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REVIEW

Malt-induced premature yeast flocculation: current perspectives

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Abstract Premature yeast flocculation (PYF) is a sporadic problem for the malting and brewing industries which can have significant financial and logistical implications. The condition is characterised by abnormally heavy (and sometimes early) flocculation of yeast during brewery fermentations. The resulting low suspended yeast cell counts towards the end of the fermentation can result in flavour defects and incomplete attenuation (fermentation of sugars to alcohol). Despite several decades of research into the phenomenon, its precise nature and mechanisms have not been fully elucidated. In part this is because the term PYF has become a 'catch-all' syndrome which can have multiple origins. Furthermore, there are complex interactions in the malting and brewing processes which together mean that the PYF status of a malt sample is hard to predict at a generic level. Whether or not PYF is observed depends not only on barley quality, but on process factors in the maltings and to a substantial extent on the brewing yeast strain concerned. This article highlights the significance of PYF, and reviews current knowledge relating to the origins of this complex phenomenon.

Keywords PYF · Brewing · Fermentation performance · Yeast flocculation · Malt quality

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Abbreviations

PYF	Premature yeast flocculation
PYF+	A premature yeast flocculation positive sample (barley, malt or wort giving rise to PYF)
PYF−	A premature yeast flocculation negative sample (control barley, malt or wort sample yielding normal flocculation characteristics)
ns-LTP	Non-specific lipid transfer protein
T-RFLP	Terminal restriction fragment length polymorphism

Introduction

Premature yeast flocculation (PYF), its origins, detection and impacts for the brewing industry form the primary focus of this review. However, in order to discuss abnormal flocculation it is first necessary to consider current knowledge related to the flocculation of brewing yeast strains.

Yeast flocculation

Yeast flocculation is a reversible, asexual and calcium-dependent process in which cells adhere to one another to form flocs [7, 13, 61, 66]. Lager yeasts (*Saccharomyces pastorianus*), which account for the majority of modern beer production, separate from the fermenting medium by sedimentation, whilst ale yeasts (*Saccharomyces cerevisiae*) rise to the surface of open or dish bottom fermentation vessels by coalescing around gas bubbles [9, 65, 66, 78]. Flocculation is of considerable importance to the brewer as it provides an effective, environmentally friendly, simple and cost-free way to separate yeast cells from green beer at the end of fermentation [57, 78]. Brewing yeast disperses,

replicates, ferments as single cells and then flocculates rapidly following the depletion of nutrients (sugars in particular) in the wort [62]. Early or premature flocculation leaves unattenuated sweet beer, whilst late or poor flocculation requires yeast cells to be removed by fining, filtration or centrifugation [9, 62, 65], which are time-consuming and expensive procedures [13]. Consequently, the timing of flocculation is an important factor influencing finished beer quality [3]. The flocculation characteristics of yeast strains are of major significance in brewing [9, 47, 59, 78] as the number of suspended yeast cells in wort during both primary and secondary fermentation affects the speed of fermentation, flavour formation, maturation and filtration [18, 20, 59]. A fit for purpose yeast for the modern brewing industry should therefore exhibit strong flocculation characteristics towards the end of fermentation [78]. The efficiency of flocculation is determined by the timing of flocculation onset as well as by the rate of flocculation in conjunction with the ratio of flocculent to non-flocculent cells [63, 64]. Flocculation, usually a property of the late exponential or stationary phase [37], is under genetic control [22, 49, 63]. Although desirable, flocculation is therefore a complex process strongly influenced by the expression of specific genes, including *FLO* genes, cell wall protein genes (*CWP*, *TIR* and *DAN* genes), and mitochondrial genes [65, 67, 78]. The *FLO* family includes 12 genes, 5 of which have been recognized as dominant zymolectin-encoding (structural) genes (*FLO1*, *FLO5*, *FLO9*, *FLO10* and *FLO11*) [9]. *FLO1* is a dominant gene situated at the right arm of the chromosome 1 [78], whilst *FLO5* and *FLO9* are highly homologous to *FLO1* [48, 51]. *FLO8*, originally reported as a structural gene, is currently identified as a transcriptional activator of *FLO1* and *FLO11* [32, 73, 78], whilst *FLO2* and *FLO4* are allelic (copies) to *FLO1*, *flo3* is semi-dominant, and *flo6* and *flo7* are respectively recessive to *FLO1* [73].

Yeast flocculation mechanism

Numerous hypotheses have been proposed to explain the mechanism of flocculation in *Saccharomyces cerevisiae* [57]. These include the early colloidal theory [28], the calcium-bridging theory [37] and the lectin-like theory [35]. The early colloidal theory was based on the assumption that in aqueous solution cells behave as negatively charged colloids [28]. The observation that inorganic salts promoted yeast flocculation was explained as surface-charge neutralization leading to aggregation and sedimentation of the cells. However, the specific requirement by most yeast strains for calcium in floc formation discredited the colloidal theory and led to the bridging hypothesis. According to this theory, calcium ions (Ca^{2+}) linked adjacent yeast cells by coupling to carboxyl groups [37]. As the inhibition of flocculation by specific wort sugars (i.e. mannose) could not be explained by this theory, Miki et al. [35] proposed the lectin-like theory of flocculation. According to this theory [35] yeast flocculation occurs when the α-mannan residues of mannoproteins interact with lectin-like proteins of adjacent cells forming large aggregates or flocs. More specifically, the N-terminal part of the lectin-like proteins bind the mannose chains (receptors) that are present in the cell walls of flocculent and non-flocculent neighbouring cells [7, 24, 49, 57, 67–78]. In this adhesion process, calcium ions are thought to ensure the correct conformation of these lectins [34, 35, 66, 72, 78].

The lectin-like proteins (flocculins), which specifically bind sugars and are present only in flocculent cells [57], are synthesized by yeast in preparation for flocculation and are located on the external surface of the yeast cell. Conversely, the mannan residues (polysaccharides of D-mannose) [23], are always present on the yeast cell wall [62]. Since the mannose residues are always present in the cell wall of both flocculent and non-flocculent cells [7, 62], a critical flocculation-determining factor is clearly the presence or absence of flocculins [78]. Stratford [65] proposed that flocculation takes place when the *FLO* genes become active and the flocculins are formed. The same author [65] suggested that after growth limitation, yeast cells become fimbriated which corresponds with a sharp increase in cell surface hydrophobicity. The increase in a cell's surface hydrophobicity results in the release of agglutinin which gives rise to fimbriae-associated glutin ligands, and finally in the formation of flocs. If agitation is applied, removal and redistribution of the fimbriae may lead to more compact flocs.

Onset of flocculation

Flocculation in brewer's yeast is stimulated by nutrient starvation and/or stress conditions [53, 65, 68, 69]. Yeast flocculation occurs when the sugars in the wort have been exhausted [54], probably because prior to that, sugars (e.g. mannose) occupy the flocculin binding sites so that they can no longer bind to the mannose residues of other cells [78]. Stratford [65] and Verstrepen et al. [78] indicated that the presence of mannose and derivatives in wort inhibits flocculation, particularly with regard to the Flo1 phenotype (which accounts for the majority of lab strains and include strains containing *FLO1*, *FLO4*, *FLO5*, *FLO8* and *TUP1* genes) due to its ability to block the flocculin binding sites of the cells [33]. In contrast, efficient flocculation of yeast strains exhibiting the NewFlo phenotype, often associated with brewer's yeast [57, 78], requires the absence of mannose as well as glucose, sucrose and maltose [65, 78]. Flo1 phenotype strains are constitutively flocculent,

producing a flocculin protein that appears to be associated with fimbriae-like structures but is not an integral part of them [3], whilst brewing yeasts belonging to the NewFlo phenotype exhibit a cyclic behaviour and flocculate only in stationary phase [46, 56, 57, 60, 62]. MI (mannose insensitive flocculation) yeast strains, the third category of flocculent yeast cells, are insensitive to mannose [33, 60]. The MI phenotype is characterised by an apparent lack of binding specificity for mannose, preventing flocculation inhibition on mannose [8, 33, 39]. Recent publications [6, 12] have reported the characteristics of Flo11-dependent flocculation in wine strains of *Saccharomyces cerevisiae*. The role of Flo11 in the flocculation of lager brewing strains remains to be elucidated.

Jin and Speers [20] indicated that sugars like galactose and fructose do not inhibit flocculation, whilst Straver et al. [69, 70] suggested that there are cases where flocculation is not solely dependent on the presence of flocculins, but in addition requires agglutinins and/or fimbriae-like structures. Miki et al. [36] also reported that concanavalin A, treatment with proteinase K, and reduction of disulfide bonds from mercaptoethanol were found to inhibit flocculation.

Factors which influence flocculation of commercial yeast strains

During a particular industrial fermentation process, flocculation can be affected by multiple parameters. For a given strain, flocculation depends on a combination of four main factors: (1) genetic background (presence of flocculation [*FLO*] genes and their regulatory elements), (2) wort nutritional status (in particular the content and profiles of sugars, free amino nitrogen (FAN) and divalent cations), (3) environmental conditions (temperature, presence of alcohol, pH, dissolved oxygen, osmotic pressure and shearing forces) and (4) physiological state of cells (cell surface hydrophobicity, vitality, membrane integrity, starvation, generation number, etc.) [9, 57, 78]. A number of cellular and extracellular conditions have been shown to affect flocculation capacity including culture temperature, ethanol, specific nutrient limitation, wort composition and petite formation [31]. In the context of the current paper the primary focus is on bottom-fermenting strains of lager yeast (*Saccharomyces pastorianus*) both because these represent the vast majority of commercial beer production worldwide and because ale strains have been shown to be relatively insensitive to PYF [18]. Whilst there have been recent advances in our understanding of the genetic regulation of flocculation in *Saccharomyces cerevisiae* [55], in particular centred around the function of the five *FLO* genes in the sequenced laboratory strain S288C, knowledge related to commercial strains of *S. pastorianus* remains limited. The lack of stability of flocculation characteristics amongst brewing yeasts has been widely reported [52, 55]. The *FLO* genes have a notable capacity to evolve and diverge more rapidly than other genes, a fact which has been attributed to the existence of repetitive sequences in their coding regions. This led Van Mulders et al. [74] to suggest that industrial yeast strains may have their own personal reservoir of adhesin-encoding genes that differs from the *FLO* gene family in S288C. They concluded that the genetic variation between strains with regard to their flocculation genes hampers attempts to control flocculation behaviour in brewer's yeasts.

Premature yeast flocculation (PYF)

PYF is a sporadic, but serious problem in the brewing and malting industries [19, 23, 29, 43, 44]. It has been defined as the phenomenon whereby flocculation competent yeast settle out of the fermentation medium abnormally early and/or heavily during primary fermentation leaving a residual extract [27]. The early or premature flocculation of the yeast cells hampers complete fermentation [17, 65], and results in a poorly attenuated wort [2] and a final product with undesirable flavour characteristics [25–27, 29, 65]. The total diacetyl content of the beer will increase, resulting in beer with a detectable diacetyl flavour [17]. In many modern brewing processes, detectable diacetyl is regarded as a quality defect and commercial practice frequently involves a 'diacetyl stand' as a part of the fermentation/maturation process, whereby the diacetyl produced in primary fermentation is taken up and metabolised by yeast cells in suspension. PYF slows this process owing to the lower suspended cell counts.

Furthermore, PYF has been reported to increase susceptibility to microbial infections [21, 38], and gives rise to lower carbon dioxide evolution rates during fermentation and a final product with lower alcohol content and increased SO_2 [29]. Consequently, PYF results in financial losses to brewers [2], as the beer requires additional blending or processing and, in severe cases, disposal [29]. Axcell [3] suggested that in the incidence of PYF, brand identity may be compromised, potentially resulting in a negative consumer reaction.

The phenomenon is illustrated in Fig. 1 using data from PYF test fermentations conducted in our laboratories [44]. Besides the poorly attenuated wort (Fig. 1a) and the subsequent higher residual extract (Fig. 1b) and lower ethanol yield (Fig. 1c), PYF results in low end-of-fermentation cell counts which can cause problems with maturation processes that require green beer to be in contact with yeast. Since brewing yeast is cropped and re-pitched into

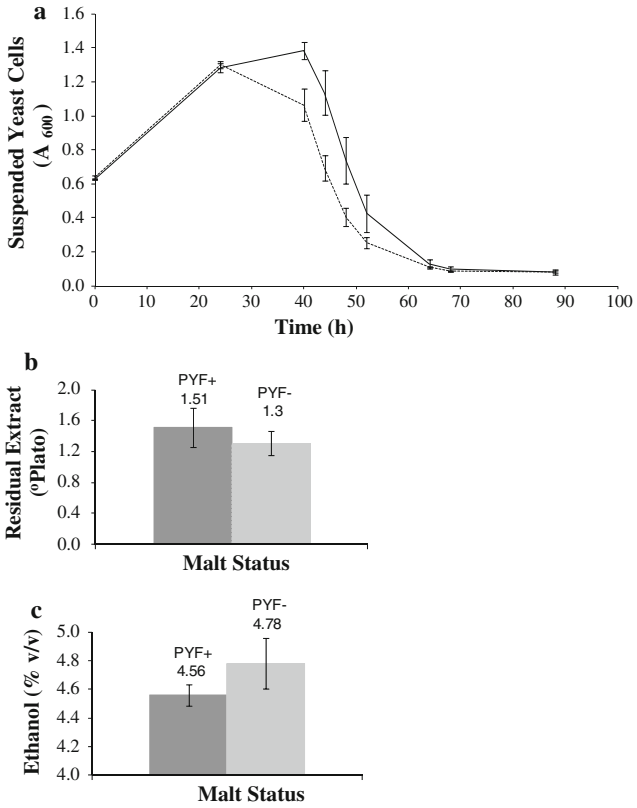

Fig. 1 Suspended yeast cell profiles (**a**), residual gravities (**b**) and ethanol yield (**c**) 88 h post-pitching of laboratory-scale (200 mL) PYF test fermentations utilising PYF+ (*dashed line*) and PYF− (*solid line*) worts. Fermentations were conducted at 15°C using SMA yeast at a pitching rate of 20 × 10^6 live cells mL^{-1}. PYF+ and PYF− data are the mean of three replicate fermentations ± SD

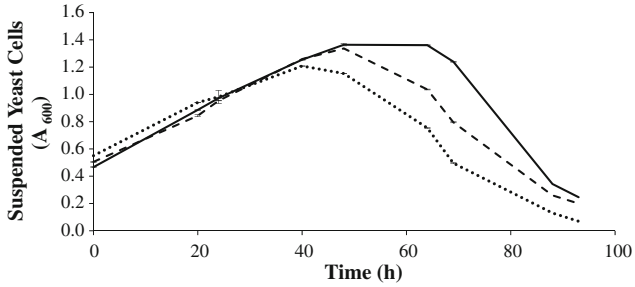

Fig. 2 Effect of re-pitching with yeast cells obtained from PYF+ fermentation. Fermentations (200 mL) were conducted at 15°C using W34/70 yeast at a pitching rate of 20 × 10^6 live cells mL^{-1}. Data are the mean of two replicate fermentations ± SD. *Dashed line* PYF+ wort, *solid line* PYF− wort, *dotted line* PYF− wort fermented with yeast cells cropped from a previous PYF+ fermentation

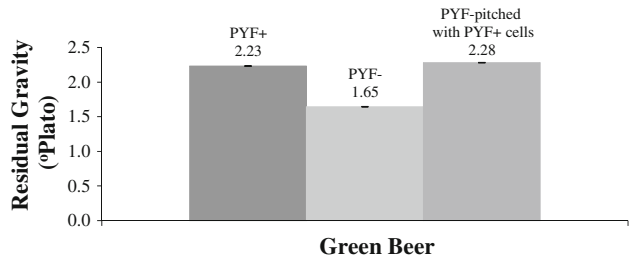

Fig. 3 Residual gravities 96 h post-pitching of laboratory-scale (200 mL) PYF test fermentations utilising PYF+ (*dashed line*) and PYF− (*solid line*) worts. Fermentations were conducted at 15°C using W34/70 yeast at a pitching rate of 20 × 10^6 live cells mL^{-1}. Data are the mean of two replicate determinations

subsequent fermentations it is also significant that exposure to PYF generates issues with the reuse of the yeast (Fig. 2; Panteloglou et al. [44]). In the example illustrated, PYF− wort was pitched with yeast cropped from a prior PYF+ fermentation. Premature flocculation was heavier in this instance than in the original PYF+ fermentation, suggesting that for this particular sample, exposure to the PYF factor had caused longer-term damage to the flocculation behaviour of the yeast. Besides the heavier flocculation, PYF− worts pitched with cells cropped from PYF+ fermentations also exhibited relatively high residual gravity 96 h post-pitching (Fig. 3; Panteloglou et al. [44]).

Origins of PYF

The periodic occurrence of PYF has been associated with certain harvests, years and regions of barley production [1]. PYF arises during brewery fermentations; however, the causative factor(s) have been shown to originate from the malted barley [15]. The link between the incidence of PYF and particular harvest conditions suggested the likely involvement of barley and malt microbes in PYF [2] and since it has been shown that surface washing of PYF+ malt can diminish the severity of PYF [19], the action of microbes on the barley husk has been a key focus of research. Van Nierop et al. [75] provided clear evidence of this linkage by showing that removal of the husk from PYF+ malts not only removed PYF but also delayed flocculation; in addition PYF activity could be induced by treating barley husk with fungal enzyme extracts.

Two main theories have been proposed to account for this phenomenon. These have largely been based upon the characterisation of purified extracts from PYF+ malts, which retain PYF activity, coupled with process knowledge and theories as to how the isolated factors might arise. Here we shall refer to these theories as (1) the bridging polysaccharide mechanism and (2) the antimicrobial peptide hypothesis.

Bridging polysaccharide mechanism of PYF

The isolation of purified fractions from malts which exhibit PYF activity has led to the identification of putative factors

which are acidic polysaccharide fractions of varying molecular size. The proposed mode of action is that the polysaccharides form cross links between developing yeast flocs, increasing their size and rate of sedimentation [25]. Herrera and Axcell [15] reported a factor which was larger than 100 kDa in size and which consisted of arabinose (27%) followed by xylose, mannose and galactose (16–17% each) and some glucose and rhamnose (12–14% each) together with an acidic sugar component. Herrera and Axcell [14] used immunogold electron microscopy to demonstrate that their isolated high molecular weight polysaccharide bound significantly to the surface of flocculent yeast cells grown in a PYF+ wort, thus giving direct evidence in support of the polysaccharide bridging hypothesis.

Koizumi [26, 27] purified a PYF factor by using yeast as an affinity column to concentrate the factor and then fractionating the extract using anion-exchange chromatography. The purified factor was composed mainly of arabinose, xylose and galactose, with rhamnose and galacturonic acid and was described as 'pectin-like' material [26]. The molecular weight of the active polysaccharide was estimated to be less than 40 kDa and interestingly it was shown that when the factor was digested, using commercial enzymes, PYF activity was retained in fractions with molecular weights less than 5 kDa. Concanavalin A affinity chromatography was used to identify the minimum digested unit that possessed PYF activity. Koizumi [27] studied the linkage structure of this fraction and concluded that it was a complex polysaccharide consisting of glucuronoarabinoxylan associated with arabinogalactan protein and rhamnogalacturonan I as seen in maize and rice seed. A putative structure for the factor and its concanavalin A binding site were reported.

From the data published from several authors, it appears that molecular weight of the proposed factor is less significant in determining PYF activity than is the presence of specific recognition factors or patterns of charge (glucuronic acid residues) which facilitate interaction with the developing yeast flocs. The molecular weight range of the active factors in a particular PYF+ sample would be anticipated to depend upon the specific cocktail of husk-degrading enzymes generated by a particular malt microflora; hence, it is not surprising that different molecular weight ranges of the bridging polysaccharide have been associated with PYF activity.

Antimicrobial peptide hypothesis of PYF

Although commonly referred to as the 'antimicrobial peptide' hypothesis [75, 76], the origin of such peptides implicated in PYF has never been categorically proven [29]. Barley in the field and/or in the maltings responds to microbial attack by producing basic peptides (i.e. thionins, defensins and non-specific lipid transfer proteins, [77]) with antimicrobial properties. Thionins, defensins and non-specific lipid transfer proteins are cationic antimicrobials which are relatively small (5–10 kDa), stable owing to multiple disulphide bridges and capable of persisting through both malting and the brewing process. Van Nierop et al. [75] proposed that the antimicrobial peptides are not only active against the barley microflora, but may also have anti-yeast activity. Amphipathic polypeptides are able to disrupt membrane integrity and function and may impair sugar uptake by yeast during industrial fermentations, thus contributing to abnormally high residual extract and problems with poor attenuation. Van Nierop et al. [76] reported a 96-well plate assay for anti-yeast activity based upon the inhibition of yeast growth on MYGP broth caused by extracts of malts. The assay could differentiate nine malt samples according to their anti-yeast activities and malts which were associated with PYF fermentations and/or gushing, a quality defect of finished beer long associated with poor microbial quality of barley, showed the highest anti-yeast activities. The extracts used in the study were shown to contain peptides, tentatively identified as α-thionin, LTP-1a and other ns-LTPs.

The original evidence of such anti-yeast activity as a component of PYF was reported by Okada et al. [40–42] who purified an anti-yeast basic peptide of molecular weight 9.8 kDa which caused yeast cell death in minutes at a concentration of 4 ppm. However, on tenfold dilution of the factor uptake of sugars was inhibited, but without lethal effect. Axcell et al. [2] speculated that the factor reported by Okada et al. [40–42] had properties reminiscent of a barley lipid transfer protein. They also proposed a mechanism whereby the antimicrobial peptide associated with yeast at the point of flocculation, due to the yeast cell surface negative charge, and linked via charge interaction to the acid polysaccharides implicated in type I PYF to form a bridging network. This hypothesis remains to be proven, but was based on the observation by Okada et al. [42] that it was mainly flocculent yeast that were susceptible to the anti-yeast factor and that sugar uptake in PYF+ fermentations was not markedly different from control fermentations prior to flocculation onset.

Stanislava [50] reported that thaumatin-like protein (TLP) and ns-LTP1 purified to homogeneity from malting barley inhibited brewer's yeast growth, fermentation and respiration. Aging yeast cells from a brewery were more susceptible to these factors than the same yeast strain propagated in the laboratory. It was commented that the anti-yeast activity of such antimicrobial compounds isolated from wort needed to be established because TLP becomes inactive against yeast due to thermal denaturation

and the properties of ns-LTP1 are changed, for example by Maillard and oxidative reactions during malting and brewing.

PYF: a 'catch-all' syndrome?

It is apparent that microbial quality of barley and malt is a factor in each of the two most accepted theories of PYF. Enzymes secreted by microorganisms growing on the barley husk can break down arabinoxylan and pectin-like husk materials to generate bridging polysaccharides, whilst their very presence may induce a stress response from barley and the generation of 'anti-yeast' antimicrobial peptides. If the two factors operate independently then it is clear that a range of interactions between the two are feasible, dependent on the actual enzymes being secreted and the severity of stress response mediated by specific microorganisms growing on barley. Furthermore, both factors are classes of compounds and not individual activities which can be clearly ascribed and/or prevented.

One of the problems with the PYF phenomenon has been that it is poorly defined [29]. In some incidences flocculation is both early and rapid. In other instances flocculation onset is normal but uncharacteristically heavy [5]. Sometimes fermentations become stuck at residual extract values as high as 6°P and must be re-pitched in order to attenuate. In other instances the main observed effect is the low suspended yeast cell counts, which give rise to maturation abnormalities, yet the difference in residual extract is relatively small as compared with control brews ('secondary PYF' as defined by Evans and Kaur [10]). Furthermore there are likely occasions when brewers categorise an abnormal fermentation profile as being PYF when the symptoms are not necessarily those of classic PYF and may be associated with poor quality worts and the depletion of specific nutrients. In a recent publication Patel et al. [45] argue that the exact mechanism of yeast flocculation is still a point of controversy in the scientific community and that changes in flocculation observed during industrial brewing were due to changes in yeast cell surface hydrophobicity. They noted that PYF fermentations resulted in yeast cells with a significantly lower zeta potential and proposed a physical mechanism for PYF involving electrostatic interaction between wort particles (positively charged towards the end of the fermentation) and negatively charged yeast.

Impact of yeast strain on incidence of PYF

One factor which has complicated the PYF discussion concerns the variable impact of this phenomenon on different yeast strains. Thus, a PYF+ malt can be dispatched in apparently identical conditions to two different breweries—one of which will experience severe PYF, whilst the other may observe no negative impacts whatsoever. Such discrepancies are largely attributed to varying susceptibilities of yeast strains to PYF; however, other process differences (e.g. in mashing, mash separation or wort boiling) upstream of fermentation may also impact on the severity of PYF observed in a particular brewing process. Jibiki et al. [19] assessed the sensitivities of six ale and nine lager yeasts to PYF using a laboratory fermentation test. They concluded that no ale yeast strains (whether flocculent or non-flocculent) were sensitive to PYF, whereas all of the lager strains tested exhibited a degree of sensitivity. Armstrong and Bendiak [1] commented on the apparent impact of yeast strain and concluded from retrospective analysis of brewery trend data (mainly with regards real extract of bright beer) that the more flocculent yeast strains involved definitely showed more susceptibility to PYF than the less-flocculent or non-flocculent strains. Evans and Kaur [10] observed that "it is the associations between the preferred yeast strains of the major brewing companies and PYF susceptibility which have resulted in problems for brewers such as Kirin, Asahi, SABMiller and Anheuser Busch (now AB InBev)".

Diagnostic tests for PYF

Standard malt analysis is unable to predict the 'hung' or 'stuck' fermentations synonymous with PYF [4, 17, 49], and therefore the principal way of predicting abnormal fermentations is the use of a small-scale fermentation test (e.g. the 'Kirin' test [11, 16, 17, 30, 38, 43, 44, 75]). These tests measure suspended yeast cell counts (e.g. Fig. 2a) and residual gravity (e.g. Fig. 2b), and depending on experimental conditions (i.e. yeast strain, pitching rate and fermentation temperature) take several days to be completed. However, Panteloglou et al. [43] suggested that monitoring suspended yeast cells, using either the absorbance at 600 nm or microscopic cell counting after a number of serial dilutions, is a more accurate predictor than residual gravity for predicting the PYF potential of malts.

The 'Asahi test' [19] is one such laboratory test which is widely used in industry. A 50-mL fermentation conducted at 21°C allows PYF+ malts to be distinguished from negative controls on the basis of suspended yeast cell counts after 2 days. Lake et al. [30] reported a miniaturised 'test-tube-based' fermentation assay using a 15-mL fermentation volume which successfully predicted the PYF status of malts in agreement with the Asahi test. One current drawback is that there is no standard method for a PYF laboratory fermentation assay, which makes results

from different research groups harder to compare. Selection of yeast strain is just one significant aspect which should be standardised. The lager yeast SMA is one strain which has been proposed as suitable, based upon its susceptibility to PYF [43, 44, 58]. Variability in the performance of malt samples in PYF tests can also arise because of a lack of homogeneity in samples submitted. Samples containing a high proportion of fines and husk material, through breakage, give more PYF+ test results than samples which have been aspirated to remove such material (Michael Voetz, VLB; personal communication).

Although they can distinguish between malts inducing PYF and malts exhibiting normal fermentation profiles, the diagnostic tests are time consuming and in some cases they do not predict the real performance of malt samples in the brewery. A positive result with the fermentability test does not necessarily translate into a problem in the brewery [3, 5]. Besides that, fermentation tests cannot determine the compounds causing PYF but rather can only indicate fermentation performance, which may not contain PYF-inducing factors [29]. However, the results obtained from the fermentability test can give useful information about potential problematic malts [3]. Koizumi and Ogawa [25] reported a rapid (3 h) assay which involved the extraction of barley or malt samples with water, precipitation of high molecular weight material with ethanol and then resuspension of the material in water. The PYF activity of such extracts was assayed using a suspension of late-logarithmically growing yeast cells in a cuvette, with the ratio $A_{600\ \mathrm{sample}}/A_{600\ \mathrm{water}}$ 3 min after resuspension of yeast being used as an index of PYF status. Results were correlated against a laboratory-scale fermentation test ($R^2 = 0.85$).

The implication of barley and malt microbes in PYF led Kaur et al. [23] to propose an assay based upon T-RFLP screening of microbial populations. A test set of 32 malt samples (including 18 PYF+ malts) were included in the study and microbial community fingerprint patterns were generated by T-RFLP analysis (based on 16S rRNA and 26/28S rRNA genes for bacterial and fungal communities respectively). The resultant data were analysed using multivariate statistical techniques and correlations sought between microbial strains and PYF status. Some fungal taxa were reported to be strongly associated with PYF+ assignments made using conventional fermentation tests.

Strategies for alleviation or prevention of PYF

Several studies have concluded that the PYF factor(s) are water extractable and consequently may be easily removed from the surface of the grain by simple washing [2, 5, 19]. Jibiki et al. [19] reported that surface washing and drying of malts led to a substantial improvement in the suspended yeast cell counts of PYF+ fermentations, although these were still only around 50% of the cell counts for the PYF− control. In agreement with this observation, Axcell et al. [2] proposed that wet milling of malt before mashing as well as the discarding of steep water may alleviate the problem. Where available, the use of a washing screw or washing drum in the maltings prior to steeping can clean the surface of the grain and reduce the microbial loading entering the malting process.

In addition to issues surrounding barley quality and surface washing of the grain, the PYF status of malts has been reported to be sensitive to process conditions in the maltings [5]. Irrespective of the origins of PYF this should not come as a surprise because the operational conditions employed in a maltings (e.g. process temperatures, airflows, hydrostatic pressures) have a strong influence on both microbial growth and the stress experienced by malting barley and its consequential stress response in the form of antimicrobial peptides. Axcell et al. [5] investigated a situation where the incidence of PYF was specific to the maltings at which South African barley (variety *Clipper*) was malted. By transferring samples between two maltings at various steps of the process it was ascertained that in this specific instance the problem originated in the steeping process at Caledon Maltings. It was hypothesized that high pump pressures during steep-out might trigger the leakage of a factor which might otherwise have remained in the kernel and been metabolised during germination. Walker et al. [79] commented on the significance in the maltings of maintaining aerobic conditions, particularly through adequate CO_2 extraction during air-rests, and through maintaining fresh (as opposed recirculated) air during germination.

On the basis of the observation that turbid worts (i.e. those with higher lipid content) offered some protection against PYF relative to the use of very bright worts, Axcell et al. [2] proposed that wort fatty acids might bind to the amphipathic antimicrobial peptides and effectively 'titrate' them out. In our research group we have been unable to reproduce this effect using 200-mL-scale laboratory fermentations which were prepared so as to be either deliberately turbid, or supplemented with additional fatty acids. In fact, when we supplemented worts with linoleic acid (6 ppm) the impact was to increase the rate of flocculation in PYF+ fermentations and thus to shorten the assay time required to differentiate between PYF+ and PYF− malts [41].

Other practical strategies available to the brewer faced with a consignment of PYF+ malt include the option to blend. Results presented by Jibiki et al. [19] indicated that blending of PYF+ wort with PYF− wort alleviated the severity of PYF in some instances and at low ratios of PYF+ malt (10 or 25%). It was an interesting feature of their results that ability to blend away the issue satisfactorily was highly dependent upon the specific PYF+

sample utilised. Nakamura [38] commented that where the practical blend ratio of PYF+ malt had been limited to 5%, the 'dead-stocks' of PYF+ malts at his brewery had swollen. As a practical measure to brew acceptable quality beer with higher blend ratios two steps were recommended. Firstly a protocol labelled 'green transfer' wherein brews based on over 50% PYF fermentation were mixed after 7 days of fermentation in a ratio of 3:1 with PYF− fermentation 3 days post-pitching. This protocol increased suspended yeast cell counts during maturation and eased problems with vicinal diketone (VDK) maturation. Secondly, an increase in fermentation temperature (from 10 to 12.5°C) was reported to improve assimilation of VDK and hence offer another potential practical strategy for brewing with higher proportions (40%) of PYF+ malt.

According to the bridging polysaccharide hypothesis the induction of PYF is associated with interactions between lectin-like proteins located on the yeast surface and part of the polysaccharide inducing PYF [11]. Thus, Axcell et al. [2] proposed that the rousing of the yeast cells and/or the increase of pitching rate might leave sufficient normal yeast cells to complete the fermentation. In this context it is interesting that Armstrong and Bendiak [1] noted, in their practical experiences of brewing with PYF+ malts in New Zealand, that the same malt which presented PYF in industrial-scale batch fermentations could perform normally in another brewery which operated a stirred continuous fermentation (Coutts') process. In the same paper it is stated that rousing of yeast after the incidence of PYF achieved nothing—the yeast appearing 'turned off' and no longer interested in the remaining fermentables! Whether this statement applies to all instances of PYF is not clear and may well depend upon the type of PYF encountered.

Sugihara et al. [71] reported the use of tannic acid to alleviate PYF issues in brewery fermentations. The mode of action was not related to wort clarity, but appeared to be linked to the ability of tannic acid to bind to the yeast cell surface during fermentation and thus disrupt flocculation. Addition rates of 25–100 mg L^{-1} were effective in increasing suspended yeast cell counts and lowering residual extract in fermentations using two PYF+ malts, each blended at 30% of grist. In addition, Axcell et al. [5] suggested that because of the risk involved, the purchase of malt from a supplier whose malt repeatedly gives poor ratings should be avoided wherever possible.

Conclusions and future prospects

PYF is a sporadic problem affecting brewery fermentations which can have a major impact on beer quality, ethanol yield and process logistics. Although it arises in the brewery, the causative factor(s) originate from malted barley. For that reason, PYF is of major importance both to the maltster and the brewer. Despite systematic investigations in recent decades, progress towards the effective detection and control of PYF has been hampered by the lack of a universal diagnostic method. Thus, the establishment of a universal and reliable test, using a common yeast strain, and the sharing of information and samples between industry and the various research labs are key goals in furthering our understanding of the mechanisms underlying PYF. Furthermore, developments in knowledge of the genetic and epigenetic regulation of flocculation in commercially relevant lager brewing strains should help to explain some apparent inconsistencies observed in the incidence of this phenomenon.

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