt	-	+	-	-
7w/7wt	-	-	+	-
8w/8wt	-	-	-	+

Note: 1w/1wt* – to compare the indicators characterizing the extraction rate of various groups of polyphenols, hops were introduced into water and a similar procedure was carried out as with wort – these are samples 1wt.

Table 2 Polyphenolic compound content in MW and AS at various hop addition modes.

Indicators content	Value, mg.L^{-1}, at the introduction stage (reliability limit p <0.05)									
	wort boiling		boiling time during hopping, min						dry-hopping	
	befor	after	45		30		15			
			AS	MW	AS	MW	AS	MW	AS	MW
					Magnum hop variety					
POL	196.8 ±17.7^{a}	147.6 ±13.2^{a}	8.2 ±0.74^{a}	131.2 ±11.8^{a}	10.3 ±0.93^{a}	139.4 ±12.6^{a}	82.0 ±7.38^{a}	123.0 ±11.0^{a}	65.6 ±5.90^{a}	196.8 ±17.7^{a}
ANTH	13.48 ±0.94^{a}	8.55 ±0.6^{a}	nf*	6.4 ±0.45^{a}	nf	16.3 ±1.14^{a}	3.62 ±0.25^{a}	8.50 ±0.60^{a}	1.97 ±0.14^{a}	12.9 ±0.90^{a}
					Tettnanger hop variety					
POL	196.8 ±17.7^{a}	147.6 ±13.2^{a}	53.3 ±4.78^{a}	141.0 ±12.7^{a}	84.0 ±7.56^{a}	182.0 ±16.4^{a}	41.0 ±3.69^{a}	262.4 ±23.6^{a}	131.2 ±11.8^{a}	205.0 ±18.4^{a}
ANTH	13.48 ±0.94^{a}	8.55 ±0.6^{a}	3.62 ±0.25^{a}	8.55 ±0.60^{a}	4.60 ±0.32^{a}	16.22 ±1.14^{a}	1.42 ±0.10^{a}	21.15 ±1.48^{a}	8.55 ±0.60^{a}	14.0 ±0.98^{a}

Note: nf* – not finding. [a] Each value represents the mean of three independent experiments (±SD).

Table 3 Hoped aqueous solution values at different pH medium.

Indicators	Characteristics at pH value (reliability limit p <0.05)		
	4.4	5.2	7.0
Aroma	jasmine, floral, hop	grass, hop	phenolic, pharmacy, oxidized hop
Colore	lemon-mint	lemon-mint	red grapefruit
POL, mg.L^{-1}	42.0 ±3.8^{a}	41.0 ±3.7^{a}	27.5 ±2.5^{a}
ANTH, mg.L^{-1}	1.97 ±0.14^{a}	1.42 ±0.10^{a}	Nf

Note: nf – not found. [a] each value represents the mean of three independent experiments (±SD).

Table 4 The amino acids influence on polyphenol content in hoped aqua solution at pH = 7.0.

Indicators	Characteristices of indicators with applaing amino acid (reliability limit p <0.05)					
	Gly		Arg		Asp	
	5	150	10	150	20	150
Aroma	pharmacy-phenolic	oxidized hop, hay	oxidized hop	oxidized hop, hay	oxidized hop	hop, citrus, spicy
Color	deep crimson					lemon-yellow
pH	7.0					5.2
POL, mg.L^{-1}	82.0 ±0.74^{a}	147.6 ±13.2^{a}	82.0 ±0.74^{a}	164.0 ±14.8^{a}	82.0 ±0.74^{a}	139.4 ±12.5^{a}
ANTH, mg.L^{-1}	0.33 ±0.02^{a}	1.42 ±0.10^{a}	0.88 ±0.06^{a}	3.62 ±0.25^{a}	1.97 ±0.14^{a}	4.71 ±0.33^{a}

Note: [a] each value represents the mean of three independent experiments (±SD).

Table 5 The different factors influence on polyphenol content in hoped aqua solution (AS) and dry hop solution (DHS).

Indicators, mg.L^{-1}	AS characteristics with different treatment during boiling with the substances in concentration (reliability limit p <0.05)								
	pH			Amino acid			Monosugar		DHS
	4.4	5.2	7.0	Gly	Arg	Asp	Gl	Fr	
Concentration, mg.100 mL^{-1}	-			150			4000		-
pH	4.4	5.2	7.0	7.0		5.2	7.0		7.0
POL	82.0 ±7.4			147.6 ±13.2	164.0 ±14.8	139.4 ±12.5	82.0 ±7.4		131.2 ±11.8
ANTH	0.33 ±0.02	1.97 ±0.14	nf	1.42 ±0.10	3.62 ±0.25	4.71 ±0.33	nf	0.33 ±0.02	8.55 ±0.60
Isoxantogumol	2.16 ±0.21	1.90 ±0.19	4.44 ±0.44	3.94 ±0.39	3.78 ±0.38	1.99 ±0.20	3.73 ±0.37	3.55 ±0.36	0.96 ±0.1
Rutin	7.77 ±0.77	8.16 ±0.82	4.32 ±0.43	3.36 ±0.33	7.86 ±0.79	7.54 ±0.75	6.43 ±0.64	3.50 ±0.35	2.29 ±0.23
Quercetin	0.04 ±0.004								
vanillic acid*	0.19 ±0.02	0.17 ±0.02	0.20 ±0.02	0.24 ±0.02	0.19 ±0.02	0.11 ±0.01	0.18 ±0.02	0.15 ±0.02	0.16 ±0.02
syringic acid*	0.07 ±0.01	0.14 ±0.01	0.14 ±0.01	0.10 ±0.01	0.15 ±0.01	0.11 ±0.01	0.18 ±0.02	0.18 ±0.02	0.14 ±0.01
vanillin*	0.13 ±0.01	0.16 ±0.02	0.16 ±0.02	0.12 ±0.01	0.13 ±0.01	0.10 ±0.01	0.18 ±0.02	0.17 ±0.02	0.14 ±0.01
syringic aldehyde*	0.08 ±0.01	0.06 ±0.01	0.18 ±0.02	0.10 ±0.01	0.11 ±0.01	0.07 ±0.01	0.16 ±0.02	0.15 ±0.02	0.08 ±0.01
synapic acid*	0.12 ±0.01	0.11 ±0.01	0.19 ±0.02	0.18 ±0.02	0.16 ±0.02	0.11 ±0.01	0.17 ±0.02	0.16 ±0.02	0.12 ±0.01
coniferyl aldehyde*	0.34 ±0.03	0.31 ±0.03	0.45 ±0.04	0.04 ±0.004	0.08 ±0.01	nf	0.22 ±0.02	0.21 ±0.02	0.11 ±0.01
cynapic aldehyde*	0.64 ±0.06	0.71 ±0.07	0.23 ±0.02	0.09 ±0.01	0.18 ±0.02	0.62 ±0.01	0.37 ±0.04	0.25 ±0.03	0.36 ±0.04
Summ of low molecular weight phenolic acids	1.57	1.66	1.55	0.87	1.00	1.12	1.36	1.27	1.11

Note: nf – not found. Each value represents the mean of three independent experiments (±SD), *Low molecular weight phenolic acid.

It′s clear, that polyphenol′s are synergistic with several organic substances such as organic acids, amino acids, carbohydrates, etc. **(Baxter et al., 1997; Codorniu-Hernández et al., 2005; Gauche, da Silva Malagoli and Bordignon Luiz, 2010; Guerra and Yaylyan, 2014; Renard, Watrelot and Le Bourvellec, 2017)**.

For example, experiments with glycine indicated that (+) – catechin forms various adducts with this amino acid through a dehydration reaction in the ring as well as with the Schiff bases formation at the attachment point to the oxidized b-ring **(Guerra and Yaylyan, 2014)**.

Malt wort contains several amino acids in minor concentrations **(Otter and Taylor, 1976)**, as well as reducing substances represented by low-molecular dextrins, non-staining with iodine, maltose, glucose, fructose **(Floridi et al., 2001)**.

It was of interest to study the polyphenol system stabilization at pH = 7; for this purpose, the amino acids present in malt wort were used – glycine (Gly), leucine (Leu), alanine (Ala), argenine (Arg), aspartic acid (Asp.), and monosugar (glucose (Gl) and fructose (Fr)). The data are presented in Table 4 and Table 5.

According to data in Table 4 and Table 5, glycine, leucine, arginine, aspartic acid and fructose monosaccharide significantly affect polyphenol stability. Alanine and glucose use led to anthocyanogen degradation. Therefore, compounds were selected – amino acids stabilizing polyphenol′s better than others such as arginine, glycine, and aspartic acid. This fact is explained by the hydrophilic nature and ability to interact with polyphenols easier than other amino acids in the case of arginine and aspartic acid **(Higgs et al., 2008)**. There is a carboxyl group binding to the polyphenolic compounds′ active sites on the surface in the main chains of these amino acids.

An interesting effect was observed in the case of adding a monosaccharide, fructose, to the medium (data in Table 5). Considering the amount increase of anthocyanogens (proanthocyanidins, prodelphinidins, etc.) comparing with the control it prevented their oxidation binding to them in other words. This is confirmed by the literature proving the

same **(Andrade et al., 2017)**. Researchers attribute interactions with carbohydrates due to van der Waals interactions providing a degree of polymerization, molecular flexibility, number of external hydroxyl groups, or number of terminal galloyl groups **(Bordenave, Hamaker and Ferruzzi, 2014)**.

In our opinion, it is interesting to note that the anthocyanogens presence affects the aroma and color change of the aqueous extract desired while the sugar adding caused no impact on the total polyphenols content.

However, a significant effect, judging by Table 4 and Table 5, we did not observe. Therefore, it was decided to examine hydrophilic amino acids at a concentration equal to the total sum of amino acids contained in malt wort – 150 mg.100mL^{-1} **(Otter and Taylor, 1976)**, data are shown in Table 5.

According to Table 5, a distinct medium acidification effect was achieved by the aspartic acid adding since the medium pH became 5.2 which is bound to increased polyphenols and anthocyanogens content. The pH changes effect on the anthocyanogens color change has been examined indicating that the acidic to neutral medium acidity changes caused complete but reversible discoloration of the anthocyanidin molecule due to the colorless isoforms formation **(Basílio and Pina, 2016)**.

The results obtained using aspartic acid exceed the control sample obtained by boiling hops in water with a pH of 5.2, provided by the citric acid adding (Table 5). In this case, the anthocyanogens amount tripled indicating their great resistance to critical conditions of extraction from hops at the temperature of 100 °C. Terpene hydrocarbons and alcohols are the main aromatics in hops. Hydrocarbons are isomerized by the ionic mechanism in case of acidic pH values with the amino acids adding **(Bejblová, Žilková and Čejka, 2008)**. Isomers are reversibly formed, enhancing the characteristic aroma. The isomerization transformations of alcohols (linalool, etc.) are more diverse than the transformations of hydrocarbons, due to the presence of a hydroxyl group **(Basílio and Pina, 2016)**. Unsaturated terpenoids undergo irreversible polymerization when a pH close to neutral and alkaline causing an odor loss **(Bejblová, Žilková and Čejka, 2008)**, thus, when a pH close to neutral, oxidized hop tones are present; pH at 5.2 shows unoxidized hop tones and the anthocyanogens and other polyphenol′s color are lighter indicating a creep of the structure of the unoxidized compound **(Basílio and Pina, 2016)**. After all the anthocyanins color change is bound to an intramolecular binding of p-coumaric residues to the anthocyanin chromophore **(Mori, Kondo and Yoshida, 2009)**.

Table 5 data indicates the change in the polyphenol accumulation in different aqueous extracts groups under different experimental conditions. According to Table 5 data the isoxanthohumol extraction is consistent with the pH medium: the closer to a neutral and alkaline medium the higher the prenylflavanoid extractability which is consistent with the literature data **(Moens et al., 2020)**. Most of all isoxanthohumol is extracted during boiling, rather than with vigorous stirring. The polar amino acid addition affects the isoxanthohumol extraction intensity since the addition of the amino acids changes the water solution polarity and increases its dissolution **(Radzicka and Wolfenden, 1988)**. When comparing the boiling method with the cold hopping vigorous stirring at pH 7.0, the isoxanthohumol goes least of all into solution with stirring without exposure to high temperatures. This can be caused by its isomerization **(Magalhães et al., 2008)**.

The quercetin extraction did not depend on external conditions (temperature, pH, stirring), and a stabilizer was added remaining at the same level. The rutin extraction was maximum under the acidic pH value conditions provided by organic or amino acid. However, the temperature factor (t = 99 °C) allowed extracting double more rutin comparing with the extraction at t = 25 °C along with stirring during the same time.

We can say that pH values have a more significant effect comparing with other factors (thermal, the presence of sugar, stirring) regarding the recoverable low molecular weight phenolic compounds (LMPS) amount. The LMPS are most intensively extracted in the citric acid 10% solution state at pH 5.2 during boiling. The LMPS amount is somewhat lower at the same pH value achieved by adding aspartic acid.

It is also necessary to pay attention to the variability of the hopped water aroma depending on the amino acid used (Table 5). Which could hypothetically contribute to the aroma and flavor development in hopped wort made from a variety of grains. There is a wide variety of amino acids containing in the wort qualitatively and quantitatively in this case **(Zhuang et al., 2017)**. Thus, one can testify that the beer taste and aroma formation begins at the hopping stage, when amino acids and mono sugars extracted from various grain raw materials in different percentages react with the hop anthocyanogens but not at the fermentation stage as was stated before so far **(He et al., 2014)**.

CONCLUSION

The research indicated that many factors affect the hop polyphenol′s dissolution in brewing technology such as the original hop variety, hopping method, the hop contact time with a hopped liquid (wort or water), polarity and pH of the hopped liquid. According to the research, a perceptible impact on the polyphenolic substances aroma begins under unfavorable conditions when the polyphenolic molecule configuration is unstable and undergoes irreversible changes at pH values of 7 or more. Under favorable conditions, the polyphenol′s aroma is a sub-tone and cannot be clearly distinguished by sensor detection. The hop aroma is revealed most fully in the hop aroma-forming substances total amount accompanied with other compounds the wort taste fullness specifications.

Thus, the aroma formation due to the hop polyphenol′s mainly depends upon the wort pH and amino acid composition extracted during mashing from grain raw materials which is mainly hydrophilic. While transformed during fermentation, they affect the beer aroma.

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