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Advances in hop extraction with supercritical carbon dioxide

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Advances in hop extraction with supercritical carbon dioxide

SUSTAINABLE SOLUTION | In the past, hops have been extracted with supercritical carbon dioxide up to a maximum pressure of 300 bar. This year, an extraction plant will be commissioned that will operate at 500 bar of pressure. The primary motivation behind increasing the pressure to 500 bar is the energy savings gained due to the enhanced properties of CO_2 as a solvent at this pressure along with the greater contribution to sustainability. The hop extracts obtained at 500 bar are only slightly different than those extracted at 300 bar of pressure, which is the focus of this report.

DEPENDING ON THE PRESSURE and temperature, CO_2 exists in one of four states: gas, solid, liquid ore above the critical point supercritical, as depicted in figure 1 [1, p.168]. Liquid and supercritical CO_2 has a density of approximately 0.9 kg/liter. In this state, CO_2 behaves in some important ways like a fluid and is capable of dissolving and extracting certain substances in hops such as α-acids and β-acids in addition to aroma compounds. At present, extraction of these hop compounds with supercritical CO_2 is the preferred method.

Fig. 2 shows a flow diagram of an extraction process using supercritical CO_2. Hop pellets are placed in an extraction vessel and supercritical CO_2 is pumped through the vessel to extract the valuable substances in the hops. The pressure is reduced to <73 bar by means of an expansion valve and evaporated in a heat exchanger. As a gas, CO_2 loses its properties as a solvent and the extract is removed from the carbon dioxide in a separator. The CO_2 is then liquefied in a condenser, compressed with a high pressure pump to the requisite extraction pressure and heated to the target temperature in a heat exchanger. In the final step, the CO_2 flows back into the extraction vessel, effectively closing the loop in this batch process. After the process is complete, the extraction vessel is depressurized, the CO_2 is recovered and the vessel is emptied. After the vessel is filled with fresh pellets, the next extraction process commences.

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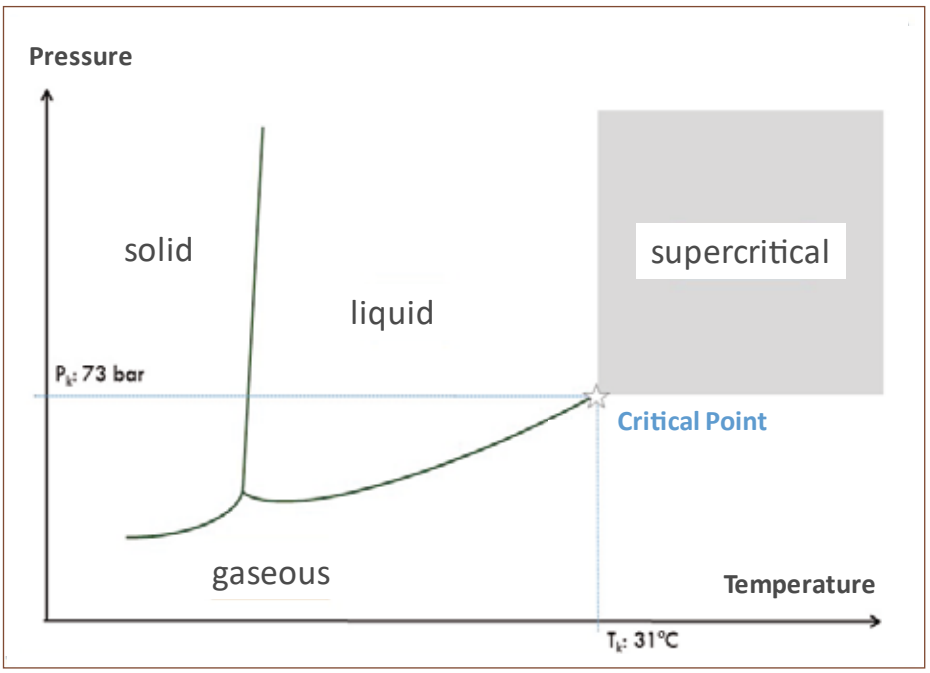


Fig. 1 Phase diagram of carbon dioxide (pressure/temperature)

All of the important substances in hops such as α-acids, β-acids and aroma compounds are brought into solution using supercritical CO_2. However, the polyphenols are insoluble [1, p. 170]. The inert atmosphere and moderate temperatures in this process suppress the chemical reactions which can take place when extracting with traditional solvents [1, p. 167].

Hop extraction at 500 bar of pressure

New findings have been made in recent years regarding the CO_2 extraction of natural substances. It has been demonstrated that the substances of interest are first soluble at pressures above 300 bar, which has spurred technical developments in this area [1, p. 170]. Through advances in the manufacture of the machinery and equipment involved in this production process, there are now extraction plants available with the capability of operating at 500 bar of pressure.

The reason behind this variation in the capacity of CO_2 to solubilize compounds is that density is dependent on pressure. At typical extraction temperatures of 50 to 60 °C, the density of CO_2 is 15 % higher at 500 bar than at 280 bar, for example. Extractions performed at 500 bar are characterized as follows:

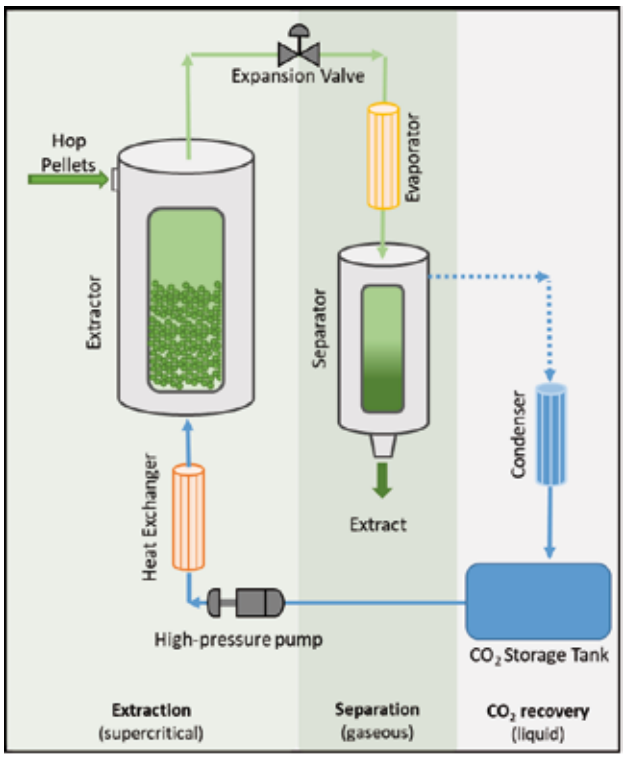
- The solvent properties of CO_2 improve at higher densities so that the quantity required for extraction can be reduced by 20 to 30 %. This, in turn, results in 20 % less energy consumption and a shorter extraction time.
- The extraction yield at 500 bar expressed in terms of mass is approximately 1 % higher based on pellets, meaning that slightly more compounds are extracted, which include small amounts of auxiliary hop bitter substances and xanthohumol.
- The 500 bar extracts are "greener" which indicates that also more chlorophyll is extracted from the hops. These extracts are also somewhat more viscous.

Comparison of 300 bar and 500 bar extracts

Exemplary for numerous comparisons of extracts, produced at lower pressures (260 to 300 bar) and at 500 bar a pair of extracts (300 and 500 bar) from a homogenous lot of Herkules pellets (2019 crop) here will be contrasted. The α-acid yield determined according to the EBC method 7.7 (HPLC) was almost identical: 97 % at 300 bar and 98 % at 500 bar. Expressed as a percentage in terms of mass, 26.2 % was measured for the 300-bar extract and 27.5 % for the 500-bar extract. The degree of enrichment can be calculated from these data: 3.82 kg pellets/kg extract at 300 bar and 3.64 kg pellets/kg extract at 500 bar. The degree of enrichment denotes how many kg of pellets are required to produce 1 kg of extract. The term "degree of enrichment" is also used to characterize the analytical metrics between extract and pellets and provides quantification of how many more times a substance recovered in the extract compared to the pellets. In an ideal situation, the degree of enrichment with regard to the mass and the substances recovered in the extract would be the same. The analysis error must be taken into consideration for each individual substance.

Essential analysis data for the pellets and the resulting extract as well as the corresponding degrees of enrichment are listed in

Fig. 2
Flow diagram of an extraction process using supercritical CO_2



BITTER SUBSTANCES IN THE PELLETS AND IN BOTH EXTRACTS...

...obtained at 300 and 500 bar of pressure (values in % by weight); degrees of enrichment (DoE; dimensionless)

Analysis criterion	Pellets	300 bar	DoE	500 bar	DoE
Weight yield		26.2	3,82	27.5	3.64
α-acids EBC 7.7	14.53	54.40		53.20	
α-acids EBC 7.9	14.69	54.27		53.34	
Average	14.62	54.43	3.72	53.30	3.64
β-acids EBC 7.7	4.50	17.05		16.00	
β-acids EBC 7.9	4.41	16.96		16.07	
Average	4.45	17.00	3.82	16.04	3.60
Xanthohumol EBC 7.9	0.72	traces	> 0	0.25	0.35
Humulinones EBC 7.9	0.24	nn		traces	> 0
Hulupones EBC 7.9	0.05	nn		traces	> 0

Table 1

CONCENTRATIONS OF INDIVIDUAL AROMA COMPOUNDS ...

... and the sums for groups of substances present in the pellets as well as in the 300 bar and 500 bar extracts; the respective degrees of enrichment are also listed

	Pellets	300 bar	DoE 300	500 bar	DoE 500
Myrcene	1013	3233	3.19	3212	3.17
β-Caryophyllene	70	279	3.99	298	4.26
Humulene	240	908	3,78	998	4.16
2-Methylbutyl-2-methylpropanoate	157	518	3.30	498	3.17
Linalool	8	28	3.50	28	3.50
2-Undecanon	11	39	3.55	39	3.55
Geraniol	13	44	3.38	43	3.31
Humulenepoxide II	7	19	2.71	18	2.57
Hydrocarbon fraction	1412	4747	3.36	4847	3.43
Oxagen fraction	384	1211	3.15	1181	3.08
Sum of all GC components	1796	5958	3.18	6028	3.36
Sum of esters	310	986	3.25	956	3.08
Sum of monoterpene alkohols	24	78	3.12	76	3.17
Sum of sesquiterpene alkohols	14	35	2.50	36.5	2.61
Sum of ketones	36	126	3.50	124.5	3.36
Sum of epoxides	11	28	2.55	27	2.45

Table 2

table 1. These data include bitter substances such as α-acids and β-acids measured using HPLC according to the EBC methods 7.7 and 7.9, their mean values and several auxiliary hop bitter substances according to the EBC method 7.9. The working group led by T. Hofmann has extensively researched and documented the positive contribution of numerous auxiliary bitter substances in hops [2, 3]. A summary of these findings can be found in [1, p. 225. The results for the enrichment expressed as a percentage by weight show a very good correlation to the degrees of enrichment determined in the individual analyses. Moreover, the values for the bitter acids between both extracts correspond closely.

Table 2 lists several individual compounds as well as the sums for some groups of aroma compounds in extracts and pellets with the degrees of enrichment. The two extracts do not exhibit any variation in the absolute concentrations of these compounds or in the individual degrees of enrichment.

The dynamic viscosities of both extracts show no remarkable differences. The 500 bar-extract is somewhat more viscous and it should be checked in each individual case whether the viscosity of 1 to 2 Pa·s, recommended for automatic dosing systems, will necessitate to slightly increase the temperature in the pre-warming step. However, the viscosity fluctuations within different varieties and crop years are higher than those between 300 and 500 bar.

A comparison of plant protection agents in pellets and extracts is provided in table 3. The transfer rates (TR) of those to both extracts based on the quantities initially present in the pellets can be calculated according to the equation below:

$$\textit{Transfer rate} = \frac{\textit{(value measured in extract)}}{\textit{(value measured in pellets)}} x \; \textit{degree of enrichment} \; x \; 100\,\%$$

The transfer rates may exhibit variations of up to 20 % due to the inherent error associated with the analysis. There were no significant differences observed between the 300 bar and 500 bar extracts. The plant protection agents measured showed a broad range of variation, from 0 to 95 %, depending on their chemical structure. The sums of residues for both extracts were nearly identical, measured at 150 mg/kg for the 300 bar extract and 151 mg/kg for the 500 bar extract. The transfer rates calculated from these values came to 75 % for the 300 bar extract and 79 % for the 500 bar extract. Consequently, the higher pressure did not result in any change in solubility of plant protecting agents.

Table 4 lists the concentration of several relevant metals in pellets and extracts as well as their transfer rates from pellets to the extract. Arsenic and cadmium were not detected in the hop pellets. The lead and mercury residues in the pellets were retained in the spent hops and were thus not extracted at either 300 bar or 500 bar of pressure.

In addition, most of the iron and copper present was not extracted. The transfer rates for iron and copper were below 1 % and 10 %, respectively, relative to the initial concentration in the hops. This correlates with the data reported for copper [1, p. 170], which lists a recovery rate of <15 %. No differences based on the extraction pressure could be inferred.

The nitrate values for both extracts are below the limit of detection.

Internal brewery trials

Since 2016, 11 comparative trials of beers have been performed at our 2 hl pilot brewery in St. Johann, Germany. Each of the beers received the same amount of αacids, added in the form of extract at the beginning of the boil. Lower pressure hop extracts as well as 500 bar extracts were used. The varieties Herkules, Magnum and Perle were selected for the trials. The assessment of the bitter substance yield (BSY) was carried out in two ways:

$$\textit{1. Specific BSY} = \frac{\textit{iso α-acids in the beer (mg/l)}}{\textit{α-acids in the hop addition (mg/l)}} \text{ x } 100\,\%$$

Measurements were performed with HPLC according to the EBC method 9.47 in beer and the EBC methods 7.7/7.9 in hop extract.

$$\textit{2. Unspecific BSY} = \frac{\textit{bitterness of the beer (IBU)}}{\textit{bitter substances added-LCV (mg/l)}} \text{ x } 100\,\%$$

IBU in the beer was determined according to the EBC method 9.8, while the lead conductance value (LCV) in the extract was measured according to the EBC method 7.6.

There were no significant differences recorded in the bitter substance yields. Besides trace amounts of isoxanthohumol in the

beers brewed with the 500 bar hop extract, no analytical differences between the beers could be observed.

Comprehensive tastings and numerous triangle tests (n=316) showed no significant sensory differences between the beers, neither in total nor in the individual comparisons.

External brewery trials

In addition, 300 and 500 bar extracts from a homogeneous lot of pellets produced from the Herkules variety were tested parallel to the pilot brewery trials in a 20 hl brewery. The basic recipe was for a bottom-fermented all malt beer with a bitterness of approximately 25 bittering units. The malt used to brew each batch of beer in both breweries was identical.

The bitter substance yields measured in both brewhouses in the preliminary trials deviated from one another. The BSY in the smaller 2 hl wort kettle was significantly lower when extracts were used. Consequently, the required hop addition at the beginning of the boil in the external trial (20 hl) was found to be 6.50 g/hl of α-acids, while at St. Johann, it was 9.55 g/hl. The 20 hl brewhouse is equipped with an internal calandria and operates at an overpressure of 90 mbar. At St. Johann, the wort is also boiled using an internal calandria; however, the boil is conducted at atmospheric pressure. The duration of the boil in the 20 hl brewhouse was 64 min with an additional 5 min to release the overpressure. In the 2 hl kettle, the wort was boiled for 70 min. A special mix of yeast is used at the external brewery, while at St. Johann, strain W34 was employed. The 20 hl batch of beer was stabilized with PVPP (10 g/hl) and silica gel (30 g/hl) prior to filtration with diatomaceous earth. The beers brewed at St. Johann were not stabilized. In order to facilitate a sound statistical evaluation, three batches of beer were brewed with the 300 and 500 bar extracts.

The three individual trials were analyzed separately. The mean values for the three batches and their standard deviations are presented in table 5. Besides the original gravity, alcohol content and pH, the bittering units, the iso α-acids and the α-acids analyzed using HPLC according to the EBC method 9.47 as well as the isoxanthohumol content and foam values are listed in the table. The very low standard deviations for the three batches fall within the range for the standard deviations from triple determinations, i.e. within the analysis error according to MEBAK [4]. The reproducibility of the batches can thus be characterized as very good. The respective mean values for the attributes analyzed in the 300 and 500 bar beers were consistent across both breweries.

The specific and unspecific bitter substance yields were calculated as per table 6. In this case as well, no substantial differences between both types of extract can be inferred.

The beers brewed in the 20 hl brewhouse were sensorially evaluated by an extended tasting panel from BarthHaas, HVG and St. Johann. In a tetrad test [5] the 29 participants classified the 300 bar and 500 bar beers correctly 11 times and 18 times incorrectly. This means that no significant differences have been assessed between both sets of beers. A triangle test with 32 participants yielded similar results with 10 correct and 22 incorrect groupings, an outcome far off any significance regarding differences between them [6].

The beers from St. Johann were evaluated by the regular tasting panel. A tetrad test was conducted with 17 participants who classified the beers correctly eight times and incorrectly nine times. These results also

CONCENTRATION OF PLANT PROTECTION AGENTS RESIDUES ...

... in hop pellets and hop extracts in mg/kg along with their transfer rates (values in extract : pellet dosage) expressed in % relative to the initial concentration

	Pellets	300 bar	TR	500 bar	TR
Ametoctradin	0.93	0.35	11	0.70	21
Boscalid	10.7	26	71	29	75
Dimethomorph	0.48	0.75	23	1.0	57
TFNG	1.15	-		-	
Flonicamid	1.1	-		-	
Fluopicolide	0.030	0.095	83	0.090	83
Mandipropamid	25.3	80.0	84	79.5	86
Metrafenon	8.4	30.5	98	28.5	93
Myclobutanil	3.08	8.95	78	8.50	76
Pyraclostrobin	1.28	3.55	78	3.65	78
Spirotetramat incl. metabolites	0.2	-		-	
Sum / Average of TR		150.2	75	150.9	79

Table 3

CONCENTRATION OF METALS IN HOP PELLETS AND HOP EXTRACTS IN MG/KG ...

... as well as their transfer rates from pellets to the extract expressed in % relative to the initial concentration

Metal	Pellets 300 bar	Extract 300 bar	TR	Extract 500 bar	TR
Arsenic	< LOQ	< LOQ		< LOQ	
Lead	0.08	< LOQ		< LOQ	
Cadmium	< LOQ	< LOQ		< LOQ	
Iron	77	1.15	0.4	2.4	0.9
Copper	8.4	2.9	8.9	2.8	9.1
Mercury	0.01	< LOQ		< LOQ	

< LOQ = below limit of quantitation; < LOD = below limit of detection

Table 4

MEAN VALUES FOR SOME OF THE ANALYSIS RESULTS ...

... (Ø = average for three batches of wort) and the standard deviations (SD) for both breweries

		20 hl				2 hl			
		300 bar		500 bar		300 bar		500 bar	
		Ø	SD	Ø	SD	Ø	SD	Ø	SD
Original gravity	% w/w	11.61	0.01	11.59	0.01	12.66	0.34	12.86	0.20
Alcohol	% ABV	5.00	0.00	5.00	0.00	5.69	0.11	5.77	0.08
pH		4.62	0.03	4.65	0.08	4.57	0.03	4.61	0.06
Bitterness	IBU	26.7	0.3	27.0	0.0	22.3	0.6	22.3	0.6
Iso-α-acids	mg/l	27.8	1.1	27.3	0.7	22.7	0.4	22.9	0.9
α-acids	mg/l	1.4	0.2	1.2	0.1	2.8	0.8	2.4	0.8
Isoxanthohumol	mg/l	nn		Spur		nn		0.1	
Foam (Nibem)	s	251	5	257	5	221	18	216	21

Table 5

SPECIFIC AND NON-SPECIFIC BITTER SUBSTANCE YIELDS OBTAINED IN BOTH BREWERIES

		20 hl		2 hl	
		300bar	500bar	300bar	500bar
Dosed α-acids	mg/l	65.0	65.0	95.5	95.5
Dosed Lead conductance value (LCV)	mg/l	71.6	74.1	105.2	108.9
Iso-α-acids	mg/l	27.8	27.3	22.7	22.9
Bitterness	IBU	26.7	27.0	22.3	22.3
Specific bitter substance yield[1)]	%-rel.	42.8	42.0	23.8	24.0
Unpecific bitter substance yield [2)]	%-rel.	37.3	36.4	21.1	20.5

1) Iso-α-acids in beer: αacids dosed x 100 %
2) Bitterness (IBU) in beer : LCV dosed x 100 %

Table 6

CONCENTRATIONS OF α-ACIDS AND β-ACIDS EXPRESSED IN PERCENT ...

... as well as the sum of all aroma compounds detected with gas chromatography in hop extract stored for more than 51 months at 20 °C

	CO_2 280 bar	CO_2 500 bar	CO_2 280 bar	CO_2 500 bar
	HKS aged	HKS aged	HKS normal	HKS normal
α-acids	99	98	98	97
β-acids	100	100	99	99
Sum of all aroma compounds	99	100	84	84

Table 7

showed there was no significant difference. The triangle test yielded a comparable outcome, with 18 participants classifying the beers correctly seven times and incorrectly 11 times. According to the DLG tasting scheme, the beers were assessed as comparable, both visually and sensorially. The total score for the evaluated beers varied between 4.35 and 4.47 points. The mean values for the three 300 bar beers and the three 500 bar beers were virtually identical at 4.42 and 4.41 points, respectively. The Kramer rank sum test [7] showed that no significant preference for any of the beers existed, and the beers were almost equal (53 % vs. 47 %) in order of preference.

Furthermore, no differences in hop aroma were discovered in a tasting conducted according to the guidelines of the CMA for hop-accentuated beers. Most especially, none were apparent in the harmony of the bitterness (7.8 vs. 7.6 points) or in the intensity of the bitterness (both scored 5.0 points).

Hop extracts and aging

Four CO_2 hop extracts were stored over several years at 20 °C. Since the trials have not been completed, only the most relevant results to date are presented here.

A comparison of extracts of the variety Herkules was performed with two-year old pellets and fresh pellets extracted at 280 and 500 bar, respectively. The duration of the 20 °C storage trials has reached 51 months (± 1 month) so far. The following analyses were carried out:

- α- and β-acids according to the EBC methods 7.7 and 7.9;
- determination of aroma compounds using gas chromatography, adapted from MEBAK.

A total of 81 identified and quantified aroma compounds encompass over 99 % of the areas under the peaks (= the sum of all GC compounds).

Table 7 presents the percentage change in the stored samples compared to the original values.

The following observations have been derived from the results:

- A relative loss in α-acids of 1.5 % was calculated for the 280 bar extracts, while the relative loss was 2.5 % for the 500 bar

extracts. In both cases, these fall within the acceptable range for analysis error.

- The β-acids in all of the extracts are also stable. Oxygen is required for them to react [1, p. 161].
- The sums of the calibrated aroma compounds using a gas chromatograph were almost identical for both pressures.
- The CO_2 extracts could not be differentiated by their α-acids nor by their aroma compounds in the storage trials. Whether they were extracted at 280 bar or 500 bar did not play a role in the stability of the extract.

Summary

Supercritical CO_2 is the preferred solvent for extracting hop compounds at this time. The extraction process is normally carried out at 300 bar. Since equipment has now been developed to allow this process to be performed at 500 bar, a large industrial facility will be commissioned this year to carry out hop extraction at that pressure. Because the density of CO_2 is higher at 500 bar, this enhances its solvent properties. Therefore, the amount of CO_2 circulating through the system and the time required for the extraction can be reduced. This results in energy savings of around 20 %; however, the investment in the equipment is higher. Substances can be extracted with CO_2 at 500 bar which are insoluble at 300 bar. Besides containing more chlorophyll – the extract is "greener" – the extracts are slightly richer in xanthohumol and contain more positive auxiliary bitter substances from the hops. The extract yield based on the quantity of pellets is almost 1 % higher at 500 bar than at 300 bar.

Numerous brewing trials have shown that the bitter substance yield achieved with both extracts is practically identical. From an analytical standpoint, the beers can only be differentiated according to the slightly higher amount of isoxanthohumol in the 500 bar extract. All tasting results indicate that no significant differences exist in the sensory profile of beers brewed with the 300 bar and 500 bar extracts. This is, however, logical, since the differences between the two are already marginal. How the process is carried out has not changed at all – the extraction has simply been optimized. ■

Literature

1. Biendl M.; Engelhard B.; Forster A.; Gahr A.; Lutz A.; Mitter W.; Schmidt R.; Schönberger C.: "Hops – Their Cultivation, Composition and Usage"; Fachverlag Hans Carl, Nuremberg, 2014, ISBN: 978-3-418-00823-3.
2. Haseleu, G.: "Sensorische, strukturanalytische und quantitative Studien zu Bitterstoffen aus Hopfen (Humulus Lupulus L.) und deren Beitrag zum Bittergeschmack von Bier", doctoral thesis, TU München, 2010.
3. Intelmann, D.: "Molekulare psychophysikalische und rezeptorbasierte Studien zum Bittergeschmack von Bier", doctoral thesis, TU München, 2010.
4. MEBAK, "Würze, Bier, Biermischgetränke", in: Brautechnische Analysenmethoden, Selbstverlag der MEBAK, Freising-Weihenstephan, 2012.
5. Ennis, J. M.; Jesionka, V.: "The Power of Sensory Discrimination Methods Revisited", Journal of Sensory Studies 26 (2011), pp. 371-382.
6. MEBAK Methodensammlung, "Sensorik", 2013, 3.1.3 Dreieckprüfung (according to DIN EN ISO 4120:2007-10), pp. 59-65.
7. Kramer, A.: "Chemical Senses and Flavor", 1 (1974), pp. 121-133.

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