Korean J. Applid. Microbiol. Beong
Vol. 3 *No.* 1 *January* 1975.

Evaluation of Batch Fermentation Conditions on Beer Flavor Development

M. Y. Pack

Department of Biological Science and Engincering

Korea Advanced Institute of Science & Technology, Seoul, Korea

(Received December 5, 1974)

麥酒의 香味形成에서 본 回分醱酵條件의 評價

朴 茂 榮

韓國科學院 生物工學科

Abstract

Brewer's worts were fermented under five different conditions in each of which one of the five elemental factors involved in the conventional batch fermentation, i. e., fermentation period, heterothermal condition, spontaneous agitation, stratification, and foam covering, was forced to alter remaining other factors unchanged. The resulting beers were analyzed for their flavor components gas-chromatographycally and all of the five factors were found to be necessary for the development of the characteristic flavor of traditional beer.

Introduction

For the purpose of increasing the efficiency of beer fermentation, various trials such as higher pitch rates (1), higher fermentation temperatures (2), agitation (3), and continuous processes (4, 5, 6, 7, 8, 9, 10, 11, 12) have been practiced by numerous investigators. However, none of the attempts have been fully successful in reproducing the characteristic flavor of the traditional beer (13, 14, 15, 16, 17, 18). The fact suggests a necessity of evaluating various factors involved in the conventional batch fermentation on the standpoint of flavor development in the beer before we attempt to alter the existing fermentation method. Some of the factors would be essential for the beer flavor and should be included in the new method of fermentation, whereas the factors of less important may be neglected thereafter. The fermentation condition for the batch beer may be considered to be composed of many individual factors such as fermentation period, heterothermal condition, spontaneous agitation, stratification, foam covering, etc. Keeping other factors unchanged, each individual factor was altered experimentally and its response on the syntheses of flavor compounds was examined gas-chromatographycally.

Materials and Methods

Fermentation In order to relate the experiments closely to the practical fermentation of beer, one of the 45, 000-liter open batch fermentors in a commercial brewing plant was selected for this

study. For the normal operation of this plant the wort pitched with yeast is filled in the fermentor to about two meters deep and is subjected to ferment for nine to ten days before the green beer is transferred to the aging tanks. A part of beer samples used for this study were taken from this fermentor. A long plastic tube of 120cm in length and 5 cm in diameter was also used as a small fermentor. The details for each particular fermentation will be given in the result section.

Gas-chromatographic analyses The flavor components in the fermenting wort or green beer were extracted with carbon disulfide and were submitted to a single quantitative dual-column gas-chromatographic analysis described by Powell and Brown (19) with a slight modification in the temperature programing and attenuation. Fifteen peaks of alcohols, acids, esters, and unidentified metabolites were clearly resolved by this method along with a peak of an internal standard (2-

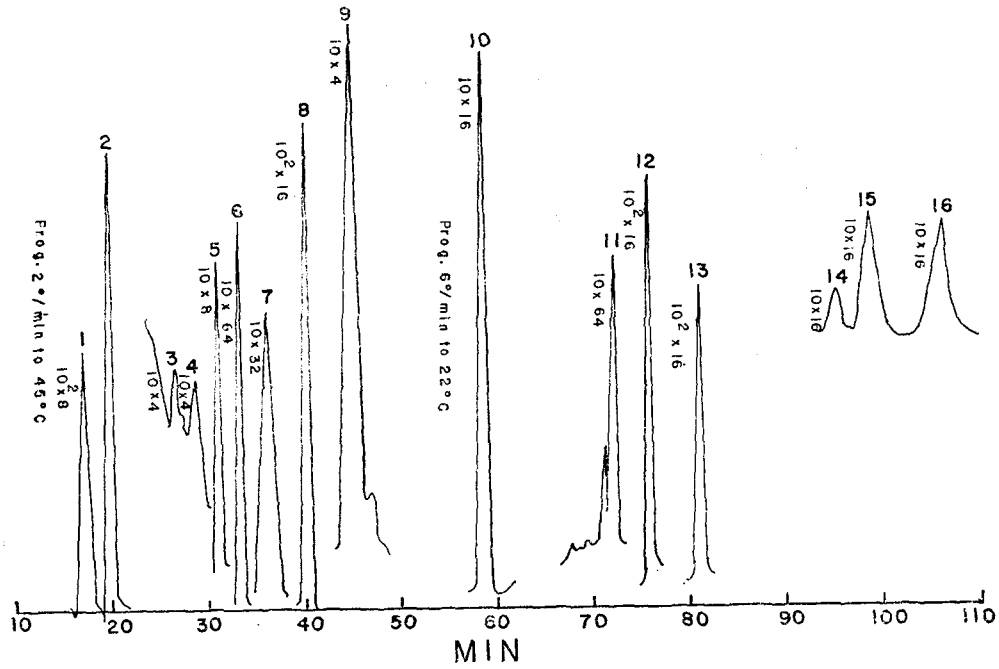

Fig. 1. Chromatogram of 9-day Fermented Green Beer. Attenuation Changes for each Peak are indicated. For identity of peaks, see Table 1.

Table 1. Identity of Peaks in the Chromatogram (Fig. 1), after Powell & Brown (19)

Peak No.	Compound	Peak No.	Compound
1	Ethyl acetate	9	Ethyl caproate
2	Ethanol	10	Ethyl caprylate and/or Acetic acid
3	Isobutyl acetate	11	β-Phenylethyl acetate and/or Caproic acid (Hexanoic acid)
4	n-Propanol and/or Ethyl butyrate		
5	Isobutanol	12	β-Phenylethanol
6	2-Pentanol (internal standard)	13	Caprylic acid
7	Isoamylacetate	14	Unidentified
8	Isoamyl alcohol and/or 2-Methyl butanol	15	Capric acid (Decanoic acid)
		16	Unidentified C_{10} acid

pentanol) as shown in Fig. 1. For the internal standard, 0.20 ml of 2-pentanol was diluted in 100 ml of distilled water, and 1 ml of the solution was added in the 70 ml of chilled beer sample before the analysis.

The identity of the peaks was also followed after Powell and Brown (19) and listed in Table 1. Using the average value of duplicate analyses the relative peak area (RPA) for each metabolite was calculated as follows;

$$RPA=\frac{\text{Metabolite peak area}}{\text{Internal standard (2-pentanol) peak area}}\times 100$$

Results

Fermentation period The change in the relative concentrations of main metabolites detected in the fermenting wort against the fermentation time is shown in Fig. 2. Most of them were formed to the maximum level within four to five days with two exceptions; in one case, ethyl acetate continually increased, and in the other, one unidentified C_{10} acid increased rapidly from the fourth day, reached to the maximum after eight days, and followed by a slow decrease thereafter.

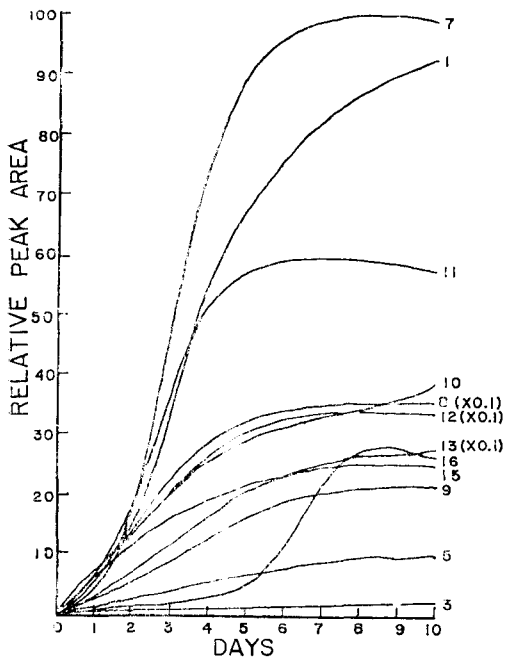

Fig. 2. Effect of Fermentation Period on the Formation of Flavor Compounds. Fermenting wort samples were taken from the plant batch fermenter once a day during the 10-day period of fermentation and were analyzed gas-chromatographycally. The numerals indicate peak numbers and for their identity, see Table 1.

Heterothermal condition In the conventional method of beer production the fermentation usually starts by filling batch fermentors with the cool wort in which yeast is pitched. The wort temperature at this time is around 9°C. As the fermentation proceeds the metabolic energy of the yeast raises the wort temperature gradually up to the maximum of about 14°C. in three or four days. Then a slight loss of temperature occurs until the cooling coil turns on, usually at the eighth day of the fermentation. The cooling process brings the wort temperature down rapidly to approximately 2°C. before the fermentation terminates. The overall change of the temperature in the plant batch fermentor during the 10-day period of fermentation is given with a solid line in Fig. 3.

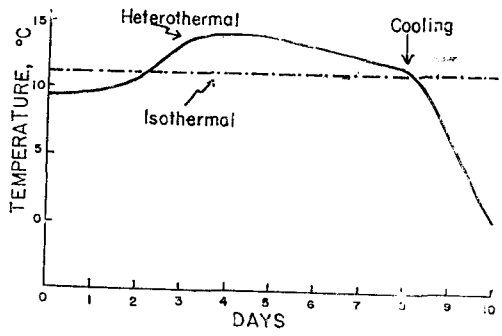

Fig. 3. The changing Pattern of Fermentation Temperature in the Plant Batch Fermentor (heterothermal) and the Constant Temperature in the Experimental Fermentor (isothermal) during the 10-day Period of Fementation.

At the start of the plant fermentation four plastic tubes of 120 cm high and 5 cm in diameter were filled with the yeast pitched wort. Two of the tubes were remained in the plant fermentor submerging the wort-filled part into the fermenting

wort so that the temperature in the tube to be changed with the surrounding plant fermentor temperature during the whole period of fermention (heterothermal) as it is shown in Fig. 3. The other two tubes were placed in a room where the temperature was controlled at 11. 5°C. (isothermal). Among the 15 metabolites detected in the worts fermented for five and ten days, those metabolites which showed significant differences between the two types of fermentations were selected and shown graphically in Figs. 4 and 5.

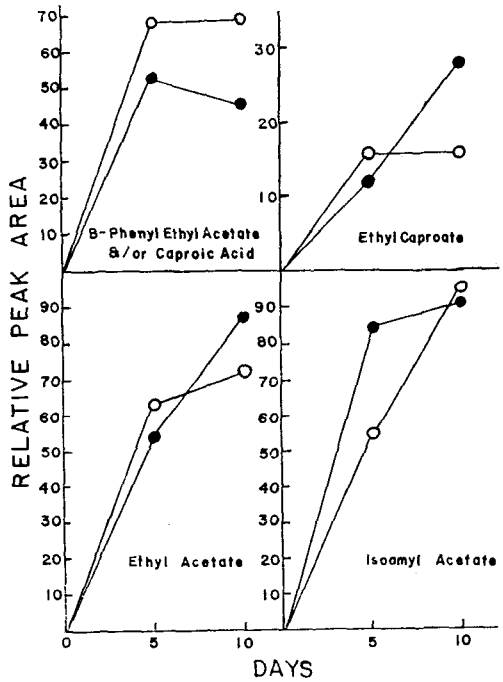

Fig. 4. Effect of heterothermal Condition on Ester Formation. -○-; Isothermal, 11. 5°C., -●-; Heterothermal, fermenting wort temperature in a plant batch fermentor.

Although the mode of influence is dependent on the kind of metabolite formed, the results suggest a good possibility of changing the characteristic flavor of the traditional beer by breaking the unique pattern of temperature accompanied with the conventional batch fermentation.

Spontaneous aqitation During the course of conventional batch fermentation, especially in the early stage, the wort in the fermentor is well agitated by the upward movement of carbon dioxide bubbles generated by the actively growing

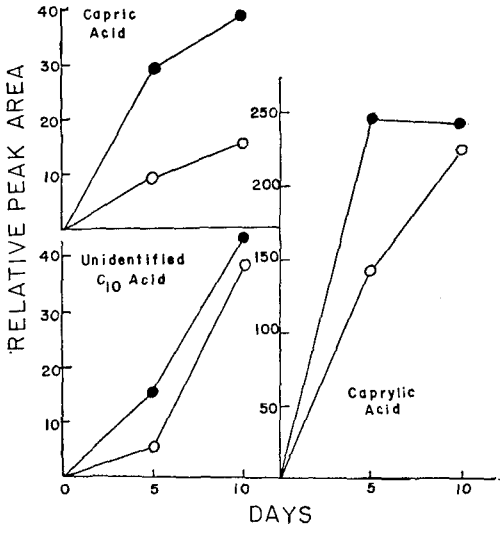

Fig. 5. Effect of heterothermal Condition on Acid Formation. -○-; Isothermal, 11. 5°C., -●-; Heterothermal, fermenting wort temterature in a plant batch fermentor.

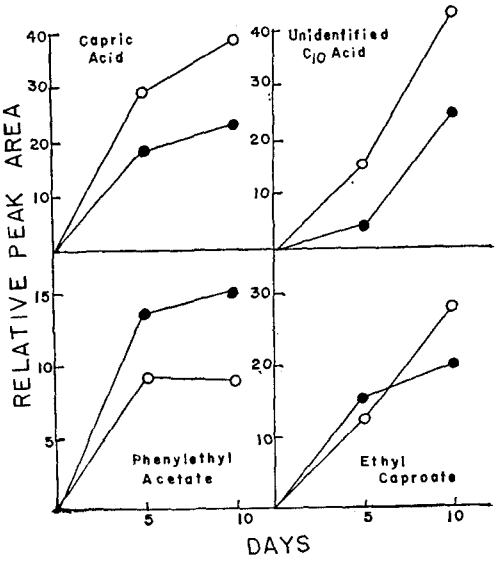

Fig. 6. Effect of Spontaneous Agitation on Acid and Ester Formation. -●- ; In the plant fermentor (spontaneous agitation), -○- ; In the tube fermentor (spontaneous agitation restricted).

yeast cells. This type of spontaneous agitation was partially blocked by fermenting the same wort in the narrow plastic tube suspended in the plant fermentor as did for the heterothermal fermentation. The analytical data of this beer were compared with that of free-moved beer in the plant fermentor in Fig. 6. The spontaneous agitation seems to be unfavorable for the syntheses of capric acid and the unidentified C_{10} acid, whereas the formation of phenyl acetate was favored.

Stratification The spontaneous agitation keeps the fermenting mass uniform during the early stage of the batch fermentation. However, as the substrate in the wort exausts the carbon dioxide generation ceases and the yeast cells starts to settle down as it is shown in Fig. 7. Thus the homogeneity of the wort exists no longer and a kind of stratification within the fermentor occurs. In this stage the fermenting body in the fermentor is sandwitched between the two different phases, i. e., the air at the top and a settled yeast layer at the bottom. Thus the suspended yeast cells near the air and those near the settled yeasts are subjected to ferment under different environmental conditions. In order to evaluate this stratification

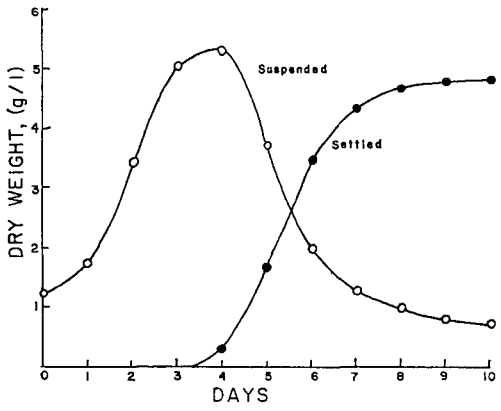

Fig. 7. Distribution of Yeast Cells in the Fermentor during the Plant Batch Fermentation. The settled yeast was estimated by subtracting the suspended yeast from the maximum total amount of yeast which was 6 gram per liter. -○- ; Suspended yeast, -●- ; Settled yeast

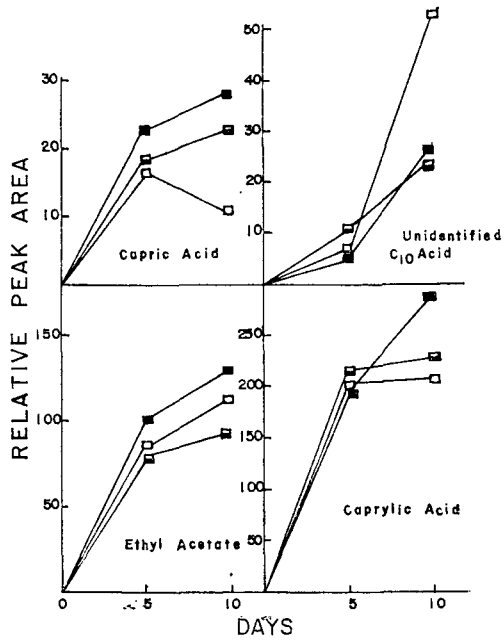

Fig. 8. Effect of Stratification on Acid and Ester Formations. -□- ; Top, -▣- ; Middle, -■- ; Bottom of the plant fermenter.

factor on the metabolite synthesis, the 5-day and 10-day fermented wort samples were collected from the bottom(2 m deep), middle(1 m deep), and the surface of the plant fermentor. Each sample contained different amounts of capric acid, caprylic acid, ethyl acetate, and the unidentified C_{10} acid(Fig. 8). The difference is more critical in the 10-day fermented stage when the stratification was more evident than the 5-day stage. The fact suggests that the stratification phenomenon accompanied by the batch fermentation also has a positive effect on the development of the beer flavor.

Foam covering During the plant batch fermentation the wort surface is covered by a layer of foam. The foam starts to accumulate at the top of the wort a few hours after the pitching and reaches a maximum thickness of more than 30cm in three or four days. The height of the foam layer then reduces gradually until some part of the liquid surface is exposed to the air just before the termination of fermentation. To reproduce

the similar condition, a 120 cm plastic tube fermentor was filled with the wort to 90 cm level leaving 30 cm for a headspace in which the foam may accumulate to cover the fermenting mass. One more tube was completely filled withwort so that the foam overflowed automatically and the liquid surface was exposed to the air throughout the fermentation. Both tubes were suspended in the plant batch fermentor as usual and fermented for eight days under the heterothermal condition. As it is shown in Table. 2, the foam covering factor also has some significance on the flavor development. The syntheses of ethyl acetate, isoamyl acetate, and capric acid appeared to be favored by the foam covering, whereas the unidentified C_{10} acid preferred exposure to the air.

Table 2. Effect of Foam Covering during the Fermentation on the Formation of Esters and Acids

Metabolites	Relative Peak Area	
	With Foam	Without Foam
Ethyl acetate	127	87
Isoamyl acetate	145	97
Capric acid	23	15
Unidentified C_{10} acid	22	31

Discussion

Drowert and Tressl (20), reviewing a number of analytical reports, listed 136 compounds as flavor components of the lager beer. More compounds will be added in the list as research proceeds. The present chromatogram of 15 peaks, therefore, may represent only one tenth of the total feature of the beer flavor. It may not be logical to guarantee the flavor of beer with the chromatogram having less number of resolution peaks than the actual number of flavor components. However, one may safely assume a possibility of flavor defect by observing any significant change appeared on the normal pattern of the chromatogram with less number of resolution peaks. At least four or five peaks among the 15 resolutions in the chromotogram were found to be altered significantly when any one of the five main fermentation factors had been forced to change experimentally. Therefore, all of the five fermentation factors tested, i. e., fermentation period, heterothermal condition, spontaneous agitation, stratification, and foam covering, are logically evaluated to be essential for the development of the characteristic flavor of the conventional batch fermented beer.

In most of the new methods of beer fermentation which have been tried by many workers, some or all of the above conditions are overlooked. In the agitated continuous fermentation, for example, none of those conditions such as the stratification, the heterothermal condition, and the sufficient fermentation period can be expected, whereas a certain degree of the foam covering and the spontaneous agitation may be retained if the agitation is gentle. In the case of continuous beer fermentation using a tower fermentor where the mechanical agitation is not applied, a stratification effect similar to the batch fermentation may be also expected in addition to that of the spontaneous agitation and the foam covering (21, 22). This may be one of the reason why the tower type continuous fermentation is more successful than the fully agitated homogeneous continuous fermentation in reproducing the typical flavor of the batch fermeted beer(17). Although there remained many other factors related to the batch beer fermentation to be evaluated, at least the above five factors should be fully considered in any modification on the existing method of fermenting beer.

要　約

麥酒의 재래식 回分醱酵에 따른 5가지 條件, 즉 醱酵期間, 變溫, 自動攪拌, 成層, 被泡現象 가운데, 한가지 條件씩을 실험적으로 변경시켜 麥汁을 醱酵하고, 생성된 麥酒를 개스크로마토 그래피로써 分析해 본 결과, 이상의 5가지 醱酵條件이 모두 麥酒의 香味形成에 필요하다는 것을 알았다.

References

1. Parsons, R. H. Method of Fermentation of Wort. U. S. Patent 3, 396, 031 (Aug. 6, 1968)

2. Coutts, M. W. Process for the Manufacture of Beer, Ale and the like. U. S. Patent3, 234, 026 (Feb. 8, 1966)
3. Pollock, J. R. A. Production of Beer. U. S. Patent 3, 207, 605 (Nov. 13, 1961)
4. Williams, R. P. Continuous Fermentation Process for Beer Production. U. S. Patent 3, 207, 606 (Sept. 21, 1965)
5. Ash, M. E. Continuous Fermentation Process with Sedimentable Microorganisms. U. S. Patent 3, 227, 557 (Jan. 4, 1966)
6. Karsch, W. Fermentation Process. U. S. Patent 2, 155, 134 (Apr. 18, 1939)
7. Portno, A. D. Continuous Fermentation Apparatus. U. S. Patent 3, 528, 889 (Sept. 15, 1970)
8. Huppmann, F. H. L. Apparatus for the Continuous Fermentation and Curing of Beer. U. S. Patent 3, 407, 722 (Oct. 29, 1968)
9. Coutts, M. W. Process for the Production of Fermented Malt Beverages. U. S. Patent 3, 157, 583 (Nov. 17. 1964)
10. Royston, M. G. Fermentation Process for the Production of Beer. U. S. Patent 3, 310, 407 (Mar. 21, 1967)
11. Hough, J. S. and A. D. Rudin. J. Inst. Brew. **64**, 404(1958)
12. Hough, J. S. and R. W. Ricketts. J. Inst. Brew., **66**, 302 (1960)
13. Enebo, L. and U. Palmqvist. Svennsk Bryggeritidskrift **76**, 1/2. 16: abstrcted, Wallerstein Lab. Comm. **24**, 85, 339(1961)
14. Sandegren, E. and Enebo, L. Wallestein Lab. Comm. **24**, 269(1961)
15. Nordstrom, K. European Brewery Conv., Proc., Stockholm, 195, (1965)
16. Watson, T. G. and J. S. Hough, J. Inst. Brew., **72**, 547(1966)
17. Thorne, R. S. W. Brewers Digest, 50, Feb. (1968)
18. Makinen, V. and T. M. Enari. Brewers Digest, 72, May (1969)
19. Powell, A. D. G. and I. H. Brown, J. Inst. Brew., **72**, 261(1966)
20. Drawert, V. F. und R. Tressl. Brauwissenschaft, **22**, 5, 169, (1969)
21. Poyston, M. G. : Guardian, Feb. (1966)
22. Royston, M. G. ; Process Bioch., July(1966)