

PROPERTIES OF BELGIAN ACID BEERS AND THEIR MICROFLORA

H. VERACHTERT and A. DEBOURG

I. THE PRODUCTION OF GUEUZE AND RELATED REFRESHING ACID BEERS

H. VERACHTERT and D. ISERENTANT

Laboratory of Industrial Microbiology and Biochemistry,
Department of Food and Microbial Technology,
KU.Leuven, Kardinaal Mercierlaan 92,
3001 Heverlee, Belgium

It is well known that one of the most common forms of beer infection is acidification by lactic acid bacteria. It may be realistic to assume that most (small) breweries once in a while may suffer from the unwanted growth of these bacteria in wort, during bottling or in casks.

However acidification by lactic acid bacteria is a very natural process to adjust the pH of the malt or the wort (Derdelinckx et al., 1994) and the presence of lactic acid in beer is not always to be considered as rejectable. Lactic acid gives to the beer a special refreshing taste. Thus we find in Europe several types of so called acid beers: in Belgium these are of the gueuze type or the Rodenbach type, in Germany the Berliner Weissbier. In England and Ireland a low concentration of lactic acid in porter, ale, scotch and stout is reported to be appreciated by the consumers.

The Belgian gueuze types deserve some special consideration as they are the only beers, which on an industrial scale are still produced by spontaneous fermentation of the wort. The Rodenbach beers are also special, considering the fact that during the long fermentation periods used for some types, yeasts considered as "wild yeasts" by the brewers, are also involved together with lactic acid bacteria.

1. LAMBIC (LAMBIK) AND GUEUZE (GEUZE)

Lambic and gueuze beers are traditionally produced around Brussels although good results have now also been obtained in other parts of Flanders. The raw materials that enter the composition of the grist are barley and wheat. By law wheat must be present (not less than 30%) and wort strength should be at least 11°P. The total acidity should at least correspond to 30 meq of NaOH. Acid beers that have been obtained by spontaneous fermentation may be named GUEUZE or GUEUZE-LAMBIC. There is no

clear agreement between the brewers whether the beer should result from 100 % wort subjected to spontaneous fermentation or whether only part of the fermented wort must include a spontaneous fermentation step. It is suggested that in that event the label should mention the percentage of spontaneously fermented wort. In fact it is up to the consumer to decide, on organoleptic or other grounds, where his preferences are and how interesting the market for the different types will be.

Traditionally gueuze is the product of a refermentation in the bottle of lambic. This is another point of controversy as many gueuzes are lambic beers, saturated with carbon dioxide and filtered. Traditional gueuzes are beers with a clear deposit of culturable micro-organisms, mainly yeasts and lactic acid bacteria. It is quite amazing to notice that many top fermented beers have evolved to include a bottle refermentation step, probably because the quality could be improved but also because it gives the consumer an impression of buying a "natural" and "traditional" product. Gueuze used to be the most classical example of such a bottle refermented beer but in a period of growing interest towards bottle refermentation, many gueuze brewers on the contrary turned to filter their beers. Amazing indeed. It is quite evident that over-simplifications of the production of a very special beer can only open the door to many imitations and the idea of adding some lactic acid to top fermented beers has ever been considered sufficient to call a beer gueuze. This might result in one more step of a tendency to homogenize tastes and flavors. Finally another characteristic of traditional gueuze brewing is the use of high doses of aged hops to avoid the interference of a bitter taste with the vinous character contributed by the lactic acid bacteria. The high hop doses act, if not as a bacteriostatic component, at least as a component with some effects on the composition of the mixed microbial flora.

2. GUEUZE BREWING

All methods of brewing are used. Traditionally it was intended to obtain a highly dextrinous wort, more appropriate to sustain a long fermentation leading to the typical lambic flavor. After boiling, the wort is cooled overnight in large shallow trays. During the cooling period micro-organisms from the brewery environment are introduced, and the infected wort is pumped into wooden casks (250-700 l or larger). The spontaneous fermentation starts and may last for two years. The product is called lambic. Re-fermentation in the bottle gives gueuze. It is a tradition to state that lambic can only be brewed around Brussels and that "... the special microclimate of the river Zenne accounts for the special mysterious microbial flora necessary for lambic brewing". Translated into scientific terms this means that the brewery environment has become a source of appropriate micro-organisms and that lambic can be brewed at other locations provided the brewer is prepared to enter a long period of trial and error. We have shown (Verachtert, not published) that microbial counts of inside and outside air are very low (10-1000 cfu's per m³) for yeasts and even lower for enterobacteria and lactic acid bacteria. Table 1 (at the end of the article) shows the identity of 89 yeasts isolated from the air. *Brettanomyces* considered as a typical lambic yeast is only identified once. However on exposing in the brewery 250 ml of wort or wort with antibiotics to enhance its selectivity, in open petri dishes, most lambic micro-organisms were found (Table 2 at the end of the article). To detect lactic acid bacteria a very rich medium was needed. These experiments also indicated that the infection takes place in a rather heterogenous manner. They also proved that during a one night wort cooling period, the micro-organisms already increase in numbers (Verachtert et al. 1990) and that they probably occur as small colonies adsorbed to inert particles. During the cooling period cells may multiply and during pumping of the wort into the casks cell aggregates may break up so that finally all casks receive an appropriate inoculum.

3. MICROBIAL ASPECTS OF LAMBIC WORT FERMENTATION

The lambic fermentation is characterized by the growth of a mixed culture of yeasts and bacteria. In general we may consider 5 different phases: the enterobacterial phase, the *Saccharomyces* phase, the acidification phase, a ripening phase and when gueuze is produced, the bottle re-fermentation phase. A typical profile is given in figure 1 (Verachtert et al. 1990).

a. The enterobacterial phase

Immediately after (and during) wort cooling there is strong development of enterobacteria. *Enterobacter cloacae* and *Klebsiella aerogenes* were most frequently recognized. Other species are *Escherichia coli*, *Hafnia alvei*, *Enterobacter aerogenes* and *Citrobacter freundii*. Total numbers may be above 10⁸ cfu after a few days. In this period very low numbers of lactic- and acetic acid bacteria are found but yeasts are easily detected. These are mainly actidione-resistant and maltose non-fermenters such as *Kloeckera apiculata*, *S. globosus* and *dairensis*. Due to their metabolic activity they disappear after about one month due to

glucose exhaustion of the wort. Due to a lowering of the pH the enterobacteria also are killed. Bacteria such as *Staphylococcus sp.* or *Streptococcus sp.* were never found.

A pure mixed culture of *Klebsiella aerogenes* and *Kloeckera apiculata* shows the same behaviour.

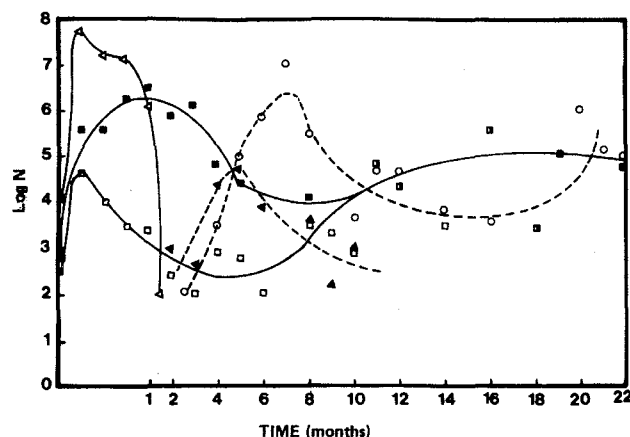


Figure 1
General microbiological profile of a lambic fermentation.
△ Enterobacteria ■ Total yeasts □ Actidione-resistant yeasts ▲ Acetic acid bacteria ○ Lactic acid bacteria

b. The *Saccharomyces* phase

At around 1 month the main microbial population consists of *Saccharomyces* yeasts: *cerevisiae*, *bayanus*, *uvarum*, *inusitatus*. They are responsible for the main ethanolic fermentation.

c. The acidification phase

After about 4 months lactic acid bacteria are on the increase. Most frequently these belong to the genus *Pediococcus*, a homo-fermentative organism producing D(-) and L(+) lactic acid. However in some instances (some breweries, very large casks) *Lactobacillus* species are active. Due to the previous metabolic activities, sugar exhaustion, acidification, *Saccharomyces* disappears and the yeast population becomes dominated by *Brettanomyces* yeasts, again actidione-resistant yeasts. Table 3 (at the end of the article) shows that almost all *Brettanomyces* species are represented in lambic, *Br. bruxellensis* and *Br. Lambicus* being frequently found, but also *Br. custersii*, *anomalous* and *intermedius*.

d. The ripening phase

After around 10 months the lactic acid bacteria may decrease and much later also the *Brettanomyces* yeasts. As shown in the next section the wort attenuation is still being reduced.

During the last three phases acetic acid bacteria are always present. Especially in warmer summer months they may produce high numbers.

e. The bottle re-fermentation phase

Figure 2 shows a profile of a bottle re-fermentation phase. At the time of bottling many film forming yeasts were present albeit at low numbers: *Candida*, *Torulopsis*, *Hansenula*, *Pichia* and *Cryptococcus*. They do not multiply and disappear after 10 months. They originated probably from a thick film of yeasts that develop in the casks during the long fermentation.

Brettanomyces yeasts however increased from around 10^2 to 10^5 cfu per ml and lactic acid bacteria from around 10^2 to 10^6 cfu per ml. After 14 months in the bottle only lactic acid bacteria were mostly detected.

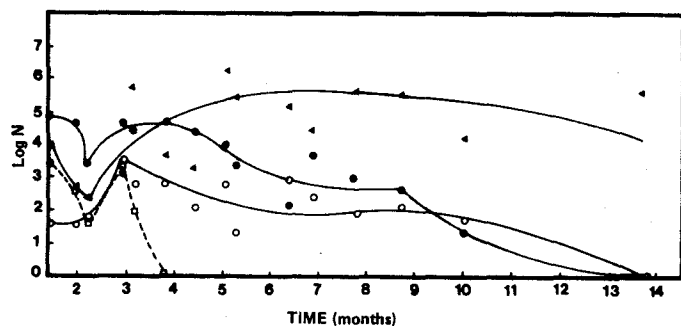


Figure 2
General microbiological profile of a lambic bottle refermentation
● Actidione-resistant yeasts ○ oxidative yeasts
□ Acetic acid bacteria ▲ Lactic acid bacteria

4. METABOLIC ACTIVITIES DURING LAMBIC FERMENTATION

a. The enterobacterial phase

In this phase an attenuation of around 15 % is realized, a decrease of about 1 pH unit, the production of minor amounts of ethanol, acetic and lactic acids, the production of 1 g per liter of butanediol, and DMS to levels of 500 ppb. In this period around 250 ppm of formic acid may be detected.

b. The *Saccharomyces* phase

In this phase most of the ethanol is produced together with the typical higher alcohols and esters. The content in DMS is reduced. It was found that *Saccharomyces* strains may also form formic acid.

c. The acidification phase

This is characterized by a strong increase in lactic acid and ethyl lactate, a slow increase in acetic acid and ethyl acetate. Diacetyl levels increase to more than 200 ppb. The pH decreases to less than 4 and the attenuation slowly increases. Caproic, caprylic and capric acid formed in the previous phase remain present but ethyl caprylate and ethyl caprate show a tendency to appear after 4 months. In this phase a cell-bound esterase (found in all *Brettanomyces* strains) may be responsible for the synthesis of ethyl lactate and more ethyl acetate, until equilibrium concentrations are formed as shown in the second part of this paper. This same esterase may hydrolyze the *Saccharomyces* iso-amyl acetate to low equilibrium concentrations. *Brettanomyces* is also capable of producing more acetic acid and may also be responsible for the appearance of iso-butyric acid. During the acidification phase lactic acid bacteria may induce the formation of slime (Van Oevelen and Verachtert, 1979).

d. The ripening phase

This is mainly characterized by a further attenuation and the disappearance of high molecular weight dextrins as shown in figure 3. DMS and diacetyl - Fig. 3 - decrease to respectively around 100 ppb and 80 ppb.

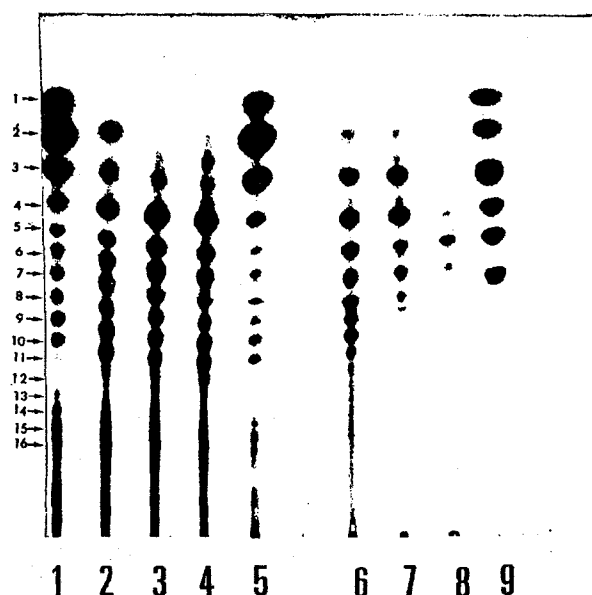


Figure 3
Carbohydrate profile of fermenting lambic
Lane 1: lambic wort diluted 4x / Lane 2: lambic after 7 weeks of fermentation / Lane 3: lager A / Lane 4: lager B / Lane 5: wort of lager A diluted 4x / Lane 6: lambic attenuated to 3°P / Lane 7: lambic attenuated to 1.5°P / Lane 8: lambic attenuated to 1°P / Lane 9: reference of saccharides C₁-C₅ and C₇

e. The refermentation phase

Traditionally bottle refermentation requires the mixing of younger lambic with older types, mainly to provide dextrins, from which, after around 6 months, 6-7 g of CO₂ per liter is formed. Table 4 (at the end of the article) shows some interesting data on the carbohydrate profile of young and old lambic and the mixture of both after bottle fermentation. It confirms the picture given in figure 3.

5. THE ACID BEERS OF ROESELARE (RODENBACH)

These beers are produced by an alcoholic fermentation due to a mixture of *Saccharomyces cerevisiae* strains and a lactic acid fermentation due to *Lactobacillus* and *Pediococcus* strains. Wort is inoculated with yeast "contaminated" with lactic acid bacteria, as a result of long term re-utilization. Several steps can be described: (1) a primary fermentation of about 7 days at 21°C with a strong alcoholic fermentation. (2) a secondary fermentation of about 5 weeks at 15°C with an increase of lactic acid to 500-600 ppm and a development of *Pediococcus* at the expense of *Lactobacillus*, present in low numbers in the first phase. (3) a tertiary fermentation in very large casks with a development of *Pediococcus* in the first 7 months and the appearance of *Brettanomyces* yeast at 7-12 months. In the second year the microflora consists essentially of *Pediococcus parvulus* and *Brettanomyces lambicus*. Very high attenuations are achieved as in lambic brewing. These beers are sold as Rodenbach (a mixture of young and old beer) and Rodenbach Grand Cru (mainly old beer) (Martens, H., not published).

6. CONCLUSION

Mixed cultures have disadvantages when undesired species grow at the expense of useful species: the infection of classical beers being a good example, although in some ales low amounts of lactic acid add flavour. Acid beers

are a good example of a mixed culture fermentation resulting in very special beers and flavours. In this field more research is needed: first of all concerning the factors that affect the yeast-yeast-bacterial interactions, secondly concerning the special and maybe new flavours which can broaden our rich arsenal of beer types.

TABLE 1

Yeasts in a lambic brewery air

YEASTS*	OUTSIDE	INSIDE
Schizosaccharomyces	0	2
Kloeckera**	0	3
Sacharomyces	8	18
Saccharomyces	1	2
Hansenula	0	1
Torulopsis	6	38
Candida	1	7
Brettanomyces**	1	1
	17	72

*

Identity of 89 isolates

**

involved in lambic fermentation

TABLE 2

Microbial species detected in a lambic brewery air

MEDIUM*	INCUBATION CONDITIONS AND TIME IN DAYS**		CFU/ML	SPECIES
Lambic wort	SA	1	1.7x10 ⁴	Enterobacteria
	SA	9	8.4x10 ⁵	Saccharomyces
			2.4x10 ³	Brettanomyces
Id. + Streptomycine (exp. 1)	SA	6	1.0x10 ⁸	Kloeckera
		10	6.6x10 ⁸	Kloeckera
			2.8x10 ⁵	Saccharomyces
Id.+Streptomycine (exp. 2)	SA	6	4.0x10 ⁷	Saccharomyces
		9	3.0x10 ⁷	Saccharomyces
		23	1.4x10 ⁶	Brettanomyces
Id.+Streptomycine ___+Actidione	SA	44	9.7x10 ⁴	Brettanomyces
Id.+Pimaricine	SA	1	5.6x10 ²	Enterobacteria
		9	1.8x10 ⁸	Enterobacteria
Id.+Pimaricine	A	9	4.0x10 ⁷	Acetic ac. bact.
UBM	A	2	6.0x10 ²	Acetic ac. bact.
		9	5.0x10 ⁷	Acetic ac. bact.
UBM	AN	23	2.8x10 ⁸	Pediococci

* Lambic wort + selective antibiotics. UBM : Universal Beer Medium

** SA = semi-anaerobic; A = Aerobic; AN = anaerobic

TABLE 3

Actidione resistant yeasts in lambic wort fermentation

Brettanomyces sp.	Months of fermentation time and number of isolates						Percentage of total
	0-1	1-4	5-8	9-12	13-18	19-24	
abstinens	0	0	0	1	0	0	0.6
anomalus	3	6	8	2	2	6	15.8
bruxellensis	0	0	12	9	3	0	14
custersianus	1	0	2	1	1	3	4.7
custersii	0	0	6	15	14	14	28.6
intermedius	0	0	8	11	4	3	15
lambicus	0	0	8	19	3	5	20.4
naardenensis	0	0	0	0	0	2	1.2
nananaus	- in - bulk - -sample - - - -						
number of	2	1	6	7	6		
Bret. species							
Kloeckera	26	4	0	0	0	0	

TABLE 4

Distribution of Carbohydrates in bottle refermented lambic

Wort type*	Total Carbohydrates	Carbohydrate Fractions**			
		Above 20,000	1000-20,000	Below 1000	Reducing sugars
1-year old	38	30.8(81)	1.52(4)	5.70(15)	3.75(10)
2-year old	6.3	4.6(73)	0	1.70(27)	1.46(23)
mixture 1+2 year old	8.9	0.26(3)	4.5(50)	4.18(47)	2.60(29)

*1-year old, 2-year old and a mixture of both after 75 days of bottle refermentation

**all data in g per liter estimated by the anthrone method and ultrafiltration separations.

Initial contents were 40 g per liter for 1-year type, 7 g per liter for 2-year type and 25 g per liter for the mixture. Numbers in parentheses are percentages of total carbohydrate.

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