Composition of Bitter Substances of Hops and Characteristics of Beer Bitterness[1]

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ABSTRACT

On the basis of available information on the chemical nature of the bitter components of beer, a technique was established for evaluation of beer bitterness. A new concept, "the constitution of beer bitterness," was proposed for evaluating the quality and quantity of beer bitterness. This evaluation is based on both the contents of the bitter components of beer (measured by ion-exchange chromatography) and the bitterness of the individual components (judged by the taste test). The bitterness of various domestic and imported beers was evaluated in this way. Results showed that the bitterness intensity and characteristics of beers by reference to bitterness unit and the iso-α-acids content could be evaluated from the constitution of beer bitterness. The freshness of hops and beer and the type of bittering agent used could also be assessed in this way.

Key words: *Bittering agent, Evaluation, Ion-exchange chromatography, Iso-α-acids, Oxidation products.*

Previously, we found by ion-exchange chromatography that at least 90 components contribute to the bitterness of beer. Besides the well-known compounds, α-acids, iso-α-acids, and hulupones, many new components such as lupoxes a, b, and c (5,8), lupdoxes a and b (5,8), lupdeps (9), and lupdols (9) which cause beer bitterness have subsequently been isolated and characterized. Recently, we confirmed by ion-exchange chromatography that the compounds reported by Ashurst and Elvidge (2), Verzele and Vanhoey (11), Connet and Elvidge (3), Laws and McGuinness (10), and Howard and Slater (4) were also included among these 90 components[2], and most of the major bitter components of beer have now been characterized. Therefore, based on these findings we attempted to establish a new technique for evaluating beer bitterness, and we propose a new concept, "the constitution of beer bitterness," for evaluating beer bitterness. The evaluation is based on the contents of bitter components and the bitterness of the individual components, providing a more accurate assessment of beer bitterness than other criteria used so far.

EXPERIMENTAL

Beers

Commercial beers were purchased in July, 1974. Other beers were prepared using our pilot brewing facilities (on a 90-liter scale) by a standard procedure using commercial unhopped wort which was boiled for 90 min with the test bittering agents. The amounts of bittering agents added were 2.4 g/l. for natural hops, 140 mg/l. for pure α-acids and their decomposition products, and 120 mg/l. for pure β-acids and their decomposition products. Samples of deteriorated hops were prepared by storing fresh hops at room temperature for 1 and 3 months. Pure α-acids and β-acids were isolated from the hops by the conventional method. The decomposition products of α-acids were prepared by heating pure α-acids in hexane at 50°C for 4 days, and those of β-acids were prepared by standing β-acids in petri dishes in contact with air at 40°C for 2 days.

Determination of Bitterness Units and Iso-α-Acids Content

Bitterness units were determined by the standard method of ASBC (1) and iso-α-acids were determined by ion-exchange chromatography as described previously (6).

Extraction and Determination of Beer Bitter Substances

Bitter substances were extracted from the acidified beer with chloroform and the extracts were weighed to measure the apparent content of bitter substances (7). Unhopped beer was extracted in the same manner and an average of 58 mg of extract (average of 5 determinations) per liter was obtained from unhopped beer. Therefore, the true content of bitter substances in beer (in mg/l.) was calculated by subtracting the blank value, 58, from the apparent content.

Fractionation of Beer Bitter Substances

The bitter substances extracted from beer were applied to a column of silicic acid (Mallinckrodt Chemical Works, 100 mesh, 1.7 × 25 cm). The column was eluted successively with mixtures of ethyl acetate and hexane in ratios (v/v) of 1:15 (250 ml), 1:3 (200 ml) and 1:1 (200 ml). The three fractions thus obtained were named fractions S-15, S-3, and S-1. The extract obtained from unhopped beer was also fractionated in the same way.

Subfractionation of Beer Bitter Substances

The above fractions were rechromatographed semiautomatically on an ion-exchange resin column (Bio-Rad AG1-X4, 200–400 mesh, acetate form, 0.7 × 50 cm) with a stepwise increased concentration of acetic acid in aqueous 80% methanol. The absorbance of the eluate was recorded continuously at 280 nm. Table I shows the solvents used and the subfractions obtained. The content of each subfraction as a percentage of the total content of bitter substances was calculated from the peak area on the chromatogram. Then, the content of each subfraction was determined from the true content of bitter substances, estimated previously, and the percentage content of the subfraction. The ion-exchange chromatogram was somewhat disturbed by substances derived from unhopped beer, so the areas corresponding to the peaks of substances in unhopped beer were subtracted.

Taste Test

Ten tasters who were sensitive to bitterness were selected by preliminary screening. The serial dilution method was used for evaluating bitterness intensity of subfractions. This procedure is briefly described below.

TABLE I
Solvents Used in Ion-Exchange Chromatography and Subfractions Obtained

Eluting Solvent		Subfraction in		
%AA[a]	Abbreviation	Fraction S-15	Fraction S-3	Fraction S-1
0.00	a	A (a–g)	G (a–b)	M (a–l)
0.05	b			
0.1	c			
0.5	d			
1.0	e			
5	f		H (c–g)	
10	g			
20	h	B	I (h–i)	
40	i	C		
60	j	D	J	
80	k	E	K	
100	l	F	L	

[a]%AA = acetic acid in 80% aqueous methanol.

[1]Presented at the 42nd Annual Meeting, Milwaukee, May 1976.

[2]M. Kowaka and E. Kokubo, unpublished data.

All of the subfractions were dissolved in 4.0% aqueous ethanol so they would be the same concentration. Bitterness intensity of test solution was compared with that of the solution of subfraction D by means of a two-glass test. If a significant difference was found between the two solutions, the subfraction D solution was diluted successively until no significant difference was found. Thus, the relative intensity of the subfraction tested was determined, based on the dilution ratio of solution of subfraction D at that time.

Results were expressed taking the intensity of subfraction D (mainly iso-α-acids) as 10. The characteristics of bitterness were standardized by training the tasters on beers with various qualities of bitterness, and were expressed as follows: sharp, mild, dull, lingering, dilatory, astringent, harsh, rough, and stimulating bitter taste.

RESULTS AND DISCUSSION

α-Acids and β-Acids as Bittering Agents

It is well known that α-acids, β-acids, and their transformation products, which are the main components of hop resins, contribute to bitterness. These components of hops are decomposed even when the hops are kept under suitable storage conditions, so deterioration of hops inevitably affects beer bitterness to some extent. The contributions of α-acids and β-acids, which are major bittering components of hop resins, were therefore examined.

Beers were brewed with α-acids and their decomposition products, respectively, and the compositions of bitter substances of these beers were examined chromatographically. The ion-exchange chromatograms in Fig. 1 show that the bitter substances in beer brewed with pure α-acids consisted mainly of iso-α-acids with some uncharacterized compounds (peak Nos. 28, 29, 30, and 31′), while those of beer brewed with decomposition products of α-acids consisted of lower amounts of iso-α-acids and larger amounts of components such as Ashurst's compound (peak No. 58), Laws' compound (peak No. 61), Connet's compound (peak No. 62), and humulinones (peak No. 63). Their components were formed on decomposition of α-acids and transferred to wort and present in finished beer.

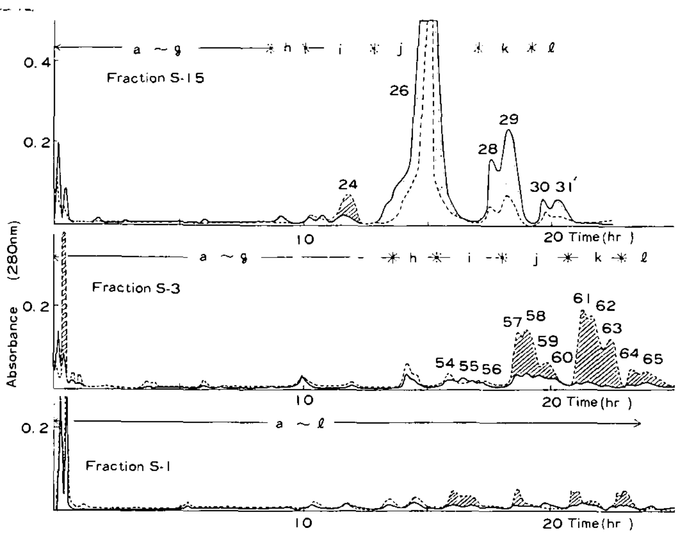

Fig. 1. Ion-exchange chromatograms of the bitter substances of test beers. Continuous line—fraction S-15 (25 mg), fraction S-3 (12 mg), and fraction S-1 (5 mg) obtained from beer (1 liter) brewed with α-acids; and dotted line—fraction S-15 (10 mg), fraction S-3 (24 mg), and fraction S-1 (7 mg) obtained from beer (1 liter) brewed with decomposition products of α-acids. The shaded and dotted areas in peaks indicate the increase and decrease in quantities after decomposition of α-acids, respectively. Peak No.: 20 (α-acids), 24 (humulinic acids), 26 (iso-α-acids), 58 (Ashurst's compound), 59, 60 (abeo-iso-α-acids), 61 (Laws' compound), 62 (Connet's compound), 63 (humulinones), 28, 29, 30, 31, 54, 55, 56, 57, 64, and 65 (uncharacterized compounds).

A similar experiment was made on β-acids and their decomposition products. The chromatograms in Fig. 2 show that beer brewed with β-acids contained few detectable bitter components, whereas beer brewed with decomposition products of β-acids contained small amounts of hulupones (peak No. 31), lupoxes a, b, and c (peak Nos. 70, 44, and 45), lupdoxes a and b (peak Nos. 46 and 47), lupdeps (peak No. 22), lupdols (peak No. 39), and uncharacterized compounds (peak Nos. 57′ and 58′).

These pilot beers were then submitted to taste tests and measurements of their bitterness units and iso-α-acids contents. The results are shown in Table II. The beer brewed with α-acids was classified as "sharp" and "lingering bitter," and that brewed with decomposition products of α-acids as "mild" and "astringent bitter." On the other hand, the beer brewed with β-acids was assessed as "dull" and "harsh bitter," and the beer brewed with decomposition products of β-acids as "mild" and "dull bitter." Thus, these differences in the composition of the bitter substances, caused by differences in storage conditions of the bittering agents, clearly affected the organoleptic bitterness. Therefore, the ion-

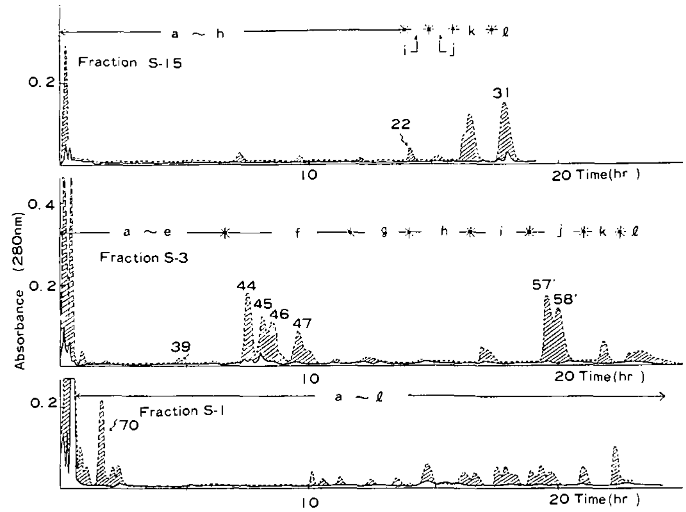

Fig. 2. Ion-exchange chromatograms of the bitter substances of test beers. Continuous line—fraction S-15 (2 mg), fraction S-3 (3 mg), and fraction S-1 (3 mg) obtained from beer (1 liter) brewed with β-acids; and dotted line—fraction S-15 (6 mg), fraction S-3 (18 mg), and fraction S-1 (10 mg) obtained from beer (1 liter) brewed with decomposition products of β-acids. The shaded areas in peaks indicate the increase in quantity after decomposition of β-acids. Peak No.: 22 (lupdeps), 31 (hulupones), 39 (lupdols), 44 (lupoxes b), 45 (lupoxes c), 46 (lupdoxes a), 47 (lupdoxes b), 70 (lupoxes a), 57′ and 58′ (uncharacterized compounds).

TABLE II
Bitterness Units and Iso-α-Acids Contents of Test Beers

Beer Brewed with:	Bitter-ness Units	Iso-α-Acids Content mg/l.	Bitterness Characteristics[a]
α-Acids	18.5	20.4	Sharp, lingering
Decomposition products of α-acids	18.0	14.0	Mild, astringent
β-Acids	2.0	1.0	Dull, harsh
Decomposition products of β-acids	9.5	1.5	Mild, dull

[a]See text.

exchange chromatogram proved to be useful for evaluating both the intensity and characteristics of beer bitterness.

Constitution of Beer Bitterness

Bitterness must be evaluated as quantitatively as possible. Accordingly, we introduced the concept of "the constitution of beer bitterness." The procedure for evaluating the constitution of beer bitterness is explained below, taking a domestic beer as an example.

Chromatograms of the bitter substances in the domestic beer are given in Fig. 3. Since the bitter substances of beer consist of at least 90 components, it is time-consuming to determine all the components on the chromatograms independently. So, in practice, the bitter substances were divided into 13 subfractions, as shown in Table III, on the basis of the similarities in organoleptic bitterness and contribution to beer bitterness. The subfractions obtained were submitted to the taste test so their bitterness intensity and characteristics could be evaluated. The bitterness intensity differed in each subfraction or fraction, but the characteristics of bitterness could be classified into three groups, corresponding to fractions S-15, S-3, and S-1. The bitterness intensity of each subfraction (Y_i), here including the bitterness characteristics, was evaluated by the taste test. The content of each subfraction (Z_i) was determined by multiplying the true content of bitter substances by the percentage content of each subfraction divided by 100. Accordingly, the bitterness contribution of each subfraction can be calculated by the following formula:

$$\text{Bitterness contribution (\%)} = \frac{(Y_i \times Z_i)}{\sum_{i=A}^{M} (Y_i \times Z_i)} \times 100$$

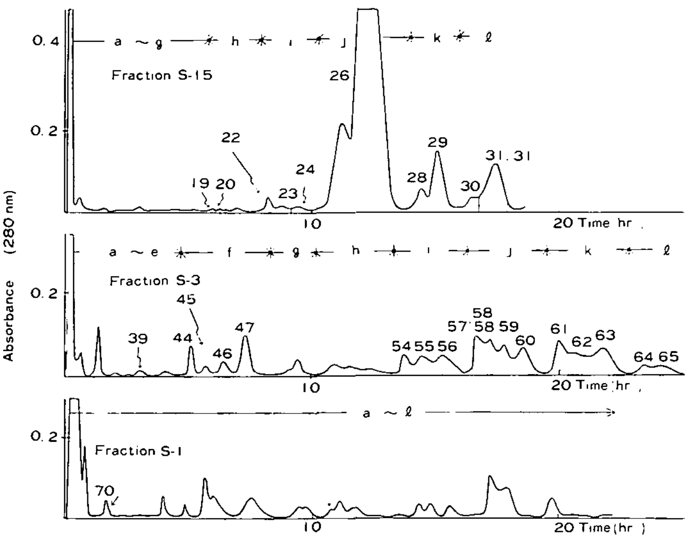

Fig. 3. Ion-exchange chromatograms of the bitter substances of domestic beer. Sample: fraction S-15 (36 mg), fraction S-3 (20 mg), and fraction S-1 (13 mg) obtained from 1 liter of the beer. Peak No.: see Figs. 1 and 2.

The constitution of beer bitterness of the domestic beer, calculated in this way, is shown in Table IV. Iso-α-acids account for 67.3% of the beer bitterness, hulupone for 5.1%, and other bitter substances for about 25%. Therefore, attention must be paid to the contributions not only of iso-α-acids but also of various other bitter substances.

Furthermore, the denominator in the formula given above shows the sum total of bitterness intensity of beer. We believe that this value, the so-called total bitterness intensity (T.B.I.), can indicate bitterness intensity of beer quite precisely. So, the relationship between this value and the bitterness unit will be examined in the near future.

Evaluation of Bitterness of Domestic Beers

The bitterness of two kinds of fresh beers, of beers stored at 0°C and 50°C, respectively, for 4 weeks and of beers brewed with hops of different degrees of deterioration were examined and results are given in Table V.

Beers I and II showed typical results for fresh beer in terms of "constitution of beer bitterness," bitterness units, and iso-α-acids contents. Their bitterness units were slightly higher than their iso-α-acids contents, but this difference was similar in the two beers. The bitterness contributions of the different fractions and subfractions in beers I and II were nearly identical. Both beers were assessed as

TABLE III
Constituents of Beer Bitter Substances and Bitterness of Subfractions

Fraction	Subfraction[a]	Major Constituents of Subfraction (Peak No.)	Bitterness of Subfraction[b]: Characteristics		Intensity
S-15	A	Unknown compounds	Sharp	Sharp	5
	B	Shaw's compound (19)	Sharp		4
		α-Acids (20)	Sharp		
	C	Lupdeps (22)	Sharp		5
		Humulinic acids (24)	No bitter		
	D	Iso-α-acids (26)	Sharp		10
	E	Unknown compounds (28,29)	Harsh		4
	F	Hulupones (30)	Sharp		5
S-3	G	Lupdols (39)	Astringent	Astringent	5
	H	Lupoxes b, c (44,45)	Astringent		3
		Lupdoxes a, b (46,47)	Astringent		
	I	Unknown compounds (54,55,56)	Astringent		2
	J	Ashurst's compound (58)	Astringent		2
		Abeo-iso-α-acids (59,60)	No bitter		
	K	Laws' compound (61)	Astringent		3
		Connet's compounds (62)	Astringent		
		Humulinones (63)	Astringent		
	L	Unknown compounds (64,65)	Astringent		3
S-1	M	Lupoxes a	Harsh		1

[a]See Table I.
[b]See text.

"sharp" and "pleasant bitter." The bitterness intensity seemed to be related to the characteristics of the bitterness: beers with high bitterness units and iso-α-acids content were assessed as "sharp bitter" and those with low values as "mild bitter."

The storage temperature of beer considerably affects the bitterness units, "constitution of beer bitterness," and bitterness characteristics. The "constitution of beer bitterness," bitterness unit, and iso-α-acids content of beer stored at 0°C were quite similar to those of fresh beer, so it is reasonable that it was assessed as "sharp" and "pleasant bitter" like fresh beer. On the other hand, the beer stored at 50°C for 4 weeks was assessed as "mild bitter," probably because its bitterness units and iso-α-acids content were less; fraction S-15 (subfraction D) was diminished, and fractions S-3 (subfraction I) and S-1 (subfraction M) were increased.

The bitterness units of beers brewed with deteriorated hops were almost similar regardless of the degree of deterioration of the hops used, but the iso-α-acids content differed because it decreased during deterioration of the hops. The sharp bitter taste was due to the presence of nearly 20 mg/l. of iso-α-acids, and the dilatory bitter taste to the increase in subfractions J (Ashurst's compound), K (Laws' compound, Connet's compound, and humulinones), L (unknown compounds), and M.

As shown above, analysis of the "constitution of beer bitterness" provides an effective means of evaluating both the intensity and characteristics of beer bitterness.

TABLE IV
Constitution of Beer Bitterness of a Domestic Beer

Fraction	Sub-fraction[a]	Bitterness Intensity[b]	Content of Subfraction mg	Bitterness Contribution, %	
S-15	A	5	2.2	3.2	80.5
	B	4	0.3	0.4	
	C	5	0.6	0.9	
	D	10	21.1	67.3	
	E	4	2.9	3.6	
	F	5	3.2	5.1	
S-3	G	5	2.7	5.1	16.0
	H	3	1.5	1.7	
	I	2	2.8	2.2	
	J	2	3.2	2.4	
	K	3	2.7	3.2	
	L	3	1.2	1.4	
S-1	M	1	16.0	3.5	

[a]See Table I.
[b]See test.

Evaluation of Bitterness of Imported Beers

The bitterness of several kinds of imported beers was evaluated and results are shown in Table VI. Ion-exchange chromatograms of Australian beer II and American beer II are also given in Figs. 4 and 5, respectively, because the compositions of their bitter substances differed remarkably from that of domestic beer.

The bitterness units of the beers ranged from 9 to 38. No distinctive difference was found in the bitterness contributions of the different fractions, except with American beer II. In general, all the beers examined seemed to be deficient in freshness at the time of use for the experiments. The bitterness of each beer was described as follows.

The constitution of beer bitterness of Czechoslovakian beer was found to be fairly similar to that of domestic beer. Its "sharp" and "lingering bitter" taste is accounted for by its high bitterness unit. The "rough bitter" taste identified by most of the panelists can probably be attributed to the contributions of subfractions J and K. The "mild bitter" taste of American beer I can be explained by its low bitterness unit, and the "pleasant bitter" taste is also

TABLE V
Constitution of Beer Bitterness of Domestic Beers

		Bitterness Contribution, %					
Fraction	Subfraction[a]	Fresh beer I	Fresh beer II	Beer stored at 0°C for 4 weeks	Beer stored at 50°C for 4 weeks	Beer brewed with deteriorated hops I	Beer brewed with deteriorated hops II
S-15	A	3.2	3.1	2.7	2.3	4.9	2.0
	B	0.4	0.5	0.4	0.5	0.5	0.7
	C	0.9	1.0	1.1	2.7	2.7	1.1
	D	67.3	68.0	65.6	48.1	55.9	50.1
	E	3.6	3.7	3.9	5.0	6.1	5.9
	F	5.1	5.1	5.2	5.4	5.4	6.3
	Summation	80.5	81.4	79.9	64.8	75.5	66.1
S-3	G	5.1	4.8	5.0	6.5	4.0	5.0
	H	1.7	1.6	1.5	0.6	1.3	1.6
	I	2.2	2.0	2.1	5.5	1.8	2.7
	J	2.4	2.6	3.0	6.8	3.8	5.8
	K	3.2	3.1	3.1	4.0	4.5	6.2
	L	1.4	1.3	1.5	1.8	3.0	3.3
	Summation	16.0	15.4	16.2	25.2	18.4	24.6
S-1	M	3.5	3.2	3.9	10.0	6.1	9.3
Bitterness units		26.0	29.0	28.0	24.0	27.5	29.0
Iso-α-acids content (mg/l.)		23.5	25.9	25.2	15.5	20.3	17.6
Bitterness characteristics[b]		Sharp Pleasant	Sharp Pleasant	Sharp Pleasant	Mild Harsh	Sharp Dilatory	Dilatory Sharp

[a]See Table I.
[b]See text.

understandable from the similarity of the constitution of beer bitterness to that of domestic fresh beer, though the contributions of subfractions J and K were slightly different. In Australian beer I, the contribution of subfraction D (iso-α-acids) was low and those of subfractions C (lupdeps and humulinic acids), E (peak Nos. 28 and 29), F (hulupones), and I (peak Nos. 54, 55, and 56) were high. The bitterness of this beer is probably due to boiling the hops in dilute alkaline solution; complicated components are produced by this procedure, as described above. This beer has a "sharp" and "rough bitter" taste. Australian beer II differed appreciably from domestic beer in that the contribution of fraction S-15 was increased and those of fractions S-3 and S-1 were decreased. This specific feature of the constitution of beer bitterness, not seen when natural hops are used, reflects the usage of an isomerized hop extract with a high content of iso-α-acids as bittering agent. However, the contribution of subfraction D was unexpectedly low and that of subfraction E rather high, probably because the beer was deficient in freshness. "Mild bitter" is attributed to low bitterness unit and "astringent bitter" to higher contributions of subfractions E and F. American beer II was exceptional in having a large difference between bitterness units and iso-α-acids content. In this beer, the contributions of the three fractions and the

TABLE VI
Constitution of Beer Bitterness of Imported Beers

Fraction	Subfraction[a]	Bitterness Contribution, %				
		Czechoslovakian	American I	American II	Australian I	Australian II
	A	3.1	3.3	0.9	3.9	3.4
	B	0.5	1.0	61.0	0.5	2.2
S-15	C	4.4	4.8	0.6	5.5	4.3
	D	61.1	65.2	24.4	55.2	59.7
	E	7.3	5.3	0.5	7.2	10.7
	F	4.2	2.7	0.9	8.7	4.9
	Summation	80.6	82.3	88.3	82.0	85.2
	G	5.1	7.5	7.4	5.5	3.9
	H	1.0	0.9	0.3	0.9	1.2
S-3	I	1.6	3.0	1.2	3.8	1.5
	J	2.5	1.3	0.5	2.6	1.9
	K	3.0	1.7	0.1	1.5	1.6
	L	2.3	0.9	0.1	0.6	2.0
	Summation	15.5	15.3	9.6	13.9	12.1
S-1	M	3.9	2.4	2.1	4.1	2.7
Bitterness units		38.0	9.0	18.5	18.5	16.5
Iso-α-acids content (mg/l.)		35.9	9.1	5.4	13.8	15.4
Bitterness characteristics[b]		Sharp Lingering	Mild Pleasant	Mild Dull	Sharp Rough	Mild Astringent

[a]See Table I.
[b]See text.

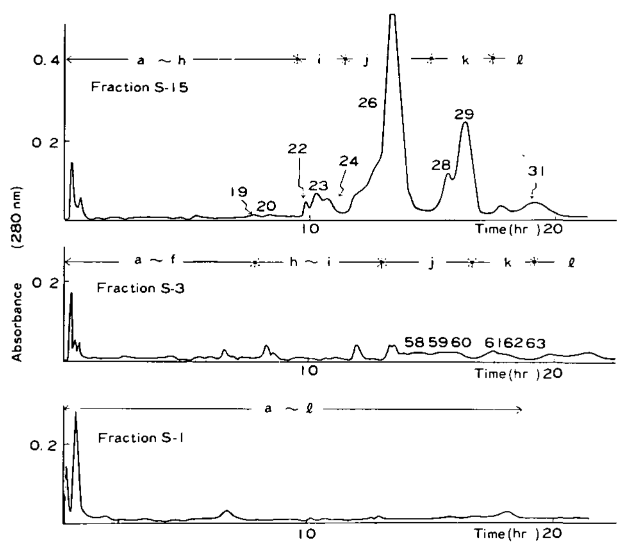

Fig. 4. Ion-exchange chromatograms of the bitter substances of Australian beer II. Sample: fraction S-15 (25 mg), fraction S-3 (9 mg), and fraction S-1 (6 mg) obtained from 1 liter of the beer. Peak No.: see Figs. 1 and 2.

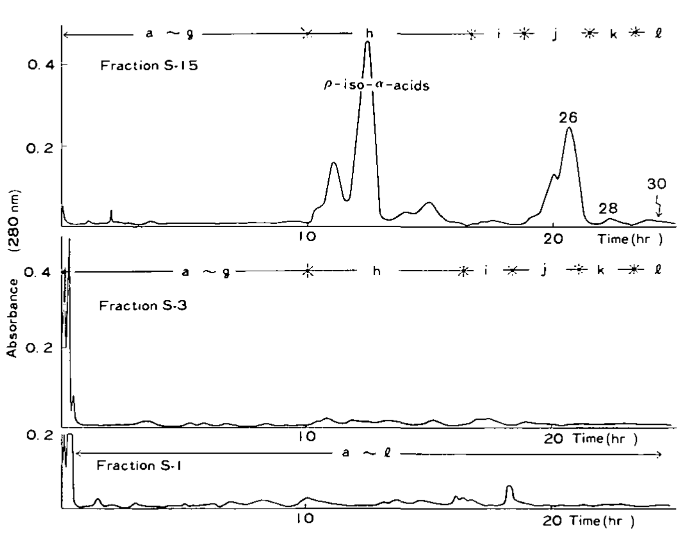

Fig. 5. Ion-exchange chromatograms of the bitter substances of American beer II. Sample: fraction S-15 (25 mg), fraction S-3 (6 mg), and fraction S-1 (5 mg) obtained from 1 liter of the beer. Peak No.: see Figs. 1 and 2.

subfractions differed considerably from those in most beers. The contribution of fraction S-15 was the highest of the beers examined, while those of fractions S-3 and S-1 were the lowest. Specifically, subfraction B contributed 61.0% of the bitterness, whereas subfraction D contributed only 24.4%. A separate experiment showed that the main component of subfraction B was rho-iso-α-acids. Thus the bitterness of this beer seems to be due to rho-iso-α-acids. The bitterness characteristics were rather mild and dull, although a sharp and pleasant bitter taste was expected from the analysis. It is probably because the relative intensity of rho-iso-α-acids is lower than that of iso-α-acids.

CONCLUSION

A new method for evaluating beer bitterness, from the constitution of beer bitterness, was developed and used to evaluate various beers. The results showed that the different kinds and compositions of bitter substances in different types of bittering agents affect the constitution of beer bitterness, and that deterioration of beer also affects the constitution of beer bitterness. By assessing the constitution of beer bitterness in connection with bitterness units and the iso-α-acids content of beer, it was possible to evaluate the bitterness intensity and characteristics of bitterness, to judge the freshness of hops and beer, and to deduce the type of bittering agent used.

Acknowledgments

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