JOURNAL OF BIOSCIENCE AND BIOENGINEERING
Vol. 106, No. 4, 317–323. 2008
DOI: 10.1263/jbb.106.317



# REVIEW

# Beer Volatile Compounds and Their Application to Low-Malt Beer Fermentation

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Received 27 August 2007/Accepted 24 June 2008

**Low-malt beers, in which the amount of wort is adjusted to less than two-thirds of that in regular beer, are popular in the Japanese market because the flavor of low-malt beer is similar to that of regular beer but the price lesser than that of regular beer. There are few published articles about low-malt beer. However, in the production process, there are many similarities between low-malt and regular beer, *e.g.*, the yeast used in low-malt beer fermentation is the same as that used for regular beer. Furthermore, many investigations into regular beer are applicable to low-malt beer production. In this review, we focus on production of volatile compounds, and various studies that are applicable to regular and low-malt beer. In particular, information about metabolism of volatile compounds in yeast cells during fermentation, volatile compound measurement and estimation methods, and control of volatile compound production are discussed in this review, which concentrates on studies published in the last 5–6 years.**

[**Key words:** beer fermentation, esters, higher alcohols, yeast metabolism]

Low-malt beers, in which the amount of wort is adjusted to less than two-thirds of that in regular beer and the malt carbon source is compensated by adding sugar syrup, are popular in the Japanese market. Moreover, new alcoholic beverages that taste like beer, in which a nitrogen source extracted from materials other than wort and sugar syrup is used, are acceptable. The liquor tax on Japanese alcohol beverages depends on the percentage of malt. Therefore, beverages with low concentration of malt are taxed at lower rates than beer made from all malt wort.

In low-malt beer, volatile compounds produced by yeast metabolism are important for approximating the flavor of regular beer. During low-malt and regular beer fermentations, as by-products of yeast metabolism, sugars in wort are converted to ethanol and volatile compounds, such as higher alcohols and esters. The volatile compounds are different from aromatic compounds in malt and hops, and many of them cause undesirable flavors when their concentration exceeds certain thresholds. Some volatile compounds are reduced in concentration during maturation, which follows fermentation. However, a fraction of higher alcohols, esters, and other carbonyl compounds remain, thereby affecting the flavor of the final product. Therefore, it may be necessary to keep the concentrations of volatile compounds in the final product below their taste thresholds so that they do not produce salty, sweet, bitter, or acid flavors.

A major difference between low-malt and regular beer fermentations is that the free amino acid nitrogen (FAN) concentration in low-malt beer wort is low at the beginning of the low-malt beer fermentation process. After nitrogen source depletion, proliferation of yeast cells ceases. Thus, the amount of FAN in the wort affects the number of yeast cells proliferated. To make fewer yeast cells consume the same amount of carbon sources than those in regular beer fermentation, yeast cell activity has to be maintained by increasing the temperature. However, high temperature fermentation can result in higher concentrations of volatile compounds. Therefore, it is important to know the production characteristics of volatile compound biosynthesis.

There are few published articles about low-malt beers; however, volatile compound production has been investigated in regular beer brewing from the viewpoint of gene functions in yeast metabolic pathways, measurement and estimation methods, and control procedures. In the production process, there are many similarities between low-malt and regular beer. For example, the yeast used and the basic fermentation procedure in low-malt beer fermentation are the same as in regular beer production. Therefore, the results from investigations in regular beer production are applicable to low-malt beer production. To creatively improve the fermentation process in low-malt beer and develop dif-

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TABLE 1. List of beer flavors associated with various compounds (1, 2, 4)

| | Flavor in beer | Organoleptic threshold (ppm) | Concentration in Japanese beer (ppm) |
|---|---|---|---|
| Higher alcohols | | | |
| Propan-1-ol (*n*-propanol) | Alcohol | 800 | 8–15 |
| 2-Methyl propanol (isobutyl alcohol) | Alcohol | 200 | 7–14 |
| 2-Methyl butanol (active amyl alcohol) | Alcohol, banana, medicinal, solvent | 65 | 46–71 |
| 3-Methyl butanol (isoamyl alcohol) | Alcohol, banana, sweetish, aromatic | 70 | |
| 2-Phenyl ethanol | Roses, sweetish, perfumed | 125 | 20–27 |
| Esters | | | |
| Ethyl acetate | Solvent, fruity, sweetish | 30 | 10–20 |
| Isoamyl acetate | Banana, apple, solvent, estery | 1.2 | 1.3–2.5 |
| 2-Phenylethyl acetate | Roses, honey, apple, sweetish | 3.8 | 0.4–1.3 |
| Ethyl caproate | Sour apple | 0.21 | |
| Ethyl caprylate | Sour apple | 0.9 | |
| Carbonyl compounds | | | |
| Acetaldehyde | Green leaves, fruity | 25 | 2.9–3.4 |
| 2,3-Butanedione (diacetyl) | Butter-scotch | 0.15 | <0.01–0.06 |

ferent alcohol beverages with original flavors, the results from regular beer brewing investigations are useful. Thus, several studies, which are applicable to both regular and low-malt beer, relevant to the metabolism of volatile compounds in yeast cells during fermentation, related to measurement and estimation methods, and pertinent to the control of volatile compound production are compiled in this review, which focuses on research studies published in the last 5–6 years.

## CHARACTERISTICS OF VOLATILE COMPOUNDS

Recently, many different volatile compounds responsible for beer flavor have been identified. They may be classified into five main groups: flavor compounds derived (i) from ingredients such as barley and hops, (ii) from roasting malt and boiling wort, (iii) as by-products of yeast metabolism, (iv) from contaminant microorganisms, (v) from the effects of oxygen and sunlight during product storage. In this article, we focus on the third group, particularly on the higher alcohols, esters, and other carbonyl compounds, which were selected as representative compounds responsible for the low-malt beer flavor. The volatile compounds discussed in this review are listed in Table 1 with their characteristic flavor, taste threshold in beer and average concentrations in several brands of Japanese beer (1, 2). Studies focusing on volatile compounds during beer maturation have been reviewed previously (3).

Volatile compounds are structurally divided into higher alcohols, esters, carbonyl compounds such as aldehydes and ketones, and sulfur-containing compounds. In the higher-alcohol groups, amyl alcohol is reported to be the most quantitatively significant flavor compound (1). Active amyl and isoamyl alcohols are sometimes considered as one and represented simply as amyl alcohol. Active amyl alcohol is usually one-fifth to one-quarter of the total amyl alcohol, and it affects drinkability because beer flavor is considered heavier as amyl alcohol concentration increases (1). Isobutyl alcohol has an undesirable effect on beer quality when its concentration exceeds 20% of the total concentration of three alcohols: *n*-propanol, isobutyl, and amyl. Beer flavor becomes fruity and undesirable when the concentrations of esters are high. Representative esters in beer are ethyl acetate, isoamyl acetate, ethyl caproate, ethyl caprylate, and phenyl ethyl acetate (4). Of these esters, ethyl acetate is typically present in the highest concentration and has a fruity and solvent-like flavor. There are experimental data that indicate correlations between ethyl acetate and isoamyl acetate productions and ethanol and isoamyl alcohol productions, respectively (5); however, the correlations were affected by several environmental factors, including oxygen, unsaturated fatty acids, fermentable sugars, and nitrogen. More detailed information is presented in a published review (4).

Concentrations of carbonyl compounds in beer are comparatively low. Even acetaldehyde, the predominant carbonyl compound in beer, is present at no more than 10 ppm. Diacetyl is produced during primary fermentation and its concentration is often used as an index of the completion of beer fermentation or maturation (6). Another volatile compound, 3-methylbut-2-ene-1-thiol (MBT), which is a sulfur-containing compound, has recently been the focus of studies for elucidation of its synthesis pathway (7, 8). MBT is produced not by yeast metabolism, but by the effects of light, and gives off a reported skunk-like odor at concentrations of a few tens of ng $l^{-1}$.

## METABOLISM OF VOLATILE COMPOUNDS DURING FERMENTATION

Generally, bottom-fermenting yeast, *Saccharomyces carlsbergensis*, which is used in regular beer fermentation, is also used in low-malt beer fermentation. *S. carlsbergensis* has been reclassified as *S. pastorianus*, as a natural hybrid between *S. cerevisiae* and *S. bayanus*. Thus, bottom-fermenting beer yeast possesses portions of two genomes: *S. cerevisiae* (Sc)-type and Lager (Lg)-type (9, 10). To investigate gene functions related to biosynthesis of volatile compounds, *S. cerevisiae* is often used because it possesses a wide experimental background and is easy to manipulate in gene-recombination technology. In this review, most of the results discussed are from *S. cerevisiae*.

**Gene functions related to biosynthesis of higher alcohols**

There are two metabolic pathways for higher alcohol production in *S. cerevisiae* (Fig. 1). One is from amino acids

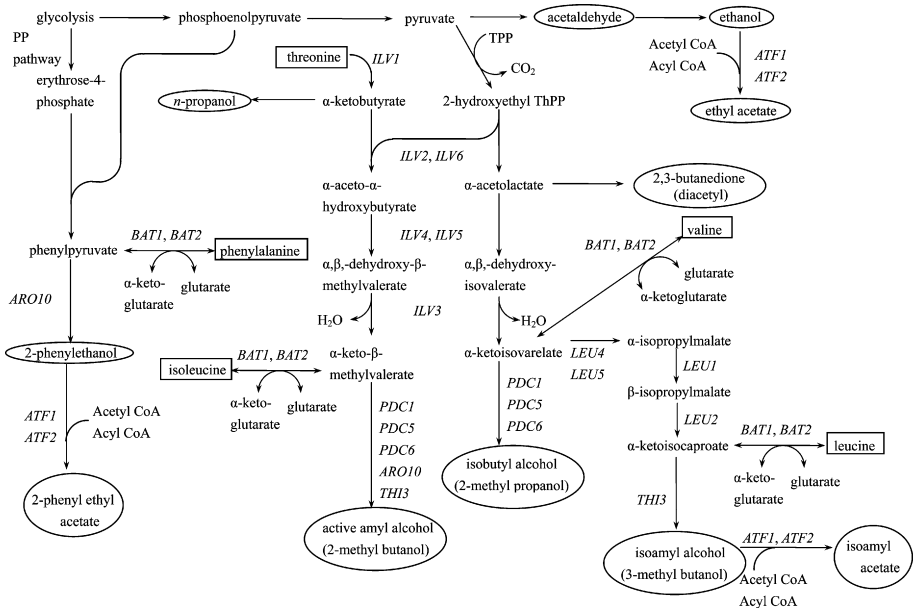


FIG. 1. Biosynthetic pathways for volatile compounds formation in *Saccharomyces cerevisiae* (6, 16).

and the other from glycolysis. In both cases, α-keto acids are produced, decarboxylated to aldehydes, and dehydrogenated to form the corresponding primary alcohols. In the first or Ehrlich pathway, the amino acids are converted to α-keto acids by aminotransferase. An ester is synthesized from a higher alcohol and acyl-CoA or acetyl-CoA, and ester production is closely related to precursor higher-alcohol production. Therefore, it is important to elucidate higher-alcohol production and the mechanism for conversion of higher alcohol to esters in the study of alcoholic beverage flavors.

The production of higher alcohols has been examined using many types of recombinant yeast, which were generated based on information derived from the metabolic pathway. Eden *et al.* focused on a branched-chain amino acid aminotransferase. The *ECA39* and *ECA40* genes in *Saccharomyces pombe* (*BAT1* and *BAT2* in *Saccharomyces cerevisiae*) have been reported to encode mitochondrial and cytosolic branched-chain amino acid (BCAA) aminotransferases (11). These workers generated *S. cerevisiae* mutants, in which the genes encoding BCAA aminotransferase were deleted. These modifications had no effect on *n*-propanol production; however, the *eca40* mutant showed markedly reduced isobutyl alcohol production (12). Unexpectedly, a higher isoamyl alcohol concentration was observed in the *eca39*/*eca40* double-deletion mutant. In another study, it was observed that the *bat2* single- and *bat1*/*bat2* double-deletion mutants grown on glucose can produce isoamyl alcohol (13). However, these strains were incapable of producing isoamyl alcohol when ethanol was the sole source of carbon. From their gene expression analysis, in which *BAT2* gene expression was strongly enhanced in the wild-type grown in ethanol as a carbon source, the authors suggested that *BAT2* expression is essential for isoamyl alcohol formation in ethanol-containing media whereas *BAT1* is essential for isoamyl alcohol production in glucose-containing media. *BAT2* overexpression was examined using a transformant with multicopy plasmids containing *BAT2*, and it was observed that overexpression resulted in 1.3-fold and 2.2-fold increases in isoamyl alcohol and isobutyl alcohol productions, respectively (14). Furthermore, a similar result was observed in *S. cerevisiae* laboratory strain BY 4742 (15).

Five genes, *i.e.*, *PDC1*, *PDC5*, *PDC6*, *ARO10*, and *THI3*, encode pyruvate decarboxylase activity (16). In the presence of such activity, α-keto acids can be directly converted to higher alcohols (Fig. 1). The role of these five genes during leucine catabolism has been investigated (17, 18). The *pdc1*/*pdc5*/*pdc6*/*thi3*/*aro10* quintuple-deletion mutant did not produce isoamyl alcohol and the *pdc1*/*pdc5*/*pdc6*/*thi3* quadruple-deletion mutant showed a markedly reduced isoamyl alcohol production, while the *pdc1*/*pdc5*/*pdc6* triple-deletion mutant produced more isoamyl alcohol than the wild-type strain. It has been reported that *THI3* is mainly responsible for the decarboxylation of α-ketoisocaproate to isoamyl alcohol.

In valine catabolism, the *pdc1*/*pdc5*/*pdc6* triple-deletion mutant showed a reduced isobutyl alcohol production, and *thi3* had no effect on such production (19). In isoleucine catabolism, only the *pdc1*/*pdc5*/*pdc6*/*thi3*/*aro10* quintuple-deletion mutant could not produce active amyl alcohol. It appears that the participation of the five genes mentioned above is significant in active amyl alcohol production (20).

In phenylalanine catabolism, it has been reported that the production of 2-phenylethanol decreased when *pdc1*/*pdc5*/*pdc6*/*aro10* quintuple-deletion mutant was used (18, 21). By measuring the enzyme activity of phenylpyruvate decarboxylase and the production of 2-phenylethanol, Vuralhan *et al.* demonstrated that *aro10* exhibits phenylpyruvate decarbolylase activity (21).

Microarray analysis and automated high-throughput screening have been performed to identify target genes related to higher alcohol production. Schoondermark-stolk *et*

*al.* identified seven genes related to the isoamyl alcohol pathway, *i.e.*, *LAT1*, *PDX1*, *THI3*, *ALD4*, *ILV3*, *ILV5*, and *LEU4*; four genes related to NAD metabolism, *i.e.*, *BNA2*, *BNA3*, *BNA4*, and *BNA6*; and five genes related to the TCA cycle and glutamate metabolism, *i.e.*, *MEU1*, *CIT1*, *CIT2*, *KDG1*, and *KDG2*. The expressions of these genes markedly changed when media pH was adjusted to the optimal pH values for isoamyl alcohol production (pH 3.0) and cell growth (pH 5.0) (22).

Abe *et al.* reported on some high-pressure growth (HPG) mutants capable of growth at high hydrostatic pressures (23). *HPG1* mutants with a defect in their Rsp5 ubiquitin ligase showed enhanced activity in the uptake of amino acids including leucine, and produced isoamyl alcohol and isoamyl acetate yields 2–3-fold and 4–8-fold, respectively, than that of the wild-type strain (24). This suggests that the leucine permeases Bap2 and Bap3 are likely stabilized by the *HPG1* mutation. In brewing yeasts with a constitutive *BAP2* expression, similar results were observed (25). Thus, *BAP2* overexpression accelerates the assimilation of leucine and valine, and such an yeast strain would increase isoamyl alcohol production.

**Gene functions related to biosynthesis of ester** Esters confer a fruity flavor to fermented beverages. The mechanism of ester production has been investigated along with that of higher alcohols. Experimental results showing the effects of the artificial recombination of genes and several industrial parameters for yeast ester formation have been reviewed (4). Volatile esters are produced via an enzyme-catalyzed reaction from a higher alcohol and acetyl-CoA. Several different enzymes are involved in the formation of esters, but the ones most investigated are alcohol acetyl transferases I and II that are encoded by *ATF1* and *ATF2*, respectively. However, research groups have used different yeast strains and different fermentation conditions for the study of ester production, so it is difficult to compare the results in different groups directly. The effects of the deletion or overexpression of *ATFl* and *ATF2* on ester production have been studied in the same yeast strain. It has been reported that *ATF1* and *ATF2* expression levels (mainly *ATF1*) affect the production of ethyl acetate, isoamyl acetate, and the other acetates. Upon *ATF1* deletion, yields of ethyl acetate and isoamyl acetate were reduced by 60% and 20%, respectively, compared with those in the wild-type strain. On the other hand, *ATF1* overexpression resulted in 30-fold ethyl acetate and 190-fold isoamyl acetate production increases despite a 50% reduction in isoamyl alcohol production (26). Furthermore, the expression levels of *ATF1* and the *ATF1* homologue, *Lg-ATF1*, were investigated under different environmental conditions during fermentation. In that study, the expression of *HPS12*, which was known to be repressed by the Ras/cAMP/PKA signaling pathway, was also investigated. *ATF1* and *Lg-ATF1* expression levels rapidly increased with glucose addition and remained constant when a nitrogen source was present in the medium. Under ethanol and heat shock stress conditions, *ATF1* and *Lg-ATF1* were transiently induced, and *HPS104* expression, which is an indicator of heat and ethanol stress response, was transiently repressed, *i.e.*, *HPS104* showed an expression profile opposite those of *ATF1* and *Lg-ATF1*. It is well known that most genes that are induced by the Ras/cAMP/PKA pathway are repressed by stress. These results indicate that *ATF1* is neither stress-inducible nor controlled by the Ras/cAMP/PKA pathway (27).

The localization of *ATF1* expression was determined using *ATF1::GFP* fusion and the fluorescent dye, Nile red, which is a highly selective chromogen for lipid particles, and is used to determine the localization and quantity of neutral lipids and polar lipids. UV fluorescence microscopy revealed that the *ATF1::GFP* fusion protein localizes in organelles with high amounts of neutral lipids or sterols stained by Nile red. Furthermore, purified lipid particles are reported to show alcohol acetyl transferase activity (28). More detailed analysis of the relationship between lipid compounds and the role of Atf1p is needed.

**Gene functions related to biosynthesis of other volatile compounds** To reduce the concentration of acetaldehyde existing as an intermediate to ethanol formation, a mutant with a disrupted alcohol dehydrogenase II gene (*ADH2*) was investigated (29). *ADH1* encodes a protein that acts as the main catalyst of acetaldehyde reaction, whereas *ADH2* predominantly encodes a protein for ethanol consumption and ethanol transfer to acetaldehyde. Although there were no differences in cell growth and concentrations of other volatile compounds during fermentation between the parent and mutant brewing yeast strains, the final acetaldehyde concentration from the mutant strain decreased to approximately one third that of the parent strain.

## MEASUREMENT AND ESTIMATION OF VOLATILE COMPOUND CONCENTRATIONS IN THE FERMENTATION PROCESS

**Measurement of volatile compound concentrations in beer** To measure volatile compound concentrations in beer, gas chromatography (GC) or gas chromatography/mass spectrometry (GC/MS) are widely used. Direct injection is not suitable for the quantitative analysis of beer samples because they contain large amounts of nonvolatile compounds that may damage the column. Although the headspace (HS) sampling technique has an advantage over direct injection in which only the volatile compounds in the sample can be injected, its sensitivity is low. Therefore, several extraction methods, which can be performed before injection, have been examined for the measurement of volatile compound concentrations in beer. Recently, two main types of extraction method have been investigated: solid-phase microextraction (SPME) and single-drop microextration (SDME). Both extraction methods may be used in combination with the HS method for beer samples. In SPME, the analyte in the sample is adsorbed to an immobilized polycoat fiber bound to a fine needle and removed by heating in the inlet of the GC or GC/MS device; this is a fast, sensitive, and solvent-free method. Furthermore, many types of alcohol, ester, and carbonyl compounds in beer can be measured simultaneously (30–32). Another study used SPME and gas chromatography-olfactometry, an analytical technique for evaluating the aroma characteristics of volatile constituents separated by GC (33). As an important component of sensory quality, the intensity of the ester based aroma characters in lager beer was evalu-

TABLE 2. Control parameters for volatile compound production

| Parameter | | Production of higher alcohols and esters | Reference |
|---|---|---|---|
| Amino acid | Leu | Addition promoted (amyl alcohols and esters) and no effect on isobutyl alcohol | 25, 44 |
| | Val | Addition promoted (only isobutyl alcohol production) | 25 |
| | Ile | Addition promoted (only amyl alcohol production) | 25 |
| | Asp | Addition promoted (ethyl acetate and *n*-propanol) and repressed (isobutyl alcohol) | 45 |
| Metal | Zn | Addition promoted (higher fermentation rates was obtained.) | 44 |
| Lees oil | | Addition promoted (isoamyl acetate) and no effect on ethyl acetate | 44 |
| EDTA | | Addition did not significantly promote | 44 |
| Fatty acid | C18:2 | Addition repressed (only acetate esters) | 44 |
| Gravity | | Promoted (from 12 to 20° Plato media) and repressed in higher-gravity media | 44, 46 |
| Temperature | | Promoted as temperature increased | 47 |
| Top pressure | | Repressed as top pressure increased | 47 |
| Oxygen | | Repressed during aeration prior to pitching | 48 |
| pH (4.9–8.5) | | Promoted (isoamyl alcohol and isoamyl acetate) and repressed (ethyl acetate) | 44 |
| Serial repitching | | Promoted as the number of repitchings increased | 44, 49 |

ated using SPME and GC data (34).

In SDME, a microdroplet is held in a large flowing aqueous phase or above an aqueous sample solution, and the analytes in the sample are extracted into the droplet. SDME is simple and is inexpensive when compared with the SPME method. To date, in beer, only alcohols have been quantified (35, 36). However, many applications using other samples have been reported (37). A comparison of SPME and SDME has been reported in detail elsewhere (37, 38).

Phenyl ethanol is difficult to analyze due to its high boiling point (220°C) and low volatility. To measure, consistently and accurately, phenyl ethanol concentrations, a stable isotope dilution analysis of beer has been performed. Deuteriated phenyl ethanol was synthesized with lithium aluminum deuteride and the phenyl ethanol concentrations in several beer samples were calculated from the ratios of labeled to unlabeled compounds in GC/MS (39).

**Estimation of volatile compound concentrations during fermentation** During fermentation, it is difficult to determine accurately the volatile compound concentrations produced by yeast metabolism. Regular and low-malt beer fermentations are performed under static conditions and the media used do not mix. Therefore, the concentrations of volatile compounds in the media are not uniform. There are many predictive models of volatile compound formation based on fundamental knowledge of biochemical pathways (40, 41); however, the models cannot be applied to actual production sites because fermentation progress indicated by sugar uptake cannot be estimated *in situ*. Although cell growth cannot be predicted, changes in the specific gravity of wort, an index of fermentation progress, by monitoring $CO_2$ concentration in exhaust gas has recently been developed (42). Such a $CO_2$ concentration parameter is valuable because it is measurable during static fermentation. $CO_2$ concentration in exhaust gas has also been applied as a parameter to estimate the concentrations of the formed volatile compounds (5, 43). It has been experimentally demonstrated that the amount of $CO_2$ produced was proportional to the levels of higher-alcohol and ester production, and their levels were easily estimated from the amount of $CO_2$ produced (5). Using these relationships, *n*-propanol, isobutyl alcohol, isoamyl alcohol, ethyl acetate, and isoamyl acetate productions have been estimated during beer fermentation by measuring the amount of $CO_2$ produced.

## CONTROL OF VOLATILE COMPOUND PRODUCTION

As mentioned above, in low-malt beer fermentation, the low FAN concentration levels affect the proliferation of yeast cells. To consume the same amount of carbon sources, which are at the same level in regular and low-malt beer worts, yeast cells are activated by keeping the temperature higher, *i.e.*, in the range of 9–21°C. However, higher temperature fermentation can result in higher concentrations of volatile compound production. Therefore, it is necessary to control volatile compound production more accurately in low-malt beer fermentation than in regular beer fermentation. Higher-alcohol and ester production can be promoted or repressed by the methods shown in Table 2 (25, 44–49). Previously, various methods of controlling ester productions were reviewed (4). In many cases, ester production was closely related to the production of higher-alcohol precursors, and the control of higher-alcohol production has been regarded as significant. The characteristics of the bioconversion of L-phenylalanine to 2-phenyl ethanol were investigated in fed-batch and chemostat cultures (50, 51). However, 2-Phenyl ethanol production was not included herein because this production is completely growth-associated.

Diacetyl production has been investigated in wort that contains different valine concentrations (6). In fermentations in low valine concentration wort (83–115 mg/*l*), the uptake rate of valine increased and valine was exhausted during fermentation compared with a high valine concentration wort (146–211 mg/*l*). In the low valine wort, a double peak was shown in the total diacetyl profile and the residual diacetyl concentration was high at the end of the fermentation. In the case of a high valine concentration in wort (146–211 mg/*l*), the rate of valine uptake became slow and valine was still present at the end of the fermentation. However, the diacetyl concentration at the end of fermentation decreased and a single peak in the total diacetyl profile was observed. Diacetyl concentration at the end of fermentation was lower in the single peak diacetyl profile (high valine wort) than the one in the double-peak profile (low valine wort).

There are many yeast strains with different volatile com-

pound production characteristics and production during fermentation differs between strains and batches. To control volatile compound production, brewers change physical factors, such as temperature and top pressure, during fermentation. Along with the development of estimation methods for volatile compounds, a computer simulation system has been developed (52). Such simulations suggest that simultaneous control of the fermentation progress and the production of *n*-propanol, isobutyl alcohol, and isoamyl alcohol, which are quantitatively significant compounds, can be realized with temperature shifts during low-malt beer fermentation. By measuring the concentration of $CO_2$ in exhaust gas, fermentation progress and volatile compound production can be monitored. Furthermore, an optimal temperature profile indicating the time for, and extent of, temperature change can be determined, and a control system with a temperature profile and a feedback control system can be constructed, taking cell activity estimates into account. The practicality of such a volatile compound control system has been demonstrated using real wort (5).

## FUTURE PERSPECTIVES

In 1996, the *S. cerevisiae* genome was sequenced, and this information is widely available through the world wide web. Many of the studies mentioned in this review used this genome information. Furthermore, DNA microarrays for bottom-fermenting yeasts have been developed (Nakao, Y., Kodama, Y., Fujimura, T., Rainieri, S., Nakamura, N., Ito, T., Hattori, M., Shiba, T., and Ashikari, T., 11th International Congress on Yeast, Rio de Janeiro, Brazil, 2004). Using these information sources, future studies using not only the ale yeast strain but also the lager brewing strain are expected. Additionally, the development of new analytical tools resulting from the recent rapid progress in chemistry, is expected. Such tools, along with the available DNA information, will provide novel information on low-malt and regular beer brewing. It is believed that these studies will permit further improvements in low-malt and regular beer development.

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