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Influence of hop bitter acids and their derivatives on beer foam stability evaluated using customer-oriented Foam Collapse Time (FCT) method

We successfully developed new automated 'FCT (Foam Collapse Time)' system for the evaluation of visual foam stability in order to get a useful knowledge for improving practical foam stability. The FCT value is defined as the required time for a reduction of the 40 % of glass hight (32 mm) of foam layer to a single layer in a prescribed glass. The improved FCT pouring apparatus was coupled with automated turntable for 6 glasses, electronic balance, and CCD-cameras (side and upper) for measuring the foam layer (mm) and the collapse point. Based on automatically collected data, the FCT value could be statistically calculated by specialized software on the control PC. Using this system, we confirmed an importance of hop bitter acids and their derivatives, which could affect the strength of foam membrane, for visual foam stability evaluating by FCT. From comparison between FCT, Sigma, and NIBEM methods, it was suggested that detectability for foam membrane strength was more sensitive in FCT and NIBEM than Sigma, and that foam membrane strength could also affect foam lacing.

Descriptors: beers, hops, bitter acids, isohumulone, Rho-isohumulone, tetrahydro-isohumulone, foam stability

1 Introduction

Foam is one of the important factors of beer quality. When beer is poured from bottle/ can into a glass, many bubbles are generated by vaporization of carbon dioxide and the bubbles are moving to upper part in the glass (Fig. 1A, see page 142). The stabilized foam layer is formed with these bubbles and clearly separated from beer layer (Fig. 1B, see page 142). Simultaneously, the foam layer is destabilising by various factors and finally collapsing (Fig. 1C, see page 142). Figure 2 (see page 142) summarize various physical phenomena which occur in beer foam layer. Hydrophobic components in beer liquid are adsorbed into a surface of each bubble and concentrated in liquid layer in stabilized foam. In the liquid layer, foam positive components, for example, proteins, hop bitter acids, polysaccharides, and metal ions, are concentrated and formed complexes which contribute to foam stability. On the other hand, destabilising effects are occurring in the foam layer. At the surface of the layer, carbon dioxide gas diffuses and induces to collapse of foam membrane (liquid layer). Gas diffusion and membrane collapse are occurring not only at the surface but also in foam layer [1]. Collapse and fusion of liquid membranes are also occurring. The liquid is draining through these foam membrane (liquid layer) to beer layer. From this summary, it is thought that foam stability could be mainly affected by three factors, collapse of foam membrane, gas diffusion, and liquid drainage rate.

For evaluating beer foam stability, Sigma [2], NIBEM [3], and Rudin [4] methods are well-known and have been widely used all around the world [5] (Fig. 3, see page 143). Several minor methods were proposed for a long time [6-7]. Most of these methods for foam stability are based on measurements of the weight or volume of the liquid collapsed from the foam [2, 4, 6-7]. There are three main criticisms of these methods, as follows: 1) The beer temperature is 20 – 25 °C. This is higher than the serving temperature for the most beer, and the physical properties of the beer may be different from those found at lower temperatures. 2) Foam is generated in an atypical way, so that the properties of the resulting foam are different from those of foam produced by typical beer-pouring methods, for example, directly pouring from a bottle/can to a glass. 3) Liquid drainage is only one of the factors related to foam stability. Using drainage alone is insufficient for the evaluation of foam stability. Consequently, values measured by conventional drainage methods can give a misleading evaluation of foam stability [8, 9].

For these reasons, we constructed a new 'FCT (Foam Collapse Time)' system for the evaluation of visual foam stability in order to get a useful knowledge for improving practical foam stability [10]. Our basic concept was to develop a customer-oriented approach, so that measurement should be performed in a consumer-use situation. Foam is generated with newly developed pouring apparatus simulating a consumer-use situation. The FCT value is defined as the

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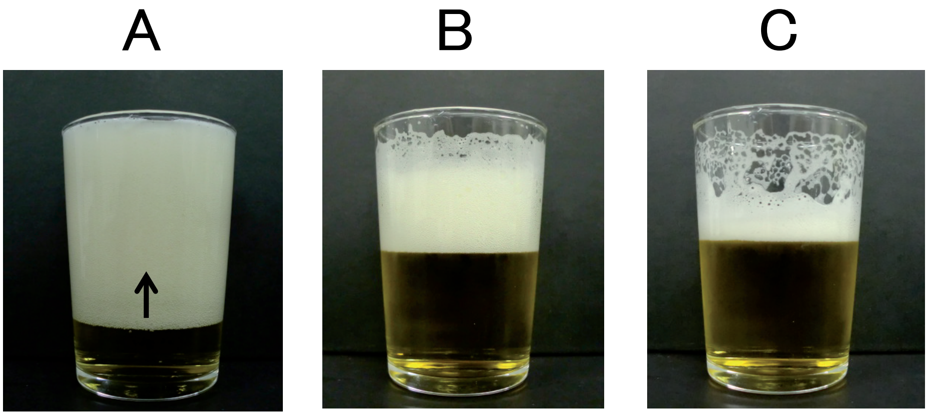

Fig. 1 **Photogram of beer foam from generation to collapse: A, pouring to glass; B, stabilized foam; C, after collapse**

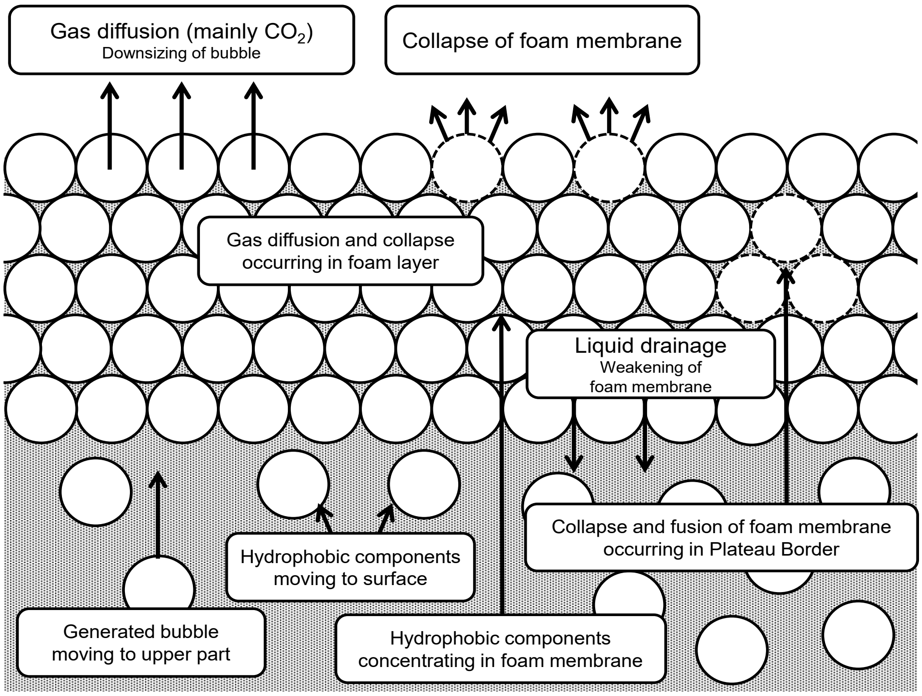

Fig. 2 **Schematic diagram of beer foam from generation to collapse**

required time for a reduction of the 40 % of glass hight (32 mm) of foam layer to a single layer in a measuring glass. In previous report, we compared Sigma, NIBEM, Rudin, FCT values, and visual foam evaluation score by panels. Using several statistical analyses, we confirmed that the visual impression of foam stability by panels is consistent with the FCT value, and that conventional values don't have a close statistical relationship to the visual impression [10]. In this report, we introduce our improved automated FCT system and try to evaluate effects of hop bitter acids, so-called iso-α-acids (isohumulones), and their derivatives on foam stability using the FCT, Sigma, and NIBEM methods.

2 Materials and methods

2.1 Hop raw materials and hop products

Commercially available hops (crop year 1997 – 1998) were used for pilot scale test brewing. Isohumulones, rho-isohumulones, and tetrahydro-isohumulones were obtained from Kalsec Inc. (Kalamazoo, MI).

2.2 Pilot-scale brewing

Beers were made with the same recipe according to the standard method of the pilot-scale plant in Sapporo Breweries, Ltd.. Briefly, the wort was prepared using commercially available 67 % malts, 33 % adjuncts (starch, corn, and rice), and hops in a 400-L pilot-scale apparatus. Boiling period was 90 minutes. For unhopped trials, no hops were added. For hopped trials, all hops were added at the beginning of boiling. The hop dosage was adjusted in each trial, so that the bitterness unit (BU) in the finished beer was at approximately 22. Each cooled wort was collected to fermentation tank (400 L/tank). Subsequently, the fermentation was started by adding 15.0 x 10^6 cells/ml lager yeast (*Saccharomyces pastorianus*) to the wort. The temperature of the fermentation was maintained at 10 – 12 °C (primary fermentation). After transferring the fermented wort to another storage tank under a CO_2 atmosphere, the maturation was carried out at 13 °C for 8 days, then at 0 °C for 2 – 3 weeks. Kieselguhr filtration and bottling were done using the pilot-scale equipment under anti-oxidative conditions. The alcohol contents of all test-brewed beers were at approximately 5.0 %. The unhopped beers used for the spiking studies (see 3.2 and 3.3) and the hopped beers used for evaluating the effect of homologues of hop bitter acids (see 3.4).

2.3 Chemicals

Cobalt (II) Chloride Hexahydrate was purchased from FUJIFILM Wako Pure Chemical Co., Ltd. (Osaka, Japan). DCHA-Iso, ICS-I (Iso-α-acids (isohumulones) standard), DCHA-Rho, ICS-R (Rho-iso-α-acids (rho-isohumulones) standard), and Tetra, ICS-T (Tetrahydroiso-α-acids (tetrahydro-isohumulones) standard) were obtained from the International Hop Standards Committee (IHSC).

2.4 Analysis of bitterness units, hop bitter acids, and their derivatives

The analysis of bitterness units (BU) was performed according to the Analytica EBC [11]. The quantification of hop bitter acids and their derivatives (isohumulones, rho-isohumulones, and tetrahydro-isohumulones) were carried out according to the standard HPLC method [12-15].

2.5 Analysis of beer foam stability

2.5.1 Sigma and NIBEM methods

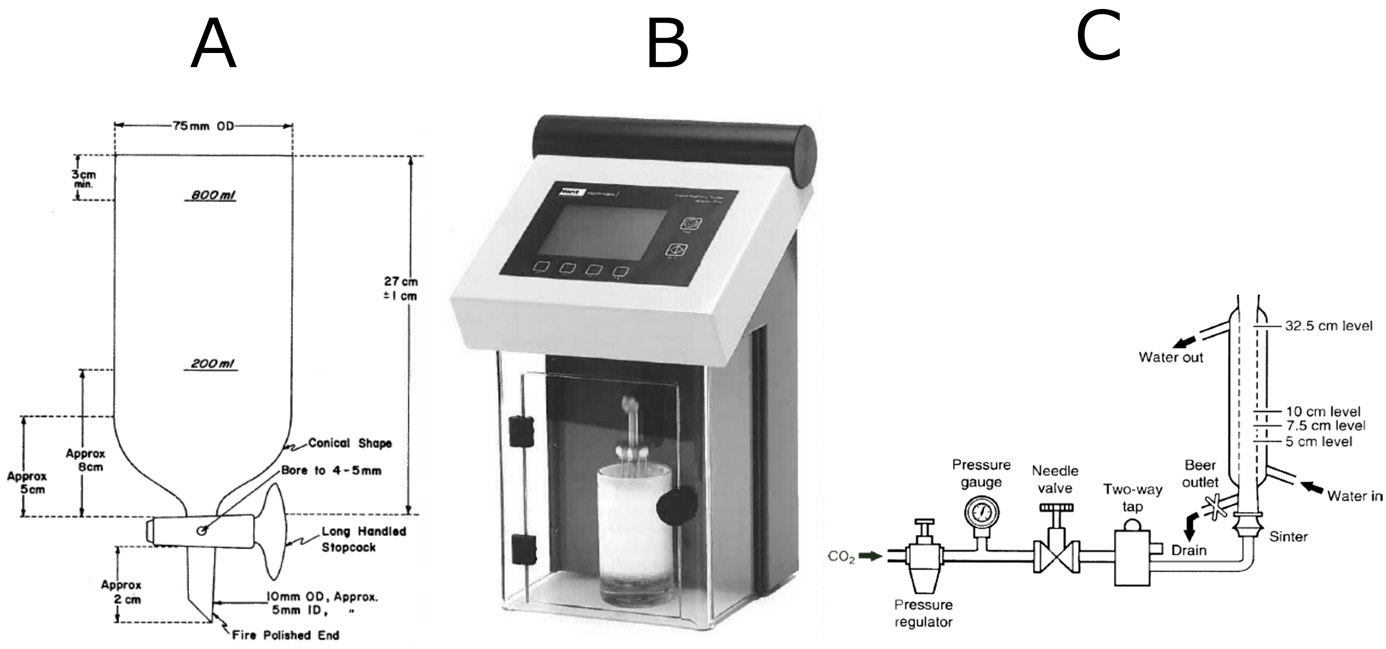

Fig. 3 **Traditional measuring apparatuses for beer foam: A, Σ method; B, NIBEM method; C, Rudin method (original figures and photo were published in ref 4)**

The analysis of Sigma method was performed according to the ASBC method [2], except that the water bath and room temperature were both at 20 °C, because almost experimental rooms are generally maintained at 20 °C in Japan. The analysis of NIBEM method was carried out according to the Analytica EBC [3]. Each beer sample including commercial and test-brewed beer was bottled into a Japanese standard bottle generally containing 633 ml of beer. Using this type of bottle, both Sigma and NIBEM methods could be measured three times a bottle.

2.5.2 FCT (foam collapse time) method

The FCT system consists of three steps: foam generation, measurement of foam amount and foam collapse time, and calculation of foam stability value (FCT value) [10]. Foam is generated by simulating a Japanese style consumer-use situation using our newly developed and improved FCT pouring apparatus (Fig. 4). The measuring condition of this method was determined according to the data of manual pouring practices, as follows (see 2.5.2.1).

2.5.2.1 Determination of measuring conditions of FCT method

According to the beer drinking situation in Japan, the temperature of beer was adjusted to 6 °C, simulating the temperature of refrigerator. The room temperature was 20 °C. The glass used (60 mm i.d., 90 mm hight) was almost the same size used in Japanese-style pubs (so-called 'Izakaya') and in most homes in Japan. The foam amount required for estimating FCT value was 40 % of glass hight. The percentage was determined on the basis of the customer-oriented concept. We gathered video data of manual pouring practices (33 people, 11 brands, for a total 292 glasses) in a consumer-use situation which showed that the average foam amount was 40 % at 10 seconds after the pouring sequences. As a result, a 40 % foam amount was equivalent to a 32 mm foam layer thickness in the defined glass. Each dispensed beer weight was 150 ± 5 g. The time for pouring beer was

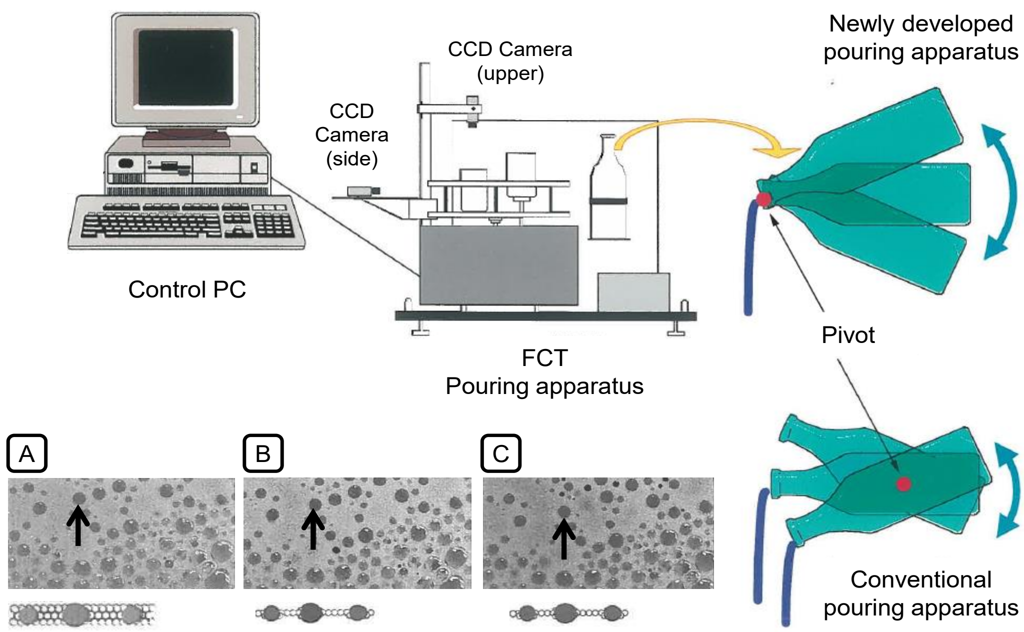

Fig. 4 **Summary for improved Foam Collapse Time (FCT) method: A, Transition of the foam surface at 5 seconds before collapse point; B, collapse point; C, 5 seconds after collapse point**

5.5 ± 0.5 seconds. Beer weight and pouring time were also determined by the pouring practice data.

Subsequently, the foam amount was determined after the end of the manual pouring sequence. The profile of the data in a glass was recorded by a CCD-camera. The image of foam profile was captured, then the percentage of foam layer was measured using a computer software program. The foam collapse time from each glass was defined as the time from the end of the pouring sequence to the reduction in the foam layer to be a single layer. The moment of the reduction to a single layer was judged by the operator's observation. The dark colour of the beer cannot be seen until the foam is reduced to a single layer. The foam condition 5 seconds before the collapse point is shown in figure 4A. The collapse point is the moment when the foam layer is reduced to a single layer (Fig. 4B). At this moment, the foam layer has not reached a single layer yet, so the bubbles have a grey gradation in diameter. The foam condition 5 seconds after the collapse point consists of many bubbles of roughly the same size, so that some entirely black bubbles are visible (Fig. 4C). Therefore, we can obtain a precise foam collapse time by observation of the foam layer. Each foam collapse time is accurate to within 3 seconds. In developed system, the FCT value is the required time for a reduction of the 40 % of glass hight (32 mm) of foam layer to a single layer in a prescribed glass. This FCT value is regarded as the estimated foam stability value. The word 'foam collapse time' written out in small letters indicated a measured value from each glass [10].

2.5.2.2 Measurement scheme of automated FCT method

In the early phase of this study, we have used proto-type FCT pouring apparatus for controlling a pouring sequence [10]. The foam layer (mm) was measured using photo data in a pouring point by software. The poured beer weight (g) are measured manually by an electronic balance. The collapse points also judged with visual check by an operator. These processes are very time-consuming and complicated. Therefore, we developed improved pouring apparatus coupling with automated turntable for 6 glasses, electronic balance, and CCD-cameras (side and upper) for measuring the foam layer (mm) and the collapse point. Using the Japanese standard bottle, FCT method could be measured three times a bottle. The obtained data were collected to the control PC and analysed by specialized software (Fig. 4).

A customer-oriented approach is a basic concept of FCT; therefore, foam is generated by simulating a consumer-use situation. Our developed pouring apparatuses, both proto-type and improved system, have three important features.

The first feature is the proper placement of the pivot (Fig. 4). In a customer's way of pouring, the pivot of the pouring motion is the lip of the bottle. Accordingly, our system has the pivot at the lip of the bottle, in spite of mechanical difficulties. In contrast, conventional pouring machines have their pivot at the bottle's centre of balance, which makes it difficult to keep a steady flow of beer.

The second feature is control of pouring motion. We simulate a customer's pouring action using the programable control unit. Each bottle is manually fixed to the bottle holder of pouring apparatus using rubber belt. The lip of the bottle is automatically adjusted to the starting position (the pivot). During pouring motion, the position of the bottle lip don't move, and the bottom of the bottle could move as drawing the circle.

Table 1 Comparison of measuring conditions, vessels and, protocols among various foam analysis methods

			Conventional method		
		FCT	Sigma	NIBEM	Rudin
Beer Temperature	(°C)	6	25	20	20
Room Temperature	(°C)	20	25	20	20
Measuring vessel type		glass	funnel	glass	colmn
Measuring vessel size	(mm i.d.)	6,0	7,5	6,0	2,7
	(mm hight)	90	270	120	350
Sample		bottled/canned beer	bottled/canned beer	bottled/canned beer	degassed beer
Foaming apparatus		used	manual pouring	used	used
Foaming gas		–	–	CO_2	CO_2 or N_2
Foaming protcol		directly pouring from bottle/can to measuring glass	directly pouring from bottle/can to measuring funnel	foaming from bottle/can to measuring glass	foaming in measuring colmn
Measuring beer in vessel	(ml)	approx. 150	approx. 200	approx. 200	approx. 100
Foaming (pouring) Time	(seconds)	5.5 ± 0.5	12 – 13	3 – 4	60 ± 5
Initial foam Layer	(mm)	32	> 200	120	> 32.5
Measuring princple		foam collapse time	liquid draining rate	foam collapse time	liquid draining rate
Affecting factors	mainly subsequently	collapse of foam membrane gas diffusion (liquid drainage)	liquid drainage (collapse of foam membrane) (gas diffusion)	collapse of foam membrane gas diffusion liquid drainage	liquid drainage (collapse of foam membrane) (gas diffusion)

The third feature is the control of foam generation. Pouring height is adjusted to obtain a prescribed foam amount. Therefore, the difference in foamability is reflected in the difference in pouring height of each bottle. Consequently, other factors that determine foamability, such as differences in carbon dioxide gas pressure, and their influence on the evaluation of foam stability are eliminated in our system.

As described in 2.5.2.1, the FCT value is defined as the required time for a reduction of the 40 % of glass hight (32 mm) of foam layer to a single layer in a prescribed glass. Even when using a precisely controlled pouring sequence, it is difficult to get a foam amount of exactly 40 % and a beer weight of 150 g. Therefore, each FCT value is determined by nine replicates using statistical analysis. Each point has the value of percentage of foam, beer weight, and foam collapse time. Using these three parameters, the FCT value is estimated by multiple regression analysis [10]. Improved pouring apparatus (Fig. 4) can automatically obtain these three parameters and a FCT value can be estimated by multiple regression analysis using specialized software on the control PC.

2.6 Spiking studies

For evaluating the effect of hop bitter acids and metal ion, we spiked 10 and 20 mg/L of isohumulones and additively 10 µM of metal ion (Co^{2+}) to the unhopped beer and analysed foam stability values using FCT, Sigma, and NIBEM methods. For evaluating the effect of hop bitter acids and and their derivatives, we spiked 10 mg/L of isohumulones, rho-isohumulones, and tetrahydro-isohumulones to unhopped beer and analysed foam stability values using FCT, Sigma, and NIBEM methods. The measurement of each method was repeated as described in 2.5.

3 Results and discussions

3.1 Effect of foam measuring principle and conditions on resulted foam stability value

Table 1 shows a comparison of measuring conditions, vessels and protocols among various foam analysis methods. In previous report, we compared FCT value, conventional foam stability values (Sigma, NIBEM, and Rudin methods), and visual foam evaluation score by panels. As a result, we confirmed that the visual impression of foam stability by panels is consistent with the FCT value, and that conventional values don't have a close statistical relationship to the visual impression. We also compared FCT and Sigma values between hopped and unhopped beers. The unhopped beer had lower FCT value than the hopped beer, whereas there is no difference in Sigma values between the hopped and unhopped beers [10].

We firstly tried to compare these foam stability values using unhopped beer used in this study. We brewed unhopped beer in the pilot apparatus and spiked commercial iso-α-acids (isohumulones) as adjusting concentrations to 10 and 20 mg/L. In spiked samples, the concentrations were determined with HPLC at 10.0 and 20.6 mg/L respectively. As a result (Table 2), the unhopped beer had lower FCT value than spiked beers and the FCT value increased depending on the concentration of spiked isohumulone, whereas there is no difference in Sigma values between hopped and spiked beers.

Table 2 Comparison of foam stability in unhoped beer and isohumulone-spiked beers between FCT and Sigma methods

		Control (unhopped beer)	Isohumulone 10 mg/L	Isohumulone 20 mg/L
FCT	(seconds)	105	121	129
Sigma		127	126	128

The principle of the Sigma value is mainly based on the liquid drainage rate [2, 5]. It is well-known that isohumulones are one of the important factors contributing to the strength of foam membrane [5]. However, as described above, the Sigma value wasn't affected by isohumulones. From these results, it is suggested that the strength of foam membrane couldn't be correctly evaluated by this method.

As summarized in table 1, each method bases on a different principle. It is thought that each method has various merits and/or demerits depending on each principle and specific measuring conditions. Of all methods, we decided to omit the Rudin method for following investigations, because the measuring conditions of this method, for example, degassed beer, foaming with N_2, etc., is very different from consumer-use situation. In the following part, we tried to evaluate the effect of hop bitter acids on foam stability values using FCT, Sigma, and NIBEM methods.

3.2 Evaluation of the effect of hop bitter acids and metal ion on foam stability

An unhopped test-beer was newly brewed for following spiking studies, because factors affecting beer foam should be at same conditions, for example, content/composition of malt-derived proteins [5, 16-17], mineral/metal ions [5, 18], short-chain fatty acids [5, 19-21] and various fermentation by-products [22-23], and so on. We spiked 10 and 20 mg/L of isohumulones and additively 10 µM of metal ion (Co^{2+}) to this unhopped beer and analysed foam stability values using FCT, Sigma, and NIBEM methods.

As a result (Table 3), the FCT and NIBEM values increased depending on the content of spiked isohumulones. However, the

Table 3 Comparison of foam stability induced with isohumulone and metal ion (Co^{2+}) among various foam analysis methods

		Isohumulone 10 mg/L – –	Isohumulone 20 mg/L – –	Isohumulone 20 mg/L $CoCl_2$ 10 µM
FCT	(seconds)	108	126	138
Sigma		119	121	126
NIBEM	(seconds)	196	217	230

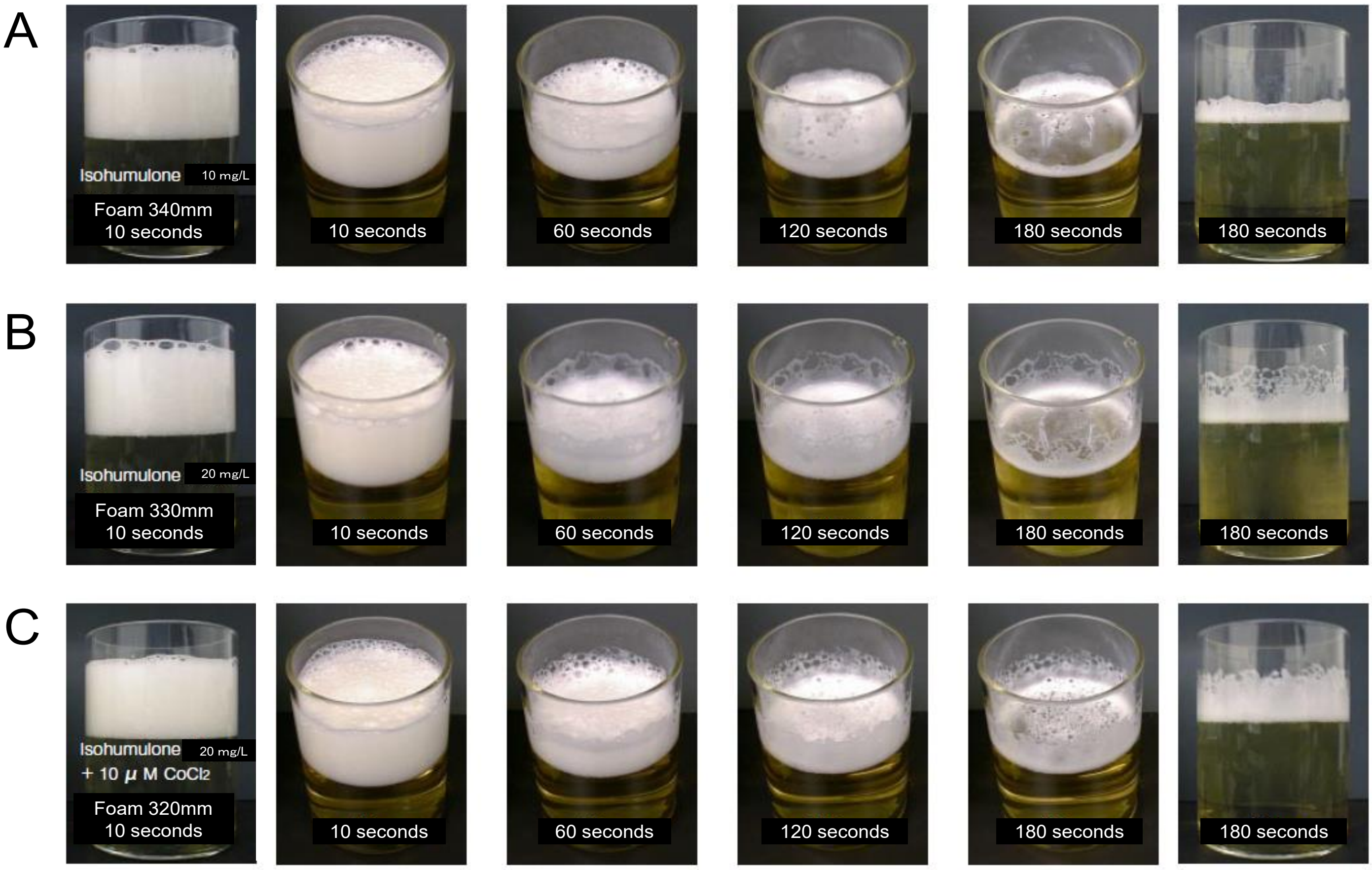

Fig. 5 **Time-course photographs during foam collapse in FCT measuring glass: A, adding 10 mg/L of isohumulone; B, adding 20 mg/L of isohumulone; C, adding 20 mg/L of isohumulone and 10 μM $CoCl_2$**

Sigma values didn't almost change depending on the contents of isohumulones as supporting the results in 3.1. Before performing this spiking test, we investigated the relationship between foam stability and several metal ions. Of all metal ions tested, Fe^{2+} and Co^{2+} had a positive effect on both FCT and Sigma values, whereas Cu^{2+}, Ni^{2+}, and Mn^{2+} ions showed no effect (data not shown). So, we selected Co^{2+} ion for this test. When both 20 mg/L of isohumulones and 10 μM of Co^{2+} were spiked, all foam stability values clearly increased.

Figure 5 showed time-course (10, 60, 120, and 180 seconds after pouring) photographs during foam collapse in the FCT measuring glass. In figure 5A (10 mg/L of isohumulones; FCT, 108 seconds) and 5B (20 mg/L of isohumulones; FCT, 126 seconds), foam layers almost reached to collapse point at 120 seconds, whereas in 5C (20 mg/L of isohumulones and 10 μM of Co^{2+}; FCT, 139 seconds), foam layer more retained than 5A and 5B at same time. In addition, foam lacing also clearly increase depending on contents of isohumulones (Fig. 5A and 5B) and additive metal ion (Fig. 5C).

It is well-known that hop bitter acids could interact to hydrophobic surface of proteins in beer, and that certain metal ions have chelating effect towards hop bitter acids [5]. The interaction between proteins and hop bitter acids could positively affect a strength of beer foam membrane, and coexistence of metal ions could also enhance foam membrane strength. It is assumed that the foam membrane strength could also affect a liquid drainage in foam layer, because complex structure of foam layer could be retained for a longer time. The results shown in table 3 and figure 5 suggested that detectability for foam membrane strength was more sensitive in

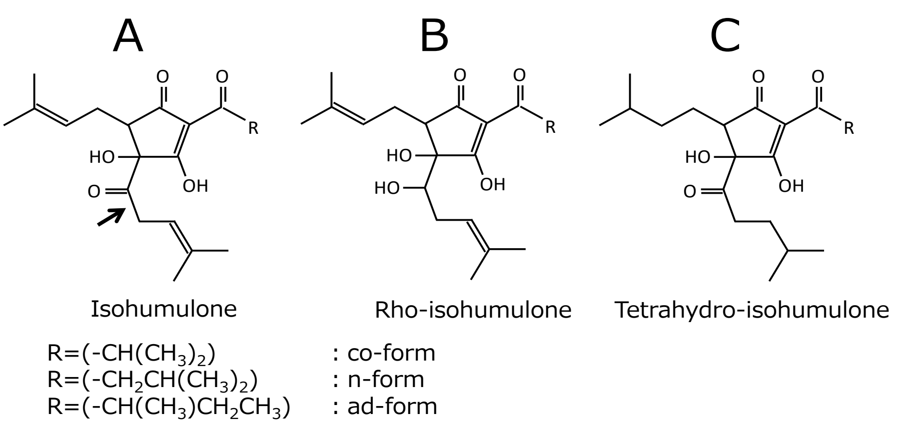

Fig. 6 **Isohumulone and their derivatives: A, Isohumulone; B, Rho-isohumulone; C, Tetrahydro-isohumulone; arrow, cleavage point for precursor of 3-methyl-2-butene-1-thiol (lightstruck off flavour)**

Table 4 **Comparison of foam stability induced with isohumulone and their derivatives among various foam analysis methods**

		Isohumulone 10 mg/L	Rho-isohumulone 10 mg/L	Tetrahydro-isohumulone 10 mg/L
FCT	(seconds)	108	114	147
Sigma		119	119	134
NIBEM	(seconds)	196	201	266

FCT and NIBEM than Sigma, and that foam membrane strength could also affect foam lacing.

3.3 Evaluation of the effect of hop bitter acids and their derivatives on foam stability

For preventing so-called 'lightstruck off flavour' derived from hop bitter acids, several derivatives have been developed and commercially used [24-27]. In figure 6, the structures of original isohumulones and two derivatives are summarized. In rho-isohumulones (Fig. 6B), a catalytic hydrogenation is performed in a carbonyl group. In tetrahydro-isohumulone (Fig. 6C), catalytic hydrogenations are performed in two double bonds in sidechains. The hydrophobicity of each molecule is changed by hydorogenation, and foam stability could be changed using these derivatives. Therefore, we next spiked 10 mg/L of isohumulones, rho-isohumulones, and tetrahydro-isohumulones to unhopped beer and analysed foam stability values using FCT, Sigma, and NIBEM methods.

As a result (Table 4), there was little difference between isohumulones and rho-isohumulones in all foam stability values, whereas tetrahydro-isohumulones induced drastic increase in all values. Figure 7 showed time-course (10, 60, 120, and 180 seconds after pouring) photographs during foam collapse in the FCT measuring glass. In figure 7A (10 mg/L of isohumulones; FCT, 108 seconds) and 7B (10 mg/L of rho-isohumulones; FCT, 114 seconds), foam layers almost reached to collapse point at 120 seconds, whereas in 7C (10 mg/L of tetrahydro-isohumulones; FCT, 147 seconds), foam layer more retained than 7A and 7B at same time. In addition, foam lacing also clearly increase using tetrahydro-isohumulones (Fig. 7C).

As described in 3.2, the interaction between proteins and hop bitter acids could positively affect a strength of beer foam membrane. It is thought that the foam membrane strength could be affected by hydrophobicity of hop bitter acids and their derivatives. In rho-isohumulones, a catalytic hydrogenation changes a carbonyl group into a hydroxyl group. Both groups are polar residues. On the other hand, in tetrahydro-isohumulones, two catalytic hydrogenations added four hydrogens into two sidechains, so that hydrophobicity of resulted compounds increase. The results shown in table 4 and figure 7 suggested that foam membrane strength could be enhanced with tetrahydro-isohumulones, and these compounds could also drastically affect foam lacing.

By the way, some methods for evaluating foam lacing have been proposed [28]. As shown in figure 5 and 7, foam lacing after measuring FCT could be evaluated by analysing side view of the

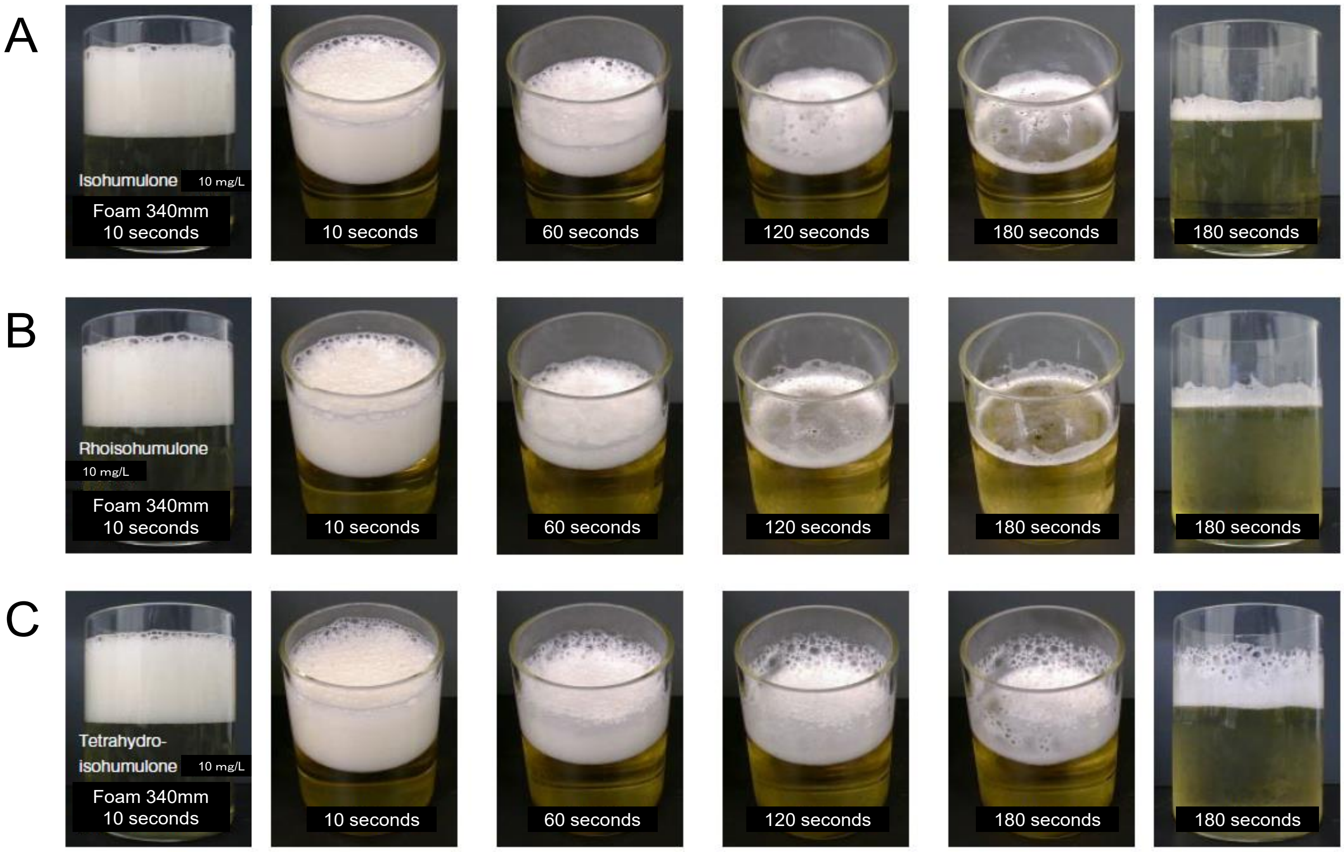

Fig. 7 **Time-course photographs during foam collapse in FCT measuring glass: A, adding 10 mg/L of isohumulone; B, adding 10 mg/L of rho-isohumulone; C, adding 10 mg/L of tetrahydro-isohumulone**

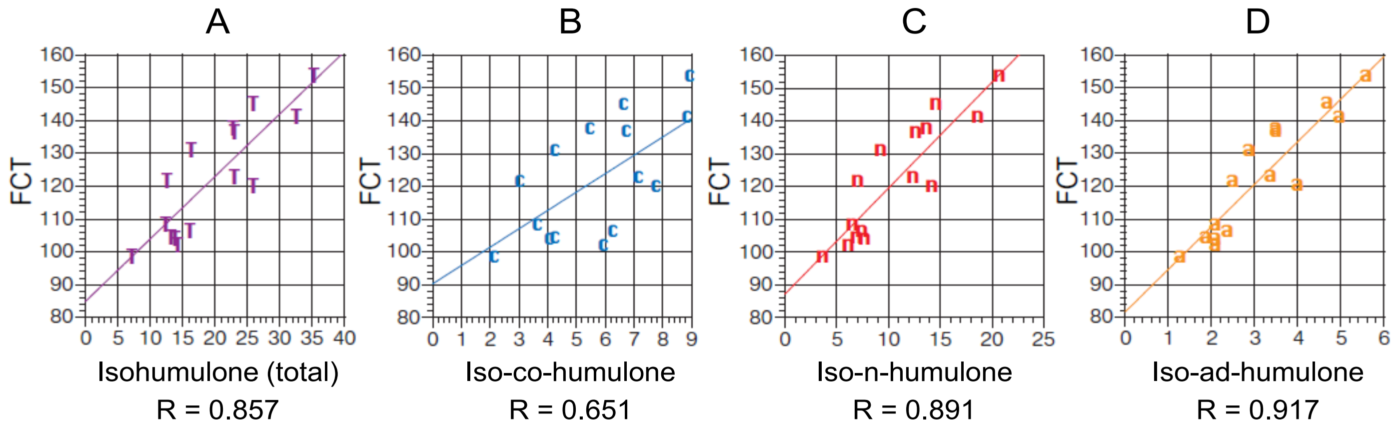

Fig. 8 **Scatter plots between FCT values and isohumulones concentrations: A, isohumulone (total); B, iso-co-humulone; C, iso-n-humulone; D, iso-ad-humulone**

FCT glass. The FCT value is defined as the required time for a reduction of the 40 % or 32 mm of foam layer to a single layer in a prescribed glass, and the side view area of initial foam layer of FCT is almost the same. It is thought that a lacing area after measuring FCT could be exactly evaluated as percentage of lacing area against total foam layer area. According to this concept, we further improved the FCT system as automatically evaluating not only visual foam stability but also foam lacing (data not shown).

3.4 Evaluation of the effect of homologues of hop bitter acids on visual foam stability

It is well-known that isohumulones consists of three homologues, iso-n-humulone, iso-co-humulone, and iso-ad-humulone (Fig. 6). Of all homologues, co-form has shorter side chain than other two forms. So, a hydrophobicity of co-form is weaker than other forms [24-27]. For example, the difference of hydrophobicity between co-form and other forms could detect by concentrating these forms into beer foam layer. We analysed beer and foam layers after measuring of Sigma method (Table 3) with HPLC. As a result (Table 5), the concentrations of total isohumulones in foam layer increased to approximately 400 % in comparison with those in beer layer. The concentration rates in n-form and ad-form were higher than those in total and co-form. Especially, the concentration rates were very low in co-form, approximately 220 %. In Sigma method, the detectability of hydrophobicity of isohumulones was good, because of over-foaming condition in this method.

The results shown in 3.2 and 3.3 suggested that FCT method have good detectability for foam membrane strength which is derived from isohumulones. Therefore, we tried to evaluate the difference of hydrophobicity among isohumulone homologues with FCT method. We had many test-brewed beer samples using various commercial hops including traditional aroma and bitter hops. The compositions of isohumulone homologues in these beers were varied by various test conditions, for example, hop varieties, fermentation conditions, and so on. Therefore, we evaluated FCT values and analysed the compositions of isohumulone homologues by HPLC. As a result, there was a good correlation (R = 0.857) between FCT values and total isohumulone concentrations (Fig. 8A). In the case of iso-n-humulone and iso-ad-humulone, the correlation factors also showed good relationship between FCT and each compound, 0.891 in n-form and 0.917 in ad-form respectively (Fig. 8C and 8D). On the other hand, the correlation factor between FCT values and iso-co-humulones was low (R = 0.651) (Fig. 8B). This result suggested that FCT method could detect small difference of hydrophobicity among isohumulone homologues, and that

Table 5 **Concentration rates of isohumulone in foam layer remaining after measurement of Sigma method: beer, original beer; foam, collected foam layer after measurement of Sigma method; concentration rate, calculated by isohumulone contents in original beer and foam layer**

			Isohumulone 10 mg/L – –	**Isohumulone 20 mg/L – –**	**Isohumulone 20 mg/L $CoCl_2$ 10 μM**
Isohumulone (total)	beer	(mg/L)	10,0	20,6	19,9
	foam	(mg/L)	40,3	80,4	86,8
	Concentration rate	(%)	403	390	437
Iso-co-humulone	beer	(mg/L)	2,3	4,8	4,7
	foam	(mg/L)	5,3	10,1	10,9
	Concentration rate	(%)	226	210	230
Iso-n-humulone	beer	(mg/L)	7,1	14,6	14,0
	foam	(mg/L)	32,5	65,4	70,7
	Concentration rate	(%)	459	447	506
Iso-ad-humulone	beer	(mg/L)	0,6	1,1	1,2
	foam	(mg/L)	2,5	4,8	5,2
	Concentration rate	(%)	426	421	448

the composition of these homologues could affect visual quality of beer foam.

4 Conclusions

We developed new automated 'FCT (Foam Collapse Time)' system for the evaluation of visual foam stability in order to get a useful knowledge for improving practical foam stability. It is well-known that isohumulones including their derivatives and/or metal ions have a positive effect on foam stability [5, 18, 24-27]. However, the knowledge has not been sufficiently investigated in the viewpoint of visual foam quality of beer. In this study, we confirmed an importance of hop bitter acids and their derivatives, which could affect the strength of foam membrane, for visual foam stability evaluating by this method.

As described above, it is thought that the strength of foam membrane is a very important factor contributing to visual foam stability. From comparison between FCT, Sigma, and NIBEM methods, it was confirmed that NIBEM could also evaluate foam membrane strengthening factors, because the sensor of NIBEM measuring apparatus could directly trace lowering of foam surface, in which foam collapse occur. Indeed, FCT method is a better way for analysing visual foam quality, however, NIBEM method is also useful for evaluating some factors which could strengthen foam membrane.

The quality of beer foam consists of many factors, including initial foaming, head retention, lacing and so on. Various approaches for revealing beer foam stability have been reported [28-33]. The FCT method is one of the such approaches. In the late 1990s, the options of sensors requiring for this method were restricted, and the power of PC and software were poorer than those in the 2020s. The concept of this method is still useful for evaluating visual foam quality. Now, successors and/or expansions of the FCT method could be developed easier than in the 1990s. The knowledge of this study could contribute to the further understanding of beer foam science.

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