Bioflavoring and beer refermentation

Genetically modified yeasts with improved flavoring characteristics

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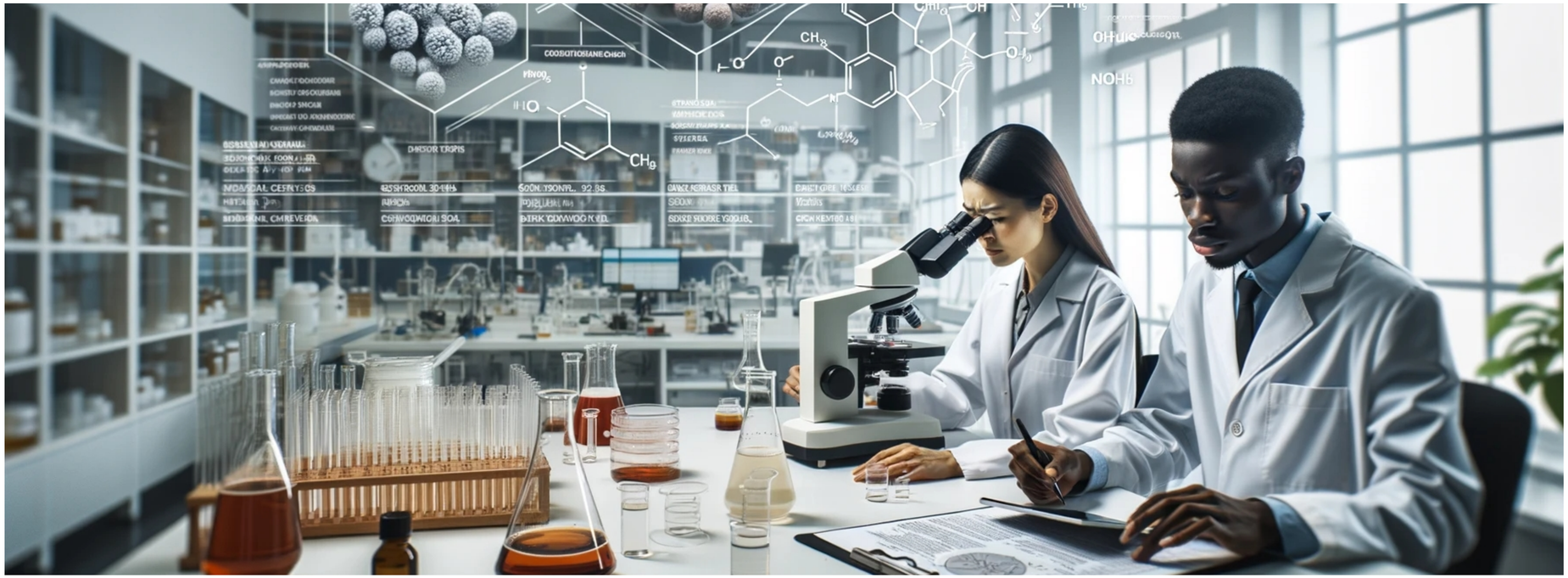
Introduction

The development of gene technology has opened up the possibility of engineering an organism’s metabolism and thus flavor production. Over the last 20 years, many examples of metabolic engineering have been described. In this paragraph, a few characteristic examples of the flavor improvement of brewer’s yeast are described.



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Ester Profiles

Who are you? *(click one)*

Beer Enjoyer 

Beer Maker 

Biochem Whiz 

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Beer Enjoyer

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Volatile aroma-active esters, such as ethyl acetate, isoamyl acetate, ethyl caproate, and ethyl caprylate, are responsible for the fruity flavors of fermented beverages (Meilgaard 2001; Nyknen 1986). However, especially in the modern beer industry, where **high-gravity worts** and **cylindroconical fermentation** vessels are used, the ester balance is often disturbed (Anderson and Kirsop 1974; Meilgaard 2001; Palmer and Rennie 1974; Younis and Stewart 1999).

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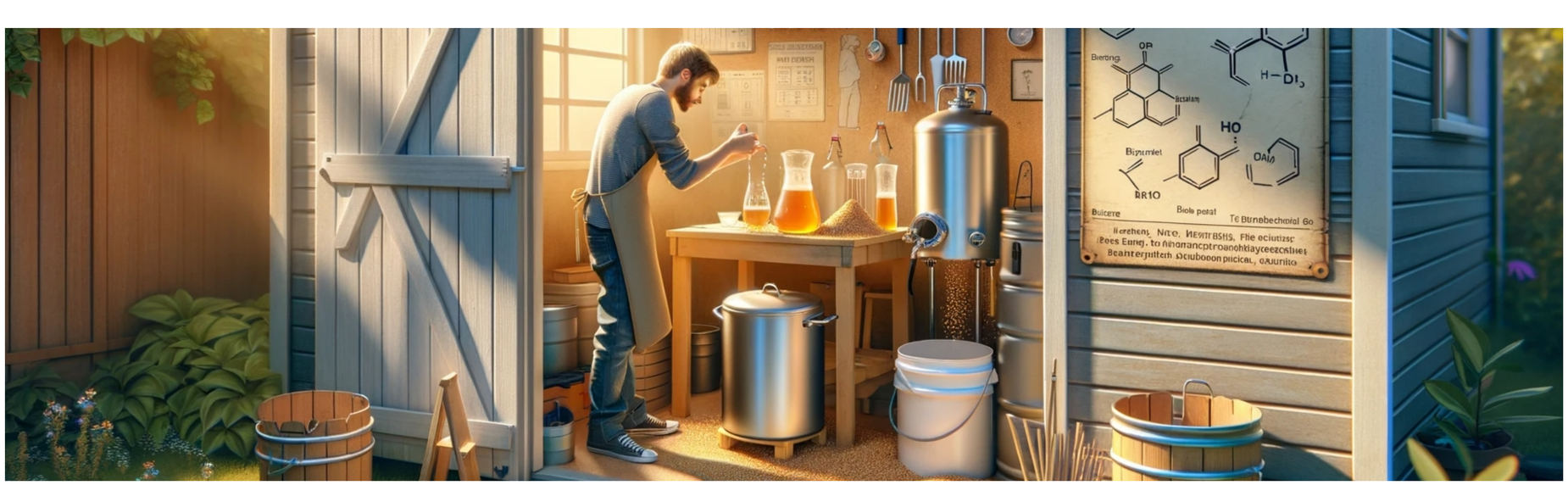
One way to alter the ester production is to change the synthesis rate of a certain **fusel alcohol**, which is in turn responsible for an increase in the corresponding ester level. This method was successfully used by Hirata et al. (1992). To increase **isoamyl acetate** levels, they introduced extra copies of the **LEU4 gene** into the **S. cerevisiae genome**. This results in an increased production of **isoamyl alcohol** and its corresponding **acetate ester**, isoamyl acetate. A comparable **Saccharomyces uvarum** mutant was isolated by Lee et al. (1995). The mutants, which have an altered regulation pattern of **amino acid metabolism**, produce more isoamyl acetate and **phenylethyl acetate**. However, since this method also disturbs amino acid metabolism and fusel alcohol levels, it is not an optimal procedure with which to alter the ester levels.



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An alternative approach is the **overexpression** of one or more of the ester synthesis genes. Experiments have shown that overexpression of the **ATF1 gene** results in a strong increase in the levels of **ethyl acetate**, isoamyl acetate, and **2-phenylethyl acetate** (Fujii et al. 1994; Lilly et al. 2000; Verstrepen et al. 2003b; Verstrepen et al. MS submitted). Using a combination of these mutant strains and the wild type yeast should make it possible to really modify the ester concentration in beer to a desired profile.

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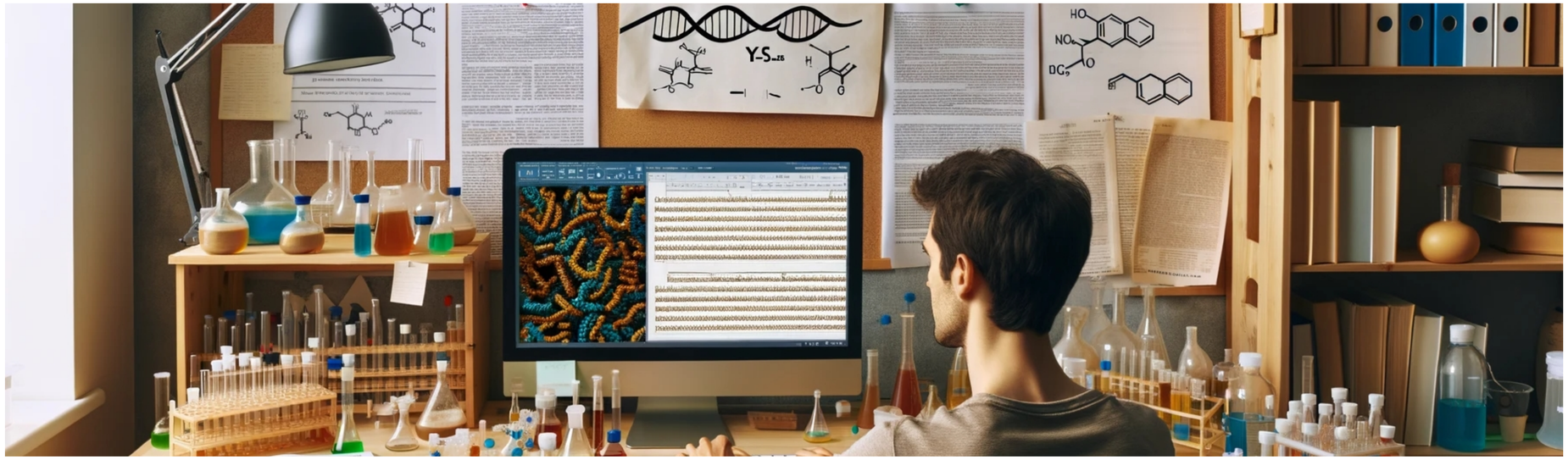
Ester formation by **S. cerevisiae** is quite a complex process. Firstly, two different co-substrates are needed for **ester synthesis**: alcohols and acids. In yeast, the acid co-substrates are activated fatty acids, and the alcohols are either ethanol or more complex **fusel alcohols**, which are derived from **amino acid metabolism** (Calderbank and Hammond 1994). Secondly, the formation of **volatile esters** requires **enzymatic catalysis** and different groups of esters are formed by different ester synthases (Yoshioka and Hashimoto 1981, 1983, 1984). Molecular research has enabled the cloning and characterization of four different genes that encode ester-synthesising enzymes: **ATF1**, **Lg-ATF1**, **ATF2**, and **EHT1** (Dufour et al. 2002; Fujii et al. 1994, 1996; Minetoki et al. 1993; Nagasawa et al. 1998; Yoshimoto et al. 1999).



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It is however expected that S. cerevisiae possesses even more, as yet unknown, ester synthesis genes (Mason and Dufour 2000). Some fermentation parameters can be adapted in order to exert a certain influence on ester formation rates (for a review, see Verstrepen et al. 2003a), but in general the complex nature of ester synthesis makes it difficult to get a tight grip on esters in **industrial fermentations**. Therefore, much research has focused on the use of mutants and genetically modified yeasts with altered ester production profiles.

Volatile aroma-active esters

Chemical compounds that vaporize into the air, often carrying with them a distinctive scent or flavor. In the context of fermented beverages, they give the drink its unique aroma and taste.

High-gravity worts

Concentrated solutions used in brewing that contain higher amounts of fermentable sugars.

Cylindroconical fermentation

The brewing process in specially designed vessels with a cylindrical top and conical bottom, facilitating efficient yeast collection and temperature-controlled beer fermentation.

LEU4 gene

A gene in yeast involved in the biosynthesis of the amino acid leucine, playing a role in metabolic pathways and potentially influencing certain fermentation characteristics.

Overexpression

A condition where a gene is more active, producing more of its product than normal.

Fusel alcohols

Alcohols that are by-products of fermentation, often responsible for "off" flavors in alcoholic beverages.

Isoamyl Acetate

An ester with a banana-like aroma, common in certain beer styles.

S. cerevisiae genome

The genetic material of brewer's yeast, influencing its fermentation characteristics.

S. cerevisiae

A commonly used yeast in brewing, especially for ales. This yeast ferments at warmer temperatures and can contribute a wide range of flavors and aromas to beer, depending on the strain and fermentation conditions.

Ester synthesis

the production of esters by yeast during fermentation. Esters are responsible for many of the fruity aromas in beer, like banana or apple.

Fusel alcohols

Alcohols that are by-products of fermentation, often responsible for "off" flavors in alcoholic beverages.

Amino acid metabolism

Processes by which yeast metabolizes amino acids during fermentation, influencing beer’s flavor and aroma.

Volatile esters

Organic compounds formed during yeast fermentation in beer, imparting fruity and floral aromas.

Enzymatic catalysis

Enzymes that break down starches in malted grains into fermentable sugars, which are then consumed by yeast to produce alcohol and CO2.

Isoamyl Alcohol

A fusel alcohol found in beer, contributing to its flavor and aroma profile.

Acetate ester

A group of compounds produced during fermentation that contribute fruity notes to beer.

Saccharomyces uvarum

A yeast species often used in lager beer fermentation, known for producing distinct flavor profiles compared to Saccharomyces cerevisiae.

Industrial fermentations

The large-scale brewing of beer in controlled conditions using specific strains of yeast to ensure consistency and quality across batches.

ATF2

A gene in S. cerevisiae that encodes for an ester-synthesising enzyme, specifically impacting the production of volatile esters that influence beer aroma.

EHT1

A gene often found in brewing yeasts that encodes for an enzyme involved in the synthesis of ethyl esters, which contribute to the fruity aromas in beer.

Amino acid metabolism

Processes by which yeast metabolizes amino acids during fermentation, influencing beer’s flavor and aroma.

ATF1

gene in brewing yeasts associated with the synthesis of acetate esters, especially ethyl acetate and isoamyl acetate. These esters contribute to the fruity aromas of many beers.

Phenylethyl acetate

An aromatic ester that contributes floral and rose-like scents to beers and wines during fermentation.

Lg-ATF1

A variant or related gene to ATF1 in S. cerevisiae, involved in the synthesis of specific volatile esters that contribute to the unique aromatic characteristics of beer.

Ethyl Acetate

An ester that gives a fruity aroma to beer, but in high concentrations can impart a solvent-like note.

ATF1 Gene

In Saccharomyces cerevisiae yeast, it regulates enzymes that define the fruity aroma characteristics in beer fermentation.

2-phenylethyl acetate

A floral aroma compound in beer, resulting from fermentation.