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APPLICATION OF KVEIK YEASTS FOR BEER PRODUCTION®

Zastosowanie drożdży kveik do produkcji piwa®

Key words: beer, kveik, yeast.

The term kveik yeast is used to describe cultures traditionally used in Norwegian farmhouse brewing. Historically, almost every farm in Norway had its own distinct culture, of which only 24 representatives have survived to this day. From the history of their use, we can find out that these yeasts were used to ferment wort in just 2 days, using temperatures between 30-40°C. Studies of these cultures have shown that they include strains of Saccharomyces cerevisiae. Current literature indicates that these yeasts are characterized by a vigorous fermentation rate that can take place at temperatures as high as 42°C without adversely affecting the sensory characteristics of the beer. Most kveik cultures ferment the sugars present in the wort to a similar degree as commercially available cultures, ensuring adequate attenuation. Additionally, they do not produce phenolic off-flavors, exhibit a high level of flocculation, and impart a characteristic fruity aroma to the beer. Thanks to these properties, brewers are more and more willing to use these yeasts in their products. Although the knowledge of these strains is still incomplete, considerable progress has been made in this area in recent years. The article presents the current state of knowledge on the history of kveik yeast, their phylogenetic features and fermentation properties.

INTRODUCTION

Recent decades have brought great diversity to the field of brewing. The market, hitherto homogenized by large breweries, has broken up, resulting in the emergence of many new beer styles, as well as the evolution of a number of existing ones. Commercial breweries and homebrewers look for new ways to create a product with unique sensory characteristics. Since yeasts are responsible for the entire fermentation process, using a different strain seems like a natural way to produce a different product. In recent years, the yeasts traditionally used to produce beer on Norwegian farms has become increasingly popular. They are known in Norway as *gjær, gjest, barm* and *kveik*, and it is this last name Słowa kluczowe: piwo, kveik, drożdże.

Terminem drożdży kveik określa się kultury tradycyjnie używane w norweskim piwowarstwie domowym. Historycznie, prawie każde gospodarstwo rolne w Norwegii posiadało swoją odmienną kulturę, z których na dzień dzisiejszy zachowanych zostało jedynie 24 przedstawicieli. Zagłębiając się w historię ich użycia, możemy dowiedzieć się, że drożdże te wykorzystywane były do odfermentowania brzeczki w zaledwie 2 dni, przy zastosowaniu temperatur z zakresu 30-40°C. Badania tych kultur wykazały, że w ich skład wchodzą szczepy Saccharomyces cerevisiae. Aktualnie dostępna literatura wskazuje, że drożdże te charakteryzują się szybkim tempem przeprowadzania fermentacji, która może odbywać się w temperaturach nawet do 42°C, bez negatywnego wpływu na cechy sensoryczne piwa. Większość kultur kveik fermentuje cukry obecne w brzeczce w podobnym stopniu jak kultury dostępne komercyjnie, zapewniając odpowiedni poziom odfermentowania. Nie wytwarzają one fenolowych aromatów, wykazują wysoki poziom flokulacji oraz nadają piwu charakterystyczny, owocowy aromat. Dzięki tym właściwościom, piwowarzy coraz chętniej sięgają po te drożdże. Mimo, że wiedza na temat tych szczepów jest wciąż niekompletna, w ostatnich latach poczyniono w tym obszarze znaczne postępy. Artykuł przedstawia aktualny stan wiedzy na temat historii drożdży kveik, ich cech filogenetycznych oraz właściwości fermentacyjnych.

that has gained international recognizability. The history of their use goes back hundreds of years, and it is believed that until the late 1800s almost every farm had its own, often distinct yeast culture, which was used to make beer. Beer was an important part of Norwegians' lives at that time, but because of cultural and economic changes, traditional ways of production began to be abandoned, and kveik yeast fell victim to that process. By the beginning of the 21st century, only a fraction of the existing cultures have survived, while most are extinct. However, the beginning of the millennium also brought a kind of renaissance of their use. Brewers are increasingly choosing to use them because of their wide range of beneficial properties. These yeasts are characterized by

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a much faster start and rate of fermentation than commercially used cultures and are adapted to higher temperatures of the environment (traditionally these yeasts were used at temperatures in the ranges of 30-40°C). They show high flocculation, resistance to elevated alcohol concentrations and low 4-vinylguaiacol production. Beverages produced using those yeasts do not exhibit a phenolic smell and have characteristic fruit aroma. Culture analyses have shown that they consist of different strains of the Saccharomyces cerevisiae yeast. Initially, some critics believed that those were ordinary baker's yeast, but available publications contradict this. Genetic studies have shown that these cultures are clearly different from all commercially available S. cerevisiae strains [4, 13, 16]. Describing the subject of kveik yeast, it is impossible not to highlight the work of Lars Marius Garshol, who is largely responsible for the interest in this subject among the international brewing community, and has done a tremendous amount of work in, among other things, collecting cultures and researching their history.

Although knowledge of these strains is still incomplete, considerable progress has been made in recent years. This article presents the current state of knowledge on the history of kveik yeast, their phylogenetic features and fermentation properties.

HISTORY

By the mid-19th century, farms throughout northern Europe had their own yeast cultures that were used to produce beer. As mentioned earlier, most of these cultures have been lost. Nowadays, 49 distinct yeast cultures have been found preserved on northern European farms. Out of the cultures found, 2 are from Russia, 2 from Latvia, 2 from eastern Norway, many cultures are from Lithuania, but the vast majority have been found in western Norway [8]. The term kveik refers to a yeast originating from Norway. Although the term 'farmhouse ale' is mainly associated with Belgian Saison, and French Bière de Garde, northern Europe has its own unique beverages, such as Finnish sahti, Estonian kodõlu, Lithuanian kaimiškas and Swedish gotlandsdricka [16, 24]. In Norway, 'farmhouse ale' is referred to as maltøl, of which there are 3 main styles: stjørdalsøl, kornøl and vossaøl, of which kveik yeasts are currently only used in the latter two. These styles vary in alcohol content as well as color (from amber to dark) but have some common characteristics. Hops are used in small amounts. For mashing, instead of pure water, an infusion of juniper (whole twigs, needles, or berries) is used, which together with the hops provides an appropriate level of bitterness. For boiling, copper or iron kettles heated on hearth are used. These beers are not filtered or carbonated. Historically, the spices used in Norwegian brewing have been mainly juniper and hops, but also spices such as myrica gale, wormwood, caraway, St. John's wort, yarrow, and tansy, among others. In addition, historically maltal has typically been a strong, high extract beer (up to 19.25°P), and has been brewed with self-produced malts [3, 7, 20, 22].

Since there are significant historical similarities between the yeasts from the above regions, their history will be discussed along with kveik yeast. A feature that has amazed modern brewers is the usage of remarkably high yeast inoculation temperatures. Most historical sources do not give an exact range, but a description of how to recognize it. Among such descriptors the term of human skin temperature is often mentioned, which would correspond to 33.5-36.9°C. Another common term is the temperature of freshly milked milk. This would correspond to a temperature of 35-38°C. Descriptors indicating lower temperatures (but still relatively high) are also found among reports from other regions, but those suggesting temperatures in the 35-40°C range predominate among brewers in Norway. Such high temperatures are probably related to difficulties in cooling the wort, due to lack of appropriate technology. With a typical brew size of 100-150L, it took a long time to cool the wort to 40°C. Further cooling to temperature at which top-fermenting strains are pitched today (~20°C) would take too long. During this time, unfavorable microflora could dominate the wort, not giving the yeast a chance to conduct a proper fermentation [9].

High temperatures were also used during fermentation. Most brewers fermented at temperatures above 25°C, and the most commonly mentioned temperature is, as with yeast inoculation, milk temperature. These are reports suggesting that fermentations were conducted at temperatures even higher than that at which the yeasts were inoculated. This information indicates that the brewing community has been adapting farmhouse yeasts for fermentation at high temperatures for centuries [8, 9, 17].

A distinctive feature of farmhouse ales is the short fermentation time, measured from yeast inoculation to racking beer for storage. Based on historical data, mainly from the 20th century, Garshol [9] estimated that the average fermentation time was 41 hours. After this time, the beer was almost completely attenuated. This short fermentation time, along with the high yeast pitching temperature may confirm high temperature fermentations. The wort did not have much opportunity to cool down due to exoenergetic nature of fermentation process, and given the fermentation characteristics of the kveik yeast, there was simply no need to lower the temperature. Brewers usually allowed for high wort attenuation, which combined with the short process time indicates that this yeast has a fast fermentation rate. The fast pace of a process is definitely helped by the high temperature of yeast inoculation, and the fact that kveik yeast exhibit an unusually short lag phase. However, it is worth mentioning that these strains ferment faster that those commercially available even at lower temperatures [10].

The domestication of kveik yeast and its development of the characteristics discussed in this article was likely due to the tradition of collecting yeast after fermentation and using it in subsequent fermentations. Collecting yeast after fermentation is a surprisingly old concept. Although we often downplay the knowledge of medieval artisans, evidence can be found in historical sources that people of the time were certainly aware of the existence of yeast, but most likely without knowing what it was. An example is the Pliny the Elder's description written in year 77 of how the Iberians and Gauls collected foam from fermenting beer, which was subsequently used in baking. It supposedly allowed them to obtain a better bread than other peoples [8, 10].

Due to the limited sources available, it is hard to exactly determine when the Norwegians domesticated yeasts. What we do know is that they had many ways of storing them. Due

to its unusual nature, a frequently mentioned tool is a yeast ring consisting of many interlocking wooden staves forming a circle. Another way was to use a piece of perforated log, called a kveikstokk. These items had a high surface area, which allowed them to collect a large amount of yeast when dipped into the beer. However, research indicates that these tools were also often used as a decoration, unrelated to brewing, so it is hard to say if their original use was to collect yeast, or if they were simply adopted for this purpose by some farmers. Other methods were less sophisticated, such as storing yeasts on a piece of cloth, brick, juniper twigs, or even on blades of grass. The yeasts collected in this way were often coated in flour, dried, and the process repeated several times. Then they were hung to dry, or sometimes hot ash was used to speed up the drying process. Almost everyone in Norway dried their yeast, but liquid yeasts were preferred for brewing. Yeast collected in this way could be stored for over a year and could be used for both brewing and baking. The preference for selecting top or bottom fermenting strains appears to be unrelated to geographic region. One farmer might use bottom-fermenting yeast, while his neighbor might choose top-fermenting ones. Farmers usually shared their yeast freely in case, when for example, a neighbor's culture spoiled, or the beers made with it began to exhibit undesirable sensory characteristics. In many places there was a saying, "You can't refuse anyone's yeast." These reports allow us to presume, that Norwegian farmers over decades took steps to enhance desirable traits in theirs yeast cultures [8, 10, 11, 21, 23].

KVEIK STRAINS

Genome studies of the cultures found in kveik yeast have shown that they belong to a distinct subfamily than other farmhouse yeasts and commercially available strains. Currently, 24 cultures identified as kveik have been found. Cultures such as Voss, Hornidal, Ebbegarden, Stranda, Årset, Midtbust and Oslo are commercially available. They inherit names from the localities in which they were found, or from the names of the owners [8, 18].

It is important to realize that traditionally available kveik cultures are usually not one particular strain, but a mixture of different yeast strains that may additionally contain bacteria. Those commercially available, consist of pure selected strain. Preiss et al. [20] studied 9 traditional kveik cultures, where the cultures contained between 1 and 9 distinct strains of Saccharomyces cerevisiae yeast. In the study by Aasen [1], all of 10 traditional cultures tested contained genetic sequences of bacteria belonging to the species Lactobacillus brevis, Lb. backii, Lactococcus lactis and Aerobacter spp. Bacterial cultures were successfully isolated from 2 of the 4 kveik yeast strains subjected to this study. Again, all the yeast strains isolated in this study belonged to the S. cerevisiae species. Garshol [8] in his book mentions that among the cultures he analyzed, only one of them contained a small proportion of Pichia spp. yeast. These studies indicate that the currently preserved kveik consist exclusively of distinct strains of S. cerevisiae, without the participation of so-called wild yeasts, which may seem unusual at the first glance [1, 8, 20]. However, it is mainly S. cerevisiae cultures that have been used for centuries to ferment beer and make bread. Available literature indicates that this yeast possesses characteristics

that allow it to dominate the fermentation environment. In addition, it is now suspected that this yeast has likely developed several antagonistic mechanisms against other fungal species. An example is the study by Gobbi et al. [12], where the antagonistic properties of the S. cerevisiae strain against Lachancea thermotolerans used in mixed wine fermentation became apparent at temperatures above 30°C. Such high temperatures may bring to mind historically high maltøl fermentation temperatures. However, it should be noted that the decrease in L. thermotolerans biomass did not occur until after day 4 of fermentation, which is much longer than a typical *maltøl* fermentation. The ability to dominate the environment is well documented in wine fermentations, where non-Saccharomyces yeast, present in significant amounts in the grape must, develops only during the early stages of fermentation, then gets displaced by S. cerevisiae which completes the fermentation. Literature sources indicate that even in spontaneous fermentations, S. cerevisiae may account for up to 50% of the sugar consumption in the must. Thus, the presence of sole S. cerevisiae strains in kveik cultures seems to be a natural progression caused by repeated use of the same slurries [2, 6, 8, 12].

Genetic and phenotypic studies conducted by Gallone et al. [6] divided S. cerevisiae yeast into 5 clades. Most brewing yeasts used today belong to two clades, named "Beer 1" and "Beer 2". The Beer 1 clade consists of 3 subpopulations, reflecting geographically distinct German/Belgian, British and US yeast groups. These show the most pronounced domestication characteristics of all the yeasts assessed in this study. Those in the Beer 2 clade are closer to wine yeast, and no clear geographical division is apparent is this group. In a study conducted by Preiss et al. [20], the 9 strains selected by the authors from kveik cultures formed a new, distinct group within the Beer 1 clade. In the study, the authors note that phenotypes and genetic markers of domestication largely overlap with those found in commercially available ale strains. Interestingly, the authors also evaluated one strain of Lithuanian traditional yeast, which did not fit into this group. This indicates that kveik yeasts form a completely different group than the currently domesticated S. cerevisiae yeasts [6, 20].

It should be noted that not all cultures originating from Norway belong to kveik yeast. An example is the Muri yeast, previously considered to be representative of kveik group. This culture was used for brewing for the last time in 1991. Bjarne Muri tried to revive it when kveik yeast began to become popular. Krogerus et al. [15] isolated a strain from this culture that turned out to be a *S. cerevisiae* x *S. uvarum* hybrid. Interestingly, although beers brewed with kveik should be characterized by a lack of phenolic aromas, this aroma was very prominent when using this strain. The study showed that this strain did not fit into the kveik subgroup of Beer 1 clade. Garshol [8] explains, that the examined Muri strain turned out to be actually a commercially available White Labs WLP351 hefeweizen yeast culture, that was probably mistakenly multiplied by the owner, and taken for a kveik culture [8, 15].

APPLICATION OF KVEIK YEAST IN BEER PRODUCTION

The first striking feature of kveik cultures is their very rapid rate of fermentation initiation. In a study conducted by Preiss et al. [20], 24 hours after the pitching all of the strains isolated from the kveik cultures produced more CO_2 than the control strain WLP002. They were inoculated into 12.5°Plato wort at 30°C. The most efficiently fermenting strain produced 70.6% more CO_2 than the control sample. In a study conducted by Foster et al. [5], after 12 hours of fermentation at 30–40°C, 5/6 *S. cerevisiae* strains isolated from kveik cultures attenuated the wort (12.5°Plato extract) in 30%, while the control strains (Cali Ale, Vermont Ale, Kölsch and St. Lucifer) were still in a lag phase [15, 20].

All of the available literature seems also to confirm their extremely fast fermentation rate, and preference for higher temperatures of the process. Foster et al. [5] studied the fermentation rate of kveik yeasts at different temperature ranges, considering a final extract <2.56°Plato as the end of fermentation, fermenting a wort mentioned above. In the 22-40°C range, most cultures reached the desired extract level within 5 days. At temperatures of 30–40°C this time was only 3 days for most cultures. The authors showed that some strains have a wide range of tolerable temperatures, like Hornindal1 (15–42°C), while others like Laerdal2 prefer a narrower range $(30-37^{\circ}C)$. Overall, the authors suggests that the $30-37^{\circ}C$ range can be adopted as the preferred range for the kveik strains they studied. In a study by Kits and Garshol [14], the authors evaluated the fermentation rates of 3 commercially available kveik strains (Escarpment Laerdal kveik, LalBrew Voss[™] and Omega LutraTM), in the temperature range of 20–42°C against a control sample with US-05 yeast, fermenting a wort with an extract of 12.1-12.5°Plato. The temperature range of 20-33.5°C had no significant impact on the fermentation rate of the control strain, but the temperature of 37°C caused a complete inhibition of its growth. The situation was different with the tested kveik strains. All of them showed growth at temperatures of 20-42°C. Generally, higher temperatures resulted in faster attenuation, but the fastest fermentation took place at the optimal temperatures determined by the authors. These are respectively, for Escarpment Laerdal kveik: 28°C, LalBrew Voss[™]: 37°C and Omega Lutra[™]: 33.5°C. These temperatures are similar to the range presented by Foster et al. [5]. At these temperatures, complete attenuation occurred after less than 48 hours, which is consistent with fermentation times in traditional Norwegian brewing. Even fermentations conducted at less temperatures favorable for kveik yeast, were significantly shorter than those carried out with US-05. Different results are presented in the paper by Aasen [1], in which kveik cultures Ørjasæter, Gamlegrua and Gausemel (which were discovered relatively recently) were studied. In the study, wort with an extract of 19.3° Plato was fermented at temperatures ranging from 22-37°C, against a control sample with US-05. Most beers finished fermentation within 6–10 days, and significant differences between fermentation rates were shown by ANOVA test but were not apparent with Tukey's test. The cited studies indicate that fermentation time is not uniform among all kveik cultures. Among them, there are cultures that ferment very efficiently, as well as those that ferment similarly to commercially available yeast [1, 5, 14].

What appears to be a universal characteristic of kveik yeast is their significant resistance to the high temperatures of the fermentation environment. Among yeast cells, this ability is determined by a number of factors, including increased synthesis of trehalose and glycerol. In a study conducted by Foster et al. [5], kveik yeast produced 1.5-2x more of this disaccharide at 30°C and began its production much earlier than control strains. Additionally, they maintained high levels of trehalose longer than control strains. The authors suggests that this may be due to dysfunction of the trehalase enzyme. The evaluated kveik strains were also characterized by faster glycerol production during the initial 24 hours of fermentation, especially at 35-40°C. In fermentations of 15°Plato wort at 25°C, conducted by Kawa-Rygielska et al. [13] beers obtained with kveik yeast had noticeable higher glycerol content, compared to the control sample fermented with US-05 yeast (1.51 g/L and 1.12 g/L, respectively). These results suggest that these two mechanisms may be an important part of their ability to ferment at high temperatures. Additionally, in the study by Preiss et al. [20], more than half of the cultures tested showed growth in an environment with 16% of alcohol [5, 13, 17].

All available literature sources indicate that most kveik yeasts produce alcohol levels similar to those produced by commercially used S. cerevisiae, with apparent attenuation rates in the 60–90% range. Only in the study by Aasen [1] the alcohol levels produced by the kveik cultures were lower than those of the control sample fermented with US-05 at 22°C and 30°C. At 37°C it was the kveik cultures that produced more alcohol. Maltose and maltotriose are the sugars present in the wort in the highest amounts ($\sim 60\%$ and $\sim 20\%$ of all sugars available in the wort, respectively), hence their efficient attenuation is crucial if only from economic aspects. According to studies conducted by Foster et al. [5] and Preiss et al. [20], most cultures efficiently utilize maltose over a wide range of temperatures. Some strains like Ebbegarden utilize it to a lesser extent at the extremes of their temperature ranges (15°C and 42°C). In a study by Foster et al. [5] all evaluated strains utilized maltotriose, but to a lesser extent than the control strains, leaving 20-25% of the original content of this sugar. An important factor determining the utilization of this sugar was the fermentation temperature, where temperatures closer to the optimum provided higher utilization. Different results were obtained by Kawa-Rygielska et al. [13], where authors found no differences in residual maltose and maltotriose content between the kveik and US-05 strains used. Commercially available beers contain 0.8-17 g/L residual maltotriose, hence the utilization rate of this sugar by kveik cultures should not be a problem for their use in the fermentation industry. These results indicate that maltotriose consumption is a variable trait among kveik strains. Another important factor for the usage of yeast in brewing industry is the flocculation capacity. Exhibiting high levels of flocculation is considered as one of the characteristic traits of yeast domestication. In a study by Preiss et al. [20] half of the strains showed high levels of flocculation (more than 80%), and 4 of the 24 strains showed low levels (<20%). Interestingly, in most kveik cultures containing more than one S. cerevisiae strain, at least one of the strains showed high flocculation, suggesting that coflocculation may occur between these strains [1, 13, 19, 20, 25].

As mentioned earlier, beers brewed with kveik yeast are characterized by a fruity, tropical aroma. In a study by Kawa-Rygielska et al. [13] panelists preferred beers brewed with kveik due to those aromas. The authors indicate that these yeasts produce higher amounts of esters than the control strain US-05, especially ethyl capronate (tropical, pineapple), ethyl caprylate (tropical, apple), ethyl decanoate (apple) and isoamyl acetate (banana), which were present above their sensory threshold. In the case of the study conducted by Preiss et al. [20], only the first 3 mentioned compounds were present in higher amounts compared to the control sample, but the authors found no significant differences in their concentrations. Similar results were obtained by Kits and Garshol [14]. This suggests that these esters may be partly responsible for the fruity character of beers produced with this yeast. In a study by Aasen [1], all kveik cultures produced higher amounts of esters than a control sample with S. cerevisiae US-05. Studies by all the authors mentioned above agree that kveik cultures produce overall low amounts of higher alcohols. Depending on the kveik strain used, and the fermentation conditions, the levels of esters and fusel alcohols are lower or higher than those produced by commercial strains. It however should be noted, that even at high fermentation temperatures these cultures do not produce excessive amounts of esters and higher alcohols, which in high concentrations are responsible for beer sensory defects. For most commercially available yeasts, the temperatures used in in fermentations with kveik yeast are too high, which manifests itself, among other things, in exaggerated production of the aforementioned volatile compounds. This necessitates maintaining lower fermentation temperatures and cooling the wort. Such actions seem not to be necessary with kveik cultures [1, 13, 14].

Beers obtained using kveik cultures do not have phenolic aftertastes, making these cultures POF (–). As for now, β -glucosidase activity, terpenoid biotransformation ability, and sensitivity to hop-derived compounds have not been evaluated among kveik cultures [20].

CONCLUSION

The currently available literature confirms historical reports on the fermentation properties of kveik yeasts. These strains are characterized by a vigorous rate of fermentation, which can take place over a very wide range of temperatures, without negatively affecting the sensory characteristics of the beer. However, it seems that the most suitable range for most kveik strains is 30-37°C. Beverages obtained using those yeasts are characterized by a tropical, fruity aroma. These strains ferment the sugars present in the wort to a similar degree as commercially available cultures, ensuring an adequate level of attenuation. The fact that the kveik yeast are genetically affiliated to the group of S. cerevisiae strains domesticated by man, produce no phenolic off-flavors, express appropriate fermentation abilities and most of them have high level of flocculation confirms that over the centuries brewers in Norway have managed to obtain cultures with completely different properties than those previously known. Due to their properties, these cultures are rapidly gaining popularity among the homebrewing community as well as beginning to appear in commercially available products.

PODSUMOWANIE

Aktualnie dostępna literatura potwierdza historyczne doniesienia dotyczące właściwości fermentacyjnych drożdży kveik. Szczepy te charakteryzują się szybkim tempem przeprowadzania fermentacji, która może odbywać się w bardzo szerokim zakresie temperatur, bez negatywnego wpływu na cechy sensoryczne piwa. Wydaje się, że najbardziej odpowiednim zakresem temperatur fermentacji dla większości szczepów z tej grupy jest zakres 30-37°C. Napoje otrzymane z ich użyciem charakteryzują się tropikalnym, owocowym aromatem. Szczepy te fermentują cukry obecne w brzeczce w podobnym stopniu jak kultury dostępne komercyjnie, zapewniając odpowiedni poziom odfermentowania. Brak produkcji fenolowych aromatów, wysoki poziom flokulacji większości szczepów, odpowiednie zdolności fermentacyjne oraz przynależność genetyczna do grupy szczepów S. cerevisiae potwierdzają, że na przestrzeni stuleci piwowarom w Norwegii udało się uzyskać kultury o zupełnie innych właściwościach, niż te dotychczas znane. Dzięki swoim właściwościom, kultury te zdobywają szybką popularność wśród społeczności piwowarów domowych, jak i zaczynają pojawiać się w produktach dostępnych komercyjnie.

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