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MINI-REVIEW

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Yeast flocculation: what brewers should know

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Abstract For many industrial applications in which the yeast *Saccharomyces cerevisiae* is used, e.g. beer, wine and alcohol production, appropriate flocculation behaviour is certainly one of the most important characteristics of a good production strain. Yeast flocculation is a very complex process that depends on the expression of specific flocculation genes such as *FLO1*, *FLO5*, *FLO8* and *FLO11*. The transcriptional activity of the flocculation genes is influenced by the nutritional status of the yeast cells as well as other stress factors. Flocculation is also controlled by factors that affect cell wall composition or morphology. This implies that, during industrial fermentation processes, flocculation is affected by numerous parameters such as nutrient conditions, dissolved oxygen, pH, fermentation temperature, and yeast handling and storage conditions. Theoretically, rational use of these parameters offers the possibility of gaining control over the flocculation process. However, flocculation is a very strain-specific phenomenon, making it difficult to predict specific responses. In addition, certain genes involved in flocculation are extremely variable, causing frequent changes in the flocculation profile of some strains. Therefore, both a profound knowledge of flocculation theory as well as close monitoring and characterisation of the production strain are essential in order to gain maximal control over flocculation. In this review, the various parameters that influence flocculation in real-scale brewing are critically discussed. However, many of the conclusions will also be useful in various other industrial processes where control over yeast flocculation is desirable.

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Introduction

Yeast flocculation is a reversible, asexual and calcium-dependent process in which cells adhere to form flocs consisting of thousands of cells (Bony et al. 1997; Stratford 1989). Upon formation, these flocs rapidly separate from the bulk medium by sedimentation (lager yeasts), or by rising to the surface (ale yeasts). The ability of yeast cells to flocculate is of considerable importance for the brewing industry, as it provides an effective, environment-friendly, simple and cost-free way to separate yeast cells from green beer at the end of fermentation. Therefore, strong and complete flocculation is a desirable property for any brewer's yeast. However, the yeast cells should not flocculate before the wort is completely attenuated, as such premature flocculation causes sluggish, so-called "hanging", fermentations and may also lead to severe off-flavours (De Clerck 1984; Stratford 1992). The ideal brewer's yeast should therefore exhibit strong flocculation toward the end of fermentation. This flocculation behaviour should also be constant during consecutive rounds of fermenting, cropping, storing and repitching. In many cases however, the yeast strains used in industrial fermentations fail to live up to these expectations. In order to understand why flocculation is such a complex, hard to control, phenomenon, it is necessary to take a closer look at the underlying biochemical, genetic and physical mechanisms.

Flocculins

Flocculation of yeast cells involves lectin-like proteins – so-called flocculins – that stick out of the cell walls of flocculent cells and selectively bind mannose residues present in the cell walls of adjacent yeast cells. Calcium ions in the medium are needed in order to activate the flocculins (Bidard et al. 1995; Bony et al. 1997, 1998; Costa and Moradas-Ferreira 2001; Javadekar et al. 2000; Kobayashi et al. 1998; Miki et al. 1982; Patelakis et al. 1998; Stratford 1989, 1992; Teunissen et al. 1993a,

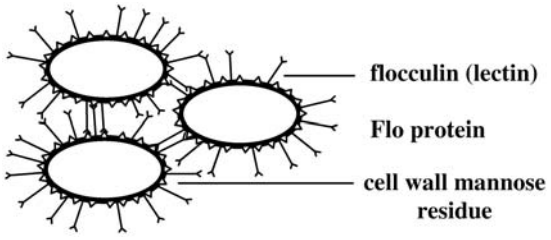

Fig. 1 The lectin model for flocculation. Lectin-like proteins (so-called "flocculins") stick out of the cell wall of flocculent cells and selectively bind to cell-wall mannose residues of adjacent cells. Calcium ions are needed in order to activate the flocculins

1993b, 1995; Van der Aar et al. 1993) (Fig. 1). Since the mannose residues are always present in the cell walls of both nonflocculent and flocculent cells, the critical factor for flocculation is clearly the presence or absence of the flocculins. Flocculation is inhibited by mannose in the growth medium, presumably because free mannose occupies the flocculin binding sites so that they can no longer bind the mannose residues of other cells. For some yeast strains, flocculation is inhibited not only by mannose, but also by glucose, sucrose and maltose. This latter flocculation phenotype, designated NewFlo, is often found in brewer's yeasts, while flocculation of most laboratory strains is inhibited only by mannose (Flo1 phenotype) (Sieiro et al. 1995; Stratford and Assinder 1991). In addition to the well known Flo1 and NewFlo flocculation phenotypes, other flocculation types have been described, suggesting that some yeast strains may flocculate through mechanisms different from the lectin model. For example, in some yeast strains, flocculation seems to be insensitive to mannose addition (Bossier et al. 1997; Masy et al. 1992; Nishihara et al. 2002). In addition, the work of Straver et al. suggests that, in some cases, flocculation is not only solely dependent on the presence of flocculins, but also requires agglutinins and/or fimbriae-like structures (Straver and Kijne 1996; Straver et al. 1993, 1994a, 1994b; Van der Aar et al. 1993). Interestingly, the co-flocculation of flocculent and non-flocculent yeasts (Nishihara et al. 2000) and even co-flocculation of bacteria and *S. cerevisiae* is established through lectin-like bonds similar to those of "pure" yeast-yeast flocculation (Lievens et al. 1994; Peng et al. 2001a, 2001b; Van den Bremt et al. 1997a, 1997b).

Genetic regulation

Like any other protein, flocculins are encoded by specific genes, the so-called *FLO* genes. The best-known flocculation gene is *FLO1*, a dominant gene situated at the right arm of chromosome 1. The 4.6 kb open reading frame of *FLO1*, which includes a large number of repetitive sequences in its central part, encodes a large (1,537 aa) Ser/Thr-rich protein (Flo1p) (Teunissen et al. 1993a, 1993b; Watari et al. 1989, 1994b). Other important *FLO* genes are *FLO2* and *FLO4*, which are in fact alleles (copies) of *FLO1*, and the genes *FLO5* and *FLO9*, which are highly homologous to *FLO1* (Russel et al. 1980; Sieiro et al. 1997). Expression of *FLO1* and its homologues causes flocculation of the Flo1 phenotype. Interestingly, lager yeasts also contain a copy of the so-called *Lg-FLO1*, which is not found in ale yeasts. It is believed that *Lg-FLO1* encodes a flocculin that binds both mannose and glucose, and is therefore responsible for the NewFlo phenotype of most brewer's yeasts (Kobayashi et al. 1995, 1998; Sato et al. 2002). *FLO8* encodes a transcriptional activator of *FLO1* and *FLO9*. In addition, Flo8p also activates *FLO11/MUC1*, a gene involved in filamentous growth, and *STA1*, encoding extracellular glucoamylase. Interestingly, the *FLO11* gene is subjected to multiple genetic regulation cascades, including the cAMP/PKA and MAP kinase pathways, suggesting a highly specific physiological role for Flo11p (Gagiano et al. 1999a, 1999b; Kobayashi et al. 1996, 1999; Pan and Heitman 1999; Robertson and Fink 1998; Rupp et al. 1999; Tamaki et al. 2000; Yamashita and Fukui 1983).

When these *FLO* genes become active, flocculins are formed and flocculation can take place (Stratford 1992). Thus, any factor that causes the cells to activate their *FLO* genes may in fact trigger flocculation (Bidard et al. 1995; Bony et al. 1998; Stratford 1992; Teunissen et al. 1995). Unfortunately, the situation is more complex than this. Firstly, the *FLO* family consists of several different *FLO* genes, each of which may be regulated through different complex mechanisms and therefore may be induced (or repressed) by different factors (Teunissen et al. 1993a, 1995). Secondly, the *FLO* gene family is very unstable, causing great differences in the flocculation profile and response between different yeast strains and even between different generations of a specific yeast strain (Reboredo et al. 1996; Sato et al. 2001, 2002; Watari et al. 1999). Thirdly, flocculation is not only a biochemical process, but also implies physical interaction: cells need to collide in order to bind to each other. Therefore, factors that influence these cell-cell interactions also play an important role, even if they do not influence the activity of the *FLO* genes. More specifically, factors that raise the collision frequency between cells, e.g. agitation of the growth medium, may promote flocculation. Factors that increase the hydrophobic character of the yeast cell walls (cell-surface hydrophobicity) or factors that decrease the repulsive negative electrostatic charges in cell walls (cell-surface charge) are also known to cause stronger flocculation, presumably because they facilitate cell-cell contact (Stratford 1992; Straver et al. 1993).

Factors influencing flocculation can therefore be divided into three groups: the genetic background of the strain, environmental factors that influence *FLO* gene expression and Flo protein activation, and factors that act upon the physical interactions between yeast cells (Fig. 2). Theoretically, by adapting these factors, brewers may be able to gain control over the flocculation behaviour of their yeast. However, in practice things appear to be quite complicated.

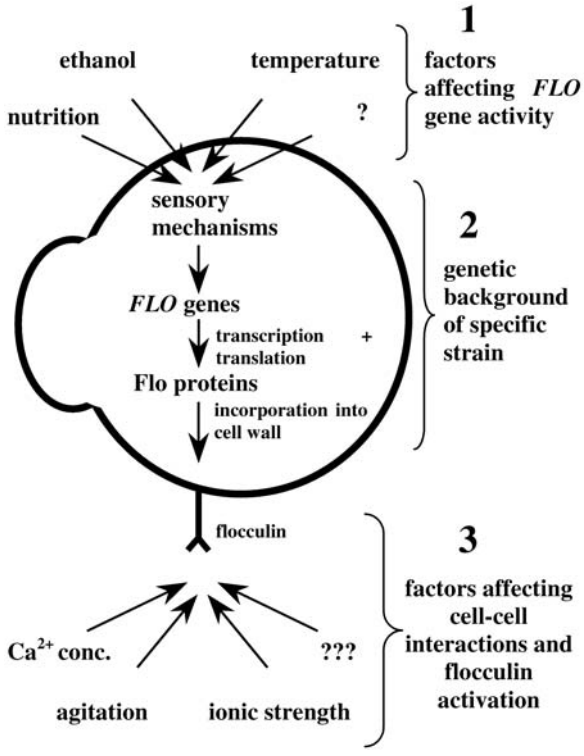

Fig. 2 Factors affecting flocculation. Three categories of factors can be distinguished according to their mode of action. Of course, some factors act through more than one mechanism

Factors that affect flocculation: a practical viewpoint

Many studies have focussed on different factors that may affect flocculation. However, the conclusions are complex and in some cases even unclear. In addition, different studies often lead to contradictions, indicating that the flocculation behaviour is highly strain-specific and depends on multiple factors. While some yeast strains show constitutive flocculation and other strains are completely non-flocculent under all circumstances, most brewer's yeasts flocculate under specific conditions (Dengis and Rouxhet 1997; Stratford 1992).

Nutrients and growth factors

It is generally accepted that flocculation in brewer's yeast is induced by nutrient starvation and/or stress conditions (Stratford 1992). Of course, in the case of NewFlo yeast strains, the absence of glucose, sucrose and maltose in the growth medium is an absolute prerequisite for flocculation, as these sugars block the NewFlo flocculin binding sites and thus inhibit flocculation. Nutrient starvation may also directly induce *FLO* genes in both NewFlo- and Flo1-type strains. It has been reported that *FLO1* is repressed by the Tup1-Ssn6 general corepressor complex (Fleming and Pennings 2001; Lipke and Hull-Pillsbury 1984; Smit et al. 1992; Smith and Johnson 2000; Stratford 1992; Teunissen et al. 1993a, 1995). The Tup1 and Ssn6 proteins are known to be involved in glucose repression in a wide variety of eukaryotes, including yeast (for a review see Smith and Johnson 2000). In addition, the promoter region of *FLO1* contains a putative GCN4-box at position 268 (Teunissen et al. 1993a). This sequence may repress *FLO1* expression under high nitrogen conditions. Several authors have indeed found that flocculation is triggered by carbon and/or nitrogen starvation and that addition of these compounds to the growth medium delays flocculation (Barton et al. 1997; Smit et al. 1992; Soares and Mota 1996; Soares et al. 1994; Stratford 1992). Therefore, it might be possible to adapt the time of flocculation onset by changing the wort carbon and/or nitrogen content. This hypothesis is contradicted by Kempers et al. (1991) and Straver et al. (1993), who showed that supplementation of wort with amino acids (up to twice the concentration normally found in wort) and maltose did not change the flocculation behaviour of the lager strain tested. However, these authors also remarked that, apart from the flocculation, yeast growth rate and maximal cell mass were also unaffected by these supplementations. This may indicate that under the specific conditions used, nitrogen levels in the unsupplemented wort were already relatively high so that extra nitrogen addition had no effect. Apart from nitrogen addition, the supplementation of glucose to the fermenting wort may also delay early flocculation. But of course, high glucose levels – especially at the end of fermentations – are unacceptable as this will most likely inhibit further maltose uptake and thus cause incomplete attenuation.

Apart from carbon and nitrogen addition, Straver et al. (1993) also tested the influence of vitamin and trace elements, but again no significant changes in flocculation behaviour were detected.

Oxygen content

In the case of NewFlo strains, flocculation onset often coincides with the arrest of cell growth. It was found that, upon pitching, cells rapidly lose their flocculation ability during the lag phase. No flocculation can be monitored during the exponential growth phase, but as soon as the cells stop dividing, flocculence gradually reappears (Soares and Mota 1996; Straver et al. 1993). As the oxygen content of the pitching wort is a major determinant of cell growth, Straver et al. (1993) investigated whether the timepoint at which cells start to flocculate could be altered by modifying the initial wort oxygen content. It was found that poor wort aeration resulted in early and incomplete flocculation, while normal saturation with oxygen both delayed and intensified flocculation. Remarkably, the poor growth and flocculation characteristics of yeast grown in de-aerated medium could be restored by addition of ergosterol and oleic acid to the medium. This indicates that oxygen probably does not act directly on flocculation, but rather indirectly through its importance for the synthesis of unsaturated fatty acids and sterols (Straver et al. 1993).

Temperature and pH

Temperature and pH have long since been recognised as important factors for yeast flocculation. Before it became clear that flocculation is the result of the binding of flocculins to cell-wall mannans, it was believed that high acidity caused a lowering of the negative cell-surface charge, so that the electrostatic repulsion between cells disappeared, thereby allowing cell-cell contact and flocculation (for a review, see Stratford 1992). More recent studies showed that flocculation can occur between pH 1.5 and 9, clearly indicating that pH is not the dominant factor causing flocculation. This does not mean that pH is of no importance whatsoever. Indeed, it has been shown that yeast flocculation is optimal in slightly acid conditions, with pH values ranging from 3.5 to 5.8 (Jin and Speers 2000; Jin et al. 2001; Soares et al. 1994; Stratford 1992). Surprisingly, some strains tend to flocculate better at higher pH values, indicating that the influence of pH on flocculation is more complicated than the lowering of cell-surface charge. It has been suggested that the flocculins may be inactive at certain pH values due to conformational changes that occur when the electrostatic charge of surface proteins changes (Jin and Speers 2000; Jin et al. 2001; Soares et al. 1994). Another possible explanation for the induction of flocculation by changes in the pH is that the pH of the medium might directly influence FLO gene activity. However, the pH optimum for flocculation seems to be highly strain-dependent, so that no general conclusions can be drawn.

As with pH, the influence of culture temperature on flocculation is rather ambiguous. Some reports state that there is little or no effect of temperature on the flocculation behaviour as long as the temperature remains within the physiological range (15–32°C) (Stratford 1992). At higher temperatures (>60°C), yeast flocs are dispersed, a phenomenon of little practical importance known as "floc melting".

However, numerous other studies indicate that the flocculation behaviour of industrial yeast strains varies markedly with temperature. Jin et al. (Jin and Speers 2000; Jin et al. 2001) found that flocculation of the NewFlo lager strain LCC125 varied between 24.1% at 5°C to 66.8% at 25°C. Other research confirmed that the flocculation of lager strains is optimal above 10°C, and decreases dramatically below 5°C (Gonzales et al. 1996). In other cases, flocculation is repressed at 25°C, and cells sediment optimally at lower (5°C) temperatures (Garsoux et al. 1993; Stratford 1992). These contradicting results clearly indicate the strain-specificity of flocculation, as well as the importance of secondary factors that are not always known or controllable.

Ethanol content

It has been reported that ethanol induces and/or enhances flocculation. On the other hand, Kamada and Murata found that ethanol inhibits flocculation (Kamada and Murata 1984), indicating that the influence of ethanol is strain-dependent, as was indeed proven by D'Hautcourt and Smart (1999). The mechanisms through which ethanol exerts its influence on flocculation are still unclear, although it has been suggested that ethanol may act upon cell wall conformation and surface charge (Jin and Speers 2000). In addition, Jin et al. (2001) reported a slight increase of cell-surface hydrophobicity with increasing ethanol concentrations. Another possibility is that stress factors such as high ethanol concentrations may induce the *FLO* genes through the numerous stress-responsive heat-shock elements that are found in the promoter region of these genes (Teunissen et al. 1993a).

Cellular size and age

Genealogically older yeast cells (cells that have produced a number of daughter cells) tend to flocculate earlier and more intensely than their younger counterparts. There are several reasons for this difference. Firstly, young "virgin" daughter cells do not have flocculins in their cell walls (Soares and Mota 1996). Secondly, and more importantly, genealogically older cells tend to be larger than younger cells, and their cell walls are more hydrophobic and "wrinkled" than those of young cells (Barker and Smart 1996; Jin et al. 2001; Powell et al. 2000; Smart 1999). The wrinkled surfaces may facilitate cell-to-cell adhesion and thus favour flocculation (Barker and Smart 1996). Additionally, older cells seem to display an increased resistance to mechanical separation of mother and daughter cells, so that mother and daughter often stay attached to each other for a longer time. These linked cells possibly provide nuclei for floc formation, explaining why older yeast populations show increased flocculation (Barker and Smart 1996).

Another aspect related to cellular size (and thus genealogical age) is cell sedimentation. Indeed, even when no flocculation occurs, yeast cells gradually sediment in the growth medium. This is because the cellular size and density of yeast cells prevents them from staying suspended due to Brownian motion (Stratford 1992). Of course, the sedimentation rate is very slow, especially when the medium is agitated, for example by gas bubbles formed during fermentation. The sedimentation rate is also dependent on the particle size; according to Stoke's law, larger particles sediment more rapidly (which is why yeast flocs sediment very fast compared to single cells). But this also implies that larger yeast cells will sediment faster than smaller cells. Therefore, older yeast cells will sediment more rapidly from the medium than younger, smaller cells (Barker and Smart 1996; Stratford 1992). While this does not have an important direct effect on brewery fermentations, there are some indirect consequences related to the difference in flocculence and sedimentation rate of older yeast cells (see below).

Yeast handling and pitching

It is known that yeast handling (pitching, cropping and storing, and the repetition thereof) can have pronounced effects on the flocculation profile. The most important factor is probably yeast storage, as the conditions in which yeast slurries are stored between consecutive fermentations influence the physiological state, overall fermentation performance and flocculation behaviour. Rhymes and Smart (2001) found that storage of the ale yeast NCYC2593 at high temperatures (25°C) and under regular agitation (shaking at 120 rpm) resulted in a significant increase in flocculation percentage. This increase in flocculence was independent of yeast starvation during storage, but when the yeast was not agitated during storage, the effect of storage at 25°C on flocculation was negligible. Storage at lower temperatures (4°C or 10°C) resulted in a reduced flocculation, independently of yeast agitation and starvation. Again, the effects of storage on yeast flocculation were highly strain-specific (Rhymes and Smart 2001). In addition to storage conditions, any treatment prior to pitching can also affect flocculation. For example, it has been shown that intensive acid washing may in some cases reduce flocculence, probably due to changes in the yeast cell wall. Strain-specific differences in cell-surface hydrophobicity, charge and conformation as a consequence of acid washing have indeed been monitored (Wilcocks and Smart 1995).

In addition to yeast storage and treatment, the specific manner of cropping can also have a significant effect on flocculation, especially when the yeast is cropped from the bottom of the fermenter, as is the case in most of today's breweries. The yeast sediment from which the cropping is made at the end of fermentation is not homogenous: older and/or more flocculent cells will sediment earlier, resulting in an enrichment of these cells near the bottom and middle part of the cone. Similarly, young and/or non-flocculent and weakly flocculent cells will be found mostly in the top layers of the yeast sediment (Deans et al. 1997). Therefore, serial cropping and repitching of discrete layers from such a yeast sediment may lead to selection for more flocculent and older cells, or for non-flocculent and younger cells. In practice, when more flocculent cells are needed, the yeast in the mid-part of the crop should be cropped for subsequent pitching (Quain et al. 2001). A rational approach to cropping may therefore offer an easy way to manage yeast flocculation in consecutive fermentations.

Jin et al. (Jin and Speers 2000; Jin et al. 2001) found that the pitching rate significantly affected the flocculation rate of the LCC125 NewFlo-type ale yeast; the flocculation rate gradually increased from 58% to 71% when the pitching concentration was changed from 1.5 to 15 million cells/ml. Of course, as the pitching rate has severe effects on fermentation speed and beer quality, in practice initial cell concentrations are normally limited to between 8 and 20 million cells/ml (equivalent to 0.5–1 kg yeast slurry/hl). Therefore, varying the pitching rate may only offer a very limited way to change the flocculation behaviour.

Generation number

In addition to the pitching rate, the number of serial cropping, storage and repitching cycles (corresponding to the so-called "generation number") also has an influence on flocculation. It was reported that in the case of an ale strain, flocculence shifted from 50% in the first generation to 100% after 9 consecutive cropping and repitching cycles. Flocculence then stayed constant for 14 generations, after which the flocculation behaviour became very unstable, with flocculence ranging between 0 and 82% (Smart and Whisker 1996). Similar trends were monitored for a lager yeast (Texeira et al. 1991). The reason for the variation in flocculation profiles is still unclear, but it has been suggested that physiological stress may be responsible for changes in properties of the yeast cell wall (Smart and Whisker 1996). In addition, prolonged cultivation may also lead to genotypic variability causing changes in the genetically determined flocculation profile of a particular strain.

In this context, it must be stressed that many flocculation genes are particularly unstable. The *FLO* genes show exceptionally high mutation frequencies, probably due to their numerous internal sequence repeats and their chromosomal position near the telomeres – known to be a hot-spot for genetic recombination (Sato et al. 2001, 2002; Verhasselt and Volckaert 1997; Watari et al. 1999). Different research groups have reported that genetic alterations in the *FLO1*, *Lg-FLO1* and *FLO5* genes occur at unusually high frequencies in both haploid laboratory strains and commercial brewer's yeasts (Jibiki et al. 2001; Sato et al. 2001, 2002; Watari et al. 1999; A. Teunissen, personal communication). A long-term study of brewing yeasts used in production plants revealed that, among the many properties of yeast that are relevant for beer brewing, flocculation is the most variable. For this study, 22 production strains derived from one common ancestor strain, but used for production in different plants, were analysed and compared to the analysis of the parental strain kept at −70°C. The strains in the different plants had been used for periods ranging from 1 to 18 years, in standard storage conditions and with frequent propagation. After analysis, it was shown that 10 of the 22 strains showed a severe reduction of flocculence, which could be linked to genetic alterations (Sato et al. 2001).

As the most common mutations in the *FLO* genes are complete or partial deletions, genetic alterations usually result in decreased flocculation. Apparently, the common industrial fermentation process selects for low-flocculent yeast, so that the low-flocculent mutant strain soon outgrows the parental flocculent cells (Gilliand 1978; Heggart et al. 1999; Sato et al. 2001, 2002; Watari et al. 1999). While variations in flocculence caused by physiological and environmental factors are usually reversible,

genetic alterations are not. It is therefore advisable to store the original production strain in glycerol at −70°C (ultrafreezer) or −196°C (liquid nitrogen) so that the original, flocculent strain can be propagated whenever the current production strain fails to flocculate sufficiently.

Premature flocculation caused by barley compounds

Several authors have reported that fermentation of wort produced from certain malt batches leads to premature yeast flocculation. This premature flocculation is caused by the presence of the so-called "premature yeast flocculation-inducing factor" (PYF), a complex and very stable carbohydrate or protein fraction that is extracted from malt husks during the brewing process (Herrera and Axcell 1989; 1991a, 1991b; Nakamura et al. 1997). The biochemical background of the formation and mode of action of PYF is not yet fully understood. However, it has been suggested that PYF is produced by barley grains as a response to microbial growth during the steeping process. It is therefore speculated that this factor may belong to a class of antimicrobial agents and that its formation can be minimised by reducing the numbers of certain micro-organisms during malting (Axcell et al. 2000). A useful predictive test that allows PYF-containing barley batches to be identified was described by Nakamura et al. (1997).

Measuring and predicting flocculation

Numerous methods have been described to measure flocculation. Flocculence can be measured on the basis of four different criteria: bond strength, morphology, extent of sedimentation and rate of sedimentation (Stratford and Keenan 1988). Bond strength can be estimated after deflocculation by mannose addition (Eddy 1955), thermal deflocculation (Taylor and Orton 1978) or by the so-called "critical cell density method" (Miki et al. 1982). Floc morphology is not suitable for quantification, but yeast floc morphology and floc size can be used to assess flocculation (Gilliand 1951; Johnston and Reader 1983; Stewart and Russel 1986; Stratford 1992).

However, the majority of current protocols (including the method recommended by the ASBC) to quantify flocculation are based on the Helm's sedimentation test (Bendiak et al. 1996; Helm et al. 1953). These tests are based on counts of free cells in a flocculating culture, which are compared to the total cell number (before flocculation or after deflocculation). The flocculation percentage is then given by the formula: [1−(free cells/total cells)] x100%. In many cases, the time course of flocculation is also monitored, so that both the extent and rate of flocculation are measured in one test. Of the many different variations and improvements of the sedimentation test, that described by D'Hautcourt and Smart (1999) is especially interesting because this method is optimised for NewFlo brewing yeasts in beer medium. Of course, the different variations of the Helm's test can generate (slightly) different results. In most cases however, extremely flocculent laboratory strains (Flo1 phenotype) will show 90–100% flocculation, while most industrial brewer's yeasts (NewFlo type) show flocculation in the range of 40–90% toward the end of fermentation. The common nonflocculent laboratory yeast strains exhibit Helm's flocculation rates between 0 and 15%.

A very interesting new method of predicting the flocculation properties of lager yeasts was described recently by Jibiki et al. (2001). These authors have developed a method based on PCR (polymerase chain reaction) amplification of the *FLO5* gene. It was found that the length of the PCR product correlates with the flocculence of the strain (or subclone). The method has been used successfully for the early detection of non-flocculating mutants of some 30 different lager yeast strains. However, it has not been extensively tested in different industrial brewing plants, so its practical usefulness remains uncertain. Another new procedure to screen yeast for flocculation performance was proposed by Mochaba et al. (2001). These authors suggest that the sensitivity of yeast flocs to the plant lectin concanavalin A may be a good predictive assay to select yeast slurries with suitable flocculation characteristics.

Genetic engineering

Since expression of the dominant flocculation genes *FLO1*, *FLO5*, *FLO9* and *Lg-FLO1* results in strong flocculation, the controlled expression of these genes may allow controlled flocculation. The first attempt to alter and control yeast flocculation through genetic modification was described by Barney et al. (1980), who introduced a large piece of chromosomal DNA derived from a flocculent strain, including the *ADE1* and *FLO1* genes, into a non-flocculent strain. The mutants showed constitutive flocculation and were therefore unsuitable for brewing purposes. Later, other more sophisticated methods using yeast plasmids were applied to introduce the *FLO1* gene, under the control of the constitutive *ADH1* promoter, into a nonflocculent strain (Ishida-Fujii et al. 1998; Watari et al. 1990, 1994a). However, as this again resulted in a constitutive flocculation phenotype, the transformed yeast strains were of no practical interest for the brewing industry. Verstrepen et al. (1999; 2001b) described the transformation of a non-flocculating yeast strain that brings the chromosomal *FLO1* gene under transcriptional control of the late-fermentation *HSP30* promoter. The transformed yeast cells showed a stable, strong flocculation phenotype toward the end of fermentation, thereby demonstrating that genetic modification can indeed be used to adapt the flocculation behaviour of weakly flocculent yeast. Moreover, as a special strategy was used to avoid the introduction of any foreign, non-yeast DNA, the transformants were in fact self-cloning strains rather than true genetically modified organisms. Self-cloning organisms are not restricted by some of the laws and guidelines regulating the use of genetically

modified organisms, and it is therefore expected that self-cloning strains may offer an easier way to obtain permission for industrial use. In the future, genetic modification may offer a valuable method to cure imperfect flocculation profiles of some brewer's strains. However, at present, genetically modified organisms are not yet accepted by the general public, so that industrial application still remains difficult (Hammond 1991, 1995; Pretorius 2000; Verstrepen et al. 2001a).

Conclusions

Flocculation is certainly one of the most intriguing and industrially important characteristics of brewer's yeast. However, the complexity and the highly strain-dependent character of flocculation makes it difficult to control the process. Of the many parameters that are known to influence flocculation, and thus offer possibilities to steer yeast sedimentation, only a few can be readily used in industrial brewing practice. Parameters such as wort sugar and oxygen content, fermentation temperature, ethanol concentration and pitching rate can be changed only slightly as they not only have an effect on flocculation, but also on other fermentation characteristics and thus on beer quality. Of course, this does not mean that they cannot be useful in certain cases. Genetic engineering may provide the ultimate way to fit the yeast properties to the brewer's demands. But as (a minority of) the public still is rather suspicious about the use of genetically modified organisms in the food industry, the large-scale implementation of genetically modified yeasts in the brewery is not yet possible.

Today, the most promising manner in which to control yeast flocculation may lie in carefully thought out yeast handling and management. Optimal yeast cropping and storage between successive fermentations may prove the key to flocculation control. However, the most important issue to keep in mind is the extremely high genetic instability of flocculation properties. It is therefore extremely important to store the original production strain at temperatures preventing genetic alterations. When the production strain shows an unwanted and irreversible decrease in flocculence, the most convenient way to overcome the problem may be to propagate a new yeast batch starting from the optimally stored master strain.

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