Features of using hops and CO_2-extract in brewing

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Abstract

Introduction. Beer that is made from hops or hop products of certain breeding varieties, varies considerably in the nature of bitterness, taste and aroma. This is due to the peculiarity of the biochemical composition of bitter substances, polyphenolic compounds and essential oil of hop of aromatic and bitter varieties. The aim of the study is to use in brewing the CO_2-extract and hop with a low content of alpha-acids, which can be the waste in the production of pellets 45, and to invent methods for its rational using.

Materials and methods. Aromatic hops varieties with low alpha-acids, CO_2-extract and beer made from them were investigated. High-performance liquid chromatography to determine the amount and composition of the bitter substances of hops, CO_2-extracts and their conversion products in the brewing process and spectrophotometric quality control methods for the bitter taste of hopped wort and finished beer were used.

Results. When we use in brewing CO_2-extract and a thin-aromatic hop with a low content of alpha-acids in optimal proportions, polyphenols of low-hops help to remove high-molecular polypeptides from the wort by coagulation with the formation of complexes. Thanks to this, a higher colloidal resistance of beer is achieved and the degree of use of bitter substances is increased by 15–20%. Beer that was prepared with the using of 40% wort, bitterness of aromatic low-hops and 60% bitterness due to CO_2-extract, has had the best flavor and aromatic qualities. Beer in which extract and aromatic hops was used in a proportion of 40:60% was not much different. Polyphenols and beta-fraction of aromatic low-hops hops positively influence the coarse bitterness of the CO_2-extract, softening and smoothing it, making the common bitterness of the beer balanced. Also, when we use these proportions, a maximum decrease in the index of high molecular weight polypeptides is observed, which predicts a high colloidal persistence of beer. When we use in brewing only a CO_2-extract, it is impossible to obtain beer with high taste qualities. However, an excessive amount of hoppy polyphenols leads to an astringent taste in beer.

Conclusions. Aromatic hop with a low alpha-acid content can be used in brewing as a polyphenol additive in combination with a CO_2-extract, taking into account the quantitative content and qualitative composition of hop products and adhering to a certain technology of making beer.

Introduction

Substances that are different in nature and chemical structure, which are part of the hops, give the beer a typical bitter taste, a characteristic specific flavor and cause many other important biotechnological properties. Hops compounds are effective agents for the deposition of high nitrogen substances from wort, involved in lighting and foaming, and exhibit bactericidal and preservative effect on the final product, increasing stability of beer in it storage [1-3].

Previous studies of national and foreign scientists [4-6] have found that beer made from hops of certain breeding varieties vary greatly in nature of bitterness, flavor and aroma. It's due with features of biochemical composition of bitter substances, polyphenolic compounds and essential oil of bitter and aromatic hop varieties. A different ratio of the components of these compounds has different effects on taste and aroma of beer [3, 5, 7-11]. Nowadays a significant part of hop with low content alpha acid is grown. Ukraine isn't an exception. The majority of hops plantation in Ukraine now occupied by traditional fine aromatic varieties for example: Clone 18, Zlato Polissya, Slovyanka, Natsionalniy. Due to difficult arid climatic conditions of recent years, that did not contribute to the synthesis of bitter substances, a significant part of hops have alpha acid content 3-4 %.

It is worth noting that regional as well as powerful breweries in Ukraine in their technology use a significant part of granulated hops. But when wort is hopping by granules containing only the AA 3-5 %, to a brewing machine is brought significant weight of hop, while there are significant losses of wort. Therefore, it was necessary to develop methods of increasing increase the content of alpha acids in granules by physical means, one of which is production of pellets type 45, which relate to preparations enriched of hop [1, 12]. This method includes additional processing of cones by cold air at a temperature of -35 ^{o}C, in which lupulin grains are getting more rigid and less resinous, which makes it possible to separate them from the petals of hop cones sifting about three times [1, 12]. Thanks to the separation of lupulin from hop cones and adding it in certain proportions to grinding hops, bitter substances content increased twice. Weight of enriched hop pellets is granulated and we get the granules of type 45. Due to the fact that it's impossible to separate lupulin from hop cones completely, there remains a certain amount of bitter substances. Therefore, there is a problem of using waste of subtly aromatic hops remaining in the manufacture of pellets type 45. Such hops, according to current standards, by the content of alpha acids is considered unusual and is not entitled to be used for direct hopping of wort, although all other indicators it meets standards.

Also in brewing along with granules is used CO_2-extracts of hops. Long-term studies show [3, 5, 11–12] that using only CO_2-extracts of hop can't get a beer with excellent taste. This is due to the fact that these extracts are mainly made from bitter hop varieties. Also, CO_2-extracts, unlike hops, doesn't contain the polyphenols of hop (PF).

Polyphenolic hop compounds contribute to protein precipitation and the formation of complex protein-polyphenolic complexes during boiling, thereby precipitating at the boil, thereby brightening the wort, and thereby preventing bitter hop substances from oxidation and loss. They are antioxidants and increase the restorative capacity of beer, affect the stability of taste. But high-molecular polyphenols with prolonged boiling cause an unpleasant astringent taste in beer. Extractable polyphenolic substances of hops affect the taste and quality of beer not independently, but in combination with bitter substances of hops, proteins and amino acids.

Salach and other researchers, analyzing the quality of hops, depending on its content of polyphenols, noted that in the Czech varieties content of polyphenols is significantly

higher than the varieties of hops other countries. Thus, the content of polyphenols in the Czech hops varieties Zhatetskyy, featuring the highest quality of 5,2–5,9%, while in the US - less than 2.6%. They considered high content of polyphenols in hops Zhatetskyy its advantage over other varieties. Nowadays scientists also claim that the best quality beer made from hops, containing about 5% of polyphenols [9,10].

Therefore, the selection of hops and products of its processing to produce beer with great bitterness and quality is an issue relevant to the Brewers Association of America [8] European brewers [4] and Ukrainian beer producers [5].

The aim of the research was to examine the features of using in brewing CO_2-extract of hops and low-alpha acids, which may be waste in the production of pellets type 45 and inventing ways of using and rational using of its valuable substances.

Materials and methods

It was used the international modern physicochemical methods of the analysis of bitter substances of hops and hop preparations and products of their transformation in the brewing process: high performance liquid chromatography, spectrophotometry, and also methods of monitoring, harmonised with the methods of the European Brewery Convention [2, 9, 10].

Methods of researches of qualitative indicators of hops and CO_2-extract

Subtly aromatic hop varieties with a low content of bitter substances were investigated. The weight of average sample for identification and biochemical studies was not less than 0.5 kg of dry hops. Alpha acids in hops were determined by conductometric EMU 7.4 [2, 13, 14]. Bitter substances of hops and the extract extracted with an organic solvent – methanol. The ratio between the weight of hop cones and extractant was 1:10. The number of α- and β-acids was determined by high performance liquid chromatography. Chromatography was performed using a liquid chromatograph Ultimate 3000 UV with the detector at 35 °C. The column 100 x 2.1 mm was used, which was filled with sorbent Pinacle DV C18 3 MK. A solution of methanol water and acetone in the ratio 38:24:38 was used as the mobile phase. International standard-a standard ICF-3 was used for the quantitative determination of the components of the bitter substances. Total polyphenolic compounds in hops were determined by the method of Folin [2].

Method of preparation samples of beer in the mini-brewery

Experimental brewing of the tested samples were carried out in the laboratory and mini-brewery of the Institute, with a capacity of 100 liters of beer per cycle, which rather adequately simulates the conditions of the real brewing industry.

In the experimental boiling preparation and filtration of the mash was performed according to the adopted at this production technology. The wort was prepared from 100% barley malt and CO_2-extract. Extract was contributed at the beginning of hopping. After the complete set, the wort was boiled for 30 min. Then in wort in each case made the experiment pawned represented hop varieties in two installments: 85% are pawned at the beginning of hopping, 15% are pawned 15 min before the end of the hopping. Duration of boiling of wort with hops was continued about 90 minutes.

Methods of researches of quality indicators of wort and beer

Bitterness of wort that is formed in the process of it boiling with the hops, as a result of extraction and isomerization of bitter substances of hops, was measured on a spectrophotometer according to the method of EMU 8.8 (International method MI). The method is based on measuring optical density of iso octane extract obtained in the extraction of bitter substances acidified hopped beer wort isooctane (2,2,4- three methyl pentane) spectrophotometer at wavelength 275 nm against isooctane. We counted quantity of bitterness, which is expressed in international units of bitterness - one EMU by index of optical density.

In the beer content of polyphenolic compounds and anthocyanins were determined on a spectrophotometer according to the EBC method 9.11 (International method MI). [4]. Antotsianoheny were determined by the method of Harris and Rikkets [14]. This method is based on the transformation of Antotsianogens in anthocyanidins upon heating with acid. Antotsianogens are completely adsorbed on the adsorbent and polyamide sorbent is dissolved in a mixture of butanol - HCl. Red pigments, anthocyanins and anthocyanidins are converted into colored form when they are heated. The intensity of color, which is determined on a spectrophotometer under the wavelength of 350 nm, determines the content of anthocyanidins.

In the prepared beer and wort was controlled the size of fractions of high polypeptides. In developing the method came from the ratio of ingredients, the method adopted in determining the fraction Lundin and Shroderhayma [15] A faction in determining macromolecular proteins.

A fraction was determined by tannin parameter that is using photoelectric by the green optical filter in the cell with the working face width of 10 mm.

Quantitative dependence of macromolecular polypeptides of fractions A content (mg / 100 ml) expressed by the equation:

$$T = -0,045 + 0,0276 \cdot A,$$

where A – A faction of content, mg / 100 ml;

T – index of tannin (optical density).

Colloidal stability of prepared beer predicted and evaluated by the "degree of ammonium sulfate precipitation" by method proposed by Basarjova, based on identifying substances that are precipitated saturated solution of ammonium sulphate using turbidimeter [16].

Preliminary, according to the formazine suspensions of known turbidity (from 0.5 to 12 units of EMU), calibration curves are constructed. In a row of tubes add 10 ml of beer, freed from carbon dioxide, and add a saturated solution of ammonium sulfate in increasing quantities. In the control, to the row of tubes with 10 ml of beer, the same amount of water is added. The turbidity at the nephelometer is measured at a wavelength of 670 nm. The measurements are carried out 15 minutes after the addition of ammonium sulfate, and the solution is transferred to the cuvette 2 minutes before the expiration of 15 minutes so that the solution in the cuvette is settled. Measurements are done against control.

Saturated ammonium sulfate solution is prepared so that at a temperature of 20 ° C in 100 ml of the solution there were 43 g of anhydrous ammonium sulfate, and after settling the solution for 48 hours, undissolved ammonium sulfate crystals remained on the bottom of the flask.

Results of discussion

There was conducted a series of experiments in brewing beer to study the effect of hop polyphenols of low-ash hop on quality indicators wort and beer, where near CO_2-extracts was currently used hops.

Characteristics of hops shown in the Table 1.

Table 1

Characteristics of hops

Hops	Contents, %			Weight of hops for hopping, g/dm^3
	Alpha-acids	Polyphenolic compounds	Antotsianogens	
Hop	1,2	7,2	5,6	4,132
CO_2 -extract	52,9	-	-	0,151

As you can see from the table, content of bitter substances in CO_2-extract is quite high and amounted to 52.9%, so its mass for hopping of wort is insignificant compared with hops (0.151 g/dm^3 for CO_2-extract and 4.132 g/dm^3 for hops). While currently hop holds 7.2% polyphenolic compounds, which does not have a CO_2-extract.

The weight of hop and extract that was introduced to the hopping for each variant of experiment, weight of hop polyphenols (PF) which were made to the wort, and also value of bitterness wort, content of total polyphenols, anthocyanogens (AG) and macromolecular polypeptides are shown in the Table 2.

Table 2

Influence of correlation of CO_2-extract and aromatic low-ash hop the on quality indicators of hopped wort

№ of sample	Was made, %		Weight of hop products, g/dm^3		Polyphenols of hop that were made mg/dm^3	Value of bitterness wort, for EMS	Content	
	CO_2-extract	Hop	CO_2-extract	Hop			polyphenols, mg/dm^3	fraction A for Lundin, mg/100 ml
1	100	-	0,1510	-	-	32,0	131,4	21,0
2	80	20	0,1208	0,826	52,3	33,5	143,6	20,7
3	60	40	0,0906	1,653	104,7	35,3	160,4	17,9
4	40	60	0,0604	2,480	157,1	37,6	188,8	14,5
5	20	80	0,0302	3,306	209,5	40,1	219,1	13,7
6	-	100	0	4,132	261,8	43,0	255,5	13,6

With an increase in the amount of hops introduced with each variant of the experiment, the amount of both the polyphenols introduced into the wort and those defined in the wort increases. It should be noted that hops polyphenols interact more intensely with wort proteins with the formation of insoluble complexes, which positively affects the colloidal resistance of beer. Proanthocyanidins are the most active compounds in the formation of polyphenolic-protein complexes. Therefore, if there are more of them in the hop preparations, much more scrap is formed when the wort is hopped and it is better illuminated. This is observed in our studies when hopping wort only carbon dioxide extract, extract and low-hops in different proportions and one low-hops hop. It was noted that in the 6th variant, in comparison with 2, the amount of hops hopped into the wort increased 5 times, although the actual amount in hop wort in version 6 increased only 1.8 times. The remaining polyphenols, obviously, precipitated with the formation of polyphenolic-polypeptide complexes. This assumption is confirmed by the fact that in the case of hopping of wort with an extract only in the absence of polyphenols, scrap hops are formed little, slightly more is formed in the second variant. We noted that in the third and fourth variants, the amount of sediment increased significantly. Most of all he was in the 5th and sixth versions. But in these cases there were also the largest wort losses with this.

Our assumption is also confirmed by the data in the table, which indicate that from 1 to 6, the high molecular weight polypeptide decreases, and this decrease is 35.2%.

Influence of correlation of beer quality which was made according to this variant and biochemical characteristic of beer are made in Table 3.

Table 3

Influence of correlation CO_2-extract and aromatic low-ash hops on beer quality indicators

№ of variant	Value of beer bitter, for EMS	Content in beer, mg/dm^3		Index of polymerization	Fraction A for Lundin, mg/100 ml	Degree of deposition $(NH_4)_2SO_4$, ml/10 ml
		PF	AA			
1	20,0	120,1	29,3	4,1	16,0	1,0
2	21,0	132,3	33,1	4,0	15,4	1,1
3	22,5	146,2	38,5	3,8	14,2	1,2
4	24,3	163,1	46,6	3,5	12,8	1,6
5	26,0	183,2	57,3	3,2	12,2	1,7
6	28,0	208,6	72,0	2,9	11,9	1,7

The table shows that increasing the dose of low-ash hop increases bitterness of wort and beer. This increase is due to the participation in its creation not only of alpha acids extract and hop and β-fractions of low-ash hops.

There is an increase of made polyphenols in wort and identified them in wort and beer. It should be noted that polyphenols of hope are more polymerized than malt phenols. And thus the rate of polymerization index in the manufacture of beer significantly increased from 2.9 to 4.1 with increase in the proportion of carbon dioxide hop extract. They also have more intensive interaction with wort proteins with creation of insoluble complexes that have a positive effect on the colloidal stability of beer.

Proantotsianidiny is most active compounds relative to the formation of polyphenol-protein complexes. Therefore, in case of a more hop preparations produced significantly more scrap at ohmelinni must and it is better illuminated. This is observed in our study with

only carbon dioxide hopping wort extract, hops extract and low-ash in different ratios and one low-ash hops. It produces a little hop scrap in case of hopping of wort only with extract without polyphenol, a little more than it produced in the second variant. We have seen that the 3 and 4th variants was significantly increased the amount of sediment. Most of it was 5 or 6th variants. But in these versions were the largest loss wort.

With increasing doses of hops low-ash reduced in the wort and beer of high rate increases the degree of polypeptides and proteins deposition saturated solution of ammonium sulfate that predicts high colloidal stability of beer.

The results of the tasting evaluation of the best by taste and aromatic qualities was beer produced using 40% low-ash bitterness of hops and 60% of bitterness due to CO_2-extract, and conversely. Compounds of low-ash hop positively affect the crude extract bitterness, softening and smoothing it, making a total bitter beer balanced.

Beer brewed only with CO_2-extract had rough, naked and residual bitterness. The reason for the poor quality of bitterness is bitter hop sorts, of which are produced CO_2-extracts and their lack of polyphenolic compounds. Beer at the stage of maturation doesn't illuminate. Was remained a significant number of high polypeptides due to lack of hop PF in the wort and it caused distraction. Moreover, an excess of high-protein, remaining in wort interacts with bitter substances of hops and reduces the extent of their use. Also complexes of bitter substances with proteins give beer a characteristic of unpleasant residual bitterness.

Quality bitter of beer was improved which along with CO_2-extract used 20% bitterness of low-ash hops.

5th and especially 6th version of beer, which used only low-ash for hopping had little astringent taste character for excessive amounts of hop polyphenols.

Obtained results testify that the formation of a full taste involved not only the bitter substances, but polyphenols, but not alone, but in conjunction with bitter substances, proteins and other specific compounds hops and wort.

Analysis of experimental brewing beer conducted by us show that polyphenols of low-ash aromatic hops contribute to the removal from the wart by coagulation macromolecular polypeptides to form complex systems, as a result we get higher colloidal stability of beer and the increased reliance on bitter substances by 15–20 %. Polyphenols of low-ash aromatic hops positively affect for gross bitterness CO_2- extract, softening and smoothing it, making a total bitter beer balanced.

Thus, low-ash hops in brewing can be used as an polyphenolic additive combined by extracts, processed it into pellets, which will cost significantly lower compared to conventional hop granules.

Technical and economic analysis shows that the use of polyphenol supplements low-ash aromatic hops in combination with CO_2-extracts in optimal conditions not only increases the quality of beer, as well as an effective means for the prevention of colloidal turbidity without using of stabilizing agents, reduces its costs and improves competitiveness.

Conclusions

1. Experimentally proved that the formation of flavor and aroma of beer mutually dependent for complex bitter core, essential and phenolic compounds hop.
2. Aroma of hop with a low content of alpha acids can be used in brewing as polyphenol supplement, if it was converted into granules and meets standards for all other indicators.

3. When we used in brewing only CO_2-extract is not possible to get a beer with high taste. It should be used in combination with aromatic or with aromatic low-ash hops, considering the quantitative and qualitative composition hops and following certain technology of beer.

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