PEER-REVIEWED ARTICLE

# The Theory and Effect of Positive and Negative Pressure on Brewing Fermentations: Pressure Effects on Beer Fermentation

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## ABSTRACT

During yeast fermentation there are many parameters that affect both yeast growth and metabolism. Manipulation of fermentation parameters including yeast strain, fermentation temperature, nutrient content, and oxygen levels allows brewers to control different fermentation attributes, including the process rate and the synthesis of volatile compounds. An additional often overlooked parameter that can be used to control volatile production is headspace pressure (either as a pressurized or reduced pressure fermentation). This paper examines how manipulation of the headspace pressure results in a variation of the $CO_2$ concentration and how this can be used to influence both the production of flavor compounds and the fermentation rate. The difference in volatile production was experimentally assessed between fermentations conducted at high and low $CO_2$ concentrations. The formation of specific volatiles was negatively correlated with the presence of $CO_2$ (most strongly: acetate esters and isoamyl alcohol). The practical applications of pressure control in the context of modern brewing systems are discussed, as well as historical perspective and specific applications.

**Keywords:** pressure fermentation, $CO_2$ solubility, open fermentation, vacuum fermentation, fermentation techniques, volatile compound production

## Introduction

Fermentation is a process that has been applied since at least 3500 BCE (ancient Mesopotamia) to produce ethanol, $CO_2$, and other organic compounds. Balling, an early beer scientist, determined that for every 100 g of sugar consumed, approximately 48.8 g of ethanol and 46.8 g of carbon dioxide ($CO_2$) are produced (4). The remaining carbon mass is used for yeast growth and secondary metabolites, including volatile flavor compounds (higher alcohols, aldehydes, ketones, esters, and sulfur compounds) (4). Fermentations done in open-to-atmosphere vessels were very popular before the advent of closed conical fermentors. In areas of present-day Germany, Austria, and the Czech Republic, it was common to ferment under cooler conditions since at least the 15th century (20). However, spoilage microorganisms such as *Lactobacillus* and *Brettanomyces* were commonly present in the brewery environments, leading to the undesired formation of volatile compounds associated with off-flavor in beer (lactic acid, vinegar, horse blanket, sweat, leather, wet wool, burnt plastic, etc.) (24). This resulted in the consumption of beer generally close to its production time and location. An additional consequence of the open-style fermentor was a higher oxygen availability and lower $CO_2$ concentration within the wort during fermentation. The reduced $CO_2$ was beneficial

to yeast health, but this was usually offset by the challenges of open-fermentor brewing. Currently, contamination issues are tackled with modern methods, aseptic techniques, and equipment such as the sealed cylindroconical fermentor. During fermentation, the use of low temperatures results in "cleaner" beers but is associated with a slow (non-optimal) yeast growth rate (30) and lower concentration of volatiles in the final product. With the current understanding of microbiology, even open fermentations can be conducted without significant contamination, allowing brewers to choose the temperatures of the microorganisms that suit the product they are looking to create. Similarly, a current understanding of headspace pressure and its association with $CO_2$ levels can be used to give brewers the freedom to manipulate aspects of their fermentations. The specific objective of this article is to examine the effect of $CO_2$ on the concentration of volatiles during brewing operations and to discuss the industrial applications and historical significance. The information presented in this article will help brewers and fermentation-related experts to make informed decisions regarding the effect of fermenting at different pressures and their corresponding $CO_2$ concentrations.

### Definition of Fermentation Pressure

Currently, industrial fermentation processes are typically performed at atmospheric or near-atmospheric pressure conditions. This level of hydrostatic pressure does not have a significant effect alone on yeast and other microorganisms. Comparable to most microorganisms, *Saccharomyces* spp. will survive and proliferate unimpeded at exceedingly high hydrostatic pressures, ultimately requiring levels over 15,000 psi (~1,000 atm) to inactivate (this is the level of pressure used in hyperbaric

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pressure processing (8). Similarly, low hydrostatic pressure has little impact on yeast down to the point where liquid water will begin to boil (31). However, when $CO_2$ gas is used to create hydrostatic pressure above water, a significant amount will dissolve into the liquid at equilibrium. As yeast is sensitive to concentrations of $CO_2$ dissolved within wort, the presence or absence of $CO_2$ will impact yeast behavior and the formation of fermentation products (10). As significant amounts of $CO_2$ are constantly produced during fermentation, the amount of $CO_2$ dissolved within the wort will correlate with the pressure of $CO_2$ trapped in the sealed vessel as per Henry's law. Hence, the simplest way to manipulate the concentration of $CO_2$ within wort is to control the release of $CO_2$ from a sealed vessel.

### Considerations of Gases During Fermentation

When deciding if and how to brew under variable pressure, brewers must consider the interaction between various gases and the wort during typical operations. Air at normal atmospheric conditions exerts about 14.7 psi (1 atm) of total pressure made up by the components of the air. This is referred to as 14.7 psi absolute (psia) or 0 psi gauge (psig), as would be read on a standard pressure gauge. With ideal gases, the pressure exerted by each component and the volume are directly proportional to the amount of each component (the molar ratio). This is important as every gas has different interactions with wort when in direct contact; for example, $N_2$ is an inert gas with very low solubility, thus having a trivial effect on wort. This is why using $N_2$ to blank or degas beer is very common in the industry. The solubility of $O_2$ is higher than $N_2$ (25), and it is typically added to saturation wort at the beginning of fermentation for yeast to produce the sterols and fatty acids necessary for healthy growth. Brewers avoid any trace of $O_2$ during or after fermentation to prevent any oxidation reaction that could generate off-flavors (5). $CO_2$ is even more soluble compared with the other two gases, and it is produced in large quantities during fermentation. This results in a continuous increase in the concentration of $CO_2$ dissolved in the medium as a partial pressure equilibrium is established within the fermentor. Hence, manipulating the pressure of the headspace during the fermentation by removing or restricting the gas leaving the system will result in a variable concentration of $CO_2$.

### Henry's Law

Henry's law states that the quantity of dissolved gas within a liquid at equilibrium is proportional to the partial pressure of the gas in contact with the liquid. This is often expressed as equation 1:

$$P_{CO2} = H_c \times C \tag{1}$$

where $P_{CO2}$ is the partial pressure of $CO_2$ in contact with the liquid in absolute pressure units (not gauge), $H_c$ (Henry's constant) is a proportionality coefficient, and C is the concentration of $CO_2$ dissolved in the medium. $H_c$ is highly dependent upon numerous factors (28), including temperature, gas, and liquid properties. Commercial variations of carbonation level calculations commonly use approximated $H_c$ values to create tables and equations that are reasonably accurate (but not perfect) for brewing applications, as shown in Table 1.

The concentrations of $CO_2$ dissolved in the beer at 77°F (25°C) shown in Table 1 were calculated using the American Society of Brewing Chemists (ASBC) $CO_2$ solubility chart (3), which uses a value for $H_c$ of approximately 20.12 psia vol/vol or 0.7 L atm/g at this temperature. Since $H_c$ is highly temperature dependent, this coefficient increases as the temperature drops. Table 1 also shows the $CO_2$ concentration based on the $H_c$ corrected for brewing at 54°F (12.2°C). $H_c$ was corrected for temperature using the van't Hoff correlation to a value of 13.81 psia vol/vol or 0.48 L atm/g.

These values can also be determined using several different $CO_2$ solubility charts (although brewers usually must convert psia to psig by subtracting 14.7 psi). Note that during standard fermentation processes at temperatures of 54–77°F (12.2–25°C) using a sealed tank without manipulation of pressure (14.7 psia or 1 atm), the amount of $CO_2$ dissolved within the beer ranges from 0.73 to 1.06 vol/vol (1.43–2.08 g/L). However, when the pressure is manipulated, the range can be from 0.18 to 2.13 vol/vol (0.36–4.17 g/L). At these concentrations of $CO_2$, an inhibitory effect on yeast growth, health, and metabolism has been shown (10). When dissolved in wort, some of the $CO_2$ will form carbonic acid dependent upon the dissociation constant. This does not appear to substantially impact the pH of most wort; however, dissolved $CO_2$ has been shown many times to negatively impact microorganisms beyond the effects of pH alone (10). Thus, using $CO_2$ is very common in food systems as a hurdle to microbial growth, and it is routinely added to food packaging. The effect on brewing yeast is less severe, but as reported by Guadalupe-Daqui and MacIntosh (13), reducing $CO_2$ within wort during fermentation has a positive impact on growth rate and total yeast counts during fermentation. The opposite effect has also been observed in which fermentations under $CO_2$ pressure negatively impacted the growth of the yeast (10,27).

Furthermore, multiple studies have shown that an increased concentration of dissolved $CO_2$ has an inhibitory effect on volatile ester and higher alcohol production (1,15,18,22,26). Dissolved $CO_2$ has been implicated in reducing the amount of amino acid uptake (27) and, therefore, the concentration of corresponding higher alcohols. Ester formation, specifically esters of acetate, appears to be suppressed by $CO_2$ in that alcohol acetyltransferase (acetyl-CoA), the enzyme responsible for acetate ester synthesis, is inhibited by $CO_2$ (26). The inhibition of alcohol dehydrogenase is in part because dissolved $CO_2$ decreases the formation of acetyl-CoA in addition to being inhibited by unsaturated fatty acids and ergosterol (26,33). This is important as the reduction of acetaldehyde to ethanol is catalyzed by the enzyme alcohol dehydrogenase as well as the conversion of fusel aldehydes into fusel alcohols (9,21).

## Experimental Fermentation Data Under Low and High $CO_2$ Concentrations

An experiment was conducted to highlight the differences in specific volatile production between two pilot-scale (30 L) lager fermentations performed at 15°C (59°F). The formulation (grain bill and mashing regime) chosen was the same as described in Guadalupe-Daqui and MacIntosh (13). This formulation was

**Table 1.** Concentration of $CO_2$ dissolved in beer at different temperatures and pressures using Henry's coefficient approximated from commercial tables (28)

| Pressure | CO₂ concentration at 77°F (25°C) | CO₂ concentration at 54°F (12.2°C) |
|---|---|---|
| 3.5 psia (0.25 atm) | 0.18 vol/vol (0.36 g/L) | 0.27 vol/vol (0.52 g/L) |
| 14.7 psia (1.0 atm) | 0.73 vol/vol (1.43 g/L) | 1.06 vol/vol (2.08 g/L) |
| 29.4 psia (2.0 atm) | 1.46 vol/vol (2.86 g/L) | 2.13 vol/vol (4.17 g/L) |

expected to result in a high concentration of esters and higher alcohols, making it ideal to assess the differences due to $CO_2$ concentration. Two pilot-scale fermentations (~30 L) were performed at two different pressures (vacuum and atmospheric) in an Applikon BioPilot 130 L bioreactor. Temperature was maintained at 15°C and controlled by a temperature probe included in the bioreactor used. Wort was oxygenated through an inner sparge pipe using pure oxygen. Wort was agitated at 100 rpm during oxygenation, and saturation was monitored using a Mettler Toledo dissolved oxygen sensor. A *Saccharomyces pastorianus* strain, commercially available as Diamond Lager yeast, was provided by Lallemand. The target inoculation level was $15 \times 10^6$ cells/mL and was confirmed using the Yeast-4 method described by the ASBC (2). One fermentation (vacuum pressure fermentation) was conducted at a lower $CO_2$ level of 0.23 vol/vol (0.46 g/L), whereas the other (atmospheric pressure fermentation) was maintained at a $CO_2$ level of 0.98 vol/vol (1.92 g/L). Both carbonation levels were reached within the first 8 h of fermentation, which lasted ~69 h for the low $CO_2$ configuration and ~95 h for the high $CO_2$ configuration. Samples collected at the end of each fermentation were vacuum filtered through Whatman 595 filter paper and frozen at –20°C until analysis. The volatile organic compounds were assessed in duplicate using a "purge and trap" technique to collect the volatile compounds, gas chromatography (GC) to separate, and mass spectrophotometry to identify.

The volatile components from 5 mL of each sample were purged using a nitrogen flow at a 40 mL/min rate for 20 min within a Teledyne Tekmar Atomx XYZ automated purge and trap unit (Mason, OH). After collection, these compounds were desorbed at 180°C and directed to the injection port of the GC. The Agilent GC was equipped with a Zebron ZB-WAXplus column (30 m × 250 μm × 0.25 μm) (Phenomenex, Torrance, CA) for separation, and a quadrupole mass spectrometer detector (5975C MSD) was used to identify the compounds. The GC oven temperature was held initially at 50°C for 5 min. The oven temperature was then increased to 118°C at a rate of 5°C/min, then to 160°C at a rate of 4°C/min, and finally to 240°C at a rate of 30°C/min, where it was held for 3 min. Each peak was com-

pared with the 2011 National Institute of Standards and Technology mass spectral library for identification. Nonanol was used as the external standard to semi-quantify volatile organic compounds.

## Results and Implications

The volatiles identified at the end of both fermentations were grouped into three different categories based on their functional group including carbonyl compounds, esters, and higher alcohols. The flavor threshold of each compound identified was used to determine the potential impact on the beer quality when fermenting at both $CO_2$ concentrations. The concentration of each volatile compound and the total concentration of each functional group at the end of each fermentation were measured and statistically analyzed using GraphPad Prism version 8.00 for Windows (GraphPad Software, La Jolla, CA). Data were subjected to an analysis of variance to determine significant difference with the Tukey post-hoc test on a significance level of $P < 0.05$. Concentrations measured in duplicate were averaged, and the standard deviation of the readings was calculated as shown in Table 2. A comparison of the total concentration of carbonyl compounds, esters, and higher alcohols synthesized after both fermentation configurations is shown in Figure 1.

When fermented at low $CO_2$ concentrations, the total amount of esters and higher alcohols increased approximately 103% and 60%, respectively, compared with typical $CO_2$ concentrations obtained during atmospheric fermentations. The total concentration of carbonyl compounds did not vary significantly between fermentations under both concentrations of $CO_2$. The majority of the volatiles generated at both $CO_2$ concentrations exceeded the threshold limit, as shown in Table 2. When assessed individually, some compounds were unaffected by the $CO_2$ concentration (carbonyl compounds, for example), whereas others were heavily influenced, particularly the acetate esters. This is consistent with the previous studies regarding the inhibitory effect of $CO_2$ on the synthesis of volatile compounds (15,17,26). While the inhibitory effect of $CO_2$ on the synthesis of volatile compounds has not been well defined, different explanations of

**Table 2.** Final volatile concentration identified at high and low $CO_2$ concentration and their flavor threshold. Data were collected in duplicate and presented as an average with the standard deviation.

| Compound | Aroma | Concentration in beer (ppm) | | Flavor threshold (ppm) | References for aroma and threshold |
|---|---|---|---|---|---|
| | | High $CO_2$ beer | Low $CO_2$ beer | | |
| Carbonyl compounds | | | | | |
| Acetaldehyde | Green leaves, fruity | 3.1 ± 0.5a | 2.0 ± 0.1a | 10–25 | 16, 29, 32 |
| Isoamyl aldehyde | Chocolate, peach, fatty | 3.0 ± 0.6a | 4.9 ± 0.3a | 0.6 | 19, 29 |
| Isobutanal | Grainy like germinating malt | 2.0 ± 0.2a | 1.8 ± 0.1a | 1.0 | 19, 29 |
| Esters | | | | | |
| Isoamyl acetate | Banana, estery | 46.6 ± 3.7a | 171.9 ± 10.7b | 1–2 | 6, 16, 22, 23, 29, 32 |
| Phenethyl acetate | Flowery, fruity, roses, honey | 4.6 ± 0.2a | 19.9 ± 0.8a | 3.8 | 6, 16, 22, 23, 29 |
| Ethyl acetate | Sour apple, solvent, fruity, sweetish | 237.7 ± 6.1a | 348.3 ± 13.6b | 20–40 | 6, 16, 22, 23, 29, 32 |
| Ethyl caproate | Sour apple, fruity | 16.3 ± 1.0a | 36.0 ± 1.0a | 0.15–0.25 | 6, 16, 22, 23, 29, 32 |
| Ethyl caprylate | Apple, anise | 13.2 ± 3.4a | 51.8 ± 8.7b | 0.9 | 6, 16, 22, 23, 29 |
| Higher alcohols | | | | | |
| Linalool | Flowery | 5.2 ± 0.3a | 6.8 ± 0.4a | 0.08 | 29 |
| Butyl alcohol | Alcohol, fusel oil, varnish | 4.8 ± 0.0a | 16.4 ± 0.0a | 450 | 29 |
| Isoamyl alcohol | Alcoholic, banana | 1,255.1 ± 9.1a | 2,014.8 ± 29.3b | 70 | 6, 16, 22, 29, 32 |
| Isobutanol | Solvent | 209.7 ± 6.8a | 243.3 ± 1.7b | 200 | 16, 22, 29 |
| 2-Phenylethanol | Roses, sweetish, perfumed | 14.9 ± 0.9a | 26.1 ± 3.2a | 125 | 16, 22, 29, 32 |

this effect have been discussed previously. Both Shantha Kumara et al. (26) and Kruger et al. (17) agreed that the inhibitory effect of $CO_2$ was based on the acetyl-CoA inhibition due to the $CO_2$ sensitivity of this reaction. Acetyl-CoA is one of the precursors of acetate esters; thus, repressing the synthesis of acetyl-CoA will then result in a low production of acetate esters. This would explain the lower concentration of esters, especially ethyl acetate and isoamyl acetate, obtained when fermenting at typical $CO_2$ concentrations during atmospheric fermentations. Knatchbull and Slaughter (15) have shown that a high concentration of $CO_2$ negatively affects the amino acid intake by yeast during fermentation. They reported that when fermenting at concentrations of $CO_2$ above the limit typically found at atmospheric pressure, the absorption of branched-chain amino acids was reduced. These amino acids included valine and leucine, which are precursors of isobutanol and isoamyl alcohol, respectively. This result agrees with the increased concentration of specific higher alcohols obtained when the fermentation was performed at low $CO_2$ concentrations. It is also noteworthy that higher alcohols are also precursors to specific esters. It is important to note that most of the compounds identified and reported in Table 2 were present in a concentration above the threshold. This means that even the high $CO_2$ fermentation used in this experiment as the control process will have similar flavor compounds compared with the process performed at a low $CO_2$ concentration. For example, the same esters were present in both control and low $CO_2$ fermentation; however, the concentration was doubled under low $CO_2$ conditions (Fig. 1).

### Vacuum and Open Fermentations (Low $CO_2$ Concentration)

The findings of this and previous studies help to explain historical differences between open and closed fermentations, as well as anecdotal findings of many brewers concerning open fermentations. The classic open fermentation is also known as open-to-atmosphere fermentation and denotes numerous challenges to brewers, including maintaining a sterile environment and controlling the interaction of $O_2$ with the wort. This technique exposes wort directly to air at atmospheric conditions, which consist of 78% nitrogen ($N_2$), 21% oxygen ($O_2$), and very small amounts of $CO_2$ (~0.05%) as well as a few other trace
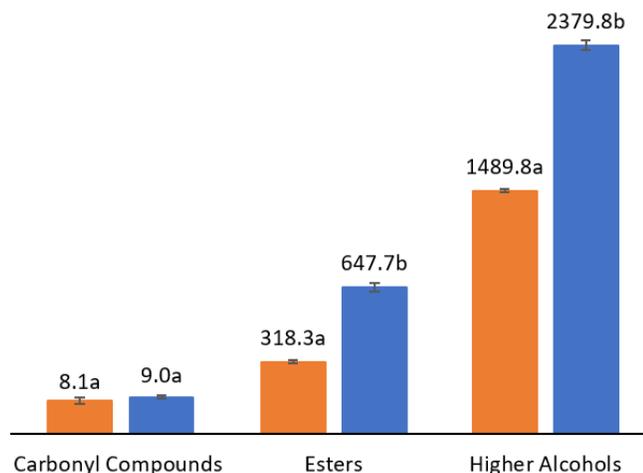


**Figure 1.** Total concentration (ppm) of each functional group after the fermentation at both high (orange) and low (blue) $CO_2$ concentrations. Data are presented as an average, and error bars represent the standard deviation. Different letters next to each fermentation within a functional group represent statistical difference between both fermentations.

gases. A clear advantage of this technique is that the amount of dissolved $CO_2$ within the fermenting beer would be similar to a vacuum or reduced-pressure fermentation within a sealed vessel (due to the lower partial pressure of $CO_2$). This would result in less stressed yeast and potentially more rapid fermentation with a higher ester production. Most modern breweries use sealed conical vessels to reduce or avoid any microbial contamination risk; however, there are a few breweries at present still using open fermentors because of the abovementioned advantages. Vinnie Cilurzo, the co-owner of Russian River Brewing Company, is quoted below (7):

> When we built our brewery in Windsor, CA, Natalie and I opted to install 6 open-top fermenters (OTF's) from Gresser Tanks from Germany. This is an old fashion way to ferment beer and it certainly adds extra work to the overall brewing process, but we love the results (it is also a great way to show our guests at the brewery an actual fermentation). With that said, what we've learned is that fermenting in an OTF is not better or worse, just different…. Because our OTF's are a 1x1 ratio and there are no lids on the top of the OTF's, the yeast is less stressed, and to me, this helps create a cleaner beer. Often brewers pigeonhole OTF beer styles to just German Weizen or maybe a Belgian style. Of course, Weizen and Saison's do great in OTF's, but we've found across the board that any style from Helles to Pilsner to even an IPA can be fermented in OTF's where we will see healthier fermentations because the yeast is in a much better place without the static head pressure and the different tank geometry.

British breweries are somewhat unique in that many have retained their open-style fermentors. Thus, British style ales have a particularly large volatile spectrum that tends to be ester forward, as less $CO_2$ pressure leads to "happy" yeast, which results in high growth and plentiful volatile formation (32). Due to the aforementioned difficulties with open fermentation, they tend to be rare in the U.S. craft market. British-style ales seem like a potential beer to explore the effect of fermenting at reduced $CO_2$ concentrations (either produced via an open fermentation or under vacuum).

To accomplish fermentation at a reduced $CO_2$ concentration within a sealed vessel, the pressure within the brewing vessel can be reduced through the application of a vacuum constantly generated using a water aspirator. This would not remove substantially more volatile compounds (including ethanol) than regular fermentation, as the volatile compounds will establish an equilibrium at the new pressure level. Alternatively, the fermentation could be flushed with another gas (e.g., $N_2$) to reduce the $CO_2$ partial pressure. This will result in the removal of volatile compounds, making this option more suitable outside of the brewing industry (biofuel, pharmaceutical, distillation, or continuous fermentation). Similar to open fermentations, reduced pressure fermentations have been shown to result in higher numbers of yeast cells, faster fermentation rates (12), and higher volatile production (13). The reduction of this stress factor due to vacuum pressure has also been shown to increase the degree of fermentation by yeast during very high gravity fermentation, compared with standard atmospheric pressure conditions where yeast died before completion (14). Fermentations under increasing partial vacuum reduce the equilibrium $CO_2$ concentration. This approaches a classical open-to-atmosphere fermentation, as these conditions will have lower partial pressures of $CO_2$ in the headspace (open air would establish an equilibrium with the $CO_2$ in air ~ 500 ppm). The benefits of reduced $CO_2$ concentrations can be used to push the limits of traditional fermentation by alleviating a source of yeast stress. This may facilitate higher gravity or temperature

brewing than would otherwise be possible; it may also assist in the efficient production of yeast biomass in other industries. Similar to pressurized fermentation, vacuum fermentation conducted in a vessel not rated for such activities can result in damaged equipment and an unsafe workplace environment.

## Pressurized Fermentations (High $CO_2$ Concentration)

Most modern fermentations are completed in sealed fermentation vessels with 14.7 psia (0 psig or 1 atm) of gas (often air or $CO_2$) in the headspace and a "pressure release valve" or "water trap" that allows gas to be released as $CO_2$ is produced. Gases in the headspace of the fermentation vessels (any $N_2$ or $O_2$) are quickly displaced with nearly pure $CO_2$ as the fermentation progresses, resulting in ~14.7 psia (1 atm) of nearly pure $CO_2$ in the headspace. There are numerous advantages of the modern cylindroconical fermentation vessel, including a sealed aseptic environment, good tank geometry (resulting in complete internal mixing from $CO_2$ evolution), good surface area to volume ratio for jacketed heat removal, ability to crop and cool yeast separately from the bulk product, efficient space considerations, and so on. If designed for the appropriate pressure, these vessels are ideal for exerting maximum control over fermentation conditions, as brewers can raise or lower the total internal pressure to adjust the $CO_2$ concentration.

There are several reasons a brewer may choose to ferment at conditions above atmospheric pressure despite the negative effect on yeast health and fermentation rate. These include the reduction in specific flavor compounds, particularly higher alcohols, commonly produced at high temperatures (21). Other reasons include the higher carbonation level at which the product will leave the fermentor, as well as a reduced foam production (due to the lower rate of $CO_2$ leaving the wort). Several home brewers are using a combination of high temperature with high pressure (above atmospheric) mainly to speed up the fermentation process while controlling the formation of volatile compounds. Additionally, this combination reduces the need for cooling, as the pressure offsets the additional flavor compound production that would otherwise be present at higher temperatures. The inherent difficulties reported with this approach include matching styles, reduced yeast health, a lower number of cells, and a slower fermentation speed compared with unpressurized fermentations at the same temperature (data not shown). During fermentation, yeast is exposed to a myriad of stressors including oxidative stress, osmotic stress, anaerobic shifts, nutrient limitations, ethanol stress/toxicity, and exposure to dissolved $CO_2$ (11). Ultimately, any additional stress (including higher $CO_2$ concentrations from pressure) placed upon yeast will be deleterious to health and the ability to successfully repitch. Additionally, $CO_2$ is known to affect cell growth, enzyme activity, and yeast cell size (1,18). While fermentation under pressure will result in additional stress, this may not measurably impact the yeast viability if other stress factors are minimal. The authors were unable to find any published studies on the effect of repitching yeast that has been fermented under pressure. Finally, all brewing vessels have maximum operating pressures that are determined by the manufacturer, and regulations concerning pressurized vessels vary between regions. Therefore, workplace safety must be considered when operating fermentation vessels at higher than atmospheric pressures.

When choosing beers to be fermented under pressure, lager beer styles have been a popular choice. Lagers are typically fermented under cold temperatures, which in turn reduces yeast growth, resulting in a slow fermentation with a reduced concentration of some volatile compounds. Additionally, this type of

beer takes longer to condition, as diacetyl rest times and sulfur reduction need to take place as these volatile compounds are reduced more slowly at colder temperatures. Another reason some breweries such as nano-size breweries might like to experiment with fermenting at conditions above atmospheric pressure is when having limitations to maintain consistent fermentation temperatures. This is extremely important to obtain good quality beer, especially when considering lager beers. If a brewer chooses to ferment a lager at high pressure, typically an additional 14.7 psi or 1 atm (for fermentation at 29.4 psia or 2 atm absolute), the fermentation temperature can be increased up to 68°F (20°C), aiming to obtain a similar flavor profile and rate compared with atmospheric pressure (7). This means that brewers will have to experiment with their recipes and systems to see if they find the sensory differences between their typical fermentation process (atmospheric) and pressurized fermentations acceptable. Lager-like ales such as Kölsch and Blond ales are typically fermented cooler (62°F/16.6°C) and are other styles of beer that could be influenced positively by fermenting at high pressure. Fermentation of Kölsch beers often takes about 21 days. Hence, increasing the pressure could allow brewers to ferment at higher temperatures to increase fermentation kinetics (and tank turnover) without increasing the production of volatiles, provided yeast health can be maintained.

Hazy IPAs and Kveiks are two relatively new beer styles that could potentially benefit from the use of high-pressure fermentation. The volatile compounds that yeast itself creates (esters and higher alcohols) may not be as important to the overall sensory and aroma of this style of beer. This brings about the possibility for brewers to explore fermenting under pressure with high temperatures, allowing faster fermentations and possibly less interference of yeast-derived volatiles. Kveik beer style is fermented at warm temperatures (85–95°F/29.5–35°F) to create its desired volatiles. The strain used for Kveik is already being used to push the boundaries of traditional fermentations, and the possibility of brewing at variable pressure may positively affect the final characteristics of these beers.

## Summary

Fermenting at both high and low $CO_2$ concentrations offers unique advantages and disadvantages. Brewers need to be able to understand the implications of a major decision such as the switch between open and sealed fermentation vessels or adjusting the pressure within a sealed vessel throughout a fermentation. Some brewers may use combinations of both to optimize their processes with regard to fermentation rate and volatile composition of their products. Due to the low detection thresholds of volatiles that were found to be affected by $CO_2$ (most strongly, acetate esters and isoamyl alcohol), small differences in the pressure can have a significant effect on the final beer. Ultimately, $CO_2$ concentration is just one additional variable alongside recipe design, yeast strain, fermentation temperature, and pressure, all of which also impact the production of volatile compounds. Manipulation of any of these variables can result in positive or negative changes depending upon the style of beer. For example, increasing the headspace pressure in conjunction with an increased temperature could result in a low ester formation (for the temperature) without affecting the fermentation rate. conversely, reducing the headspace pressure during fermentation may be an avenue for novel brewing techniques, or for replicating open fermentor styles under more controlled conditions. Note that the fermentation performed at different headspace pressures can also be of importance to industries outside of the brewing industry (e.g., the biofuel and

pharmaceutical industries) where volatile production is secondary to kinetics, yeast health, and ethanol production.

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