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Review

Comparison Review of the Production, Microbiology, and Sensory Profile of Lambic and American Coolship Ales

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Abstract: Sour beers have been traditionally brewed with spontaneous fermentation. This has been occurring in Belgium for hundreds of years, and more recently in the United States as the American craft beer industry has boomed. Belgian sour styles include lambics, which are mirrored in a burgeoning style called the American coolship ale (ACA). American beers have much more creative leeway than their Belgian counterparts, as American craft brewing tends to incorporate more contemporary techniques and ingredients than their traditional European forebears. This review paper will summarize the history, production methods, fermentation, microbiological profiles, and sensory profiles of Belgian lambics and American coolship ales.

Keywords: lambics; flavor; microbiota; American coolship ales

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1. Introduction

Spontaneous fermentation relies upon brewery microbiota. Belgian lambics are the most well-known spontaneously fermented beer currently produced. American coolship ales (ACAs) are the lesser-known American version of spontaneously fermented beer [1]. Lambics are weakly carbonated beers that are spontaneously fermented for one to three years prior to bottling [2]. In general, these spontaneously fermented beers are fermented with a wide variety of microorganisms over longer timeframes and are characterized as having a diverse flavor profile that is sour, acidic, and at times astringent, but generally lacking the bitterness associated with hops in other traditional beer styles. Lambics and newer ACAs are a result of the specific history, variety of microbiota, and distinct sensory profile which will be described in this paper.

2. History

2.1. Lambics

Lambic is one of the oldest styles of beer still brewed in the western world [3,4], with origins dating to the 13th century [2]. Beer in this era almost certainly utilized spontaneous fermentation. Brewers who used this process would expose the wort to the air, allowing it to be inoculated with wild-born yeast and other microorganisms present in the air. The first documented bottled lambic was sold in 1844 [5]. Only a 500 square kilometer area around Brussels and the Payottenland, a valley on the Senne River on the western side of the city, has the right combination of airborne microorganisms necessary to create lambic beer consistently through spontaneous fermentation [2]. The concentration of fruit orchards and traditional farmhouse breweries provide an ideal environment for wild yeast and bacteria to become airborne in order to reside in the breweries that make lambic beer [6].

2.2. American Coolship Ales (ACAs)

Unlike its European forebears, American sour beer is a recent phenomenon: the first commercial American sour was produced in 1995 [7]. In 1999, New Belgium Brewing released their iconic La Folie, and in 2002, the Great American Beer Festival (GABF) introduced the style for the first time [7]. Since then, the popularity and prevalence of American sour beers have exploded. In 2002, there were 15 total entries in the GABF; by 2015, there were 238 sour entries split into five different categories [7]. In the early stages of American sour brewing, most examples closely followed historical European examples. However, as breweries developed their own methods, and consumers their own preferences, American sour production began to diverge from traditional Old World examples [7].

3. Production Methods

3.1. Lambics

Lambic, gueuze, and similar styles are complex beers made from a few key components including malted barley, unmalted wheat, aged hops, and fruits. While the ingredients play a vital role in the brewing process, the fermentation process plays a key role in the uniqueness of this style of beer.

One unique characteristic of lambic brewing is the utilization of a turbid mash. This mash contains high levels of proteins and complex carbohydrates that allow microorganisms to thrive. Additionally, aged hops are used because they impart less bitterness than fresh hops while retaining their antibacterial properties [2]. The wort is allowed to cool overnight in open shallow trays (cooling tun or coolships) and is inoculated by the natural microflora present in the surrounding environment [4,8]. Nights need to be cool enough to allow for the wort to reach a temperature of 20 °C [9]. Once the wort has finished cooling overnight and inoculation has occurred, it is pumped into metal or wooden casks and stored in non-air-conditioned warehouses. The casks are stored at cellar or ambient temperatures (15 to 25 °C) [4,8]. It takes 4 to 8 months for the wort to decrease from 12 °P down to 3 °P. This corresponds to the main fermentation [10]. This is followed by additional time where the beer continues to acidify and mature. The production time of a lambic can range from one to three years [2].

3.2. ACAs

ACA brewing is nearly identical to lambic brewing, with a few terminology changes. For example, the open vessel that cools the beer overnight and inoculates the beer with native microflora is called a “coolship” in the United States. The basics of the production process are identical to traditional lambic brewing [11].

Oak barrels and foeders play an important role in the inoculation and production of lambics and ACAs [12]. Barrels and foeders not only aid in the development of a the flavor profile for lambics/ACAs, but are also important for inoculation [13]. Unlike stainless steel, oak allows for gas exchange (micro-oxidation) during the aging process, resulting in the development of aromatic and flavor compounds. This phenomenon has been well studied in distilled spirits; however, research focusing on beer has been limited. The concentrations of flavor and aromatic compounds change over time due to enzymatic and chemical reactions in beer [14].

4. Stages/Phases of Fermentations

Lambic and ACA beers are unique in that the fermentation is caused by a combination of traditional and wild yeast, along with other microorganisms. Fermentation takes place in four distinct phases, in which microbial succession occurs in each stage: the Enterobacteriaceae phase, the main fermentation phase, the acidification phase, and the maturation phase (Table 1) [4,11].

Table 1. Microbes found during each stage of the Fermentation Process.

	Enterobacteriaceae	Main	Acidification	Maturation
Lambic	*Enterobacter*, *Hafnia*, *Escherichia*, *Citrobacter* spp., *K. pneumoniae*	*Saccharomyces cerevisiae* and *Saccharomyces pastorianus*	*Pediococcus damnosus* *Lactobacillus brevis*	*Brettanomyces* spp. dominant yeast strain within first month
ACA	*Klebsiella* spp., *Enterobacter* spp., *Pectobacterium carotovorum*, *Serratia ureilytica*	*Saccharomyces cerevisiae* and *Saccharomyces pastorianus*	*Pediococcus damnosus* *Lactobacillus brevis*	*Saccharomyces cerevisiae* to *Brettanomyces* spp. occurs at six months

The first stage of fermentation is dominated by enteric bacteria and *Kloeckera apiculata*. The enterobacterial phase begins less than a week after fermentation begins and lasts for slightly over a month. In this phase, Enterobacter spp., *Klebsiella pneumoniae*, *Escherichia coli*, and *Hafnia alvei* predominate, as well as oxidative yeasts such as Kloeckera spp. [4,15]. *K. apiculata* reaches a maximum concentration of 10^5 cells/mL within the first week of fermentation, but is quickly out-competed by the Saccharomyces species and dies off [16]. *K. apiculata* only ferments glucose. *K. apiculata* and Enterobacteriacea are fast-growing microorganisms and wort pH to decrease from 5.1 to 4.6 because of acetic and lactic acid production. During this stage, there is little ethanol production [13]. However, the microbiota of the enterobacterial phase are different in ACAs. Instead of dominance by *Enterobacter*, *Hafnia*, *Escherichia*, *Citrobacter* spp., and *K. pneumoniae*, the enterobacterial phase of ACAs is dominated by *Klebsiella* spp., *Enterobacter* spp., *P. carotovorum*, and *S. ureilytica* [4,11,15,17]. In addition to traditional lambic LABs such as *P. damnosus* and *L. brevis*, Bokulich et al. (2012) found Lactococcus and Leuconostoc in ACAs, although *Pediococcus* spp. were the dominant LAB in most batches [11].

During the main fermentation phase, typical brewing strains *S. cerevisiae* and *S. pastorianus* dominate [13]. The third stage of fermentation overlaps the second stage. This stage starts approximately three to four months after brewing. Stage three is dominated by the proliferation of lactic and acetic acid bacteria, with both peaking in cell numbers around six to eight months, which is usually in late spring to early summer, when the early morning temperatures become warmer. Warmer temperatures are required for the growth of these microorganisms [3].

In the acidification phase, which lasts six months, lactic acid bacteria are produced by LABs, such as *P. damnosus* and *L. brevis* [13]. These bacteria can convert sugars into lactic acid. The characteristic sourness of lambic beer can be attributed to the presence of this lactic acid. While some strains of *Pediococcus* are beneficial in beer, others can produce a slime layer that causes a permanent haze that cannot be removed with filtration. Unlike lactic acid bacteria, acetic acid bacteria are undesirable in this style of beer because of their ability to convert ethanol into acetic acid, causing the beer to become acidic or hard (high volatile acidity). This only becomes a problem if the cask or barrel that the beer is stored in has been damaged to allow oxygen to contact the beer. Increased oxygen nurtures the aerobic Acetomonas bacteria [2,3].

After approximately eight months, an increase in the number of yeast cells occurs, signifying the start of the fourth and final stage of the fermentation process. *Brettanomyces* spp. plays a crucial role in the development of the aromatic and flavor profiles of this beer. A large portion of the aromatic profile is composed of esters, a byproduct of *Brettanomyces* spp. metabolism. The two most influential compounds produced are ethyl lactate and ethyl acetate [2,3]. The other relevant microorganism in this phase is *Brettanomyces bruxellensis* [5]. *Brettanomyces* spp. can ferment complex carbohydrates such as maltotetraose and maltopentaose, and produces volatile compounds important in traditional lambic aroma, such as octanoic (caprylic) and decanoic (capric) acids and their esters [1], along with 4-ethylguaiacol and 4-ethylphenol [18,19].

The key differences between lambics and ACAs relate to fungal communities, particularly the emergence of *Brettanomyces* spp. *B. bruxellensis* becomes the dominant yeast strain within the first month of fermentation, while the transition from *S. cerevisiae* to *Brettanomyces* spp. does not occur until six months for lambics [12,13].

The relative stability of ACA microbiota shows the potential existence of "site-specific microbiota" that impact ACA fermentations on a local level, much like lambic fermentations in Belgium [11]. Unlike Belgian lambic breweries, most American breweries that produce ACAs also produce other styles, leading to the dominance of *S. cerevisiae* early in ACA fermentations, which may help in the selection of stable microbial communities on a batch-by-batch basis [11].

5. Microbiota

5.1. Yeast

Saccharomyces cerevisiae are the primary yeast strains in the primary fermentation. These strains have an important role in the final flavor and aroma profiles of the finished beer [20]. In lambic beer, Saccharomyces species are responsible for the main alcoholic fermentation and for most of the attenuation in the wort. For the first seven months of the fermentation process, these microorganisms dominate the microflora of the wort, reaching a population density of 5×10^6 cells/mL after three to four weeks. The overall yeast cell population, however, is still significantly smaller than that normally found in most commercial fermentations (1×10^8 cells/mL). The two main species of Saccharomyces yeast found in lambic wort are *S. cerevisiae* and *S. bayanus*. Both species of Saccharomyces can metabolize glucose, maltose, and to some extent maltotriose, the main sugars found in lambic wort [3]. Unlike other beers, lambic beers go through a secondary fermentation step which increases the attenuation beyond the normal range (63–83%). This is the result of yeast such as *Brettanomyces bruxellensis* converting the remaining sugars left in the wort [3,21].

5.2. Enterobacteriaceae

Enteric bacteria, or *Enterobacteriacea*, are Gram-negative bacteria [3]. Some of these bacteria are harmless, while others are pathogenic to humans, animals, and/or insects. A few of the following genera are included in this family and they are *Escherichia*, *Shigella*, *Yersinia*, *Morganella*, and *Samonella* [22].

Enterobacteria are not potential spoilage microorganisms in lambics, as they are in other beers. Once the wort has cooled, the enteric bacteria in the wort reach a very high cell density, reaching a maximum concentration of 1×10^8 cells/mL [3,21,23–25]. The enterobacteria population decreases once the pH of the wort drops below 4.4 and the ethanol concentration rises above 2% [25].

Enterobacteria impart flavors such as sweet, honey, fruity, vegetal, and even fecal. Similar to other brewing microorganisms, enterobacteria have the ability to metabolize glucose not only for growth, but also into organic acids (lactic acid, acetic acid), ethanol, and carbon dioxide. They are unable to metabolize other usually fermentable sugars such as maltose or maltotriose. Enterobacteria can consume amino acids and peptides that temporarily impact beer flavor. One possible reason for the slow start to the main fermentation is because of the depletion of the amino acids in the wort by enterobacteria [26].

Enterobacteria produce several different sulfur compounds, which impact the aroma and flavor profile of beer, although some of these compounds will disappear during later phases of fermentation. The compounds become trapped by the CO_2 produced during the main fermentation. Dimethyl sulfide (DMS) is a good example of this phenomenon. Within the first two weeks, enterobacteria produces DMS in excess of 450 ppb. The concentration of DMS drops to 100 ppb because most of it is stripped away by fermentation gases [3,8].

5.3. Lactic Acid Bacteria (LAB)

Lactic acid bacteria are Gram-positive nonsporulating rods or cocci [22]. These microorganisms are potentially one of the most dangerous spoilage microorganisms in beer because they are microaerophilic, able to tolerate the antiseptic properties of hops, and able to survive in ethanolic and acidic environments [16]. The major division between types of

Lactobacillus spp. is how they metabolize glucose [27]. Lactic acid bacteria are broken down into four genera: (1) *Lactobacillus* for the rod shaped organisms, (2) *Streptococcus* for the homofermentative facultatively anaerobic cocci, (3) *Leuconostoc* for the heterofermentative cocci that occur in pairs or short chains, and (4) *Pedioccus* for the homofermentative cocci that divide into pairs and tetrads [22].

Pediococcus and *Lactobacillus* populations increase upon the completion of primary fermentation [3]. After three to four months, lactic acid begins to develop. Bacterial populations peak at approximately seven months, which usually coincides with the beginning of summer [4]. The warmer temperatures in the cellars during the summer appears to be essential for the growth of these bacteria. This phenomenon is seen again in the second summer of fermentation. It has been hypothesized that lambic/gueze fermentations can be sped up by increasing the ambient temperature in the aging cellar once the main fermentation stage is complete. Unfortunately, creating temperature-controlled aging cellars would be costly for breweries because of the building ages and associated renovation costs [3].

Lactobacillus spp. have varying tolerances to the antibacterial properties of hops [22]. *P. damnosus* is impervious to the antiseptic properties of hops, allowing it to grow in hopped beer. Lactic acid bacteria are slowly growing microorganisms that have complex nutritional requirements, which is partly why lactic acid bacteria do not reach a very high cell density in the wort. *P. damnosus* not only has the ability to produce lactic acid, but also acetoin and diacetyl, both of which contribute to the aroma present in this beer [3,4,28].

5.4. Brettanomyces

Brettanomyces bruxellensis (Brett) is an asexual, nonsporulating wild yeast associated with the spoilage of red wines, beer, and ciders [29]. Brett is slow-growing and often is not detected until several months after initial fermentation [21]. Brettanomyces was first isolated in 1904 from the stock of a late fermenting English beer [30]. N. Hjelte Claussen of New Carlsberg Brewery was the first to introduce the name Brettanomyces to describe the yeast required to make the English stock ale. It was not until 1920 that Brettanomyces/Dekkera was recognized as a unique genus [31]. There are five species of Brettanomuces/Dekkera and they are *B. custersianus, B. naardenensis, B. nanus, B. anomalus,* and *B. bruxellensis* [32].

Brett is found in wine, beer, cider, wineries, breweries, vintnering and brewing equipment, and oak barrels used for aging [33]. Brett is very difficult to remove from breweries once introduced [3]. Some forms of wild yeast such as Brettanomyces are typically considered 'niche' contaminants because they require specific conditions to thrive [34].

Brettanomyces is not detected in lambics and ACAs until approximately eight months into the fermentation process. *B. bruxellensis* and *B. lambicus* are the two dominant yeast strains in lambic and gueuze beer [8,11,17]. These two strains are present in the beer for another eight months and contribute heavily to the aroma profiles of lambics and ACAs [8]. *B. bruxellensis* is the predominant strain in urban breweries, while *B. lambicus* is the predominant strain in rural breweries [3]. Brett, unlike other yeasts, ferments much more effectively under aerobic conditions than anaerobic conditions, allowing it to form a film, or pellicle, on the surface of the fermenting beer. Brewers do not allow the pellicle to be disturbed during the aging process due to the increased risk of oxidation [3]. Strains of the yeast species *B. bruxellensis, B. anomalus*, and *B. custersianus* may occur in lambic beers during the maturation phase.

The secondary byproducts of Brett metabolism play a critical role in the sensory profile, especially the aroma, of lambic beer, despite being present in low concentrations. Esters ethyl acetate and ethyl lactate are the primary compounds produced. These esters can be formed enzymatically or chemically. Esterase forms esters through a reaction between ethanol and an organic acid. *Brettanomyces* spp. displays unusually high esterase activity compared to other yeasts [35].

Brett has a unique ability to synthesize ethyl phenols (4-ethylphenol and 4-ethylguaiacol) and vinyl phenols, compounds that impart unique characteristics to lambics and ACAs [36].

Brett is the only microorganism known to produce ethyl phenols. While there are some species of lactic acid bacteria and yeast that can produce ethyl phenols in cultured media, none have been shown to produce them in a beverage system [37]. *Brettanomyces* spp. is a key player in the flavor profile of lambic and gueuze beer [4,34] and ACAs. The 'bretty' character produces aromas and flavors such as mineral, tobacco, barnyard, leather, pharmaceutical, and smoke [23,35]. Aroma compounds produced by Brett can stifle desirable fruity notes [38]. Tetrahydropyridines are responsible for the trademark horsy smell imparted by Brett [3]. Tetrahydropyridines are produced when esterase breaks down ethanol and lysine. Horsy character in these styles is generally desirable in low to moderate concentrations [3,39].

6. Sensory Profile

6.1. Lambic

Gueuzes, the non-fruit version of a lambic, can range in color from golden yellow for young gueuze to light amber for older beers. Gueuze undergoes bottle fermentation, such as champagne, to produce high carbonation levels. Foam may gush open bottle opening, ranges in color from white to yellowish. The CO_2 bubbles are larger than in sparkling wine and disappear quickly. The uniquely high volatile acidity of the style imparts a vinegary, goaty, and/or rancid aroma. Fruity aromas include apple, melon, and apricot. The aroma profile of gueuze is balanced fruity esters and woody and vanilla aromas from the wooden fermentation casks.

Sour, acidic, and sometimes astringent flavors dominate the palate of lambic beers. Little to no bitterness is found in these beers because of the aforementioned use of aged hops. Gueuzes can be dry or sweet, depending on their age. Astringency levels differ between traditionally produced and mass-fermented gueuze. Traditionally produced gueuze contains more tannins from the wheat and wood, giving it a thinner taste, while bulk-fermented gueuze is thicker and has a smoother mouthfeel.

6.2. ACA

The sensory profile of ACAs is similar to traditional Belgian lambics. The presence of 2,3-butanediol is indicative of the enterobacterial phase of fermentation, while acetic acid concentration increases dramatically between years one and two of maturation [40]. Acetic acid is indicative of *Brettanomyces* spp. fermentation, and concentration can be minimized by mixing older ales with younger ones (as in the production of gueuze) [40].

The Beer Judge Certification Program (BJCP) does not have set guidelines for the style, allowing some breweries to take stylistic liberties that would not be possible in Belgium. Therefore, there are no set sensory characteristics in ACA production, aside from the obvious similarities to traditional Belgian lambic.

7. Flavor

The most important flavor compounds in both lambic and other beers are the higher alcohols and esters, organic acids, dimethyl sulfide, diacetyl, and VDKs [16,41].

7.1. Fusel Alcohol and Esters

Higher alcohols and fusel alcohols are important byproducts of fermentation. Esters are important flavor and aroma compounds. These compounds possess strong fruity flavors. Harrison (1970) determined that iso-amyl, phenethyl, propyl, and iso-amyl (2-methylbutanal) alcohols contribute to beer flavor [42].

Higher alcohol concentrations in lambics and other beer styles are similar. However, ester concentrations are different [8]. Ethyl acetate, which provides a solvent-like fruity aroma, is found in much higher concentrations in lambic beers than other styles. The average concentration of ethyl acetate is 8–48 ppm for traditional beers, while it is 33.4–67.6 ppm for filtered gueuze and 60.9–167 ppm in unfiltered gueuze [14]. Contrastingly, isoamyl acetate is found in much lower concentrations in lambics than other styles. Ethyl lactate is a compound normally found in whiskey [43], wine, sherry [44], and cider [45]. This compound is present in lambics but almost never in other styles.

The ethyl esters of higher fatty acids, ethyl caprylate, and ethyl caprate, are traditionally found in lambic and gueuze beers. These ethyl esters are normally absent in lagers and present in only small amounts in ales [20,46]. Both ethyl caprylate and ethyl caprate are typical aroma and flavor compounds in lambic and gueuze. The ethyl esters caproic, caprylic, and capric acid give lambic and gueuze vinous and fruity flavors. Based upon the study carried out by Van Oevelen and his colleagues, the main aroma characteristics compounds of lambic beers were identified as ethyl lactate, ethyl acetate, acetic and lactic acid. Higher levels of both acetic and lactic acid are connected with higher amounts of both ethyl acetate and ethyl lactate. Lambic beers also contain high levels of caprylic (C_8) and capric (C_{10}) acids and ethyl captrate. Gueuze beer tends to also have a low level of phenethyl acetate [8,29,47].

7.2. Organic Acids

Ales and lagers have much lower acetic and lactic acid concentrations then gueuze beer [20,42]. Lambic and gueuze are well known for their high levels of acid (lactic and acetic). High levels of lactic acid are expected in lambics ranging from 2–3.5 g/L, while acetic acid has been found to vary between 0.4 and 1.6 g/L [17,19]. Most people have a flavor threshold of acetic acid at 200 mg/L while the flavor threshold for lactic acid is 400 mg/L [42,47]. Propionic, isobutyric, and butyric acid are also found in somewhat higher concentrations than in other styles of beer. The taste threshold levels of butyric, propionic, and isobutyric acid are approximately 1, 100, and 200 mg/L, respectively. Butyric acid is one of the few acids in gueuze beer that is traditionally found over its threshold level [8]. Butyric acid can be described as having a cheesy or rancid aroma [14]. Lambic and gueuze beers are known for containing high levels of octanoic (caprylic) and decanoic (capric) acids. Decanoic acid concentration in gueuze beer usually exceeds 2 ppm, which is slightly higher than the concentration found in lagers or ales [46].

8. Brewing Industry

8.1. Lambics

Lambic brewers are currently jeopardized by the very products they produce because of the time-consuming process required to produce their beer. Lambic beers are aged for anywhere from a few weeks to two years. Therefore, breweries can have anywhere from USD 100,000 to over USD 300,000 in product ageing in a barrel at any one time. The Belgian tax system, which charges taxes on beer within a year of production, is also harsh on lambic breweries, as it causes breweries to owe money before the beer is sold. Another problem is that lambic brewers are beginning to retire without brewing successors [2,3].

8.2. ACAs

Allgash Brewing is credited for producing the first ACA. Prior to 2007, no craft brewery produced spontaneously fermented beers in the United States, because of the time and effort required to produce them. Significant volumes of beer are disposed because of quality issues until an established microbiome is formed in the brewery. Jester King (Austin, TX, USA), De Garde Brewing (Tillamook, OR, USA), and Peekskill Brewery (Peekskill, NY, USA) are other prominent ACA brewers [48].

9. Conclusions

Belgian sour beers are steeped in tradition, with very little room for exploration or even geographical diversity, as lambics must be brewed in certain regions of the country. Lambics follow a microbial succession and utilize open fermentation, leading to reliable but varied microbiological profiles that utilize traditional brewers' yeasts in addition to wild yeasts and bacteria. Lambics are unique in that the base beer is very rarely consumed because it is uncarbonated. Bottled lambics are bottle-conditioned, but often, lambics of different ages are mixed to create gueuze.

American sour styles are unique in that they are interpretations of traditional European styles. The "American lambic", the American coolship ale (ACA), follows traditional lambic production steps and shares many microbiota with the traditional style.

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