



# H<sub>2</sub>S

HYDROGEN SULFIDE

*& Beer*

# INTRODUCTION

There's no mistaking the classic smell of rotten eggs, indicative of the presence of hydrogen sulfide ( $H_2S$ ). This off-flavor can be controlled — and for some beer styles,  $H_2S$  is part of the normal flavor profile.

The presence of  $H_2S$  or other volatiles derived from  $H_2S$  may result in discarding an entire batch. Yet, this off-flavor and aroma can be controlled — once it is understood.

This white paper from Lallemand Brewing will help you to understand:

- The composition of hydrogen sulfide,
- How it is produced during brewing,
- How failure to remove  $H_2S$  can result in formation of other, more stable, sulfur compounds,
- Detection methods,
- Prevention tips, and
- Techniques to remove  $H_2S$

Our experts gathered all the important literature and interviewed specialists to increase your understanding of  $H_2S$  in beer production so the natural flavor of malt and hops can shine through.

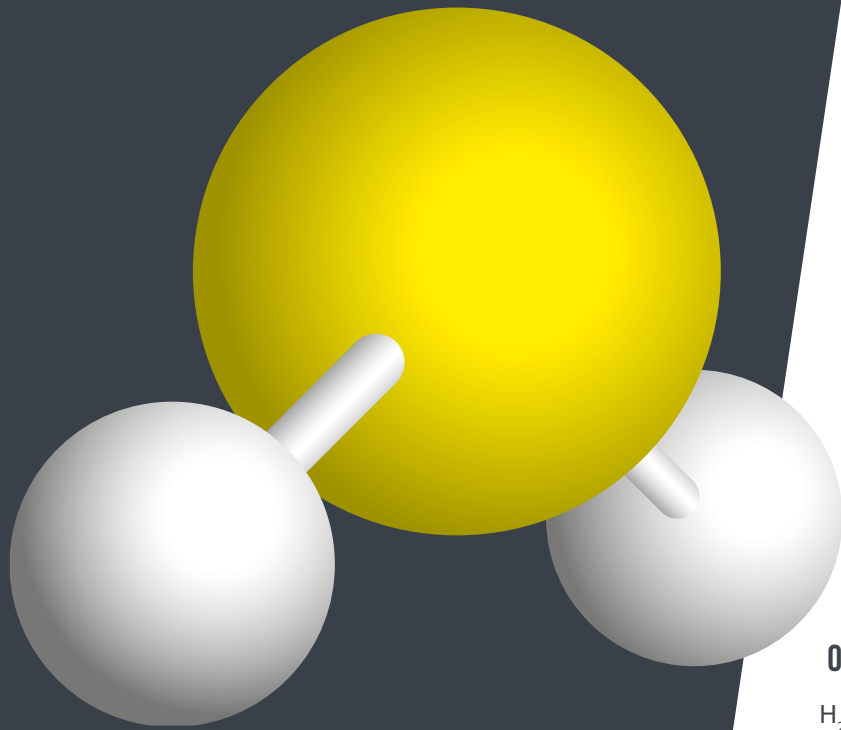
**$H_2S$  IS  
NOT ALWAYS  
AN OFF FLAVOR!**

At low levels, hydrogen sulfide contributes to the typical character of lagers. Even at higher concentrations,  $H_2S$  is considered acceptable in certain traditional beer styles such as English pale ales from Burton-on-Trent.





# WHAT IS HYDROGEN SULFIDE?



## THE MOLECULAR STRUCTURE OF H<sub>2</sub>S

Yellow = sulfur, white = hydrogen.

Brewers have been aware of sulfur compounds in brewing since 1898. Today, we know that H<sub>2</sub>S is a highly volatile compound with a low flavor threshold in beer, between 0.01 to 0.02 mg/L is detectable by most people.

H<sub>2</sub>S is produced in different concentrations by brewing yeasts depending on a variety of factors, including wort composition, fermentation conditions and genetics of the yeast. H<sub>2</sub>S can be produced:

- **By both brewing yeast as well as wild yeast contamination**
- **Bacteria contamination**
- **During fermentation or maturation**

## ONE OF MANY VOLATILE SULFUR COMPOUNDS

H<sub>2</sub>S is one of many sulfur compounds contained in beer, each with its own flavor profile. Sulfite (SO<sub>2</sub>) has an aroma of burnt matches whereas dimethyl sulfide (DMS) has an aroma of corn or cooked vegetables. Mercaptans are characterized by a foul sewer-like, burnt rubber or rotting vegetable aroma.

## H<sub>2</sub>S IS CONVERTED TO OTHER COMPOUNDS

H<sub>2</sub>S readily converts into other compounds in response to changes in pH, temperature and staling reactions, which makes it difficult to detect through analytical methods. For example, hydrogen sulfide may react with carbonyl compounds to produce pungent mercaptan off-flavors, which are very stable and difficult to remove from the beer after they are produced.

The amount of H<sub>2</sub>S produced is determined by several factors, including:

- **Yeast strain**
- **Fermentation temperature**
- **Nitrogen composition of the wort**
- **Yeast handling practices**

# WHY IS H<sub>2</sub>S PRODUCED? 1/3

H<sub>2</sub>S is produced by the normal metabolism of the yeast *Saccharomyces cerevisiae* when sulfate ions are reduced for processing into amino acids. Due to the very reductive metabolism of alcoholic fermentation, there is an accumulation of a large amount of hydrogen ions (H<sup>+</sup>) in the yeast cell. This creates an acidic environment in the cytoplasm, which is stressful to the yeast cell.

If the yeast does not eliminate these hydrogen ions, the survival of the yeast during fermentation would not be possible. H<sub>2</sub>S is produced by the transformation of sulfur by enzymatic reduction of sulfates (sulfate reductase) in order to process and remove excess hydrogen ions from the yeast cell.

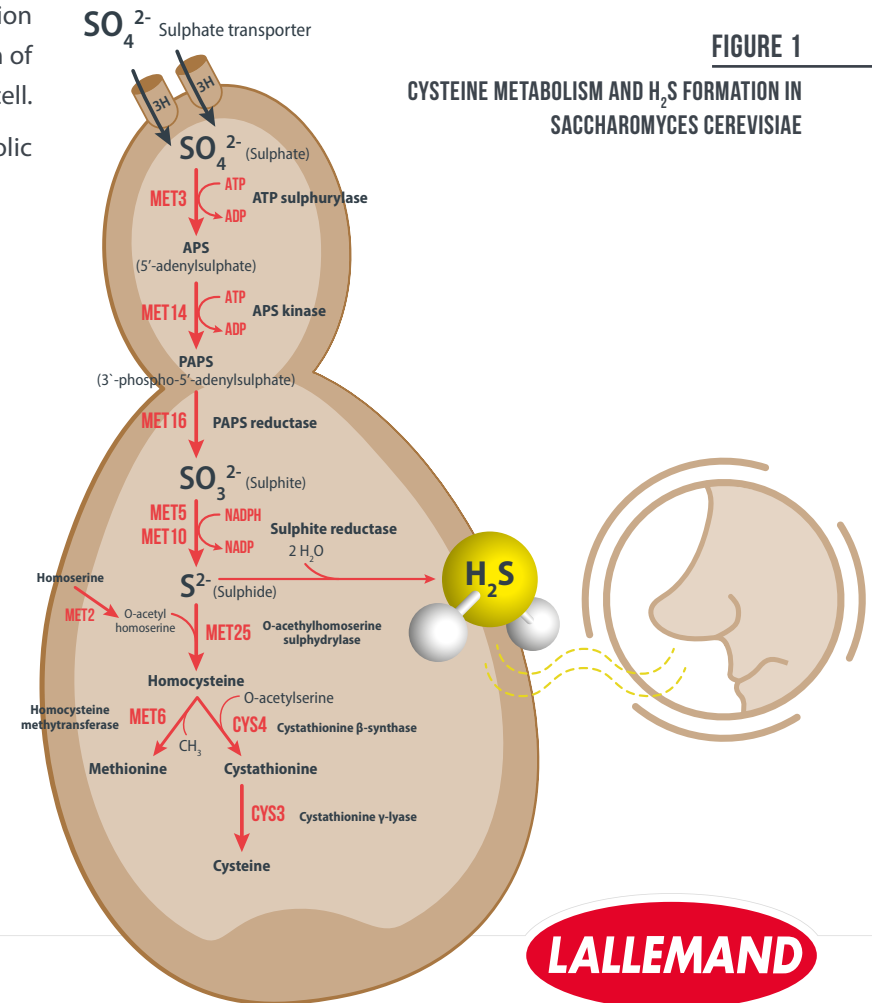
The production of abnormally high concentrations of H<sub>2</sub>S can occur especially during alcoholic fermentation with nitrogen deficiency or at lower fermentation temperatures.

## ROLE OF NITROGEN AND AMINO ACIDS

H<sub>2</sub>S is produced as a bioproduct in the formation of the amino acids cysteine and methionine, which is facilitated by the sulfur reduction sequence (SRS) enzymes (See Figure 1). When enough nitrogen is present in the medium, the precursors O-acetyl serine and O-acetyl homoserine will sequester H<sub>2</sub>S during the formation of methionine and cysteine respectively. *S. cerevisiae* yeast cultures can be induced to liberate H<sub>2</sub>S by starvation of assimilable nitrogen.<sup>1</sup> If nitrogen is limiting, insufficient precursors are available, and free H<sub>2</sub>S can accumulate in the cell and diffuse into the fermentation.

Other amino acids in the beer wort can influence H<sub>2</sub>S production. Common amino acids in the wort that can favor the production of hydrogen sulfide include cysteine, homocysteine, aspartic acid, glutamic acid, glycine, histidine, homoserine, lysine, ornithine, threonine, and serine. Yeast can also produce other sulfur compounds from these amino acids.

Vitamin deficiency can also result in higher levels of H<sub>2</sub>S. Vitamins act as cofactors for the SRS enzymes involved in amino acid metabolism. Low vitamin content in the fermentation can result in decreased levels of methionine, leading to increased levels of H<sub>2</sub>S.



# WHY IS H<sub>2</sub>S PRODUCED? 2/3

FIGURE 2

TYPICAL PATTERN OF H<sub>2</sub>S BEHAVIOR AND YEAST IN SUSPENSION DURING FERMENTATION<sup>2</sup>

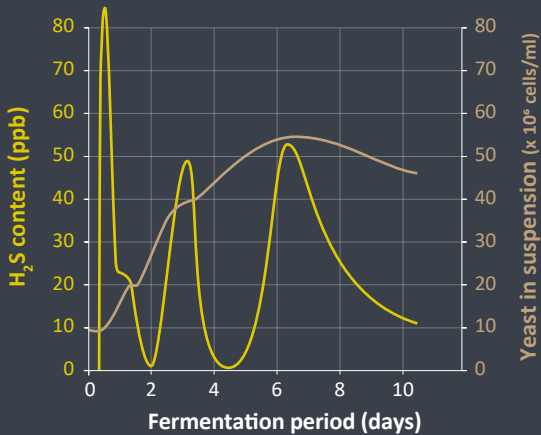
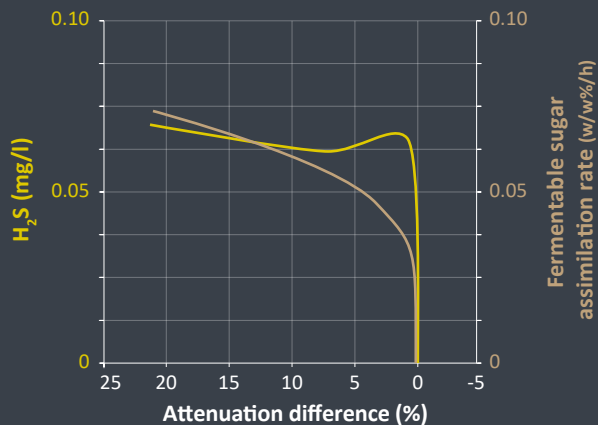


FIGURE 3

H<sub>2</sub>S BEHAVIOR AND FERMENTABLE SUGAR ASSIMILATION RATE DURING LATE-STAGE FERMENTATION<sup>3</sup>



## ENVIRONMENTAL FACTORS AFFECTING YEAST FORMATION OF H<sub>2</sub>S

Sulfur metabolism is a dynamic process and H<sub>2</sub>S levels rise and fall over the course of fermentation (Figure 2).<sup>2</sup>

During the first stage of fermentation, yeast take up sulfate and then reduce it to sulfite and then sulfide, which is used for sulfur-containing amino acid synthesis that supports yeast growth and cell division. When budding occurs, the H<sub>2</sub>S in the fermenting wort decreases very rapidly, probably through yeast uptake. When metabolism slows, sulfite and sulfide are released from the yeast cells. In the mid and late stages of fermentation after yeast peak biomass, the decrease in H<sub>2</sub>S content is said to be mainly attributed to CO<sub>2</sub> purging. As fermentation activity and CO<sub>2</sub> production slows towards the end of fermentation, other factors begin to contribute to decreasing the H<sub>2</sub>S levels in the beer.<sup>3</sup>

There is a relationship between the assimilation rates of the fermentable sugars (glucose, maltose, and maltotriose) and the rapid decrease in H<sub>2</sub>S at the end of fermentation. Once the fermentable-sugar assimilation rate drops below 0.05 w/w%/h, a rapid H<sub>2</sub>S decrease is observed suggesting that the decrease in H<sub>2</sub>S is triggered by the depletion of fermentable sugars (See Figure 3). Thus, the timing of rapid H<sub>2</sub>S decrease is associated with the fermentation reaching its attenuation limit.<sup>3</sup>

At the end of fermentation, yeast will reabsorb H<sub>2</sub>S resulting in a decrease in H<sub>2</sub>S levels in the beer. The rate of H<sub>2</sub>S reabsorption by yeast is related to the number of yeast cells still in suspension at the end of the fermentation. Extending the fermentation time prior to transferring the beer from the fermenter will provide more contact time with the yeast and a greater overall decrease in H<sub>2</sub>S.<sup>3</sup>

## CONDITIONS THAT INFLUENCE H<sub>2</sub>S LEVELS IN BEER

Increased H <sub>2</sub> S	Reduced H <sub>2</sub> S
Low nitrogen levels	Adequate level of nitrogen
Yeast stress and autolysis	Vigorous fermentations
Lager yeasts	Higher copper concentrations

# WHY IS H<sub>2</sub>S PRODUCED? 3/3

## OXYGEN

The introduction of **oxygen** at the end of fermentation during packaging or beer transfer is associated with increased H<sub>2</sub>S levels in the finished beer.<sup>2</sup> The introduction of oxygen after fermentation is complete may result in the yeast being stimulated to reactivate metabolism in a nutrient depleted environment, resulting in H<sub>2</sub>S production.

## STRESS RESPONSES

H<sub>2</sub>S formation is also associated with **stress responses** in the yeast cell. Inadequate nutrition (low nitrogen or vitamins), insufficient pitching rate and too low or too high fermentation temperature can result in the overproduction or failure to eliminate H<sub>2</sub>S from the fermenting beer. Yeast stress and autolysis will likely occur in parallel, resulting in a complex profile of off-flavors.

## H<sub>2</sub>S CAN BE FORMED:

- **During primary fermentation when yeast biomass is at its peak**
- **In the final stages of sugar consumption**

## H<sub>2</sub>S CAN BE REDUCED:

- **During yeast budding**
- **During active fermentation**
- **After achieving the attenuation limit**
- **During maturation while beer is in contact with the yeast**

## YEAST STRAINS

Different **yeast strains** vary in their response to physiological and environmental factors in the production and reabsorption of reduced sulfide. It is important to know the potential of a specific yeast strain to produce and subsequently eliminate H<sub>2</sub>S when selecting a strain for a particular beer style.

## COPPER IONS

**Copper ions** in the beer can react with H<sub>2</sub>S to form insoluble and non-volatile copper sulfide, which precipitates out of the beer. Low concentrations of copper can lead to more noticeable quantities of H<sub>2</sub>S.<sup>4</sup>

## STAINLESS STEEL EQUIPMENT MAY BE INCREASING THE PREVALENCE OF H<sub>2</sub>S PRODUCTION

Traditional brewing equipment was made from copper, which contributed copper ions to the fermenting beer. Modern brewing equipment is most often constructed from stainless steel, resulting in reduced copper concentrations in beer and a higher prevalence of H<sub>2</sub>S.

Copper is contributed by multiple ingredients in the brewing process, including water, malted barley, hops, and/or yeast.

Copper concentrations are regulated by governing bodies, such as the European Union. In a study, samples of 19 different beers all performed well below the maximum allowed concentrations.<sup>4</sup>



# HOW TO DETECT H<sub>2</sub>S?

## IS IT ON-BRAND OR AN OFF-FLAVOR?

*Train your sensory panel well to determine if H<sub>2</sub>S levels are within acceptable limits for the style (i.e. lagers). Early detection of H<sub>2</sub>S allows for the brewer to take corrective action if required.*

Some methods exist for detecting H<sub>2</sub>S in the lab by reaction with lead acetate, but these methods are non-quantitative and are typically not sensitive enough to detect trace quantities of H<sub>2</sub>S in finished beer. For these reasons, these methods are not widely used in breweries.

Humans have developed a hypersensitivity to sulfur compounds, including H<sub>2</sub>S and mercaptans. The presence of copper ions in the nasal mucus increases the binding affinity of sulfur compounds to receptors in the nose and increases the sensory threshold by up to 1000x. Sulfur compounds, in comparison to other common off flavors/defects in beer, are much easier to detect. For example, the average sensory thresholds for diacetyl (0.15 mg/L), acetic acid (90 mg/L) and lactic acid (140 mg/L) are much higher compared to H<sub>2</sub>S (0.01 to 0.02 mg/L).

## TRAIN YOUR NOSE AND FLAVOR PALATE

A trained sensory panel is the most effective tool for detecting H<sub>2</sub>S in the brewery. Brewers can train their palates to be more sensitive to specific off-flavors and other aromas, including H<sub>2</sub>S and mercaptans. Commercial sensory panel management courses and kits are available, which allow brewers to “spike” their beer sample with specific chemically pure flavor compounds.

Using this approach, a sensory panel can be trained to become increasingly sensitive to H<sub>2</sub>S, mercaptans, other sulfur compounds, and more.



**Siebel Institute  
OF TECHNOLOGY**

## SENSORY TRAINING

Ensure the quality and consistency of beer by learning how to build and manage proficient sensory panels with the Siebel Institute of Technology Sensory Panel Management course and at-home sensory training kits.

Learn more by visiting:

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# PREVENTING H<sub>2</sub>S PRODUCTION

THERE ARE VARIOUS METHODS AVAILABLE TO AVOID H<sub>2</sub>S PRODUCTION DURING FERMENTATION.

## YEAST STRAIN SELECTION

Most ale strains produce lower levels of H<sub>2</sub>S. Lager strains tend to produce more H<sub>2</sub>S than other strains. Recently, specific yeast hybrids have been selected to produce lower levels of H<sub>2</sub>S. These strains overexpress the MET10 gene, which encodes a sulfhydrylase (SHLase) capable of using either O-acetylserine or O-acetylhomoserine and have been shown to dramatically reduce H<sub>2</sub>S production under both low and high nitrogen conditions.<sup>6</sup>

## YEAST NUTRIENTS

All-malt wort usually contains everything a yeast cell needs for a healthy fermentation. Depending on your malt and water profile, your wort may be deficient in nitrogen, vitamins or minerals. Adding some nutrient might help to reduce H<sub>2</sub>S formation. The type of nutrient and timing of addition is important.

Nutrients normally contain either a mineral nitrogen source (i.e. ammonium or urea), an organic nitrogen source (yeast derived), or a combination of both. Mineral nitrogen sources such as ammonium or urea result in very fast fermentations that rapidly deplete amino acids in the fermenting beer, which may trigger H<sub>2</sub>S formation. Organic nitrogen sources are metabolized more slowly, resulting in more stable amino acid levels throughout fermentation and less H<sub>2</sub>S formation. Nitrogen is not the only nutritional factor that influences H<sub>2</sub>S production. A more complex nutrient including nitrogen, vitamins and minerals will reduce the chance of having H<sub>2</sub>S in the finished beer.

Lallemand offers a range of yeast nutrient solutions, including [YeastLife Extra™](#) - specifically formulated to provide a balanced blend of all essential elements required for a healthy fermentation - an insurance against H<sub>2</sub>S production during fermentation.

## LONG AGING/MATURATION PERIOD WITH MINIMUM OXYGEN INGRESS

Giving beer more time to age or mature in the presence of yeast can help reduce the H<sub>2</sub>S concentration by allowing more time for reabsorption by the yeast. Longer maturation periods have other flavor consequences that can be positive (i.e. reduced diacetyl) or negative (i.e. reduced hop aroma). A longer maturation period is particularly important in lagers where yeast metabolism is slower at cooler temperatures.



*Choose the right yeast strain. Some lager strains are simply more sulfuric than others and require longer maturation times. It's much easier to avoid hydrogen sulfide production during yeast selection than after the beer is brewed."*

**RICCIARDI GIANMARIA**  
TECHNICAL SERVICES MANAGER - ITALY & SLOVENIA

**LALLEMAND**

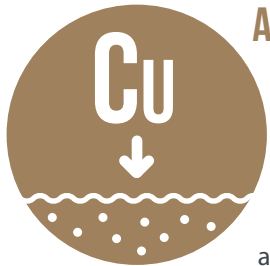


# TECHNIQUES TO REMOVE H<sub>2</sub>S



## CO<sub>2</sub> PURGING

CO<sub>2</sub> purging refers to bubbling CO<sub>2</sub> slowly through the finished beer in the maturation tank. CO<sub>2</sub> purging is effective for removing sulfur in the form of H<sub>2</sub>S, which has an aroma of rotten eggs. If H<sub>2</sub>S has reacted to produce mercaptans with aromas such as garlic, onion, cooked cabbage, green beans, dishcloth, etc., then this technique will not be effective. If there is some oxygen ingress in addition to the CO<sub>2</sub>, then this method may even be counterproductive and result in increased formation of sulphides and disulphides with very disagreeable aromas and very low sensory thresholds. CO<sub>2</sub> purging may also reduce other positive sensory characteristics such as hop aroma.



## APPLICATION OF COPPER DERIVATIVES

The most effective techniques for removing H<sub>2</sub>S from a beer after fermentation is complete are based on the application of copper derivatives, resulting in a redox reaction with the sulfur compounds.

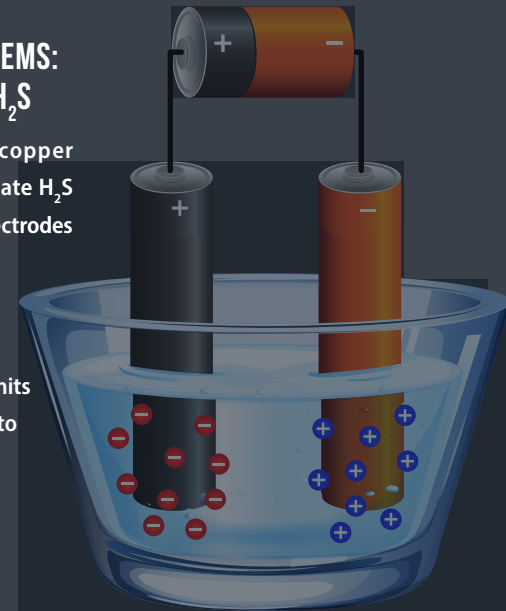
Copper sulfate added to beer quickly and efficiently removes all H<sub>2</sub>S from the aroma through the formation of insoluble copper sulfide (CuS), which precipitates out of the beer. As an alternative, beer also can be run through copper tubing or treated with a copper electrolysis system to eliminate H<sub>2</sub>S. Copper application may also remove mercaptan aromas from the beer.

Copper treatment is very effective and commonly used in cellars, but it does have some drawbacks. The most important among them is that it leaves many copper ions in solution in the media. The copper reacts rapidly with phenolic compounds causing sensory modifications in the texture of the tannins as well as dryness, metallic sensations and astringency. Copper is also a catalyst for oxidative processes, which may shorten the shelf life of the product.

## COPPER ELECTROLYSIS SYSTEMS: ANOTHER WAY TO CONTROL H<sub>2</sub>S

Researchers have designed a copper electrolysis system that can eliminate H<sub>2</sub>S from beer. The unit consists of two electrodes in a stainless steel housing.

A power supply allows adjustment of the current by changing the voltage to precipitate H<sub>2</sub>S as CuS. This system permits additions of minute amounts of copper to beer in the range of 30 µg/L or less.<sup>4</sup>



# CONCLUSION:

Keeping H<sub>2</sub>S concentrations below the detectable threshold is an important goal for producing high-quality beers. The simplest way to remove H<sub>2</sub>S from beers is to prevent its formation in the first place. This is accomplished by choosing an appropriate yeast strain, providing sufficient nutrition during fermentation and avoiding yeast stress. If H<sub>2</sub>S is detected, there are corrective measures available to help rescue the beer by reducing or eliminating this distinct aroma before it reacts to form more stable sulfurous off-flavors.

## FOR MORE INFORMATION



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In collaboration with Renaissance Yeast (Vancouver, BC, Canada), Lallemand Brewing recently launched their first 'low H<sub>2</sub>S' yeast strain, **LalBrew Farmhouse™**. This novel and innovative Saison yeast, was bred using classical, natural breeding techniques to select low and non-H<sub>2</sub>S producing strains.



From a yeast nutrition perspective, the AB Vickers nutrient, **YeastLife Extra™**, a blend of assimilable nitrogen, vitamins, minerals and specific amino acids is designed and formulated to reduce the level of sulfur compounds during fermentation.