Bottle Conditioning

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Bottle conditioning refers to a method of adding fermenting wort or yeast suspension in sugar solution into beer in its final package. Additionally denoted as bottle refermentation, this technique has been originally developed to assure beer carbonation, and has further significance related to formation of distinctive sensory attributes and enhancement of sensory stability, which are the phenomena associated with ongoing yeast metabolic activities in the final package.

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1. Introduction

The brewing industry has undergone a global transformation as a result of the ongoing craft beer revolution, associated with an increasing number of craft beer producers who have flooded the market with innovative and intriguing beer styles using novel ingredients and production methods to attract consumer interest and provide a unique sensory experience [1][2]. From customers' point of view, biological generation of flavors and use of natural ingredients appears more attractive, and therefore this approach has become a strategy of choice for beer producers as it increases the probability of the acceptance of their product on the market [3]. The induction of new flavors into beer or alteration of existing flavors by addition of new ingredients or fermenting microorganisms that are connected with the relevant technological adjustments in the brewing process is termed bioflavoring. Bottle conditioning, which can be considered as a type of bioflavoring, is a traditional method developed in order to enhance beer carbonation. Bottle conditioning, also denoted as bottle refermentation or bottle kräusening, indicates an additional fermentation in the bottle that is initiated by adding yeast and fermentable carbohydrates 4. During the saturation phase, in the first 14 days of refermentation, as a result of yeast multiplication, alcohol concentration, and carbonation increases and the concentration of various flavor-active compounds are altered, which results in changes of beer aroma and taste [5]. Due to the yeast's reducing properties and consumption of oxygen present in the final package, bottle conditioning can prevent oxidative damage, which is successively manifested by aging of beer and development of stale flavor. Thereby, the presence of the yeast during refermentation can enhance storage stability and prolong beer shelf life [6]. Other types of microorganisms apart from brewing yeast can also be employed in bottle conditioning. In those cases, pronounced differences in the production of sensory-active metabolites in comparison with brewing yeast represent another source of distinctive features of the final beverage [7][8].

2. Historical Aspects of Bottle Conditioning

Production of top-fermented beers prevailed until the second half of the 19th century. Due to the higher temperatures during fermentation, ales are typically less carbonated than bottom-fermented beers. Therefore, bottle conditioning was originally implemented as a method to enhance carbonation and thus eliminate the flat taste of beer [4]. The technology of introducing carbonation into a bottled beer beverage was inspired by the "champenoise" method for the production of sparkling wines, which has been known since the end of the 17th century and further established during the 18th century when glass bottles became widely available. Second alcoholic fermentation is one of the key steps after the first fermentation and blending of fermented grape juice from several varieties to achieve the desired balance of flavors. Sugar and yeast are added, and the blended mixture is introduced in thick-wall glass bottles and allowed to ferment at temperatures of 12–14 °C. Second fermentation adds about 1.2–1.5% of ethanol and 11.8 g/L of CO_2 . After a 15-month period of aging, bottles are disgorged, sediment of dead yeasts is removed, and a new cork stopper is used for closing [9]. As the "champenoise" method and its products had become increasingly popular, it was logical that brewers started to be interested to the implementation of those new technologies to increase the beer lifespan and attractiveness of their products.

Bottle conditioning has a long tradition in the production of Belgian acidic beers made by spontaneous mixed yeast bacterial fermentation. The biological principles of acidification were unveiled during the 18th century and acetic acid bacteria were identified at the end of the 19th century. The first bottled lambics were sold in 1844. The term for beer made by blending of lambics and subsequently refermented in bottles is "Gueuze"; however, the origin of the name is unknown. Gueuze beers appeared in the market at the end of the 19th century. The maturation of lambic and Gueuze is assured by the presence of Brettanomyces sp. and their ability to cleave higher maltooligosaccharides. In Gueuze production, sugar or sugar and pure yeast suspension can be optionally added in bottles to support refermentation [10].

Traditionally, wort was being inoculated by yeast collected from the surface of a previous fermenting batch. In the Middle Ages, brewing was predominantly in hands of abbeys, which could assure stable production of grains and manage sustainable beer production [11]. In the 10th century, addition of hops was introduced in brewing to prevent beer turning sour and conserve it for further storage. The use of hops differed from region to region, but since the 14th century it was dominantly used in European countries, a trend that was confirmed by The Bavarian Beer Purity Law in 1516 [12][13][14]. Between the 15th and 16th centuries, Bavarian monks started with spontaneous selection of bottom-fermenting yeast by lagering of beer in cold cellars. As they observed, beer that was inoculated by yeast and settled to the bottom of the barrel after storage in the cellar had much better properties and stability 15. This transition to cold fermentation was also supported by an edict of Prince Maximillian in 1533, which permitted brewing exclusively from September to April. The development of industrial refrigeration technology by von Linde has facilitated the production of bottom-fermented beers in larger manufacturing scales, regardless of the season. Nevertheless, many breweries were using natural ice cubes to cool their cellars until the first half of the 20th century. Furthermore, the construction of the thermometer and saccharometer enabled better control and understanding of the brewing process. The principle of alcoholic fermentation was elucidated by Gay-Lussac in 1810, who described the conversion of glucose to ethanol and carbon dioxide [16]. Carl Napoleon Balling defined the attenuation law in mathematical equations, which have been used until now [17][18]. Pasteur's discoveries from

1860s represent a historical milestone for the brewing industry. The role of microorganisms in beer spoilage and the approach on how to prevent it were explained in his work. Pasteur also introduced the technique of yeast acid washing to eliminate the presence of undesired contaminants during primary fermentation and described the differences of yeast aerobic and anaerobic metabolism. A few years later, in 1883, Hansen succeeded to isolate pure yeast strain and highlighted the importance of its use [16].

The evolution of bottle conditioning technology goes hand in hand with the development of packaging materials. Refermentation in wooden casks is the oldest method of conditioning applied for ale beers. Originally, the method has developed by ongoing fermentation of beer packed in the casks during transportation from breweries to pubs. As observed, beer obtained higher carbonation while on the way. The approach to beer conditioning evolved into a more sophisticated procedure; when following up primary fermentation, beer with a sufficient amount of residual extract and concentration of yeast cells around $0.1-1 \times 10^6$ per mL is racked into wooden casks and kept at lower temperatures (12-14 °C) to be allowed to mature, which generates additional carbonation and drop of gravity (1-2 °P). The method is still used in the production of traditional English cask ale [19]. Even nowadays, cask-conditioned real ale is dispensed directly from the casks using hand pumps or gravity forces. In England, a voluntary organization, Campaign for Real Ale, was established in 1971 to promote this beer style and provide guidelines and supervision on the traditional technology of cask-conditioned beers [20]. The addition of fermenting wort containing yeast into beer during maturation stage or after racking is an established method for production of krausened lager in the Czech Republic. The presence of yeast contributes to beer freshness and imparts an authentic character [21][22].

The invention of crown corks and bottle openers in 1892 by Irish engineer William Painter brought up new possibilities and enabled consumption of beer in domestic conditions apart from pubs and bars [16]. The manufacturing of glass bottles became automatized by the end of the 19th century and was followed by gradual improvement of filling technologies during the 20th century. The generic use of wooden casks was progressively replaced by packaging into aluminum and stainless-steel kegs after the Second World War [23][24]. Dramatic development of industrial brewing technology during the 20th century resulted in improved quality of produced beers but also to unification and standardization of the major produced beer styles. Currently, emerging customer demand for innovative beer flavors related to the aspect of craft production introduces the urgency for product differentiation [25][26]. Bottle conditioning in bottles or casks can bring desired distinctiveness of brewers' portfolios.

3. Principles of Flavor Enrichment by Bottle Conditioning

Additional fermentation of beer in the final package induced by addition of yeast and sugars triggers multiple changes in its chemical and sensorial properties. Beer flavor is given by raw materials used for brewing and yeast metabolism during primary fermentation to a major extent. However, concentrations of flavor active compounds can be influenced throughout the full course of the production process and the final beer quality is a result of their mutual balance. When refermentation is applied, new flavors are additionally generated by the yeast activity in bottle or cask. Yeast metabolic products, which are the main constituents of beer aroma, are higher alcohols, esters, vicinal diketones, aldehydes, and sulfur compounds [27][28].

Higher alcohols can be synthesized de novo by anabolic pathways from carbohydrates or by the Ehrlich pathway as products of amino acid catabolism [29]. The most abundant in beer are n-propanol, iso-butanol, 2-methylbutanol, and 3-methylbutanol, contributing to the enhanced alcoholic flavor and promoting a warming character. In some beers, higher content of 2-phenylethanol is desirable, imparting its rose and floral aroma [30].

Esters are the contributors of floral, fruity, and solvent-like flavors and are present in trace concentrations. The most important flavor constituents with their respective flavors are ethyl acetate (green apple), isoamyl acetate (banana), ethyl butyrate (pineapple), ethyl hexanoate (sweet apple), isobutyl acetate (banana, sweet), ethyl caproate (apple, fruity), and 2-phenylethyl acteate (roses) [31]. Esters are formed by condensation of organic acid and alcohol. By yeast metabolism, two types of esters can be formed that comprise ethyl esters from condensation of ethanol and middle chain fatty acid or acetate esters formed from acetic acid and ethanol or higher alcohol [32].

Vicinal diketones (diacetyl and pentane-2,3-dione) are formed as intermediate products during synthesis of valine and isoleucine. Subsequently, they are reduced by yeast reductases during ongoing fermentation. Increased concentrations of vicinal diketones impart a buttery flavor and are therefore undesired [33].

Aldehydes are formed as intermediates during higher alcohol synthetic pathways. Furthermore, they are also formed during beer aging by oxidation of higher alcohols. Aldehydes have lower sensory thresholds compared to their respective alcohols. The most abundant, acetaldehyde, is associated with green apple flavor. Others confer with nutty, grainy, papery, and cardboard flavors, and are overall connected to a beer stale flavor [34].

Sulfur compounds occurring in beer comprise several groups of compounds with varied chemical structures (hydrogen sulfide and sulfur dioxide, thiols, sulfides, disulfides, trisulfides and thioesters). Most of them are derived from biosynthetic pathways of sulfur-containing amino acids methionine and cysteine, or from their successive degradation [35]. Hydrogen sulfide forms as a by-product that is subsequently added on allylic alcohols from hops or onto α,β-unsaturated carbonyls from wort, forming sulfanylalkylalcohols (SA) or sulfanylalkylcarbonyls (SC), respectively. Examples of SA whose concentration increases during bottle conditioning are 3-sulfanylhexan-1-ol, imparting a rhubarb flavor, and 4-sulfanylnonan-2-ol, contributing a lemon flavor. Similarly, from the SC group, 4-sulfanyl-4-methylpentan-2-one and 3-sulfanylhexanal contribute catty and floral flavors [36]. Moreover, the concentration of free thiols during bottle conditioning is also increased due to yeast lyase activity, which cleaves present cysteine adducts [37]. The lyase activity is strain-dependent and can significantly vary in between different yeast strains [38]. Apart from sulfury notes given by the presence of inorganic sulfur compounds in beer, other sulfur-containing compounds are associated with undesired aromas resembling cabbage, corn, onion, or rotten vegetables [35].

During yeast growth phase, residual amino acids from beer are sequentially taken up and synthesis of aldehydes and higher alcohols proceeds. However, the extent of de novo formation of these compounds such as formation of esters in bottle-conditioned beer is much lower in comparison to main fermentation because of poor availability of metabolic substrates [4]. Despite that, even small changes in concentrations of flavor-active compounds can be perceivable, especially due to low thresholds of some of the esters [39].

Another pathway of new flavor formation during bottle conditioning is the release of flavor-active aglycones from their odorless glycoside-bound state by yeast enzymes with glucoside hydrolase activity, such as exo-1,3- β -glucanase (EC 3.2.1.58). The sources of these aromas are glycosides present in hops and malt, which are transferred into beer during the brewing process, with examples including citronellol, vanillin, and β -damascenone [40][41][42]. Some yeast strains are characteristic with pronounced formation of phenolic flavors, which resemble cloves, smoked meat, or medicinal odors. Due to the presence of the PAD1 gene, which encodes phenylacrylic acid decarboxylase, hydroxycinnamic acids (p-coumaric acid and ferulic acid) can be decarboxylyzed to corresponding vinylphenols (4-vinylphenol and 4-vinylguaiacol, respectively), which are the carriers of the specific aroma typical mostly of wheat beers [43][44][45].

Changes in beer flavor during bottle conditioning can be additionally caused by mutual transformations of hop terpenoids induced by yeast enzymes [46]. As an example, citronellol is generated from geraniol, and linalool concentration can increase by transformation of nerol and geraniol, which leads to alterations of beer aroma [47].

Bottle conditioning leads to an increase of concentration of carbon dioxide, which supports beer effervescence. The major amount of CO_2 in bottle-conditioned beer is generated by decarboxylation of pyruvate and formation of acetaldehyde by pyruvate decarboxylase. A certain amount of CO_2 is also produced via the hexose monophosphate pathway and through the Krebs cycle [48].

When all fermentable sugars are metabolized and beer becomes saturated with CO₂, yeast growth arrests and the cells enter the stationary phase. Progressively, the viability declines and the permeability of the yeast cell wall is disrupted, which causes autolyzes and release of intracellular content including hydrolytic enzymes, fatty acids, amino acids, peptides, and nucleotides, which can have a negative impact on the beer flavor, especially loss of fruity notes and occurrence flavors associated with beer aging [49][50]. Yeast esterases are also excreted into beer during main fermentation, and therefore it is sometimes advised to pasteurize beer prior to bottle conditioning to minimize ester degradation. The esterase activity is strain-dependent, and, generally, top-fermenting yeast possesses more active esterases than bottom-fermenting yeast. The most affected esters are isoamyl acetate, ethyl hexanoate, and ethyl octanoate, and the level of their cleavage depends on environmental conditions such as temperature, alcohol concentration, and pH [51][52]. On the other hand, some esters are newly synthesized during storage, such as ethyl 2-methylbutyrate, ethyl nicotinate, and ethyl pyruvate [51]. They impart a winey aroma and are related to stale flavor [53].

Despite unfavorable conditions in beer in the beginning of bottle conditioning, which comprise the presence of alcohol and low pH, yeast is still capable of multiplication and growth to some extent. Because the concentration of oxygen in final beer is limiting, fermentative metabolism is prevailing and only approximately 5% of added sugars are metabolized by respiratory pathway [4]. Dissolved oxygen and oxygen in the bottleneck space are also utilized for synthesis of fatty acids and sterols during yeast growth phase. Therefore, yeast is regarded as an oxygen scavenger in bottled beer, and since the role of oxygen in flavor deterioration of beer during storage is indisputable, the presence of yeast can exert protective effects and decelerate the beer aging process [54][55][56]. Fermentative metabolism generates reduced coenzymes NADH and NADPH, which are associated with yeast reducing power.

These coenzymes are subsequently employed by several aldoketoreductases and convert aldehydes present in beer to their respective alcohols [57]. During the storage of packaged beer, several reactions comprising oxidation of fatty acids, Strecker degradation, and Maillard reactions occur spontaneously and give rise to carbonyl compounds that are considered as markers of aged flavor. Flavor stability has been extensively studied and, in particular, increased concentrations of linear aldehydes (trans-2-nonenal), Strecker aldehydes (2-methylpropanal, 2-methylbutanal, 3-methylbutanal, benzaldehyde, phenylacetaldehyde, methional), and furan derivatives (furfural, 5-hydroxymethylfurfural) are connected with undesired stale flavor [34][58][59]. Bottle conditioning can assure prolonged flavor stability by reducing the concentration of carbonyl compounds associated with staling, thereby diminishing the intensity of off flavors related to beer aging [60]. Furthermore, sulfites produced by yeast metabolism enhance flavor stability as well. The anti-aging mechanism of sulfites is exerted by radical scavenging, thus inhibiting the oxidative reactions; otherwise, they can bind to present aldehydes and form adducts with hydroxysulfonate structures [61].

4. Conclusions

The inspiring idea of bottle conditioning came into the brewing industry from winemaking in the middle of the 19th century. Since then, it has been established as a traditional method for production of specialty beers, particularly in Belgium, as well as for other specific beer types in selected European regions. Nowadays, the old traditions are being reborn and innovated worldwide, following current trends in craft brewing. In our review, we demonstrated the principles and technological aspects of bottle conditioning. Furthermore, we explained how it contributes to flavor complexity and assures exclusivity of the product. Bottle conditioning can represent an opportunity for the enrichment of brewers' portfolios and, simultaneously, it can be an approach as to how to satisfy growing customer demand for beers with distinctive character.

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