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Quality control of beer hopped with reduced isomerised products

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SUMMARY. The traditional method for measuring bitterness in beer by UV absorbance (ASBC 9.6) remains a viable quality control method for normally hopped beer. However, after reduction of isoalpha acids specific absorptivity of active components change and HPLC analysis shows to be a reliable method for quality control of these materials. This work is intended to adapt mathematically the ASBC (American Society of Brewing Chemist) spectrophotometrical method and to evaluate an HPLC method to control the composition of different commercial reduced isomerised products added to unhopped wort and partially kettle hopped beer. The results we obtained showed that the HPLC method is reliable for quality control of reduced isomerised products and that the traditional spectrophotometrical method is viable for quality control of beer, also using reduced isomerised products as far as an adequate factor is used.
Key words: Hops, *Humulus lupulus*, beer.

RESUMO. Controle de qualidade do amargor em cerveja obtida pela adição de produtos isomerizados e reduzidos de lúpulo. O método tradicional de medição do amargor em cerveja por absorbância no UV (ASBC 9.6) permanece viável para o controle de qualidade de cervejas lupuladas normalmente Contudo, após adição dos ácidos iso-alfa, a absortividade específica dos componentes ativos muda e análise de HPLC mostra-se um método adequado para o controle de qualidade destes materiais. Este trabalho pretende adaptar matematicamente o método espectrofotométrico da ASBC (Sociedade Americana de Químicos Cervejeiros) e avaliar um método de HPLC para controlar a composição de diferentes produtos comerciais de lúpulo reduzido e isomerizado, adicionados ao mosto não lupulado e cerveja parcialmente lupulada na caldeira. Os resultados que obtivemos mostraram que o método de HPLC é adequado para o controle de qualidade de produtos reduzidos e isomerizados e que o método espectrofotométrico tradicional é viável para o controle de qualidade de cervejas, mesmo que utilizando produtos reduzidos e isomerizados. Desde que fatores específicos sejam estabelecidos.
Palavras chaves: Lúpulo, *Humulus lupulus*, cerveja.

INTRODUCTION

Toward the end of middle ages, hops (*Humulus lupulus*), a wild plant native to Europe and western Asia, became widely used to bitter beer. Hop belongs to the family Cannabinaceae presenting male and female flowers on separated plants. Although hops have had a traditional role in medicine and baking (1), they are used almost exclusively to provide aroma and bitternes to beer. Only female plants have the chemical constituents that are used in the brewing process. These contain small yellow granules called lupulin glands consisting of hard and soft resins, and essential oils.

The soft resins (alpha and beta acids) are converted, during wort boiling, into bitter substances in the beer. A need to produce specialty beers to attract and hold a particular group of consumers increased the use of reduced isomerised products (Dihydro-isoalpha, Hexahydro-isoalpha and Tetrahydro-isoalpha acids) as post fermentation additives and has been accompanied by substantial efforts to provide more accurate and yet simple methods of analysis for the brewer.

In addition to whole hops (hop cones) and pelletized hops, other hop products and extracts are used either as alpha acids added to the kettle or already isomerised after fermentation.

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In addition to whole hops (hop cones) and pelletized hops, other hop products and extracts are used either as alpha acids added to the kettle or already isomerised after fermentation. The first isomerised hop products became commercially available in the late 50's as an impure post fermentation bittering agent which frequently caused gushing and beer haze. They were produced by extraction with hexane, methylene chloride, ethanol, benzene or toluene. Liquid and than supercritical carbon dioxide were later applied to produce clear and less impure extracts. Ranges of percentage distribution of relevant compounds of hops and hop extracts were published by Moir (2) and are presented in Table 1.

TABLE 1
Composition of hop and hop extracts (% W/W) (2)

Component	Whole Hops	Organic Solvent ext.	Super Critical CO_2	Liquid CO_2
Total resin	12 - 20	15 - 60	79 - 90	70 - 95
Alpha acids	2 - 12	8 - 45	27 - 55	30 - 60
Beta acids	2 - 10	8 - 20	23 - 33	15 - 45
Essential oils	0,5 - 1,50	0 - 5	1 - 5	2 - 10
Hard resins	2 - 4	2 - 10	5 - 11	—
Tannins	4 - 100	0,5 - 5	0,1 - 5	—
Waxes	1 - 5	1 - 20	4 - 13	0 - 10
Water	8 - 12	1 - 15	1 - 7	1 - 5

Nowadays we find four main categories of isomerised products: isomerised hop powder/pellets, isomerised resin extract, isomerised hop powder/pellets, isomerised resin extract, isomerised post fermentation extract and reduced isomerised products. The principal reason for their development was to improve the conversion of hop alpha acids into isoalpha acids and spectifically reduced isomerised products to add also light stability. It has long been known that isoalpha acids in the presence of sunlight produce a «skunky» or «light struck» flavor in beer. This is why beer is packaged in light-proof bottles. Research on the chemical transformation of isoalpha acids (isohumulones) revealed that their capacity to produce skunkiness is lost after reduction. Practical brewing experience shows that different forms of reduced hop components differ considerably in their bitterness and also in their capacity to improve foam stability (2-3).

The ASBC (4) rapid method for determination of bitterness in wort and beer relies on the extraction of bitter substances with isooctane from acidified sample and measurement of the absorbance value at 275 nm. To provide a bitterness value in terms of bitterness units (BU), the absorbance is multiplied by a factor of 50. However, this factor is not applicable to beers bittered by hop products such as reduced isomerised extracts.

The objective of this work was to adapt mathematically the ASBC method for bitterness evaluation when reduced isomerised products are used and also to evaluate the adequacy of a HPLC isocratic method to control the composition of different reduced isomerised products.

MATERIAL AND METHODS

Sample: Samples of pure reduced isomerised products were generously provided by two different suppliers. These products are commercially avialable in various forms such as alkaline solutions with water or water/propylen glycol as carrier, or as aqueous solutions of the potassium salts of hop acids. The concentration varies from 5 to 40% hop acids (isoalpha acids (IAA), Dihydro-isoalpha acids (DHIAA), Hexahydro-isoalpha acids (HHIAA) and Tetrahydro-isoalpha acids (THIAA) in different products. Wort and beer samples were obtained from a Brazilian brewery. Isoalpha acids (TIAA) were individually and as a mixture added to unhopped wort (100% hopped with mixtures I, II, III, IV and V) and normally hopped beer (50% kettle hopped and 50% hopped with mixtures I, II, III, IV and V). We elaborated 5 mixtures with different proportions of IAA, DHIAA, HHIAA and THIAA according to Table 2. The pure products were analyzed by HPLC and bitterness of beer and wort samples were determined spectrophotometrically before and after addition of the 5 mixtures, following the ASBC method.

ASBC spectrophotometrical method: The following procedure is a rapid method for measuring bitterness in beer and wort, described by ASBC 9.6 (4). Beer and wort samples were degassed and centrifuged, respectively. To aliquots of 10 mL of each sample, 0,5 mL of HCl 6N and 20 mL isooctane were added in a centrifugal 50 mL flask. These flasks were shaken for 15 min and then centrifuged for 10 min at 3000 rpm. Finally the absorbance of the isooctane phase was spectrophotometrically measured at 275 nm.

HPLC method: the HPLC procedure was based on the method described by Buckee, 1990 (5). Each pure bittering agent was accurately weighted (0,0500 g) into a 10 mL volumetric flask, diluted to volume with a acetronitrile - 1% acid solution, pH 7,0 (40:60) and diluted at 1:24 rate in the same solvent prior to chromatography, long-pair chromatography was applied using an isocratic HPLC system, which consisted of a Lichrospher 100 RP-18 (5um - 250x4 mm) column (Merck, Germany), an HPLC pump model LC-10 AAD, UV/VIS detector model SPD-10 AV at 254 nm and an integrator model CR6-A (Shimadzu, Japan). The column was equilibrated and eluted with the mobile phase at a flow rate of 0,5 mL/min at room temperataure. The mobile phase was prepared by mixing 360 mL of aqueous buffer (3,83 g of 85 % phosphoric acid with 42 mL of tetrabytylammonium hydroxide 0,4 mol/L solution and about 900 mL HPLC grade water) at pH 7,4 and 840 mL of organic solution (160 mL acetonitrile with 900 mL methanol).

RESULTS AND DISCUSSION

ASBC spectrophotometrical method: The factor of 50 is an empirical factor, from which an implied specific absorptivity ($E_{1cm}^{1\%}$) of 400 for isoalpha acids from hop can be derived from the following formula (6):

$$50 = \frac{2}{E_{1cm}^{1\%} \times 10^{-4}}$$

This factor of 50 is used to discount the reading due to the presence of non-isoalpha acid substances in the beer which are extracted into the isooctane and also absorb at 275nm. If only pure, reduced or not, isoalpha acids are added to beer, it is inappropriate to discount the absorbance reading and the factors in Table 3 (6) must be used to give BU's. In Table 3 we can see the specific absorptivity and the bittering factor published by *Todd Johnson & Worden* (6).

The bittering factors for the five mixtures shown in Table 2 were theoretically and experimentally determined after adding them to unhopped wort and normally hopped beer.

TABLE 2
Composition of product mixtures - % (W/W)

Pure Products	Mixture I	Mixture II	Mixture III	Mixture IV	Mixture V
Isoalpha acids (IAA)	-	10 %	-	10 %	25 %
Tetrahydro -isoalpha acids (THIAA)	15 %	10 %	25 %	20 %	25 %
Hexahydro - isoalpha acids (HHIAA)	15 %	10 %	25 %	20 %	25 %
Dihydro -isoalpha acids (DHIAA)	70 %	70 %	50 %	50 %	25 %

TABLE 3
Absorptivity and bittering factors of pure bittering agents in isooctane at 275 nm

Pure Products	$E_{1cm}^{1\%}$ (Absorptivity)	Factors
Isoalpha acids (IAA)	285	70,2
Tetrahydro -Isoalpha acids (THIAA)	275	72,7
Hexahydro -isoalpha acids (HHIAA)	245	96,1
Dihydro -isoalpha acids (DHIAA)	276	72,5

Unhopped wort sample: We knew previously how much mg/L (BU) bitterness was added to each wort sample then the absorbance was measured and using the formula below the experimental factors were determined.

$$BU = A_{275} \times F$$

The theoretical factors were achieved substituting the emperical factor of 50, which multiplies the absorbance of the isooctane extract, for the known bittering factors (see Table 4). Using the proportion of each pure product in each mixture we calculated the weighted average. For an unhopped wort, the bittering will be due to the added mixture, consequently, the bittering factors (F) for the mixtures are calculated by the expression:

$$F = \frac{(\%IAA \times 70,2) + (\%THIAA \times 72,7) + (\%HHIAA \times 96,1) + (\%DHIAA \times 72,5)}{100}$$

TABLE 4
Experimental and theoretical factors of wort and beer added with the experimental isoalpha acid mixtures

Products	I	II	III	IV	V
Theor. Factors	76,1	74,7	78,5	77,0	77,9
Exp. Factors	75,8	78,5	76,4	76,8	78,0
Unhopped wort					
Average	75,84	78,54	76,35	76,83	77,98
SD	3,9	3,2	3,3	2,8	1,4
CV %	5,0	4,0	4,5	3,5	2,0
Theor. Factors	63,1	62,4	64,3	62,9	64,0
Exp. Factors	63,7	63,1	62,8	62,9	64,0
50 % Normally Hopped Beer					
Average	63,70	63,10	62,79	62,93	63,97
SD	1,6	1,3	3,1	2,5	2,2
CV %	2,5	2,0	5,0	4,0	3,5

Partially hopped beer sample

The experimental factors were achieved as described above for wort samples and the theoretical factors were achieved calculating the weighted average of the bitterness contribution of the mixture and kettle hopping. For partially hopped beers, the bitterness will be due to the both kettle hopping, which must be measured by using the conventional factor of 50, and to the added mixture. The partially hopped beer factors are calculated by the expression:

$$F = \frac{(\%\ \text{mixture} \times F\ \text{mixture}) + (\%\ \text{kettle hopped} \times 50)}{100}$$

Table 4 shows the condensed results of unhopped worts and 50 % kettle hopped beers.

The data showed that we can find the right factor for unhopped and partially hopped beers, using reduced isomerised products, so the traditional method for measuring bitterness by UV absorbance remains viable for quality control. The brewer must only know their own product composition and how he is going to brew.

HPLC method: The most common bittering agent is a mixture of the various geometric isomers and analogs of the iso-alpha acids (isohumulones), which are normally produced by addition of hops to boiling wort. The iso-alpha acids can be converted to the THIAA by catalytical hydrogenation and can be also formed from beta acids as starting material. an insufficient hydrogenation reaction can result in DHIAA where the double bond adjacent to the carbonyl group remains. The THIAA produced from beta or alpha acids can be converted to the HHIAA, which contains both the saturated double bond and a secondary alcohol group on its side chain. Figures 1

(a,b,c), 2 (a,b,c), 3 (a,b,c) and 4 (a.b.c) show their chemical structures (7).

Each isomerised product, reduced or not, has a CIS and TRANS isomer and therefore there are six different components: co-. ad-and n-humulone/CIS and co-, ad-and n-humulone/TRANS. Complete separation of the six main iso-alpha acids by HPLC is difficult and leads to long analysis time. In practice, HPLC methods usually separate only the cohomologues from the normal and ad-isomers.

Many laboratories have examined and published HPLC methods for the analysis of hops, hop products and beer bitterness. These methods revealed some disadvantages, such as, the use of gradient solvent elution, complex composition of mobile phase and relatively long analysis time.

FIGURES

1, 2, 3 and 4. Chemical structures

Figure 1

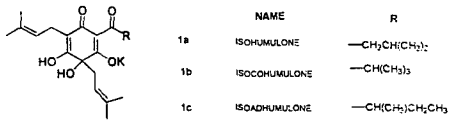


Figure 2 The reduced iso-α-acids

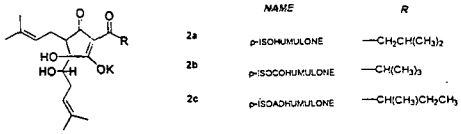


Figure 3 The tetrahydroiso-α-acids

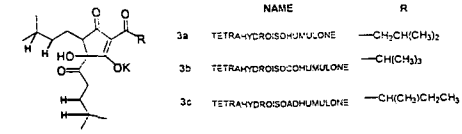


Figure 4 The hexahydroiso-α-acids

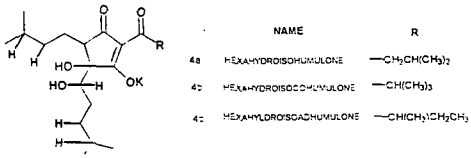
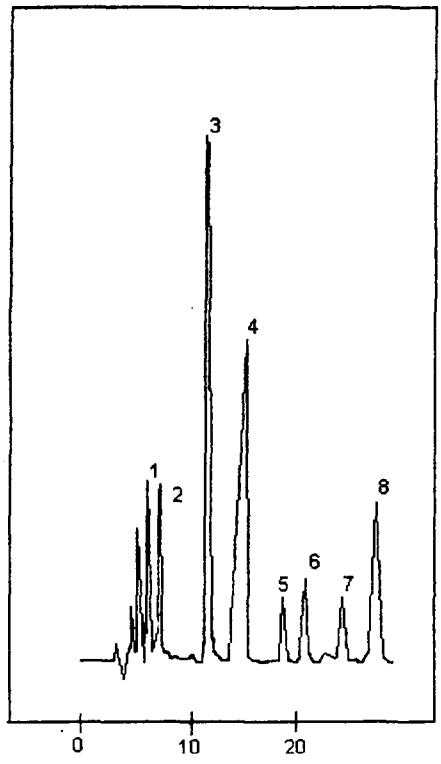


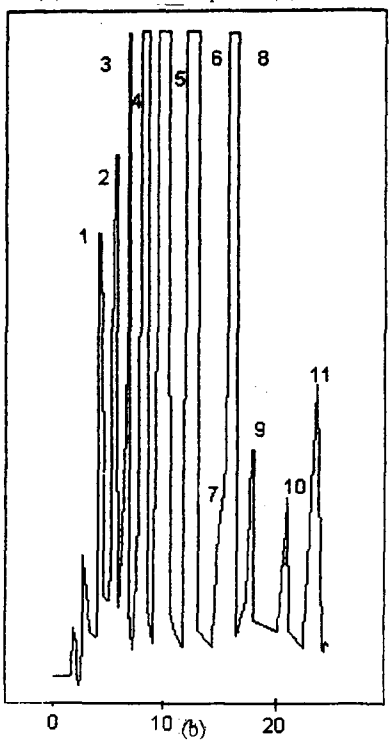
FIGURE 5

Chromatograms of the mixture: (a) IAA with THIAA; and (b) DHIAA, IAA, HHIA with THIAA



(a)

HPLC analysis (4) of IAA and THIAA from hop extract or RP-18 column. Peaks 1,2,3 and 4-IAA and peaks 5,6,7 and 8-THIAA



(b)

HPLC analysis (4) of IAA, DHIAA, HHIAA and THIAA from mixtre V (Table 2) on RP-18 columm. Peaks 1 and 2 -IAA, peaks 3 and 4 -DHIAA, peak 5 -DHIAA and IAA, peak 6 -IAA and HIAA, peaks 7 and 8 -THIAA and HHIAA, peaks 9,10 and 11 -THIAA.

Therefore, the aim was to find a system, sufficiently simple for routine analysis of commercial products composition. This is possible with an isocratic ion-pairing method on RP-18 column. Based on the procedure described by *Buckee* (4) the single products (IAA, HHIAA, THIAA and DHIAA) were adequately separated into 4 main components with a maximal run time of 30 minutes, but the method was not appropriated for mixtures of IAA, HHIAA, THIAA and DHIAA, all together. However, the mixtures commercially available do not have such a complex composition. Figure 2a shows a chromatogram of IAA whith THIAA; peaks 1, 2, 3 and 4 represent IAA and peaks 5, 6, 7 and 8 represent THIAA. So, this HPLC method can be used to measure reduced and unreduced hop acids. Figure 2b shows a chromatogram of the complex mixture V (Table 2) where we can see that the peaks 4, 5, 6 and 8 coelute with other isomers of DHIAA, IAA, HHIA and THIAA.

CONCLUSIONS

There are a large array of analytical procedures published for the analysis of the post-fermentation bittering agents and the best of these is certainly HPLC with UV detection. The HPLC method used in this work shows to be a reliable method for quality control of these materials.

The results obtaines showed that the traditional method for measuring bitterness by UV absorbance remains viable for quality control of beer using reduced isomerised products.

Nowadays breweries have greater freedom in choosing hopping procedures and in practice, mixed solutions are often adopted, using a blend of hops / processed products. Both, HPLC and the spectrophotometrical analysis, represent adequate approaches for quality control. The first for product composition and the latter for beer bitternes.

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